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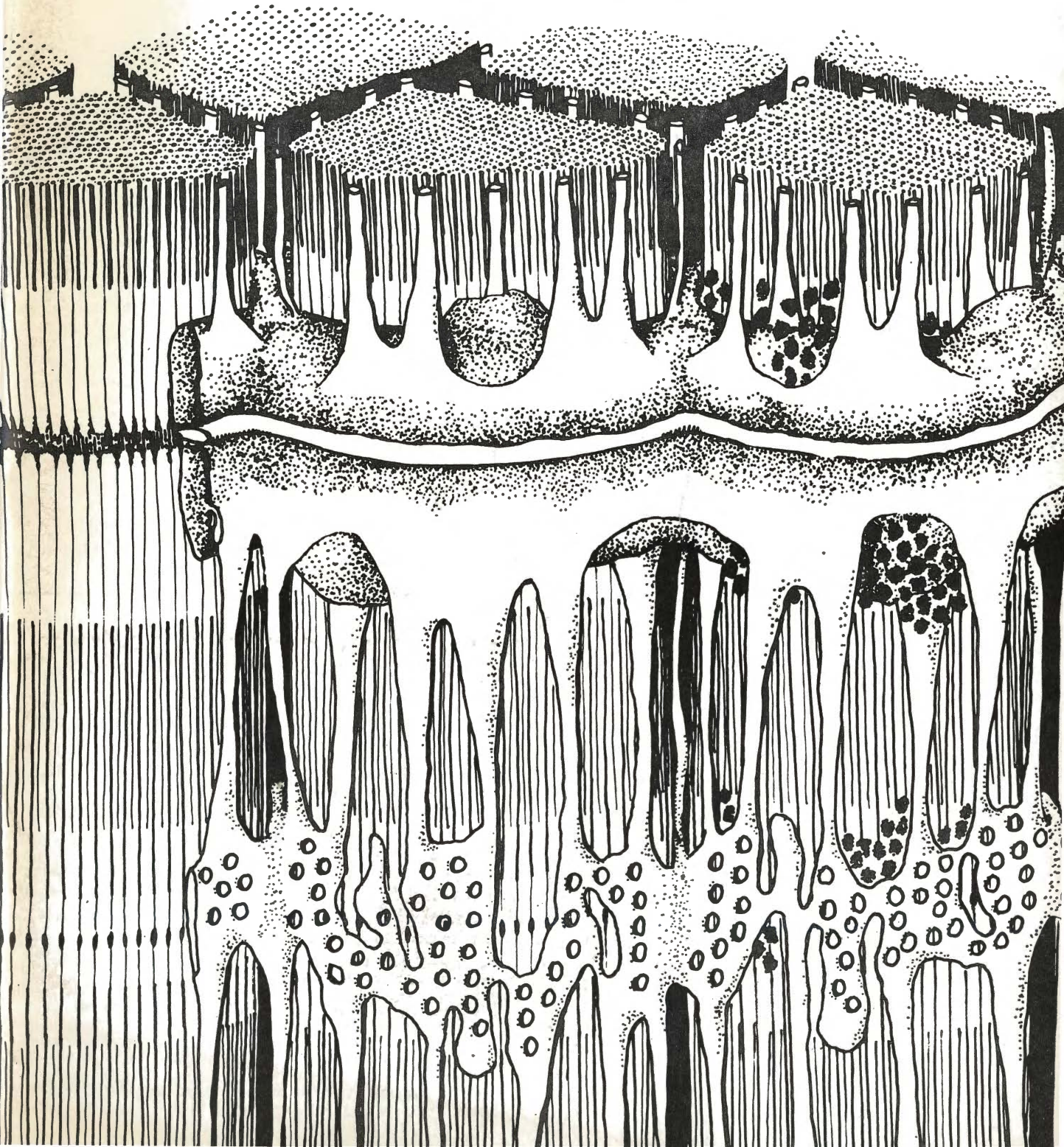
MEDICAL COLLEGE OF VIRGINIA QUARTERLY

SUMMER 1966



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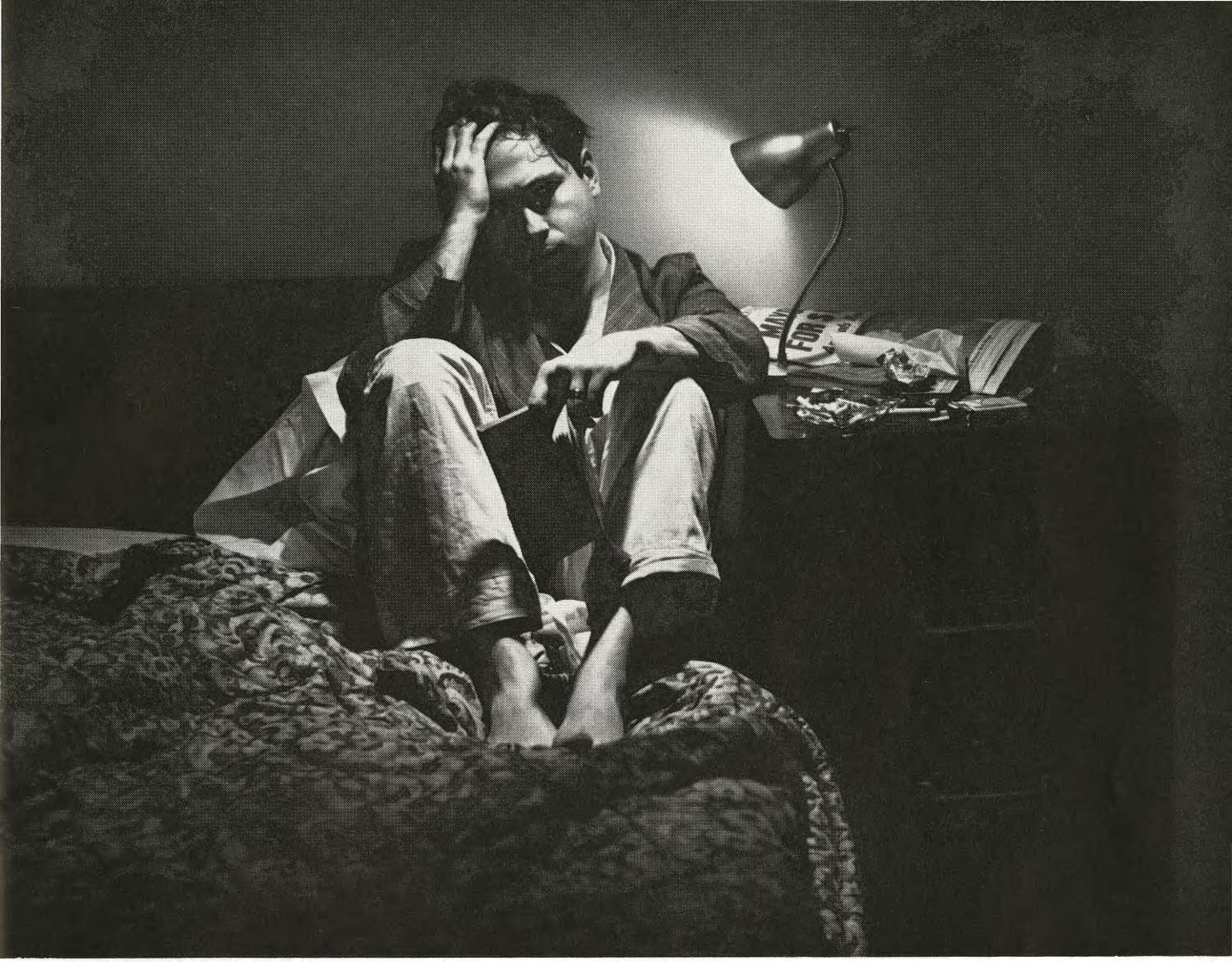

insomnia cycle

other psychotropics, particularly MAO inhibitors or phenothiazines; warn patients of possible combined effects with alcohol. Observe usual precautions in impaired renal or hepatic function, in long-term treatment and in presence of depression or suicidal tendencies. Exercise caution in administering drug to addiction-prone patients or those who might increase dosage; withdrawal symptoms, similar to those seen with barbiturates or meprobamate, can occur upon abrupt cessation after prolonged overdosage. Caution should be exercised in prescribing any therapeutic agent for pregnant patients.
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MEDICAL COLLEGE OF VIRGINIA QUARTERLY

A Scientific Publication of the School of Medicine

SUMMER 1966 • VOLUME TWO • NUMBER TWO

CONTENTS

Papers Honoring Dr. Ernst Fischer on the Occasion of His Retirement

The MEDICAL COLLEGE OF VIRGINIA QUARTERLY is designed primarily for the postgraduate education of physicians. The QUARTERLY will publish results of original research in basic and clinical sciences, and report on seminars and symposiums held at the College. Contributions from outside the MCV faculty are invited.

Manuscripts, submitted in duplicate, should be prepared according to recommendations in the *Style Manual for Biological Journals,* 2nd ed., published in 1964 by the American Institute of Biological Sciences, 2000 P Street, N.W., Washington, D. C. 20036.

Subscription rates in the USA and Canada: 1 year, $4; 2 years, $7; 3 years, $9. All other countries: 1 year $5; 2 years, $8; 3 years, $10. Interns, residents, and students: 1 year, $2.

Correspondence: MEDICAL COLLEGE OF VIRGINIA QUARTERLY, Medical College of Virginia, Richmond, Va. 23219. Phone: 703/644-9851.

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COVER: Three-dimensional reconstruction of sarcoplasmic reticulum from frog sartorius muscle (see page 90). Reprinted by permission of Lee D. Peachey and The Rockefeller University Press from The Journal of Cell Biology, volume 25, pp. 209-231, 1965.

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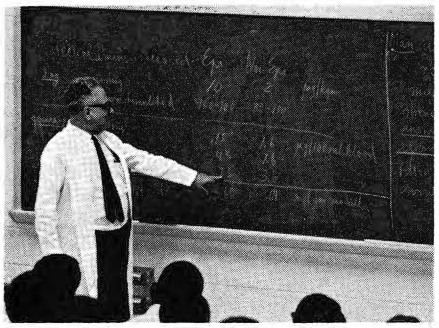
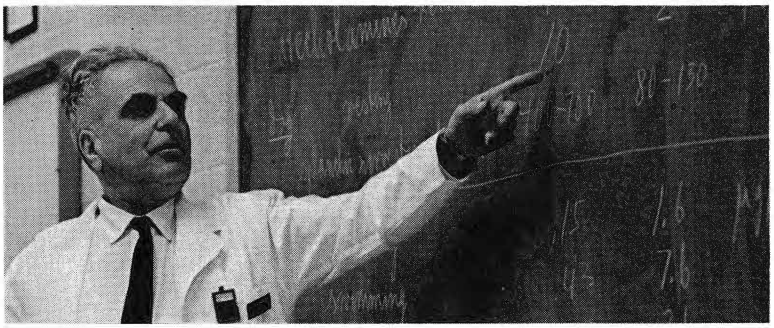
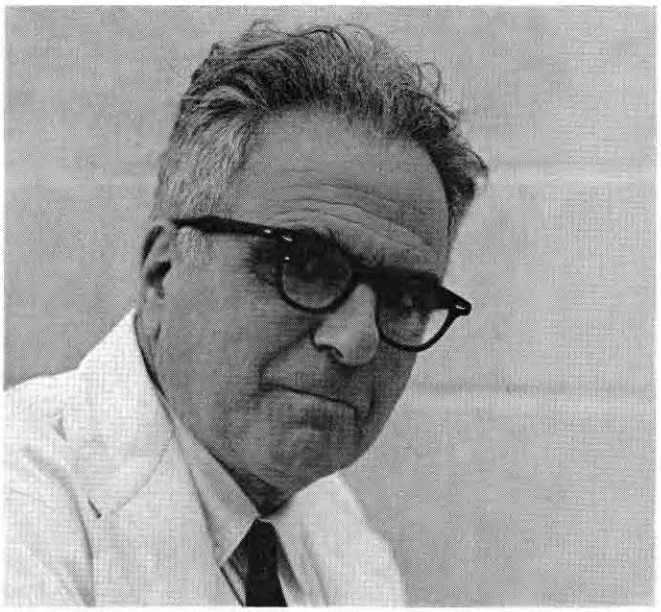
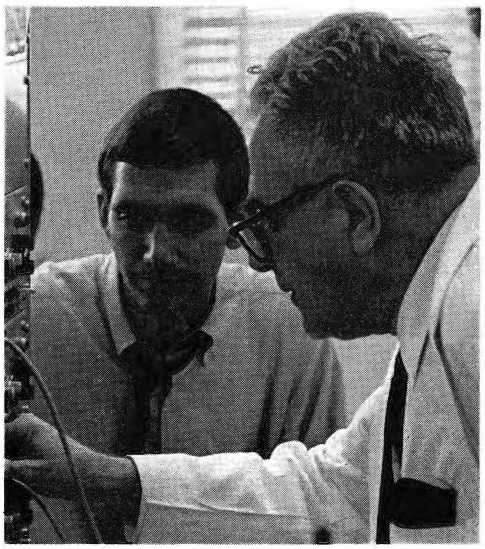




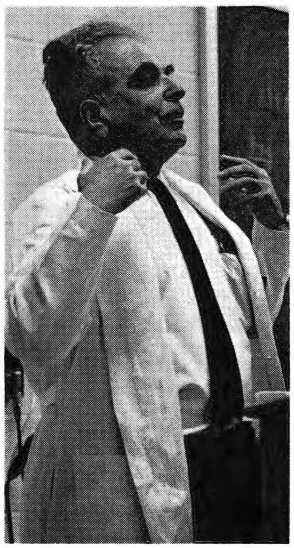




This issue is dedicated to Dr. Ernst Fischer, who recently retired after 30 years of service to the Medical College of Virginia. The QUARTERLY is pleased and proud to be able to honor Dr. Fischer in this manner.

Dr. Sydney Solomon, professor of physiology at the University of New Mexico, and formerly Dr. Fischer's associate in Richmond, suggested the idea; and Dr. Ernst Huf, once Fischer's student in Germany, gathered the manuscripts from his friends and colleagues around the world.—Ed.

Photographs by Wirt Christian



Ernst Fischer
Thirty Years of Service at the Medical College of Virginia

Born in 1896, in Breslau, Germany, Dr. Fischer received his early education, leading to the M.D. degree, in Frankfurt (Main), Germany. In 1928, he received the degree of Dr. med. habil. physiol. from the University of Frankfurt, and he made as a student of Dr. Albrecht Bethe a brilliant start in the academic career. This, however, ended abruptly in 1934. Harassed by the ruffians in Germany who took it upon themselves to decimate the rows of humanitarians, true liberals, and intellectuals, Dr. Fischer was one of the many who were dismissed from their positions. He eventually found refuge in the USA of which he became a citizen in 1944. His decision in 1935 to settle down in Richmond, Virginia was, in part, the result of the strong impression that Dr. William T. Sanger, then president of MCV, made upon Dr. Fischer, and, in part, because of Dr. Fischer's love for Richmond as "a good place to raise a family." Moreover, Dr. Sanger, with a keen sense for superior human qualities and scholarly accomplishments, found in Dr. Fischer one more good man whom he needed for his institution to develop MCV to greater heights as a teaching and a research institution. Thus began a deepening personal devotion of Dr. Fischer to the numerous functions of MCV (or, as Dr. Fischer calls it with unexcelled affection: MC-We.)

Endowed with a strong, healthy physique and an unusual mental alertness, Dr. Fischer applied these gifts unselfishly and always with great joy to the fulfillment of his many duties. A man of vision, tireless energy, zest for knowledge of professional matters as well as of human affairs, he soon became a Nestor to whom presidents, deans, colleagues, and especially students, turned for advice in times of trouble. The rewards were the many lasting friendships which he sincerely appreciates and which he nurtures through extensive world-wide correspondence. Dr. Fischer's brilliance in the art of dialectics is legendary, and is often commemorated by those of his contemporaries who argued with him. A good listener, untroubled by self-consciousness, Dr. Fischer frequently led the team of debaters to the point of no return. A smile on his face then gave the signal that he considered the dispute as having reached its endpoint. This is just one example of Dr. Fischer's refreshing attitude (so much more typical for Americans than for Germans!) to look at himself with some degree of humor, rather than with tenacious sternness.

Dr. Fischer's *teaching sessions* were characterized by great clarity of exposition, and were filled with peppery remarks about student's attitudes. He knew how to stimulate them and keep them moving. In no uncertain terms, he made it clear that students must work harder. As a counselor, he judged the students with great fairness, but never lost sight of the need for applying increasingly higher standards for performance in all the professional schools but especially in the School for Graduate Studies. Most students appreciated Dr. Fischer's judgement, they found it easy to talk things over with "their professor." Often there existed some degree of difficulty in communication which, resulted, in part, from Dr. Fischer's strong German accent. It remains a remarkable fact that a man so highly sensitive to the rapid changes in the world of ideas and the visual arts is virtually insensitive to the world of music. Forty years of teaching of acoustics and the physiology of hearing have not overcome a defect which accounts for the state of perplexity that many freshman students experienced when they attempted to jot down some notes from the spoken words of their beloved professor. This problem, however, was actually solved, once and forever, years ago by President Sanger, as recalled by Dr. Robert W. Ramsey. Dr. Sanger advised the students: "Listen carefully and grasp what you can. It is better to understand five minutes of a lecture by a wise man than to understand an hour's lecture by a fool."

As an *administrator,* Dr. Fischer was not a man who operated from his seat on the throne. He rather

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liked to see himself as a part of the whole department, the *primus inter pares*. Departmental affairs were discussed at regular staff meetings. Expressions of differences in viewpoints were encouraged rather than suppressed. If he had any prejudices, he did not show them; and if they existed, it is doubtful that they influenced his rational but mindful actions. His sense for punctuality remains unchallenged. He led his staff of coworkers largely by setting up an example of enthusiasm for work. Only occasionally was this amplified by sometimes camouflaged, sometimes not-so-camouflaged, hints that the solutions of the problems of teaching and research in the physiology department, which functions as a semi-infinite system within MCV, requires the knowledge and proper application of certain boundary conditions. Higher authority never frightened D. Fischer, who was a champion of law and order and a courageous spokesman for human rights. His persuasiveness had many triumphs from which he drew well-deserved personal satisfaction. He was a good loser on rainy days.

As a *scientist,* Dr. Fischer enjoys the distinction of being an internationally known expert on the physiology of skeletal and heart muscle. This is not the place to write about details. Suffice it to say that during his academic career he has published well over one hundred papers on muscle physiology, many of which are as significant today as they were at the time they were published. Some of the problems he explored in muscle research emerged from his deep interest in physical medicine, which is interesting because it reflects again on the inner nature of the man. As a laboratory scientist, he sought and found a way of expressing his concern for his fellow man, especially for the physically handicapped. Dr. Fischer had a wide range of interests in the large fields of physiology and medicine, even though he concentrated his efforts in the laboratory on studies of muscle. He was well read in many areas. This accounts for the fact that he was always a stimulating, and often formidable discussant at scientific meetings.

At the age of 70, Dr. Fischer left MCV, functionally speaking, a "young man." Full of vigor he will continue to teach physiology at the Hacettepe Medical Center, Ankara, Turkey, under a Fulbright Fellowship. He was the dynamic architect of a modern department of physiology that fulfills to the limits possible, the needs of a growing institution, combining several professional and a rising graduate school. It will not be easy to fill the vacancy which D. Fischer's retirement has created.

Ernst G. Huf
Department of Physiology

The Contractile Fine Structure of Vertebrate Smooth Muscle

HANS H. WEBER AND J. C. RÜEGG

The Max-Planck Institute for Physiology, Heidelberg, Germany

I. THE IDENTITY OF THE CONTRACTILE MECHANISM IN SMOOTH AND STRIATED MUSCLES

About 30 years ago, Ernst Fischer introduced a new approach to muscle research by comparing the fine structure, and the function of the contractile mechanism of smooth and striated muscle. At that time (Fischer, 1936a and b; 1938) he systematically and successfully investigated the total, the intrinsic, and the form birefringence of smooth muscles and compared his results with analogous data concerning the contractile structure (Noll and Weber, 1935) and the oriented actomyosin threads (Weber, 1935) of skeletal muscle. These investigations were especially important because the birefringence of all muscles is based on its contractile structure and functional state, and because birefringence was better understood in micellar and molecular terms* since Wiener's theory.

It was found that the total birefringence of all smooth muscles (Fischer, 1936a and b; 1938) and skeletal muscles (Noll and Weber, 1935; Weber, 1935) is composed of intrinsic and form birefringence, and that the diffraction coefficient in both striated and smooth muscle deviates little from the value of 1.5. On the other hand, Fischer (1935a and b; 1938) found considerable quantitative differences in the amount of intrinsic and form birefringence, even in the case of different smooth muscles. The differences between smooth and striated muscle appeared especially large when the volume fraction of the birefringent elements was calculated from the form birefringence. The volume fraction of the double birefringent portion appeared to be about 10 times smaller in the smooth muscles investigated than in striated muscle (Fischer, 1938). Consequently, the intrinsic birefringence of the *small* birefringent volume in smooth muscle ought to be about 10 times larger than that of the *large* volume involved in striated muscle. This follows from the fact that the birefringence of the entire muscle-volume is similar in both types of muscle. This raises some problems! On the other hand, from the qualitative similarity in the birefringence of smooth and striated muscle, Fischer was able to conclude that the contractile fine structure of smooth and striated muscle was essentially identical (1936a and b; 1938).

In the meantime, additional important evidence was obtained with chemical and other (non-morphological) methods which strongly suggested that the contractile mechanism in all kinds of muscle and even in contractile cells such as fibroblasts, amebae, and thrombocytes was identical. This consisted of five main points:

1) From all kinds of muscle, including smooth muscle (Naeslund and Snellman, 1951) and contractile cells (Hoffman-Berling 1956; 1958), a typical contractile protein can be extracted which consists of two components, myosin and F-actin (*see also* table 1).

2) The ratio of the two components is about the same in smooth and striated muscle, i.e., about 3 or 4 g myosin per one gram actin (*see* table 1).

3) Most importantly, the contractile structures of all kinds of muscle (Bohr, Filo, and Guthe, 1962; Hasselbach and Ledermair, 1958; Huxley, 1963; Weber, 1958; Ulbrecht and Ulbrecht, 1952) and contractile cells (Hoffman-Berling, 1956) contract and relax under identical conditions when they are functionally isolated by extraction of the soluble muscle components. Contraction and relaxation occur only in the presence of ATP when Mg^{++} ions are also present (e.g. in smooth muscle, Hasselbach and Ledermair, 1958). Contraction is always maximal when the concentration of free calcium ions is about 10^{-5} M, and relaxation is complete when the free calcium concentration is below 10^{-7} M (fig. 1; *see also* Hasselbach, 1964).

4) The ATPase activity is high during contraction and low during relaxation and rest (cf. figs. 1 and 2).

5) In cross-striated muscle (Hasselbach, 1964; 1960; Barany and Jaisle, 1960) and probably also in smooth muscle, this behavior of the ATPase activity depends on the association of myosin and actin

* In the 1930's, the x-ray diffraction patterns of muscle (Astbury and Dickinson, 1935; Boehm, 1931; Boehm and Weber, 1932) were unspecific because of their technical imperfection and because only wide angle diffraction patterns were possible. It is therefore not astonishing that these patterns appeared to be identical, not only in all types of muscle, but also in other fibrous protein structures such as keratin and fibrin.

during contraction, and on their dissociation during relaxation.

This physicochemical and functional similarity in the contractile complex of smooth and striated muscles has its counterpart in the similarity of its components—actin and myosin. The myosin component contains ATPase activity which is activated by Ca ions and inhibited by Mg ions in striated muscles (Barany and Jaisle, 1960) or at least not activated by Mg ions in smooth muscles (Gaspard-Godfroid, 1964; Needham and Cawkwell, 1956; Schirmer, 1965). However, the intact F-actin component has no ATPase activity in smooth muscles (Rüegg, Strassner, and Schirmer, 1965). All kinds of myosin have a similar sedimentation constant of about 6 S (in smooth muscles, Cohen, Lowey, and Kucera, 1961; Laszt and Hamoir, 1961). The myosin component of all kinds of muscle is soluble even in the absence of ATP if the ionic strength is larger than 0.3 μ, and barely soluble if it is smaller than 0.15 μ. The purified myosins of smooth (Hanson and Lowy, 1963) and striated muscle (Huxley, 1963) precipitate at ionic strengths below 0.3 μ, not as amorphous aggregates, but as "typical" myosin filaments with a polar structure.

The fibrous form of the actin component (F-actin) of smooth as well as striated muscle is transformed into the globular actin monomer (G-actin) in salt-free solutions. G-actin polymerizes again to form F-actin when salt and Mg^{++} or Ca^{++} ions are added (e.g. in smooth muscle, Carsten, 1965; Schirmer, 1965 table 2). The actin components of smooth and striated muscle are completely soluble in solutions of high and low ionic strength, in the G state as well as in the F state (e.g. in smooth muscle Hasselbach and Schneider, 1951). F-actin filaments from all types of muscle, including vertebrate smooth muscle (Hanson and Lowy, 1963; Shoenberg et al., in press) have a double helical structure of about 50 Å thickness (table 2).

The conditions for the formation of the actomyosin complex from actin and myosin of smooth and striated muscle are identical:

1) In the absence of ATP or other nucleosidetriphosphates, actin and myosin combine to form actomyosin quite independently of the ionic strength in the range of 0.1 to 0.6 μ (e.g. in smooth muscle Bohr et al., 1962; Naeslund and Snellman, 1951; Needham and Cawkwell, 1956).

2) In the presence of ATP the complex is dissociated into its components if the ionic strength is greater than 0.3 μ (e.g. in smooth muscle Bohr et al., 1962; Naeslund and Snellman, 1951; Needham and Cawkwell, 1956).

3) The formation of the actomyosin complex has the same functional consequences in all types of muscle. If ATP, Mg^{++} ions and traces of Ca^{++} are present, the actomyosin structure contracts and develops tension (fig. 2).

The contractile structures of smooth muscle (Hasselbach and Ledermair, 1958; Ulbrecht and Ulbrecht, 1952; Filo, Bohr, and Rüegg, 1965) and of striated muscle (Barany and Jaisle, 1960; Hasselbach, 1960; 1964; Weber, 1958) relax again *only* in the presence of ATP if the splitting of ATP is inhibited by a lack of Ca^{++} ions, by interaction inhibitors or by SH-

TABLE 1
Actomyosin Content of Smooth and Striated Muscle

Muscle	Actomyosin content in % of protein	Actin-Myosin Ratio	Author
Cross striated (rabbit)	52	1 : 3	Hasselbach and Schneider (1951)
Arteries (cow)	3		Rüegg et al. (1965)
Uterus (rabbit)	2 to 4	1 : 4	Needham and Williams (1963)
Taenia coli	3		Rüegg et al. (1965)
Byssus retractor (clam)	25		Rüegg (1961)

TABLE 2
Double Helices and Reversible Depolymerization of Actin in Smooth and Striated Muscle

Muscle	Actin filaments with double helical structure according to: (author)	Reversible polymerization and depolymerization according to: (author)
Cross striated (rabbit)	Hanson and Lowy (1963)	Straub (1943)
Cross striated (clam)	Hanson and Lowy (1963)	
Cross striated (crab)	Peterson (1963)	
Uterus (rabbit)	Hanson and Lowy (1963)	Carsten (1965)
Taenia coli	Hanson and Lowy (1963)	
Arteries (cow)	Shoenberg et al., in press	Rüegg et al. (1965)
Smooth adductor (clam)	Hanson and Lowy (1963)	

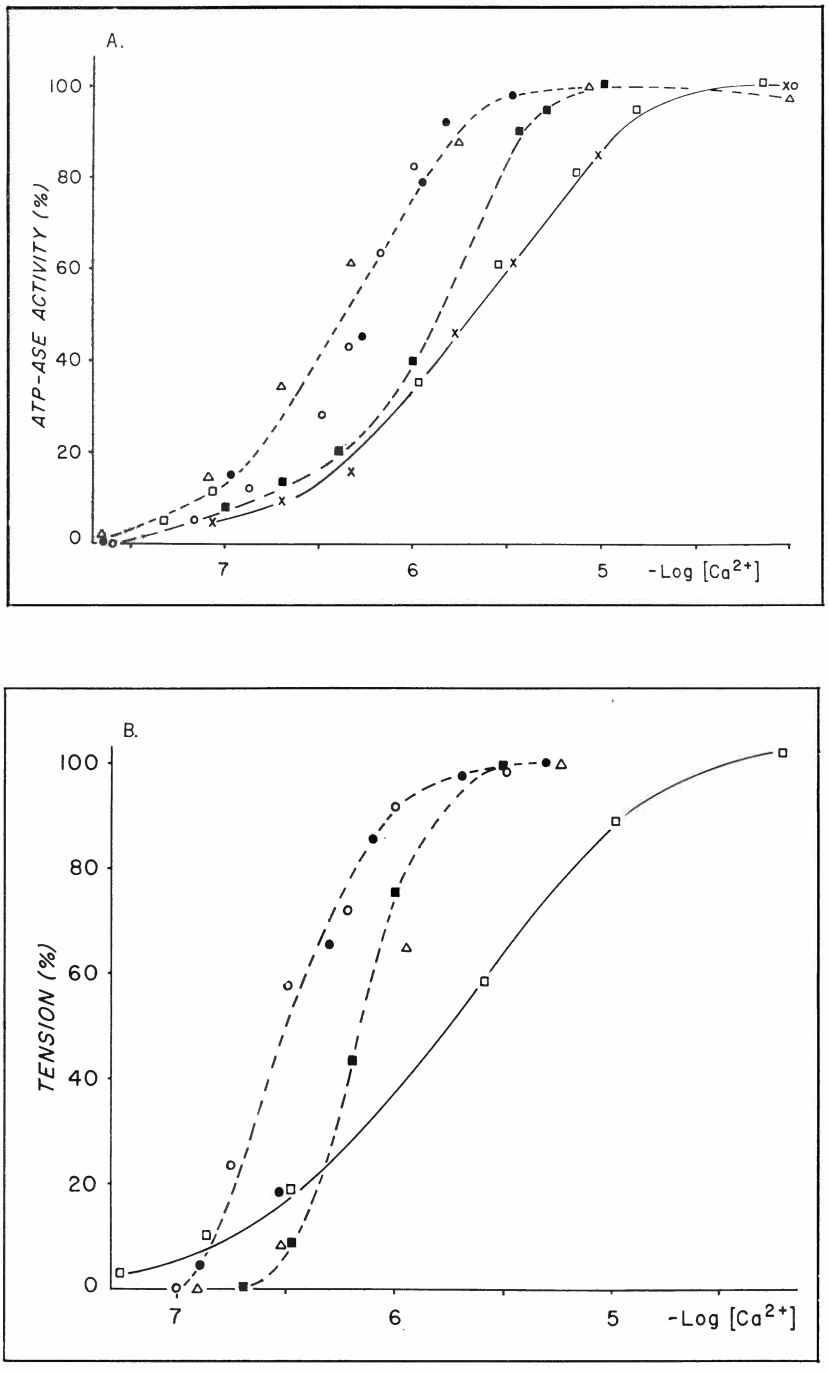

Fig. 1—The calcium requirement for the activation of smooth and cross striated glycerol extracted muscle fibers or myofibrils. Abscissa: negative logarithm of the free calcium ion concentration. Ordinate: *A,* ATPase activity; *B,* tension, both as percent of maximal activation; 0 = activity in the absence of calcium activation. Cross striated muscle is represented by the broken line, smooth muscle by the solid line. □ taenia coli of the guinea pig, △ heart of the dog, ● leg muscle of insect (Schaedler, in preparation); ■ leg muscle of the crab (Portzehl et al., 1965); X byssus retractor of the clam (Schaedler, in preparation); ○ skeletal muscle of the rabbit (*A,* Weber et al., 1964; *B,* Filo et al., 1965). Composition of solution: Mg-ATP 5 mM; Ca EGTA buffer 4mM; KCl 0.05; histidine buffer 0.02 to 0.05 M, pH 7; temperature 20 C.

reagents. The actin and myosin components from different muscles are so similar that actin and myosin from smooth and striated muscle (Schirmer, 1965) and even from thrombocytes and striated muscle (Bettex-Galland and Lüscher, 1965) may be recombined to form hybrid contractile actomysin complexes.

The functional identity of smooth and striated muscle provides strong evidence in favor of the conclusion which Fischer derived from his comparison of the double refringence of the two types of muscle, i.e. the contractile structure seems to be practically identical.

The functional identity of smooth and striated muscle may, in addition, offer an explanation for Fischer's birefringence results. From studies with skeletal muscle we know that the contribution of the (double helical) actin filaments to the birefringence of muscle is very small. The birefringence of the I-bands which contain actin but no myosin is about 10 times smaller than the birefringence of the myosin containing A-bands. Consequently we may consider total birefringence as the sum of form birefringence due to the parallel arrangement of whole myosin filaments and of intrinsic birefringence due to the parallel arrangement of myosin molecules within each filament.

II. DIFFERENCES IN STRUCTURE AND FUNCTIONAL PERFORMANCE OF ACTOMYOSINS FROM SMOOTH AND STRIATED MUSCLE

The relatively simple and uniform concept outlined in part I does not harmonize with the results of modern electronmicroscopy. The electronmicrographs of thin sections from paramyosin-free vertebrate smooth muscles show only actin filaments and no myosin filaments (Needham and Shoenberg, 1964; Hanson and Lowy,

1963; Elliott, 1964), in contrast to the electronmicrographs of striated muscles. The discrepancy between the presence of form birefringence and the absence of myosin filaments in smooth muscles may be based on the presence of birefringent paramyosin filaments in the retractor muscles of Phascolosoma and Thyone, investigated by Fischer. At least one of these muscles—retractor of Phascolosoma—gives a strong paramyosin x-ray diffraction pattern (Bear, 1945).

The lack of demonstrable myosin filaments in electronmicrographs of smooth muscle makes it difficult to apply the generally accepted concept of sliding filaments to the contractile mechanism of vertebrate smooth muscles. However, the details of contraction and chemistry (*see* part I) strongly suggest the presence of a similar contractile mechanism in all types of muscles and even in contractile cells. Consequently, information concerning the properties of myosin from smooth muscles is needed.

The filaments of all types of myosin are formed spontaneously as soon as the myosin becomes insoluble. If the ionic strength of ATP-free myosin solutions is lowered, the myosin does not form an amorphous precipitate but produces typical filaments of somewhat varying size, as outlined above (Huxley, 1963; Hanson and Lowy, 1963). It is therefore tempting to assume that under physiological conditions in the presence of ATP, smooth muscle myosin is soluble even at low ionic strength! In fact, the actomyosin of all investigated vertebrate smooth muscles is so soluble that it can be easily and completely extracted at 0.1 μ.* Skeletal muscle actomyosin, on the other hand, is completely extracted only at ionic strengths above 0.3 μ.

This unusually high extractability of smooth muscles is dependent on the presence of ATP from the extracted muscle. Unlike actomyosin from striated muscle, smooth muscle actomyosin is not only dissociated by ATP, but also the myosin component is rendered soluble by ATP even at low ionic strengths (Laszt and Hamoir, 1961; Schirmer, 1965). The dissociation of actomyosin and the solubility of the myosin component from smooth muscle can be recognized in ultracentrifugation studies, i.e. after sedimentation at 100,000 $\times$ g the pellet contains actin almost free of ATPase activity.

The myosin in the supernatant is not completely dispersed. Its aggregation is shown by a sedimentation constant of 12 S (Laszt and Hamoir, 1961; Schirmer, 1965) as compared with the normal value of 6 S. These aggregates are so much smaller than the myosin filaments of striated muscle that, unlike these filaments, they cannot be seen in the electronmicroscope when they are negatively stained (Hanson and Lowy, 1963; Shoenberg et al., in press). The high dispersion and solubility of smooth muscle myosin is due to the presence of ATP. This is shown by the precipitation of smooth muscle actomyosin or myosin at low ionic strengths (*see also* part I) when ATP is removed by dialysis (Laszt and Hamoir, 1961). Actomyosin and myosin become soluble again after addition of ATP (Laszt and Hamoir, 1961).

Consequently, the actomyosin of smooth muscle may be purified by repeated cycles of solution and precipitation by ATP addition and removal (Rüegg et al., 1965). When such purification cycles are continued for three days the actomyosin is still as soluble as in the extract (Schirmer, 1965). However, after aging for about one week, even without purification, the solubility at low ionic strengths is greatly diminished (Schirmer, 1965). The high solubility of the native myosin component under physiological conditions satisfactorily accounts for lack of myosin filaments in smooth muscle, in spite of the fact that purified smooth muscle myosin aggregates spontaneously into filaments when the ionic strength is lowered in the absence of ATP (*see* part I). However, the ability of living smooth muscles to contract in spite of the absence of myosin filaments remains puzzling, since ATP-free actomyosin gels prepared from vertebrate smooth muscles do not contract after addition of ATP but dissolve (Laszt and Hamoir, 1961; Schirmer, 1965), in contrast to actomyosin from striated muscle which superprecipitates under these conditions.

This difference between contractile living smooth muscle and noncontractile isolated systems disappears under conditions in which the high solubility of the myosin component is abolished.

Thus the actomysin system isolated from vertebrate smooth muscles contracts:

1) if the actomyosin preparation is aged for about one week,

2) if the contractile system is functionally isolated by extraction of the fiber with 50% glycerol for several days (Schirmer, 1965),

3) if the actomyosin preparation is kept at pH 6 before ATP addition (Schirmer, 1965),

4) if synthetic actomyosin is synthesized from purified actin and myosin,

5) if the actomyosin is reprecipitated several times in the presence of about 5 mM Ca^{++} ions (Filo, Bohr, and Rüegg, 1963). The high

* Since actomyosin prepared from smooth muscle can be extracted at 0.1 μ ionic strength as well as at 0.6 μ ionic strength, it was believed that there were two types of actomyosin (Laszt and Hamoir, 1961), one type dissolving as true actomyosin at 0.6 μ ionic strength, and another type, the so-called tonoactomyosin, dissolving at 0.1 μ ionic strength. However, it was found later (Rüegg et al., 1965) that the so-called tonoactomyosin is identical with the actomyosin extracted at high ionic strength and that no actomyosin can be extracted if the previous extraction of tonoactomyosin was exhaustive (Rüegg et al., 1965).

solubility disappears irreversibly with these treatments except in condition (3). If the pH of the living muscle is lowered to 6 in the presence of CO_2, the actomyosin is no longer extractable with pH 6 buffered solutions of low ionic strength. Subsequently it can still be extracted if the pH is readjusted to 7 (Schirmer, 1965).

The mechanism of contraction of isolated systems under all these conditions is almost certainly similar to the contraction of isolated actomyosin from skeletal muscle. Contraction is even possible in artificial actomyosin prepared by combining smooth muscle myosin with striated muscle actin and vice versa (Schirmer, 1965).

One wonders whether or not the high solubility of myosin and actomyosin in fresh actomyosin preparations is an artifact. Isolated actomyosin systems of smooth muscle are able to contract as soon as the high solubility of actomyosin and myosin is diminished, while within the living smooth muscle cell the actomyosin system is always able to contract. The possibility of an artifact must be seriously taken into account since a number of protein factors were isolated recently from skeletal muscles, including "native tropomyosin" (Ebashi, Ebashi, and Maruyama, 1964) and "inhibitor" (Perry, in press), both of which inhibit the interaction between actin and myosin and may also raise the solubility of myosin. Thus it seems possible (Filo et al, 1963) that smooth muscles contain similar solubilizing proteins which, however, may be located differently from actomyosin in situ so that they do not react with the contractile proteins in living muscle. After extraction these proteins would then be able to combine and react with actomyosin.

This possibility has so far not been confirmed experimentally. At least it is clear that the high actomyosin solubility in the ATP-containing extract is not caused by "native tropomyosin." The action of this factor is inhibited with about 10^{-6} M calcium ions (Ebashi et al., 1964) while the actomyosin in fresh extracts remains dissociated and soluble even after raising the Ca^{++} ion calcium concentration to about 10^{-6} M with calcium EGTA [ethyl-eneglycol bis (amino-ethyl-ether)-N, N'-tetraacetic acid] buffers. There is no evidence that another of the actomyosin contaminating proteins is the "solubilizing factor." Actomyosin reprecipitated several times by the described "ATP method" still dissolves after addition of ATP, although most of the contaminating proteins, including free tropomyosin B, are removed by this method (Schirmer, 1965). If myosin is then separated from such actomyosin in the preparative ultracentrifuge, the high solubility of myosin at low ionic strengths is diminished. However, it must be remembered that this isolation procedure requires about a week which is sufficient time to make smooth muscle actomyosin ATP-insoluble and contractile through aging even without purification (Schirmer, 1965).

Since the other actomyosin treatments mentioned above under conditions 1), 2), 4), and 5) also involve about one week's aging, two alternatives remain:

1) Aging for about one week abolishes the high solubility of actomyosin through selective denaturation which does not impair contractile function and ATPase activity.

2) Within one week a solubilizing factor is removed by either purification or denaturation of the factor.

Even if the first of these possibilities pertains, native myosin would not be totally disolved in the *living* cell. In the presence of actin the solubility of fresh myosin under physiological conditions is about 3 to 4 mg per ml (Schirmer, 1965). From the actomyosin content of smooth muscle—5 mg in 1 ml muscle (Rüegg et al., 1965)—an intracellular myosin concentration of about 20 mg* per ml of muscle cells can be calculated. Thus it would appear that about 80% of the myosin exists as a gel structure and not as a sol. The question is why this structure ("insoluble" myosin) does not form the type of myosin filaments which are formed in vitro and which are so typical for striated muscle. It remains for future research to find out the detailed structure of smooth muscle myosin within the smooth muscle cell. It is very likely that the measurement of intrinsic and form birefringence applied so successfully by Ernst Fischer will be extremely helpful in defining this structural analysis.

* This calculation is based on the assumption that the connective tissue is about 25% and the extracellular space about 40% to 50% of the muscle volume, i.e. the intracellular space containing all the myosin is about four times smaller than the volume of the whole tissue.

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"Slow" and "Fast" Muscle Fibers

ERNEST GUTMANN

Department of Physiology and Pathophysiology of the Neuromuscular System, Institute of Physiology, Czechoslovak Academy of Sciences, Prague

The great variety of structure and function of different muscles reflects apparently the process of adaptation to different functional demands, but it has remained a source of confusion, especially if an attempt is made to find a common principle of order for this variety. The terms "fast" and "slow" muscle fibers in mammals are used in reference to their faster or slower contraction times. Both these muscles, e.g., the fast M. Extensor digitorum (E.D.L.) and the slow M. soleus of the rat are "twitch" muscles, i.e., they react with propagated action potentials to nerve stimulation. Contrary to this, the slow-tonic muscle fibers of the frog respond with local non-propagated depolarizations, activating contractures (Tasaki and Mizutani, 1943; Kuffler and Gerard, 1947; Kuffler and Vaughan Williams, 1953). The normal responses of these "tonic" fibers in the body are long-lasting contractures, which they maintain in a graded fashion to depolarizing concentrations of acetylcholine (ACh) or potassium. In fact, localized responses to ACh in fast-twitch muscle fibers and slow long-lasting contractures in slow-tonic muscle fibers have already been described by Rieser and Richter (1925) and Sommerkamp (1928). Essential differences in contracture responses have been described in the fast (twitch) and slow (tonic) fibers of the frog, especially in their reactions to KCl solutions which initiate contractures by membrane depolarizations. A phasic contracture is evoked in fast fibers (Hodgkin and Horowicz, 1960), i.e., the fast fibers of the frog will relax relatively rapidly despite membrane depolarization, but the slow fibers of the frog show a sustained contracture during the whole period of reduced membrane potential (Kuffler, 1946; Fleckenstein, 1955).

Thus two distinct fiber types, i.e., fast (twitch) and slow (tonic) fibers do exist in frogs and toads and related differences have been described for the membrane potentials (Kuffler and Vaughan Williams, 1953; Kiessling, 1960), arrangement of muscle fibrils ("Fibrillen" or "Felderstruktur"), pattern of filaments and structure of the sarcoplasmatic reticulum (Krüger, 1952; Gray, 1958; Peachey, 1965), and type (focal or multiple) of innervation.

Krüger (1952) claimed that some mammalian muscles consist entirely of fibers with "Felderstruktur" and that the same distinction between muscle fibers with "Fibrillen" and "Felderstruktur" as in frog muscles could also be made in mammalian muscles. Mammalian muscle fibers with "Felderstruktur" showing only non-propagated junctional potentials in response to nerve stimulation have indeed been found in the extraocular muscles of the guinea-pig (Hess, 1961). However, in other mammalian muscles such a distinction has not been found. Moreover, no distinct structural differences could so far be detected between fast and slow mammalian muscle fibers.

Therefore, the question whether there are two fiber types or a whole spectrum of many different muscle fibers as regards structure, innervation, speed of contraction and type of electrical response in mammalian muscles is still unsettled (Huxley, 1964).

A study of the contracture responses in fast and slow mammalian muscle fibers especially during development should be rewarding. Contractures do in fact demonstrate the main features of the mechanisms of the process of excitation-contraction coupling, and the basic differences in the contracture responses to different agents in the two types of muscle fibers in the frog would suggest important modifications of this process (*see* Sandow, 1965). Are there any suggestions for such differentiation also in mammalian muscle fibers?

ACh CONTRACTURES IN E.D.L. AND SOLEUS MUSCLE OF THE RAT

Figure 1 shows that in both E.D.L. and soleus muscles of the rat (three days old), contractures to ACh can be produced. In figures 1 and 2 the contracture response and the tension developed during contracture respectively are expressed in percent of maximal tetanic tension output of the muscle. It can be seen that there is a considerably stronger contracture response in the soleus muscle than in the E.D.L. Moreover, contracture is observed at a threshold value of 10^{-7} in the soleus muscle, but at 7.10^{-7} in the E.D.L.

However, the fast E.D.L. loses the capacity to react with contractures to ACh about 20 to 25 days after birth of the animal, whereas the slow soleus muscle reacts to ACh, though to high concentrations only, with contractures at all stages of development. It is interesting to see that there are considerable differences in threshold and intensity of contracture response during the earliest stages of development that I have studied (Gutmann and Hanzlíková, in press). Very marked differences in contracture responses to ACh are, of course, known to exist between fast (twitch) and slow (tonic) muscle fibers of the frog. Similar qualitative differences exist between the fast Latissimus dorsi posterior (L.D.P.) and the slow Latissimus dorsi anterior (L.D.A.) of the chicken (Gutmann, Jirmanová and Vyklický, to be published). The L.D.P. does not react to ACh even at highest concentrations, whereas the L.D.A. reacts at relatively low concentrations of ACh with a sustained contracture.

CAFFEINE CONTRACTURES IN E.D.L. AND SOLEUS MUSCLE OF THE RAT

In the E.D.L. of animals 22 to 30 days old exposed to a 20 mM solution of caffeine, potentiation of twitch tension but no contracture is observed (Gutmann and Sandow, 1965). However, contracture responses to exposure of caffeine could be observed until the 18th to 22nd day after birth. No contracture was observed in this muscle of animals more than 22 days old. In contrast to the E.D.L., the soleus responds with contracture also in animals one month old. Thus the contracture response to caffeine is lost during ontogenesis in the fast E.D.L., but not in the soleus muscle which maintains sensitivity to this contracture-inducing drug. A similar contracture behavior is observed in the Latissimus dorsi of the chicken. The fast L.D.P. of adult animals shows no contracture, whereas the slow L.D.A. responds with a contracture to caffeine (Gutmann, Jirmanová, and Vyklický, 1966, to be published).

NERVOUS INFLUENCES AFFECTING CONTRACTURE BEHAVIOR OF MAMMALIAN MUSCLES

It is well known that during denervation the whole muscle fiber becomes sensitive to ACh (Ginetzinsky and Shamarina, 1942; Axelsson and Thesleff, 1958; Miledi, 1960; and others). Thus the denervated muscle shows the contracture behavior of muscle at early stages of development (Diamond and Miledi, 1962). A similar change in contracture behavior of denervated muscles is observed after exposure to caffeine. After denervation the E.D.L. of adult animals reacts to caffeine with contracture; it has thus gained properties of the slow muscle (Gutmann and Sandow, 1965). The student of differentiation between fast and slow muscle fibers will find many valuable clues from work comparing striated and smooth muscle, a line developed successfully, e.g., in respect to birefringence and other characteristics (Fischer, 1944). Slow muscle fibers and muscles during early stages of development resem-

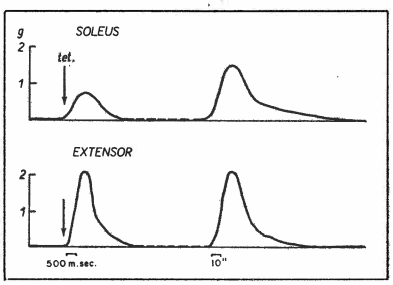

Fig. 1—Maximal isometric tetanic contraction (first curve) and contracture response to acetylcholine (second curve) added to the bathing solution at a concentration of 5.10^{-5} of M. soleus and M. extensor digitorum longus of three-day-old rats. Tetani were produced by "massive" direct stimulation in vitro by a 300-msec-long stimulus, the single stimuli being square shocks 1.0 msec in duration (*see* Sandow and Brust, 1958).

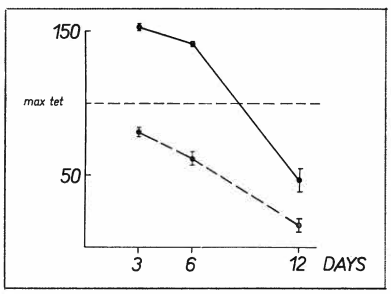

Fig. 2—Changes of contracture response of M. soleus (full line) and M. extensor digitorum longus (interrupted line) of rats 3, 6, and 12 days after birth. The contracture response is expressed in percent of the maximum isometric tetanus tension (= 100%), obtained by electrical stimulation in vitro.

ble in their reactions the smooth muscle. The comparative approach has and will certainly prove to be the most helpful concerning the problem of basic conceptions of contraction mechanisms (*see* Fischer, 1944). Hetero-innervation experiments were used to show the effect of an additional nerve supply on contracture behavior of the muscle (Gutmann and Hanzlíková, to be published). If the peroneal nerve is sutured into the soleus muscle (this is a hetero-innervation) and simultaneously the tibial nerve is crushed, hyperneurotization of the soleus muscle due to the additional supply of fast peroneal nerve fibers is achieved. The additional nerve supply affects the contracture behavior of the reinnervated soleus muscle, apparently by mediating fast nerve influences. In these experiments only the tibial nerve was crushed on the control side. Both muscles react with contracture to a solution of caffeine. Five weeks after reinnervation of the muscles, the tension developed by the contracture was 2.26 ± 0.26 g in the control muscles (reinnervated by the tibial nerve only) and 1.30 ± 0.24 g (10 animals) in the muscle reinnervated by tibial and hyperneurotized by peroneal nerve fibers. Thus the additional fast nerve influence had reduced the contracture response of the slow soleus muscle.

DIFFERENCES IN METABOLISM OF "FAST" AND "SLOW" MAMMALIAN MUSCLE FIBERS RELATED TO DIFFERENT CONTRACTURE BEHAVIOR

The differences in contracture behavior between fast and slow mammalian muscle fibers suggest that two basic groups of muscle fibers may exist, which may be somehow related to the differences of fast (twitch) and slow (tonic) muscle fibers of the frog. Are there indications of differences of metabolism between such basic groups of muscle fibers?

Our first consideration will, of course, be centered on the role of Ca^{++} ions, which have such an important role in the process of excitation-contraction coupling (*see* Sandow, 1965). It may suffice to point out that caffeine increases the capacity of the sarcoplasmatic reticulum to release Ca^{++} (*see* Sandow, 1965) and that ACh increases the inward movement of Ca^{++} following an increase in membrane permeability (Jenkinson and Nicholls, 1961). Moreover, the sarcoplasmatic reticulum, the structure which apparently mediates the process of excitation-contraction coupling, is relatively less developed in the slow (tonic) muscle fibers of the frog (*see* Page, 1965). In analogy, sensitization of the E.D.L. to caffeine contracture caused by denervation suggests alterations in the sarcoplasmatic reticulum and in its capacity to regulate the myoplasmic flux of Ca^{++} (Gutmann and Sandow, 1965).

Our next consideration concerns the well-known differences of energy metabolism of fast ("white") and slow ("red") muscles. In the former, anaerobic glycolysis, catalyzed by the enzymes of the Embden-Meyerhof chain, apparently plays the dominant role; in the latter, the oxidative processes catalyzed by enzymes of the citric acid cycle (the intramitochondrial enzymes dominate; *see* Pette, 1965). These differences are apparently related to adaptation to different functional demands (e.g., Needham, 1926; Yakovlev and Yakovleva, 1953) and are reflected in the differences of speed of contraction (Close, 1964).

However, slow (tonic) fibers of frog, toads, or chickens and slow mammalian muscle fibers alike are required for posture and maintenance of tension for long periods of time, and differences in protein metabolism of two basic types of muscle fibers related to "long-term regulations" might be expected. This is indeed the case.

Incorporation of radioactive amino acids into the proteins of the slow L.D.A. of chicken and of the soleus of rats is increased compared with that of the fast L.D.P. of chicken and the E.D.L. of rats (fig. 3). Also a higher level of ribonucleic acid content was found (mg RNA/100 mg of proteins) in the slow L.D.A. of the chicken, rectus abdominis of the frog and soleus of the rat compared with the fast L.D.P. of the chicken, sartorius of the frog and E.D.L. of the rat (Gutmann and Syrový, 1966, to be published). These are first indications of a higher turnover of proteins of slow muscles, but, of course, more data will be necessary to strengthen the assumption of a relation of speed of proteosynthesis and mechanisms concerned with maintenance of tension.

CONCLUSIONS

On the basis of the clear-cut differentiation used in fast (twitch)

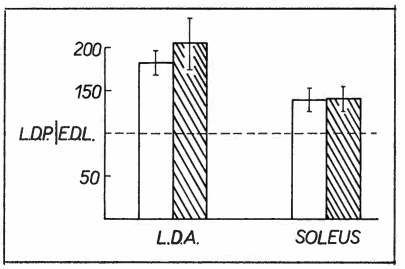

Fig. 3—Incorporation of radioactive S^{35} methionine into the proteins of the M. latissimus dorsalis anterior of the chicken and the M. soleus of rats (counts/min/mg of precipitated proteins) one hour after intraperitoneal injection of S^{35} methionine (200 μc/100 g of body weight). Specific activity is expressed in percent of activity measured in the M. latissimus dorsalis posterior of the chicken and the M. extensor digitorum longus of the rat (white columns). The levels of ribonucleic acid content (*see* Schneider, 1945) in L.D.A. of chicken and the M. soleus of the rat (γP/mg protein) are expressed in percent of the RNA content in the L.D.P. of the chicken and in the E.D.L. of the rat (black columns).

and slow (tonic) muscles of the frog, both the fast E.D.L. and the slow soleus muscle should be considered twitch muscles. However, they reveal a marked differential behavior in their contracture responses to ACh and caffeine. Moreover, all the slow muscles I have studied (i.e., the L.D.A. of the chicken, the rectus abdominis of the frog, and the soleus of the rat) show a higher rate of proteosynthesis. This may be related to the basic function of slow muscles concerned with long-lasting maintenance of tension, the extreme being, for example, the contracture responses observed in reaction to ACh. There may be a relation of rate of protein metabolism to the mobility of protein-bound Ca^{++} in the sarcoplasmatic reticulum. The differences in contracture behavior are apparent already three days after birth of the animals. All this may indicate a basic differentiation of two main groups of muscle fibers. Neural long-term influences operate in the development of this differentiation in contracture behavior of fast and slow muscle fibers. The mechanisms by which the nerve cell affects this behavior have still to be uncovered.

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Latency Relaxation: A Brief Analytical Review*

ALEXANDER SANDOW

Division of Physiology, Institute for Muscle Disease, New York

In this report I review certain aspects of the research on the latency relaxation (LR), the minute relaxation of a stimulated muscle that occurs during the latter half of the latent period, i.e., just prior to the onset of contraction (*see* fig. 1, and, e.g., Sandow, 1944). The first part of my discussion will be historical, dealing with the early, mostly descriptive work on the LR, and then I shall present a more analytically oriented attempt to indicate the significance of the LR in relation to certain aspects of the response of a muscle to stimulation. I take pleasure in dedicating this article to Professor Ernst Fischer, as he terminates his formal work at the Medical College of Virginia and starts a new career in teaching abroad. My dedication takes on a special pertience because I began my studies on the LR in close relation to some of Professor Fischer's early research, and so I shall start by telling of this connection.

Professor Fischer published the research I have in mind just forty years ago (Fischer, 1926) and—as indicated in the title of his paper: "Die Zerlegung der Muskelzuckung in Teilfunktionen"—he delineated the behavior of the segments of an isometrically contracting frog sartorius muscle as a wave of activation coursed along its length after initiation at the spot where excitation was first set up. The main point of this study demonstrated that individual longitudinal segments of the isometrically contracting muscle shortened during the response by as much as 15%, and other segments, that either were not yet excited or were by then actually relaxing, were correspondingly stretched. I read this article in the early thirties when I was just beginning my study of muscle physiology, and I was greatly impressed by the fact that in an ordinary "isometric" contraction, the individual lengthwise elements of the muscle do not contract isometrically at all —in fact, each one not only shortens considerably during one phase of its response but it also in general lengthens markedly at some other phase.

This interested me very much for it showed that a normally occurring contraction of an indirectly stimulated muscle in the body, was not at all simple but was the resultant of the generally out-of-phase mechanical sequences of its many segments. And—even more pointedly to me at that time, since I was interested in studying basic mechanisms of contraction in an excized whole muscle, such as a frog's sartorius—Professor Fischer's findings plainly demonstrated that the prevailing methods for activating a contraction in an isolated muscle yielded a mechanical response which, as recorded either at the end or at any part of the muscle, could not possibly present correctly the basic dynamics of the elementary contractile unit. To some extent this difficulty could be lessened, as Professor Fischer's results suggested, by separating the stimulating electrodes to the opposite ends of the muscle, instead of placing them in the more conventional pattern, both together at one end. But it was clear that a more radical solution of the problem was needed, and this was found in what has come to be called "massive stimulation." In this procedure, first introduced by Brown and Sichel (1936) for work on single muscle fibers and later adapted by me for work on whole muscle (Sandow, 1947), the preparation is fully immersed in a physiological medium, and is flanked by a pair of long electrodes, originally made of bright silver or of silver-silver chloride, but now much more preferably made of platinum (*see* Sandow and Isaacson, 1966). When an electric stimulus, e.g., a short (0.2 msec) square-wave shock of adequate intensity, is applied to the electrodes of this massive stimulating system, the current passing through the medium from electrode to electrode necessarily passes simultaneously through all longitudinal elements of the intervening muscle tissue. Thus, they are all thrown into excitation at the same instant and they continue their responses quite synchronously (Sandow, 1948). In this way, the distortions due to propagation of a wave of activation are effectively eliminated. Another procedure for achieving practically the same result by means of a multi-electrode assembly has been introduced by the Hill school of muscle physiology in the "all-over" method of stimulation (Hill, 1949). It should be noted that even under either of these improved conditions the fundamental response of the contractile component is still affected by the presence of invetable series elastic material (Hill, 1949). But the record now made of the

* Supported by grants from the U.S.P.H.S. (NB-04262-04) and The Muscular Dystrophy Associations of America.

massively stimulated mechanical response, by connecting one end of the muscle to a myograph (the other being fixed), is at least free of the sort of propagative distortions so beautifully demonstrated by Professor Fischer in muscles stimulated in the more usual method by means of a pair of ordinary wire electrodes placed astride the muscle.

The direct improvements resulting from recording a contraction having essentially perfect synchronization of response of all lengthwise units along each fiber were as follows: the peak tension output of the twitch was increased by about 30%, the rate of tension rise, especially at the start of a contraction, was increased several fold, the latent period was shorter, and the recorded LR was several times deeper (Sandow, 1945; Sandow and Kahn, 1952). These features of the massively stimulated contraction not only gave a truer account of the dynamical behavior of the basic contractile mechanism; but the great increase in the recorded depth of the LR very greatly reduced the difficulty of recording this minute relaxation against a background of extraneous base-line disturbances due to various amplifier noises and parasitic ambient mechanical vibrations. Hence, such improvements in myography as we now have in consequence of our present massive and all-over stimulation techniques owe much to Professor Fischer's indication in his 1946 paper that the tension course of an ordinarily stimulated muscle was a highly distorted variation of the behavior of the basic contractile component.

But, in my own case the indebtedness goes further, and this brings me to discuss the latency relaxation in detail. I first observed the LR late in 1941. It may be of interest that this occurred in consequence of my listening to some recorded music at home one evening and suddenly realizing that the crystal pickup unit of my phonograph (a piezoelectric device) might make a good mechano-electric transducer for myographic purposes. I immediately put my hunch to the test, for I removed the pickup arm from the phonograph and brought it to my laboratory where—quickly and rather crudely, to be sure—I set it up in connection with an oscilloscope as a myographic recorder. I soon found that not only did it have its expected use in myography (*see* Sandow, 1944), but, above all, it indicated unmistakably, though far from perfectly, something unexpected: the existence of a precontraction elongation of the muscle. I believed that evening that I had made the very first observation of this phenomenon. But later I recalled, and then definitely checked, that in his 1926 paper Professor Fischer, using a special elastometer, showed that a frog sartorius muscle undergoes a very small increase in extensibility during the latent period. Furthermore, Fischer stated in his paper that this was the equivalent of a precontraction elongation which had been observed for the first time by Rauh in 1922 by means of a simple, though extremely sensitive, optical lever system. I was, of course, rather disappointed that I was not the first to discover the LR. But I was also excited by the possibility that, by properly using my highly sensitive crystal pickup, I would have such a greatly improved technique over that available to Rauh, which enabled him only to detect the LR, that I could fruitfully develop studies of the LR and of the latent period as a whole in relation to the general problem of the mechanism causing activation of contraction. In the following I trace some features of this development. (Needless to say, the pickup arm of my phonograph was taken home in due time and reconnected to my phonograph, and it was replaced in my laboratory by an appropriately constructed piezoelectric myographic unit.)

It is interesting that the precontraction relaxation that Rauh had discovered did not immedately attract much attention. Probably this was due to the view held by some (*see* Rauh, 1922) that the effect was only an artifact, or to the feeling that, if real, it could hardly be studied in detail since the instruments available to muscle physiologists in the twenties could hardly do more than demonstrate its existence. The phenomenon, at any rate, was given a name, *Rausche Nase* (Rauh, himself, had called it *die Nase,* from the rather nose-like contour of the LR on his records). It is noteworthy that Professor Fischer did not refer to it in this fashion, and, in my 1944 paper, I gave reasons for discarding this term and replacing it with "latency relaxation." More substantively, however, attempts were made to account for this odd feature of a muscle's response, whether, e.g., it represented an active or passive increase in extensibility. Garten (in an appendix to Rauh, 1922) suggested that it represented a change in elastic coefficient caused by the heat which developed in an activated muscle. But this explanation was rejected by Fischer (1926; *see also* Abbott and Ritchie, 1951), and he concluded by stating: "Wir mussen vielmehr vorläufig einen noch unbekannten Umlagerungsprozess als Ursache dieses Phänomen ansehen." And, in essence, this view still holds although, as will be seen, we can now embellish our ignorance with new possibilities that are at least more alluring than those at first proposed.

For some twenty years following the earliest work on the LR there were only a few references to it (e.g., Schaefer and Göpfert, 1937; and Göpfert and Schaefer, 1941, who demonstrated the LR in mammalian muscle), but nothing further was done to elucidate its nature. Then, when there was a resumption of active interest in it, attempts were made to identify the material of the muscle that was responsible for producing it, and proposals were made that the source

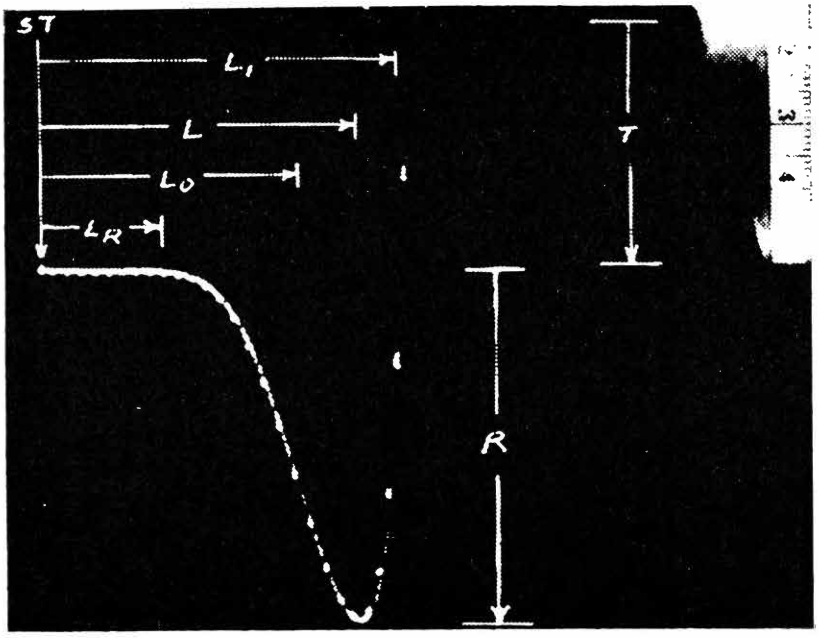
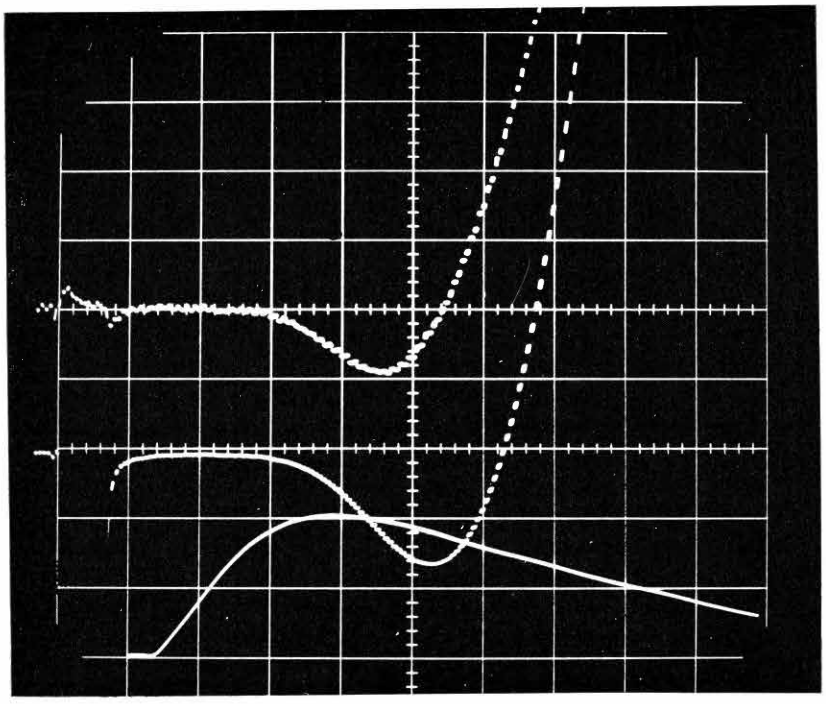


Fig. 1—Records of latency relaxation and associated changes in isometric twitch responses of the massively stimulated, curarized frog's sartorius muscle.

Upper photograph. Made at 13 C by piezoelectric transducer and cathode-ray oscillography (Sandow, 1952). *St* indicates instant of stimulation; the *L*'s indicate various latencies measured from the instant of stimulation: L_R, to onset of the LR; L_o, to point of inflection of the LR (at which contraction has onset, *see* Sandow, 1944); *L*, to end of the LR; and L_1, to onset of positive tension output above original resting tension level. *R* measures the depth of the LR, and *T*, the peak twitch tension recorded by optical myography. The dots superimposed on the LR record are timing signals at 0.2 msec intervals.



Lower photograph. Record made at 20 C by the RCA Type 5734 mechanoelectronic transducer tube and multi-channel cathode-ray oscillography. The twitch output is recorded on the lowest trace, with one horizontal box equalling 10 msec, and one vertical, 10 g. The middle and uppermost records present the earliest part of the mechanogram on the two traces of the electronically switched second beam of the oscilloscope, the lower of these giving the LR in direct form, and the upper as the time derivative (rate) of the direct tension change. For both of these traces, one horizontal box equals 0.5 msec, and the direct LR tension calibration is 50 mg for one box. Note that the direct and derivative LR traces first record the duration of the shock (0.3 msec).

was the contractile material (Sandow, 1944) or, as cogently discussed by Hill (1951), some parallel structure such as the sarcolemma. Although evidence then available could not definitely resolve this issue, the presence of the LR and the study of its variations under a variety of conditions provided new definite criteria for measuring the latent period, which permitted determination of certain kinetic features of the earliest phases of activation of a muscle's response (Sandow, 1944, 1945, 1947; Abbott and Ritchie, 1951; Hill, 1951) and indicated some underlying aspects of excitation-contraction coupling (Sandow, 1952, 1965; Sandow, et al., 1964; Sandow and Preiser, 1964; Sandow, Taylor and Preiser, 1965). Since in this article I do not intend making a comprehensive review of work on the latent period, the interested reader can refer to the listed papers for details of the indicated applications of the observations regarding latency phenomena.

I wish now to discuss the latest attempts to give significance to the LR. In the period since the aforementioned proposals were made about the nature of the LR, some new and highly important functional systems of muscle have been discovered—the sliding filament mechanism of contraction, and the internal membrane systems concerned with excitation-contraction coupling—and it is interesting to speculate about them in relation to the LR.

As is well known (H. E. Huxley, 1960, 1965), the sliding filament mechanism involves two sets of interdigitating filaments in each sarcomere; the one, thin and made up of actin, and the other, thick and made up of myosin. These engage in contraction when the heavy meromyosin side-branches of the thick filaments are activated by excitation-contraction (E-C) coupling to form cross-bridge attachments to the actin filaments, and then move, and make and break cyclically, so as to force the thin filaments further into the spaces between the thick ones, thereby tending to make the sarcomeres shorten and set up contraction in the fiber as a whole. A. F. Huxley (1957), impressed with the view (Hill, 1951) that the LR must arise in a structure parallel to the contractile system, suggested that this condition could be satisfied if either, but not both, the actin or the myosin filaments had special connections so arranged as to make each part of an element running continuously between the two neighboring Z lines of a sarcomere and under some tension in the resting fiber. The LR could then be conceived to arise as a relaxation of this tension that begins just prior to the structurally parallel formation of the cross-bridges and the resultant development of positive tension. At the time this scheme was postulated it was thought that the inner ends of the actin filaments were joined together by the so-called S filaments, and so there seemed to be a factual basis for the particular supposition that it was the actin filaments that produced the LR as described above. But there is now no visual evidence for the existence of the S filaments, nor of equivalent ones that were imagined to join the myosin filaments to the Z lines, and so there is at present no morphologically evident basis for explaining the LR as suggested by Huxley. But, as discussed by Podolsky (1964), certain physiological evidence suggests the existence of S filaments, and so there is still a possibility that the LR reflects a very early relaxation of a specially continuous type of thin filament.

If we disregard the view that the LR must take place in an element parallel to the contractile mechanism, it could be supposed that even in the resting state some cross-bridges exist and create some tension, and that the LR is produced when some very early event of E-C coupling breaks these cross-bridges. But this mechanism is inherently quite improbable, and certain features of it, which need not be detailed here, are in contradiction to experimental results. I, therefore, feel it does not merit further discussion.

We now consider the internal membrane system as a second recent major development of our knowledge of how muscles work that may have some bearing on the mechanism of the LR. This set of structures is composed of two distinct parts, the longitudinally oriented sarcoplasmic reticulum (SR) and the transverse (T) tubules. These ramify throughout the interior of the fiber, but combine to form special complex units which are in regular register with certain parts of the striations. In the skeletal muscle of the frog these units are the triads which are composed of a central T tubule flanked by a pair of lateral sacs of the SR, and which closely encircle each myofibril at the level of each Z band (*see*, e.g., Peachey, 1965a and H. E. Huxley, 1964). As recently reviewed (Sandow, 1965), the function of these structures in E-C coupling seems, in outline, to be as follows: the action potential, running its course essentially along the surface membrane of the fiber, produces an inward moving signal in the radially oriented T tubules which, on arriving at each triad, activates the two flanking lateral sacs of the SR to release some of their stores of bound calcium. The free Ca^{2+} diffuses into the neighboring myofibrils, first into the I bands near the Z lines and then toward the region of cross-bridges in the A bands where it activates the sliding filament mechanism of contraction. It is noteworthy that A. F. Huxley (1957) proposed that, at least in frog fibers, there seems to be a longitudinal spread of activation in each sarcomere, which starts at the level of the Z lines and moves through the I bands to the region of overlap in the A bands. As one of the possibilities of a mechanism for such a process, Huxley suggested the diffusion of a

substance, and this is now given a specific form in the diffusion of Ca^{2+} through the myofibrils as indicated above.

It is obvious that in this E-C coupling sequence the actions of the T tubules and the lateral cisternae, and the diffusion of the Ca^{2+} to the cross-bridges, must occur during the latent period, and it is therefore reasonable to ask whether the LR might not be an expression of some feature of these processes. There seems to be no reason why the diffusion of Ca^{2+} should produce any mechanical effect such as an LR. And propagation of the E-C coupling signal down the T tubules can also be ruled out, since this occurs very early in E-C coupling (it may even occur in part almost in synchrony with the action potential, *see* Sandow and Isaacson, 1966), and thus very likely takes place during the earlier part of the latent period before the LR develops. We are thus left with the possibility that the Ca-releasing action of the SR in E-C coupling is responsible for producing the latency relaxation.

In elaboration of this idea, it should be noted that the SR makes up a large part, 15%, of the volume of the fiber (Peachey, 1965a), and it is arranged in longitudinal columns within the fibers thus forming, in conjunction with the triadic element of the T tubules, a set of essentially continuous axially oriented mechanical systems which envelop and are in parallel with the myofibrils. Now, release of Ca^{2+} from the lateral cisternae could set up an osmotic change in the SR, i.e., the release of Ca^{2+} from the lateral sacs (especially if accompanied by some anion) should cause the osmotic pressure to decrease in the SR and cause water to flow out of it. Thus, tension within the initially stretched muscle should decrease and give rise to the latency relaxation. This decrease in tension should, furthermore, be detectable not only as the usually observed axially trecorded LR, but also as a transverse LR, if the postulated decrease in osmotic pressure results in essentially isotropic changes in shape of the SR. Such a transverse LR has, in fact, been observed (Sandow, 1947). Thus, there is the following presumptive evidence that the LR reflects a decrease in tension of the SR as the lateral sacs of this structure release Ca^{2+} during E-C coupling: 1) the LR occurs after a brief delay following excitation, but before contraction begins, and thus at a time when the SR should be releasing Ca^{2+}; 2) an osmotically produced decrease in tension of the SR may conceivably occur in consequence of the release of Ca^{2+} by the SR; and 3) the general argument of Hill (1951) that the LR must occur in a structure parallel to the contractile component is satisfactorily met by making the reticulum responsible for producing the LR. In accordance with this interpretation of the LR, we should note that certain of its parameters may serve as temporal signposts of the release and longitudinal spread of the activator Ca^{2+} in the myofibrils. Unfortunately, such signposts must involve some ambiguity because our present knowledge (Sandow, 1965) indicates that it takes some time, of the order of several milliseconds, for the activating signal in the T tubules to spread inward into the depths of the fiber. Hence, the series of reticular elements of the various triads connected to a particular T tubule will be asynchronously activated to release their Ca^{2+} and produce their supposed LR response, and there will then also be an asynchrony in the onset of tension in the corresponding myofibrils. It seems reasonable to suppose, however, that at least the temporal features of the LR represent the essential timing of the events of interest occurring in a thin layer of myofibrils which lie just inside the sarcolemma and are the first to respond. Therefore, the assumption is made in the following that L_R effectively marks the instant at which the Ca^{2+} is released

from the reticulum of this relatively superficial layer and begins its longitudinal diffusion in the neighboring myofibrils, and L_o signifies the earliest moment at which the Ca^{2+} has reached the nearest overlap spot in sufficient concentration (*see* Sandow, 1965) to activate contraction. The justification for choosing L_o for indicating the onset of contraction and not L or L_1, will be found elsewhere (Sandow, 1944).

There is a considerable amount of evidence about the variations in all the different parameters of the LR in the frog sartorius muscle as a function of muscle length (Sandow, 1944; Abbott and Ritchie, 1951; Guld and Sten-Knudsen, 1960, fig. 2), and pH and temperature (Sandow, 1947). In view of the preceding discussion (and other considerations, too) it is at present unclear how these various findings can be used to test the hypothesis that the LR has its source in the reticulum, and much more work is needed to settle the various problems which are posed by such tests. For the present, however, we turn again to the results presented in fig. 2 and note the following. Increase in length causes in general oppositely directed temporal variations in L_R, and in L_o, L and L_1 which measure, each in its own fashion, the latent period for positive tension development. This difference is consistent with the hypothesis for it might be expected that, since the LR and the positive tension development are supposed to occur in such structurally and functionally different components of the fiber, they might then show qualitative differences in the way their kinetics are altered by changes in muscle length.

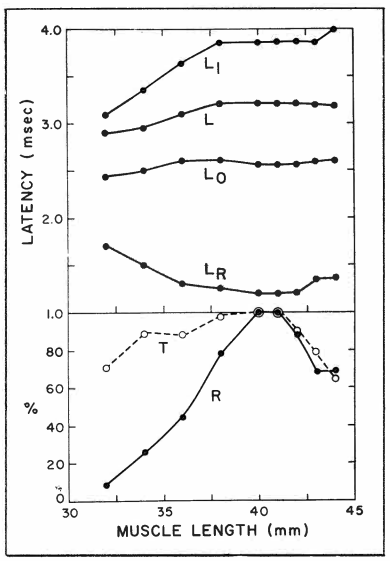


Fig. 2—Changes in latencies and tension outputs of an isometric massively stimulated curarized frog's sartorius muscle (in situ length, 33 mm) at 20 C as a function of the length of the muscle. Symbols of the various parameters are defined in figure 1.

Particular problems are posed by the specific nature of the effects of muscle length on L_R as against those on the various positive tension L's. It is not easy to see how increase in muscle length should alter the supposed behavior of the SR so as to decrease the time L_R. It is conceivable, moreover, that this decrease in L_R is, at least in part, a consequence of the decrease in transverse size due to stretching the fiber, and thus of some change in the timing of the T tubular function in E-C coupling. More meaningful, however, is the stretch-induced increase in the L's for positive tension, especially in relation to the associated decrease in L_R. Now, Guld and Sten-Knudsen (1960)—who have obtained results on single fibers like those on whole frog sartorii reported earlier (Sandow, 1944; Abbott and Ritchie, 1951) and now confirmed by the new experiments of fig. 2—ascribed the increased latency for positive tension development that is produced in stretched fibers to the greater time required for an activation process (in the general sense as proposed by A. F. Huxley (1957), but whose detailed nature they did specify) to spread longitudinally over the greater distance in the stretched sarcomeres from Z line to filament overlap region. Guld and Sten-Knudsen's analysis of their results indicated that the activation process moves longitudinally in the myofibril at 22 C with a velocity of 0.4 mm/sec. I agree with their general interpretation, and suggest, furthermore, as already indicated in the foregoing, that the essential process is the longitudinal diffusion of Ca^{2+}. As discussed elsewhere, (Sandow, 1965) the processes of E-C coupling by which Ca^{2+} is made available to activate contraction, in, e.g., a frog skeletal muscle fiber, include both radial and longitudinal diffusion through the myofibril until the Ca^{2+} reaches the overlap region of the filaments in the A band, where it activates contraction. A condensed analysis of the radial diffusion kinetics of the Ca^{2+} has been given in the previously cited work (Sandow, 1965), and our concern now is only in the longitudinal kinetics. This is a special problem in diffusion theory which will not be discussed in detail here. But, if we recall that stretch of a sarcomere causes an increase in width of only the I band (Huxley, 1960), it is obvious that the longitudinal path and associated time for diffusion of the Ca^{2+} from the neighborhood of the Z line, where it is liberated, to the region of overlap in the A band, where it acts, should be greater, the more the sarcomere is stretched. And furthermore, a measure of the time required for this to occur, as previously suggested, would be the difference in time between L_o and L_R, i.e., $L_o - L_R$. This postulated mechanism might be questioned by positing that stretch of a muscle would result in some displacement and distortion of shape of the lateral sacs that would tend to reduce or even eliminate the increase in diffusion distance with stretch. But this does not seem likely, because the detailed structure of the relatively sparse and irregularly subdivided longitudinal tubules and fenestrated collar of the SR (Peachey, 1965a and b) suggests that these elements would be pref-

erentially stretched by increase in length of the sarcomere, thus leaving the lateral cisternae in their essentially original placement opposite the portions of the I band close to the Z line.

Turning now to the results presented in fig. 2 (which were obtained from massively stimulated frog sartorii and therefore are preferable to those previously obtained (Sandow, 1944) by wire electrode stimulation), we see that the interval $L_o - L_R$ is in fact increased with increase in length of the muscle, and thus it increases with lengthening of the sarcomere, or, more precisely, with increase in the distance of longitudinal diffusion of the activator, this distance being, in essence, half the width of the I band, i.e., 0.5 I. In connection with the results of fig. 2, the average sarcomere lengths corresponding to the muscle lengths of 32 and 40 mm were very likely about 2.3 and 2.9 μ, respectively, and thus the increase in the 0.5 I distance was 0.3 μ. And the corresponding increase in $L_o - L_R$ was from about 0.7 to 1.4 msec, i.e., 0.7 msec. Thus, an apparent velocity for longitudinal diffusion of activator Ca^{2+} would be $0.3\mu/0.7$ msec $= 0.43\mu$/msec, and this is in quite good agreement with the value, 0.4 mm/sec, i.e., 0.4 μ/msec, that Guld and Sten-Knudsen (1960) found from a similar analysis of their results obtained on single muscle fibers at 22 C.

It is obviously of great interest to determine whether this apparent speed of longitudinal movement of an activating influence could be accounted for by the diffusion of Ca^{2+} along the length of one-half of an I band. Rough calculations suggest that this is the case, but the details will be omitted here. For the present, however, the main point of our analysis regarding the significance of the increase in the time interval $L_o - L_R$ with stretch of the muscle is that it suggests that the time lapse between the beginning of the LR and the earliest instant of tension production may be accounted for by the time taken for the diffusion of a substance, which I assume to be Ca^{2+}, from a point in the sarcomere near the Z line to the region of overlap of the sliding filaments. This is consistent with the view that the LR reflects a mechanical change of the stimulated fiber which is produced in consequence of the release of Ca^{2+} from the lateral sacs of the reticulum. Much more must be done, however, to test the proposed hypothesis. Thus, on the basis of the hypothesis, the ability of a muscle to produce an LR should be related to the structure of its internal membrane systems, particularly of the SR. Indicative in this connection are the peculiarities and the relative difficulty of recording the LR in certain invertebrate muscles (Lowy and Sten-Knudsen, 1963). But, before anything definite can be concluded in this regard it will be necessary to know much more about the SR, or, more generally, of the system regulating Ca flux of these muscles (but *see* Hanson and Lowy, 1961). It is also interesting that, in the amphibia, the slow muscles differ from their fast counterparts in respect to both the T tubules and the SR (Peachey and Huxley, 1962; Page, 1965) and it will be of great importance to see if the slow muscles produce corresponding variations in their LR. Finally, it should be noted that in some muscles the T tubules and hence the corresponding lateral sacs of the SR make contact with myofibrils at the level of the boundary of the A and I bands (e.g., A. Huxley, 1959; Andersson-Cedergren, 1959) and, therefore, stretch of such fibers should not cause the increase in tension latency found in frog fibers. Clearly, there are several types of experiments yet to be performed which should indicate whether the hypothesis regarding the source of the LR in the behavior of the sarcoplasmic reticulum is correct.

In conclusion, it is worth emphasizing that the LR is an undoubted feature of the response of many different types of muscles. It is provocative that there is at present no definitive explanation of it although this review indicates that either the thin member of the sliding filaments or the sarcoplasmic reticulum may provide the structural basis for producing the LR. Further research should resolve this problem and in so doing increase our comprehension of the mechanisms, in general, by which muscles respond to stimulation.

Acknowledgment

I am grateful to my associate Dr. Hanna Preiser for obtaining the results presented in figure 2.

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Some Effects of Extreme Shortening on Frog Skeletal Muscle

SIBYL F. STREET, MICHAEL N. SHERIDAN† AND ROBERT W. RAMSEY

Departments of Physiology and Anatomy, Medical College of Virginia, Richmond

In 1940 Ramsey and Street found that if isolated fibers of frog muscle were stimulated to shorten to more than one-third of the length at which maximum isometric tension was developed, some of their reactions were permanently changed. Probably the most important of these changes was an alteration in the relation of fiber length and isometric tension. Active tension developed at shorter lengths than before, and maximum isometric tensions were about 50% of normal. At that time, we believed that it was the contractile proteins that were affected because we could find no significant change in passive tension or in excitation, but this was not sufficient evidence to prove the point. Now that electron microscopy has so greatly increased the information on the fine structure of muscle, it seemed worthwhile to undertake further studies on muscle that has shortened below its normal limits. In 1940, we applied the term "delta state" to all such muscle, but in this paper we apply it only to muscle which has shortened to 35% or less of optimum tension length. This article will describe the results obtained to date in an electron microscope study of shortened muscle and will also include measurements of the resting oxygen consumption of delta state frog sartorius muscles.

* Work largely supported by PHS grant He-06389 and also by A. D. Williams funds of the Medical College of Virginia. These results were partially reported to the Federation of American Societies for Experimental Biology, April, 1966.

† Now at the University of Rochester School of Medicine and Dentistry.

MATERIALS AND METHODS

Electron Microscopy

Semitendinosus muscles (*Rana pipiens*) were isolated and equilibrated overnight in frog Ringer's solution. The next day small bundles consisting of three or more muscle fibers were dissected from them. These were stimulated to shorten partly or fully as described in the figure legends.

The bundles were fixed for two hours by adding sufficient glutaraldehyde to the frog Ringer bath to bring the concentration to 2.5% and post fixed in 2% OsO_4 with phosphate or veronal acetate buffer for three to four hours. They were then washed in distilled water, dehydrated in a graded series of acetone solutions and embedded in Araldite. Sections of 600 to 800 Å in thickness were cut with a Porter-Blum microtome (MT-2) using glass knives. The sections were stained with $KMNO_4$ (Lawn, 1960) or lead citrate (Reynolds, 1963) and viewed with an RCA EMU 3 E or 3 G electron microscope.

Oxygen Consumption

Sartorius muscles of medium sized frogs (*R. pipiens*) were measured in situ. Both muscles were dissected out, but were left attached to the pelvis and placed in frog Ringer's solution to equilibrate overnight at 4 C.

The next day tetanic isometric tension was recorded with the muscles held at the length measured as maximum in the body: for sartorius this is approximately optimum tension length. (Grass stimu-

lator: stimulus duration, 1 msec; frequency 80/sec for 0.2 seconds, temperature 18 C). Tension was recorded by a suitable strain gauge. Then both muscles were given three two-second tetani at 10-minute intervals; one muscle was allowed to shorten freely, the other was held at maximum body length.

Resting oxygen consumption was measured by standard methods. Right and left muscles from each frog were paired because resting oxygen consumption of muscles from different frogs normally varies by at least a factor of two. After the tetani were administered, the muscles were cut off the pelvis, weighed, and placed in Warburg flasks. Oxygen consumption was measured at 18 C for 4 to 5½ hours.

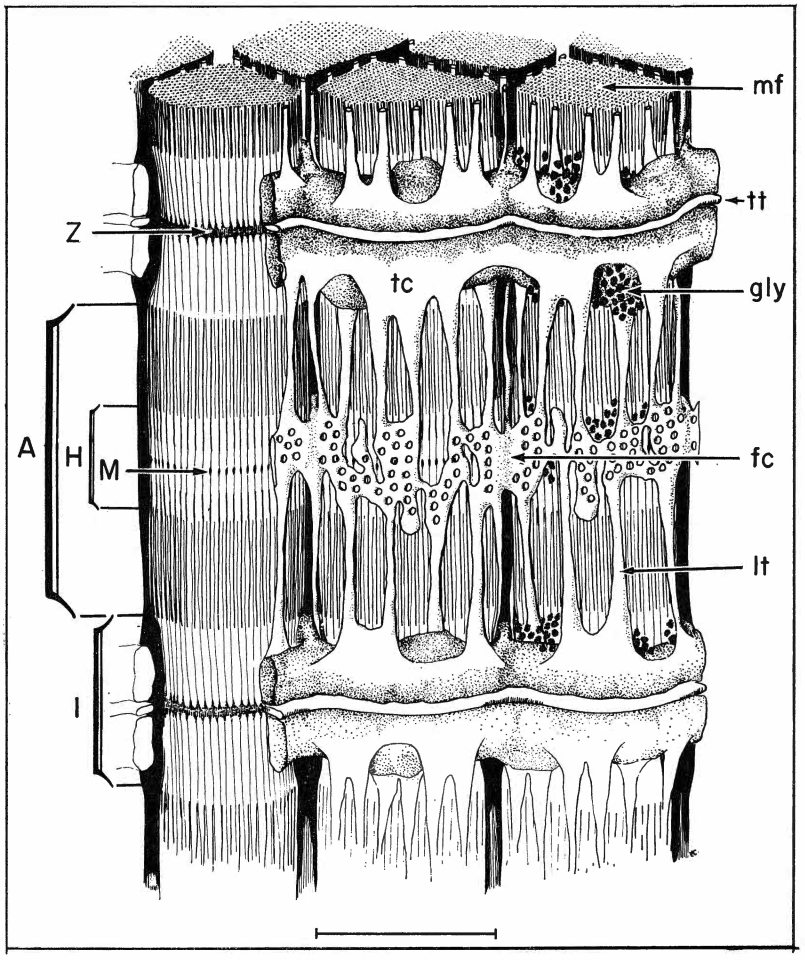

Fig. 1—Three-dimensional reconstruction of the sarcoplasmic reticulum (SR) associated with several myofibrils of frog sartorius muscle. Reproduced by permission of the Rockefeller University Press (Peachey, 1965) with our lettering added. The myofibrils (mf) show a transverse banding pattern with the Z lines at the centers of the light I bands (I). The A band (A) has a central light H zone (H) and denser outer regions where the thick and thin myofilaments overlap, and a dark M line (M) at its center. The fenestrated collar (fc), or H band cisterna, of the SR connects to the terminal cisternae (tc) by the longitudinal tubules (lt). Transverse tubule (tt). Glycogen granules (gly). Dimensions: sarcomere, Z line to Z line-2.65 μ; A band (thick myofibrils) $\sim$1.6 μ; thin myofibrils $\sim$1 μ. Bar $\sim$1 μ.

Three series were done: 1) Normal paired with delta long (the shortened muscle was slowly extended to its original length and active tension during a brief, 0.2-second tetanus was recorded at that length). 2) Normal paired with delta short (the shortened muscle was not re-extended and the last two-second tetanus was given in the Warburg flask). 3) Delta short paired with delta long.

RESULTS

Electron Microscopy

We have found evidence that it is the contractile proteins (the interdigitating filaments) which are most affected by extreme shortening and have also demonstrated that the sarcolemma is tied in to each sarcomere at the level of the M line, as well as at the Z line. Before describing these results we shall

Fig. 2—Normal, longitudinal section, a relatively smooth sarcolemma (S) is present. The usual cross banding is evident (A,I,Z, and M). Mitochondria (Mi), lipid (L) and glycogen (electron dense granulation) occupy the intefibrillar space. Triads (T) are formed by the transverse tubule centrally and terminal cisternae of the sarcoplasmic reticulum laterally. Pb stained. [Figs. 2 through 7, labeling as in fig. 1. Frog semitendinosus muscle.]

outline the presently accepted concepts of the fine structure of a striated, frog muscle fiber. (The structure of mammalian muscle fibers is essentially the same, the main differences being in the location of the transverse tubule system).

Each muscle fiber is enclosed by its sarcolemma, a four layered elastic sheath which terminates in micro-tendons; its innermost layer is the plasma membrane of the cell (Mauro and Adams, 1961). The myofibrils largely fill the interior and are believed to extend from one end of the fiber to the other. Figure 1 is a three dimensional reconstruction (Peachey, 1965). The myofibril on the left shows the interdigitating thick and thin myofilaments (H. Huxley and Hanson, 1954; A. Huxley and Niedergerke, 1954) and their relation to the Z and M lines; the others show the related sarcoplasmic reticulum (SR) and transverse tubule system. The transverse tubules form a network which transverses the fiber at the Z lines and has openings to the fiber surface. (Francini-Armstrong and Porter, 1964; H. Huxley, 1964). The SR is homologous to the endoplasmic reticulum of other cells; it sheaths the myofibrils and its network extends across the width of the fiber at each sarcomere level. In longitudinal section, the transverse tubule with the associated terminal cisternae of the SR form a "triad."

When sarcomere length is 2.0 to 2.2 μ, maximum isometric tension develops if the muscle is appropriately stimulated. Within the normal range of motion of a muscle, changes in fiber length, whether active or passive, correlate with changes in sarcomere length and with the amount of overlap of the thick and thin filaments, without change in length of the filaments. When a sarcomere has shortened to 1.6 μ, the ends of the thick filaments are touching the Z line; further shortening involves crumpling or coiling of the filaments.

Figure 2 is a longitudinal section of normal semitendinosus, showing typical cell structures, including the sarcolemma, while figure 3 shows a bit of the edge of a fiber from a small bundle that was stimulated to shorten to a sarcomere length of about 1.3 μ. Sufficient Glutaraldehyde was added to the Ringer's solution to bring the concentration to 2.5% while stimulation continued. The Z line, presumably, is obscured by material of the filaments piling up near it and the M line by the presence of a double array of thin filaments which have slid into it. The most striking feature is the festooning of the sarcolemma. Festooning at the Z line was first described about a hundred years ago (Tiegs, 1955). In so far as we know, festooning at the M line has not been observed before. Its occurrence strongly suggests that the H-band cisterna, or fenestrated collar of the SR, adheres to the sarcolemma.

Figures 4, 5, 6, and 7 are all of the same specimen. This was a bundle of three fibers which were stimulated to shorten from 14 mm to about 4 mm by three two-second tetani. Such long stimulation is not necessary for complete shortening of a small bundle of fibers; it was done so that the specimen would be comparable with the delta state sartorius muscles. The bundle was stretched slightly after shortening and fixed at a fiber length of 6.5 mm. The structures shown are indicated in the figure legends. Festooning of the sarcolemma at the Z and M lines is definite, indicating that the transverse systems have not been disrupted. The mitochondria with their cristae are intact and transverse tubules and sarcoplasmic reticulum and the triads they form are easily recognized and appear normal. However, the thick myofilaments are less than 1 μ long and somewhat disarrayed.

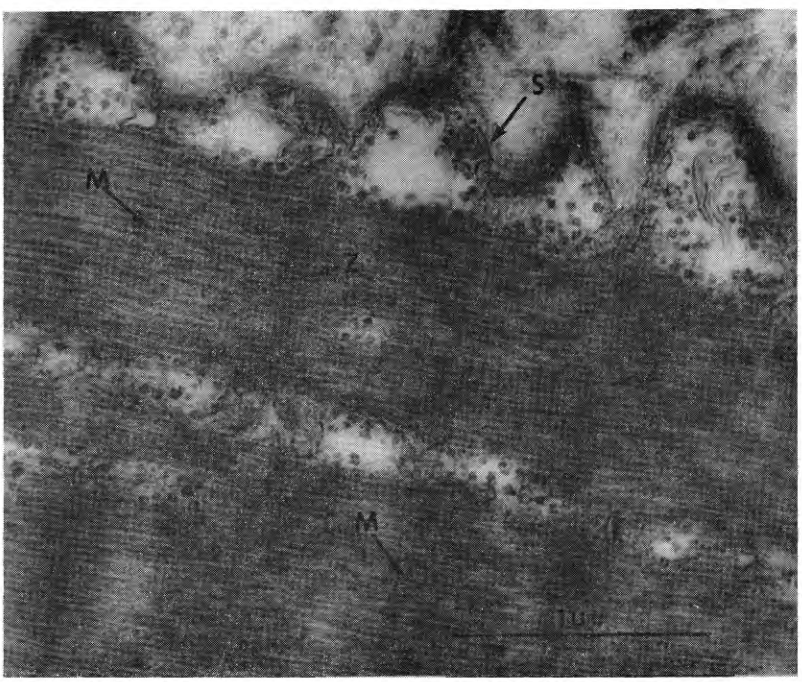

Fig. 3—Longitudinal section of the edge of a muscle fiber fixed at a sarcomere length of 1.3 μ while being tetanized. Shortening has obscured the cross-banding; Z lines are not distinct due to thick filaments of adjacent sarcomeres abutting at that point. M band is present. Note the pronounced festooning of the sarcolemma produced by shortening. This preparation indicates an attachment of the sarcolemma to intracellular structures at the level of the M line as well as at the Z line. $KMnO_4$ stained.

Resting Oxygen Consumption

In these experiments, we considered that a muscle was in the delta state if maximum isometric tension decreased at least 30% after the shortening procedure; usually it decreased 50%.

When using whole muscle it is necessary to remember that one is dealing with a population of fibers. We think the loss of tension is not due to failure of half the fibers to react for several reasons. The three main ones are:

a) Visual observation with low magnification confirms that most fibers shorten.

b) It is well known that a quick stretch of 2 or 3 mm applied to a normal muscle during a tetanus at optimum length results in a considerable increase of tension, as much as one-fourth to one-third of the maximum. Quick stretch of delta state single fibers or whole muscle results in only a slight increase of tension and never restores the maximum.

c) After shortening, the relation between length and tension is the same for sartorius muscle as for delta state single fibers of muscle (Ramsey and Street, 1940). Figure 8 shows a graph of such an experiment. Its details are explained in the legend.

The data for the resting oxygen consumption are given in table 1. There is considerable variation, but since the oxygen consumption of delta state muscle always exceeded that of its normal pair, and delta short always exceeded delta long the differences are definitely significant. The probability that these observations could occur by chance is less than 0.005 for the first and third sets and 0.05 for the second.

DISCUSSION

The finding that the sarcolemma is quite firmly tied in to the sarcomeres at the M line as well as at the Z line is an interesting one. According to Tiegs (1955), the attachment at the Z line, indicated by festooning, was observed as far back as 1859. It seems likely that the network of transverse tubules is part of this tie. He describes the M line as "exceedingly delicate," and pictures it as sometimes extending to the sarcolemma. Dorn (1965) says he has often observed invaginations at the M line as well as at the Z line in guinea pig muscle, but does not illustrate it.

While the sliding filament model of muscle structure has been accepted for years as the best one available, some aspects of mechanical coupling have never been clarified. For example, how can the unattached array of thick myofilaments stay neatly in place in the middle of the sarcomere while generating forces of 2 to 4 kg/cm^2 of *fiber* cross sectional area? A transverse network at the M line could play a role in positioning these filaments and there is considerable evidence for the existence of a complex network there. Franzini-Armstrong and Porter (1964) have pictured cross connections between the thick filaments at the M band. The cisternae of the SR, located at the middle of the sarcomere have continuity across the transverse width of the muscle fiber (Bennett, 1960; Peachey, 1965), and Bennett (1955) and Foulks (1965) have shown that they often connect to the M band. We also have seen this in our preparations (fig. 5). It seems likely that these cisternae adhere to the sarcolemma at the circumference of the muscle fiber but serial sections will be necessary to prove the point.

In some circumstances, active tension, even maximum isometric tension, is transmitted laterally from myofibril to sarcolemma and tendon (Street and Ramsey, 1965). It is possible that the transverse networks are involved in this mechanical coupling, in spite of their delicacy.

What happens to the interdigitating filaments when a sarcomere shortens below 1.6 μ is a problem we are still working on. Micro-

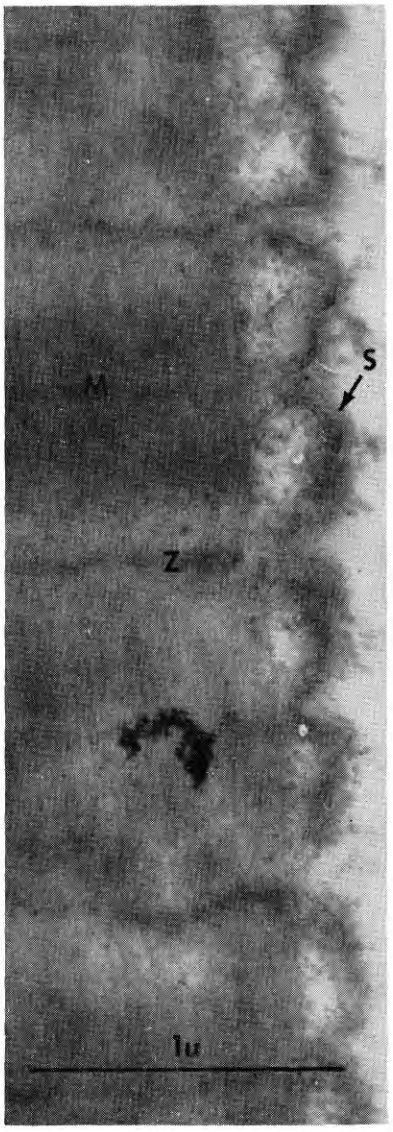

Fig. 4—The sarcolemma still shows festooning, indicating that the attachments in the regions of the Z and M lines are not broken. $KMnO_4$ stained. (Figs. 4, 5, 6, and 7 are all from the same preparation. Three muscle fibers were stimulated to shorten fully, as described in the text, slightly re-extended, and fixed at a sarcomere length of about 1 μ. Delta state muscle.)

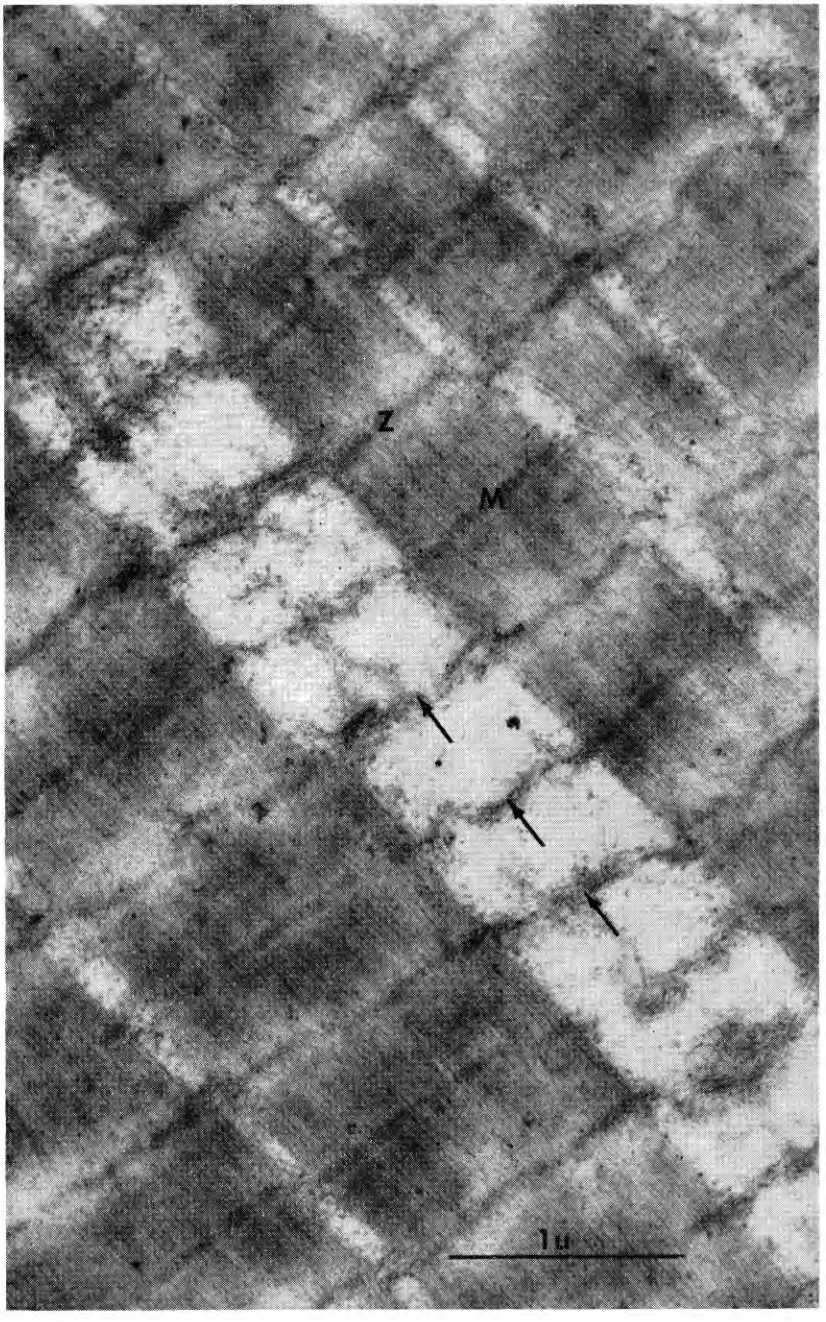

Fig. 5—This micrograph demonstrates myofibril connections at M and Z lines by strands of sacoplasmic reticulum transversing the interfibrillar space (arrows). $KMnO_4$ stained.

graphs of re-extended delta state muscle show the M line still clear and straight, which suggests the cross connections between the thick filaments are not broken. Galey (1964) has published pictures of contractures in frog semitendinosus which show thick filaments varying in length from 0.4 μ to 1.6 μ. Perhaps when the thick filaments shorten they stay short even when the muscle is re-extended.

The increase in resting oxygen consumption of delta state muscle is interesting, but so far unexplained. Fischer (1931) clearly showed that when normal muscle, contracting isotonically, shortened by 10% of its length, the activity oxygen consumption was markedly reduced. In fact, it was this early work of Dr. Fischer's that stimulated our interest in the oxygen consumption of delta state muscle.

SUMMARY

We have established that the sarcolemma of frog skeletal muscle is so firmly tied in at each sarcomere level near the M line, as well as near the Z line, that it is thrown into folds or festoons when the fibers shorten. The attachment is not broken even when the fibers shorten to 25% of optimum tension length. Such extreme shortening affects both the morphology and physiology of the muscle; the morphological change seems to be limited to the myofilaments. The physiological effects in frog sartorius muscle include an increase in resting oxygen consumption and changes in the relation between fiber length and isometric tension similar to those found in isolated muscle fibers.

Acknowledgments

We thank Miss Julia Thomas and Mr. Harvey Selden for technical assistance with the oxygen consumption experiments and Mrs. Shirley Craig for assistance with the tissue preparation for electron microscopy.

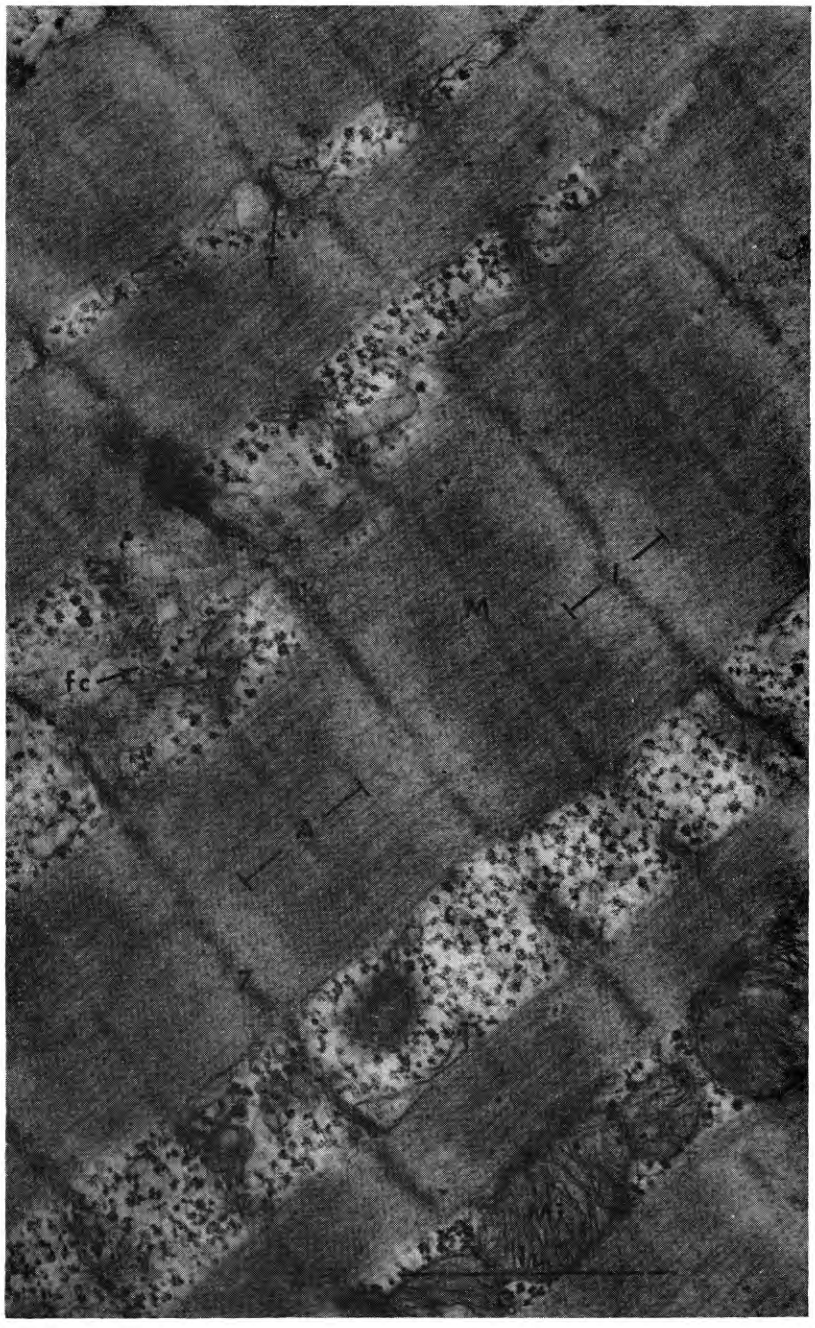

Fig. 6—Mitochondria, triads and fenestrated collar appear normal. The A band is reduced to a length of 0.6 μ in places and its outline is irregular. Pb stained.

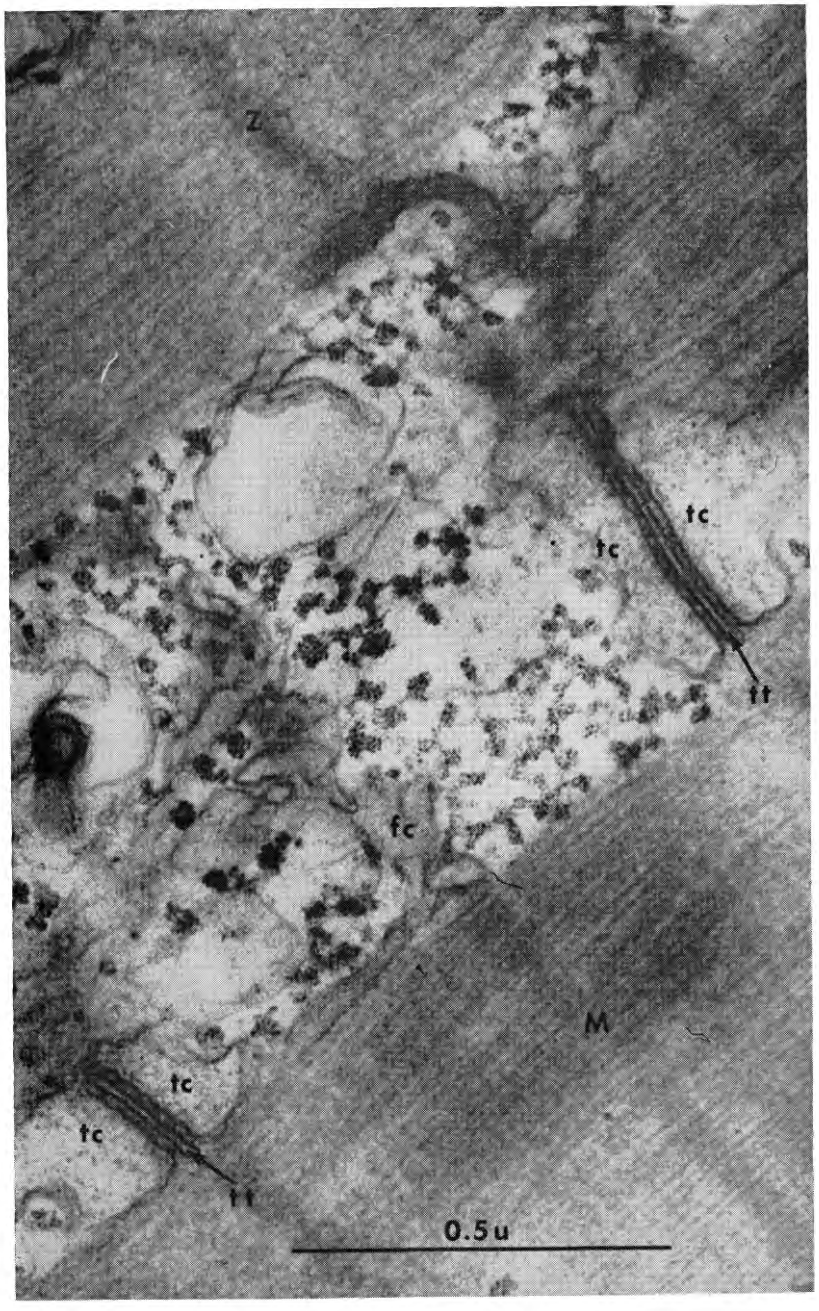

Fig. 7—This micrograph illustrates in more detail the quite normal appearance of the transverse tubules, terminal cisternae, and fenestrated collar region in delta state muscle. Pb stained.

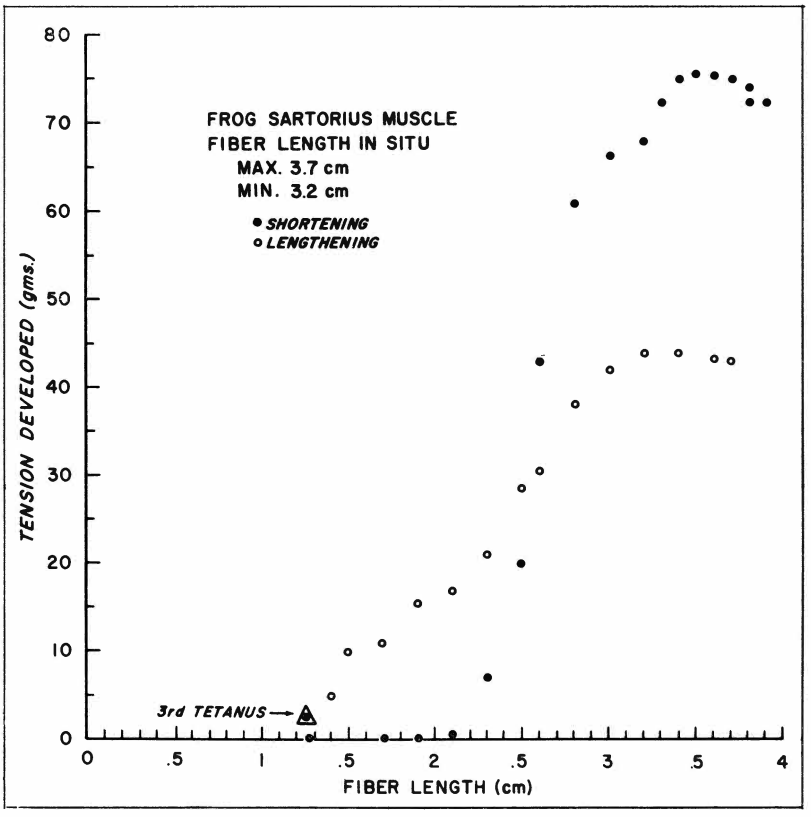

Fig. 8—Graph showing the relation between fiber length and isometric tension. All tetani were 0.2-sec except the three at the shortest length which were 2 sec each. Average sarcomere length (SL) was 2.4 μ at fiber length 3.7 cm. Calculated SL at fiber length 1.3 cm. is 0.8 μ. Shortening tensions (solid circles) and lengthening tensions (open circles) were measured in successive steps. The shape of the curve for re-extension after extreme shortening is the same as that seen in delta state single fibers of muscle. T 18 C.

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TABLE 1
Resting Oxygen Consumption of Frog Sartorius Muscle (μ l/g/hr)

Exp. No.	Normal	Delta Long	Percent
5	54.2	74.1	137
18	44.4	100.9	227
22	62.9	69.3	110
28	51.9	81.4	157
29	55.0	71.1	129
29	44.4	57.7	130
30	38.2	72.1	189
30	41.1	60.4	147
31	44.2	64.8	146
32	50.6	66.3	131
33	36.2	63.1	174
34	41.8	63.8	152
35	33.1	59.8	181
35	32.3	71.5	221
34	54.3	62.0	114

Average % $\frac{\text{Delta Long}}{\text{Normal}}$ = 156

Exp. No.	Normal	Delta Short	Percent
9	48.6	122.5	252
9	42.2	69.7	165
10	59.8	92.0	154
16	64.1	147.4	230
31	38.9	83.9	216
32	38.8	84.9	219

Average % $\frac{\text{Delta Short}}{\text{Normal}}$ = 206

Exp. No.	Delta Long	Delta Short	Percent
6	68.1	108.1	159
6	67.8	87.8	128
11	65.7	86.3	131
15	99.2	102.9	104
15	90.2	114.0	126
17	91.2	101.1	111
17	108.7	124.1	114
19	84.5	91.3	108
19	106.3	133.6	126
23	106.8	122.9	115
23	124.1	134.7	108
24	77.6	116.3	150
24	82.6	118.8	144
25	122.4	163.8	134
26	121.8	183.5	151

Average % $\frac{\text{Delta Short}}{\text{Delta Long}}$ = 127

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Plasma Volume Expansion and Proximal Tubular Reabsorption of Salt and Water by Rat Kidney*

SIDNEY SOLOMON AND HARALD SONNENBERG

Department of Physiology, University of New Mexico, Albuquerque

Venous infusion of both hypertonic and isotonic solutions has been known to lead to diuresis and natriuresis. In recent micropuncture studies, attempts have been made to identify the tubular locus at which renal functions may be altered and to identify the mechanism by which functional changes are mediated. In the rat, Giebisch et al. (1964) found suppressed fractional reabsorption of sodium at the level of the distal tubule when hypertonic saline was administered, whereas suppressed proximal fractional reabsorption was found only when excreted sodium was greater than 13% of the filtered load. On the other hand, Cortney et al. (1965) have observed reduced proximal fractional sodium reabsorption following infusion of isotonic sodium chloride. Similar results have been obtained in the dog (Dirks, Cirksena, and Berliner, 1965). To resolve this seeming discrepancy, Kamm and Levinsky (1965) have suggested that the distal tubule responds to increases in plasma sodium concentration, while the proximal tubule responds to volume expansion. It is our intent to make a detailed study of this response. The present report, however, presents preliminary data showing that the proximal tubule does, indeed, respond to volume expansion.

* Supported in part by NIH grant #HE-08477.

METHODS

Rats weighing 250 to 350 g were anaesthetized with "Inaktin" (100 mg/kg body weight). The jugular vein was cannulated. The animal was infused with a solution of 6% polyvinylpyrrolidone (PVP) in Ringer's solution. The infusate also contained H^3 carboxy inulin (250 μC/75 cc of solution). A priming infusion of 4 cc was given over the first thirty minutes followed by a sustaining infusion given at the rate of 2.4 cc per hour. The left kidney was prepared for micropuncture by conventional techniques (Marsh, Ullrich, and Rumrich, 1963). Micropuncture samples were collected from proximal tubules for no longer than 10 min. Puncture sites were later identified by microdissection of the latex casts of the tubules. Blood was collected in HCT tubes at the end of every two collection periods from the cut tail.

HCT was determined by conventional methods. Plasma and tubular fluid sodium was measured by direct flame photometry using the Beckman flame attachment. Tritium counting rates were obtained by scintillation counting (Packard Tri-Carb).

RESULTS AND DISCUSSION

Upon PVP infusion, a diuresis developed, accompanied by a sharp drop in hematocrit. The HCT fell to 20% when the "steady state" was established. No significant change in plasma sodiums was evident. Plasma sodium in control rats has been found to be 140 mEq/liter, whereas in these experiments plasma sodium concentration was 138 mEq/liter. The HCT and plasma sodium concentration data are interpreted as indicating that infusion of PVP acts to expand plasma volume. Gross changes in renal function which accompany PVP infusion will be the subject of another report.

The possibility exists that PVP could be filtered and that a solute diuresis would result. If such were the case, one would predict that the ratio of tubular fluid to plasma ($^{TF}/P$) sodium concentration would decrease along the length of the proximal tubule as has been shown to occur with mannitol diuresis (Windhager and Giebisch, 1963). Fig 1*A* shows $^{TF}/P_{Na}$ as a function of distance along the proximal tubule. No drop is evident, and the ratio has a value very close to one. It does not seem likely, therefore, that PVP has its major effect by causing a solute diuresis.

Since sodium concentration is constant along the length of the tubule, net sodium reabsorption is indicated by the product of plasma sodium concentration times volume loss. Inulin $^{TF}/P$ as a function of length was used to indicate tubular volume changes and the results of eleven localized punctures are shown in fig. 1*B*. In non-diuretic rats, $^{TF}/P$ of inulin reaches a value

of about three at the end of the proximal convolution, indicating a 66% reabsorption of sodium and water by this nephron segment (Giebisch et al., 1964; Windhager and Giebisch, 1961). In these studies, $^{TF}/P$ of inulin is reduced showing that the reabsorption of sodium and water has been suppressed. These studies indicate then that expansion of plasma volume per se is able to trigger a regulatory response whereby proximal reabsorption of sodium is decreased. This conclusion is in agreement with the suggestion put forth by Kamm and Levinsky (1965).

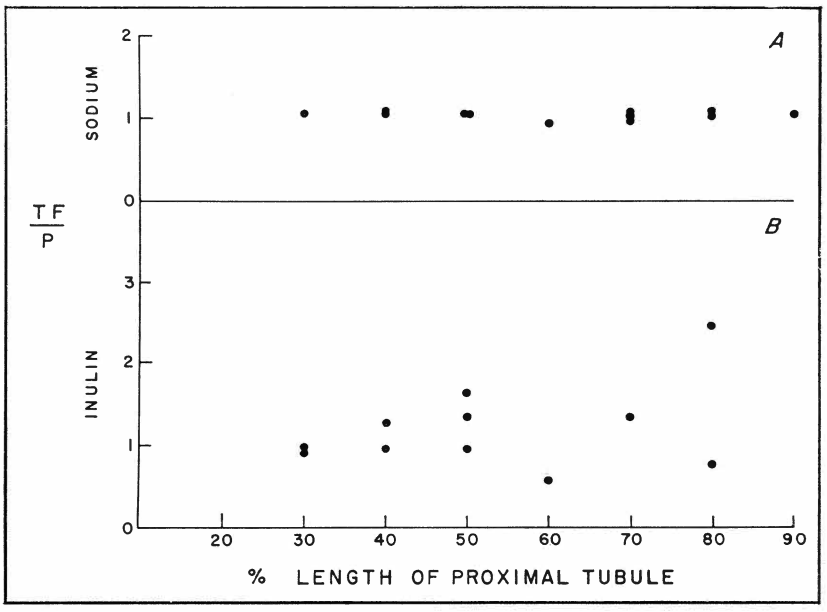

Fig. 1—Tubular fluid to plasma ratios as a function of length of the proximal tubule (measured from glomerulus to beginning of straight segment). *A* shows sodium ratios while *B* indicates inulin ratios.

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Dynamic Physical Fitness and Body Composition

ARCHIBALD W. SLOAN

Department of Physiology and Medical Biochemistry, University of Cape Town, South Africa

THE CONCEPT OF PHYSICAL FITNESS

Although the term "physical fitness" is in common use and each of us has a personal interpretation of its significance, the concept is difficult to define. Physical fitness implies not only freedom from disabling deformity or disease, but also the capacity to perform daily tasks without limitations imposed by poor function of any of the systems of the body.

Gallagher and Brouha (1943) divided physical fitness into three categories: static fitness, dynamic fitness, and motor skills fitness. It is useful to be able to assess the standard of fitness in each category and to know how to maintain, and if necessary improve, each type of fitness.

Static (medical) fitness is assessed by routine medical examination. If no evidence of disease or deformity is found, the individual is considered to be medically fit. There are, of course, degrees of fitness in this category as in the others, and our concept of what constitutes health is quite arbitrary.

Dynamic (functional) fitness is the capacity for strenuous physical exercise. Assuming that the individual is medically fit, the limiting factor in dynamic fitness is the ability of the heart and lungs to supply the active muscles with oxygen.

Motor skills fitness depends on the coordination of groups of muscles to perform purposive movements. This is achieved by the nervous system and improves with practice. The development of motor skill permits a given task to be performed with less effort by reducing unnecessary movements and so contributes to dynamic fitness. Motor skills are assessed in competitive athletics as well as by special tests and are important in all activities which depend on a high degree of neuro-muscular coordination.

Other investigators postulate various categories of physical fitness based on athletic tests. Apart from endurance, which is closely related to dynamic fitness, the main categories are strength, balance, agility, power, and flexibility (Cureton, 1947). Nicks and Fleishman (1962) subdivide the strength factor into explosive (power), dynamic, and static strength, and add speed and coordination. Many batteries of tests have been devised to assess the degree of fitness in each of these categories and from that to derive an estimate of general fitness.

HARVARD STEP TEST

The Harvard step test (Brouha, Graybiel, and Heath, 1943) is a simple test, yet fulfils the criteria for a satisfactory test of dynamic fitness. The subject works at a constant rate proportional to his body weight; large muscle groups are used so local fatigue is not as a rule the limiting factor, and the exercise requires no unusual skill. The results of the Harvard step test have a high correlation with measurements of maximum oxygen intake (Hettinger et al., 1961; Rodahl et al., 1961) currently the most fashionable expression of work capacity, but requiring complex and costly apparatus.

In the original test the subject steps onto and down from a bench or platform 20 inches high 30 times a minute for five minutes or until fatigue compels him to stop. Immediately after the exercise he sits down and his pulse rate is counted for the periods 1 to 1½, 2 to 2½, and 3 to 3½ minutes after the exercise. The fitness index (FI) is then derived from the duration of exercise in seconds ($\times$ 100) divided by the sum of the three pulse counts ($\times$ 2). Montoye (1953) showed that an almost identical FI is obtained if the first pulse count only ($\times$ 5.5) is used, as follows: FI = (duration of exercise (sec) $\times$ 100)/ (5.5 $\times$ pulse count 1 to 1½ min after exercise).

On the results of the test, individuals may be classified in three categories. An FI below 50 is considered poor, 50 to 80 average, and above 80 good.

The Harvard step test was designed for adult men, but with suitable modification it may be used for women or children. Since I found that young women performing the test on a bench 17 inches high had similar FIs to a comparable group of young men stepping onto a 20-inch bench (Sloan, 1959), I have used a 17-inch step routinely for women. However, since 1959, most groups of women I have tested have achieved lower mean FIs than corresponding groups of men.

DYNAMIC FITNESS OF YOUNG MEN AND WOMEN

During the past 8 years I have applied the Harvard step test (or

the modified test for women) to many groups of young adults in South Africa, the United States of America, and Great Britain (Keen and Sloan, 1958; Sloan and Keen, 1959; Sloan, 1959, 1961, 1963). Physical education (PE) students were compared with other medically fit students, non-athletic (NA) in the sense that they were not in regular training for any competitive sport.

The mean FI for each group is given in table 1. In Cape Town the non-athletes were medical students or student teachers: in Chapel Hill they were students of liberal arts; in England they were student teachers; and in Richmond they were students of medicine, dentistry, or physical therapy. In general the PE students had higher FI's than the others, and British men were more fit than South African, who in turn were more fit than American. There was no consistent difference in fitness between the national groups of women.

BODY COMPOSITION

Probably the best method available at present for the estimation of body composition is the underwater weighing technique for determining body density (Behnke, Feen, and Welham, 1942). Applying the time-honoured principle of Archimedes, the subject is weighed first in air and then completely submerged in water; from the loss of weight on immersion the specific gravity is calculated. Assuming that the body consists of two components (fat and lean body mass), each of known and constant specific gravity, if one finds the specific gravity of the body as a whole and makes allowance for air in the lungs and air passages, one can calculate the proportion by weight of fat in the body. The most popular formula is that of Keys and Brožek (1953):

$$\% \text{ fat} = 100\ (4.201/SG - 3.81)$$

Since the apparatus for underwater weighing is not portable and the technique is not practicable on every subject, other ways of predicting body composition have been sought. For many years obesity has been roughly assessed from the thickness of skinfolds pinched up at selected sites and measured by suitably constructed calipers. Formulae have been worked out for the prediction of specific gravity and hence of percentage body fat from skinfold measurements in young men (Brožek and Keys, 1951; Sloan, 1966) and in young women (Sloan, Burt and Blyth, 1962).

A more sophisticated technique for assessing the depth of subcutaneous fat is based on ultrasonic echoes (Hill and McColl, 1961; Whittingham, 1962). A generator produces pulses of ultrasonic waves at a frequency of 2.5 megacycles per sec, which are applied by a small probe through a thin layer of oil to the subject's skin. The probe has two crystals, one of which produces the ultrasound while the other detects the echoes reflected from interfaces between tissues of different composition. The echoes are then amplified and displayed on a cathode ray oscilloscope. The distance between the signal artifact on the oscilloscope screen and the first major peak (echo from muscle sheath) is a measure of the depth of subcutaneous fat. As with skinfold measurements, specific gravity may be predicted from ultrasonic measurements at selected sites. The most appropriate sites for this technique and the corresponding formulae have yet to be found.

BODY COMPOSITION OF YOUNG MEN AND WOMEN

In 1961 I estimated by underwater weighing the proportion of body fat in women students at the University of North Carolina (Sloan et al., 1962). Skin-fold and girth measurements were made on the same subjects and the best prediction of body fat was found to be from two skin-fold measurements (iliac crest and back of arm). This prediction had a high correlation ($r = 0.74$) with the estimation of body fat from specific gravity.

In 1964, the underwater weighing technique was applied to men students at the University of Cape Town (Sloan, 1966). On the same subjects the depth of subcutaneous fat at selected sites was measured with a skinfold caliper and by the ultrasonic technique. The best pre-

TABLE 1
Dynamic fitness of young adults. Mean fitness index on Harvard step test (men) and on modified Harvard step test (women). Physical education (PE) students and non-athletic (NA) students in South Africa (SA), United States of America (USA) and Great Britain (GB).

Date		Mean Fitness Index			
		Men		Women	
	Place	PE	NA	PE	NA
1957	Cape Town (SA)	86	62		
1958			56	77	58
1959				77	60
1960		85	65	67	40
1961	Chapel Hill, N.C. (USA)	66	63		41
	Greensboro, N.C. (USA)			57	
	Exeter (GB)	96	82		
	London (GB)			70	61
1964	Cape Town (SA)	84	80	74	59
1965	Richmond, Va. (USA)		63		51

diction was found to be from two skin-fold measurements (front of thigh and inferior angle of scapula; $r = 0.84$). The best prediction from ultrasonic measurements (front of thigh and iliac crest; $r = 0.81$) was slightly less accurate than the prediction from skin folds.

From skin-fold measurements I have estimated the body fat of women students in Cape Town and of men and women students at the Medical College of Virginia. The Cape Town women were student teachers and the Virginian women were students of physical therapy. In Virginia the ultrasonic technique was used as well as skin-fold measurements, but only the estimation from skin-fold measurements has as yet been worked out. Table 2 gives the mean proportion of body fat in each of the groups studied.

RELATION OF FITNESS TEST PERFORMANCE TO BODY COMPOSITION

Men and women students in Cape Town and men in Richmond showed a significant negative correlation between FI (Harvard or modified Harvard step test) and proportion of body fat; for women students in Richmond there was no significant correlation (table 2). In Cape Town there was no significant correlation between FI and either height or weight, except for a low negative correlation between FI and weight in women ($r = -0.250$). In Richmond there was no significant correlation between FI and height or between FI and weight in women, but a highly significant negative correlation between FI and weight in men ($r = -0.413$).

DISCUSSION

As a general rule the capacity for strenuous exertion depends on the degree of physical activity of the individual. Athletic men have higher FI's than non-athletic men (Brouha et al., 1944; Taddonio and Karpovich, 1951; Keen and Sloan, 1958) and athletic women than non-athletic women (Sloan, 1961; Skubic and Hodgkins, 1963). Systematic physical training raises the FI of athletic men (Seltzer and Brouha, 1943; Sloan and Keen, 1959; Cureton, 1963) and women (Hardy, Clarke and Brouha, 1943; Sloan, 1961). Presumably improvement in neuromuscular coordination reduces the load on the heart, and the trained heart, having a greater stroke volume, meets the demands upon it with a less prolonged increase in rate.

The higher mean FI of British than of South African and of South African than American men may be due to the different degrees of physical activity customary in the different communities. Although it would be unwise to generalise about national characteristics from the study of such small groups it seems to me that the British pattern of life involves considerably more physical exertion than the South African and the South African than the American. Furthermore the physical education training which I have observed in Great Britain and in South Africa is much more strenuous than in North Carolina, where the American PE students were tested. The least fit PE students were American women but there was no consistent international difference in fitness of non-athletic women.

The proportions of body fat in young men in Cape Town and in Richmond were lower than the 13.3% (White, 1961) and 16% (Behnke, 1961) found in American men and fall between the 8% (Le Bideau, 1959) and 10.7% (Macmillan et al., 1965) found in Europeans. The figures for young women in Chapel Hill and in Cape Town are lower than the 24.9% reported from Cornell University (Young et al., 1961) but similar to the 19.6% for young women in Stockholm (van Döbeln, 1956). The physical therapy students at Richmond seem to be the least obese group of young women so far investigated.

Most investigators have found little or no relationship between the results of fitness tests and the height or weight of the individuals concerned but there is some evidence that obesity impairs performance of most motor fitness tests (Best and Kuhl, 1955; Riendeau et al., 1957). My investigations so far support this view. Although the mean proportion of body fat in men students at Richmond was similar to that at Cape Town the range was wider in the Americans and the heavier (and fatter) subjects were less fit. In contrast to this the Richmond women, with less body fat than other groups of women, showed no influence of either fat or weight on dynamic fitness.

TABLE 2
Percentage by weight of fat in the bodies of young men and women and correlation coefficient (r) between percentage fat and fitness index (FI) on Harvard step test (men) and on modified Harvard step test (women).

Date	Place	Mean % Fat		Correlation between % Fat and FI	
		Men	Women	Men	Women
1961	Chapel Hill, N.C. (USA)		20.1		
1964	Cape Town (SA)	9.6	20.3	-0.520	-0.345
1965	Richmond, Va. (USA)	9.9	17.1	-0.533	-0.084

SUMMARY

Dynamic physical fitness may be satisfactorily estimated by the Harvard step test (modified for women) and this and other aspects of physical fitness are measured by appropriate test batteries.

The percentage by weight of fat in the body may be calculated from the specific gravity or from caliper measurements of skin folds or ultrasonic measurements of the thickness of the layer of subcutaneous fat at selected sites.

In general, physical education students are fitter than non-athletic students. British men students of physical education are fitter than American. International differences are less marked in non-athletic men and in women.

Performance of fitness tests is not as a rule influenced by the height or weight of the subjects but the more obese subjects have a poorer performance as do heavier subjects when the extra weight is due to obesity.

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Brains of Prominent People: History, Facts and Significance

WALTHER RIESE

The historian of neurology notes with no small surprise that an ever-decreasing interest in the study of the so-called brain of the elite has led in the last decades to an almost total disappearance of the subject from brain-anatomical and brain-anthropological research laboratories and from published material. The historian is able to cite several reasons for the growing unpopularity of the investigation of brains of prominent people. First of all, it is undeniable that the results of the studies of these brains, once undertaken with a naive optimism and enthusiasm, were rather disappointing and discouraging and certainly not in proportion to the time and effort invested. Furthermore, the overwhelming part of these investigations can be traced back to the time when the anatomical method used in this area of brain research was that of gross morphology or pure inspection. With the rise and growth of histological and particularly cytoarchitectural knowledge and methods, the gross-morphological study of the so-called brain of the elite lost much and, in the eyes of some investigators, even all of its credit.

Ever since cytoarchitectural areas became the new constituents or structural units of the cortex and proved to have borders that seldom coincide with the convolutions, once studied so eagerly and in such great detail as to their limits, courses, sizes, anastomoses, branches and occasional submersions, all these morphological criteria have largely lost their meaning. It is only occasionally recognized that the number of cytoarchitectural areas having a *known* physiological significance remain surprisingly small in proportion to the great number of cytoarchitectural areas distinguished in the human and mammalian brains.

Moreover, the doctrine of cerebral localization of *functions,* on which the whole idea of the brain of the elite ultimately rests, has been increasingly compelled in the last decades to retreat behind the doctrine of cerebral localization of *symptoms* which is all the physician has to deal with in his attempt to reach a regional diagnosis in life as well as in post-mortem examination. With the growing insight into the history of nervous function and the *chronogenetic* and successive involvement of wide cerebral areas in the maturation of even the apparently elementary functions, little hope remained for a circumscribed seat of nervous functions, particularly the so-called highly evolved functions, such as language, mental abilities, etc. These functions defied any attempt to assign to them a few square millimeters of brain tissue as their restricted local residences. In other words, little hope was left for a revival of the once so popular doctrine of the seat of the soul in neuroanatomical terms. Thus, at this crucial point of growing understanding the new concept of *integration,* i.e., the cooperation of *all* parts of the nervous system, if not of the whole organism, in the final activity of each single part became incompatible with the once cherished idea of the brain as a compound of *isolated* functions and structures.

In restudying the brains of emi-

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nent people, I have been fully aware of the limit set to this type of investigations, as well as to its scope and teachings. Ultimately, one deals here with the age-old problem of the interrelation of mind and body. I believe that this problem cannot be discussed solely in anatomical terms and that its solution (insofar as it is possible) belongs before another forum. The shift in method from purely morphological to histological investigations might therefore be of lesser importance for the basic problems and principles implied in these studies than one might be inclined to think. It is with all these restrictions in mind and in this spirit of humble caution that I submit in the second part of this paper the results of my findings in the brains of two prominent people.

The first to investigate brains carefully whose bearers had been known for their outstanding accomplishments was Retzius (1905). It is true that many years previously Rudolph Wagner (1860) had compared five brains of scientists from Göttingen with brains of "ordinary" people. He reached the conclusion that neither weight nor richness nor complication of the fissural pattern were criteria of outstanding mental ability.

After Retzius, Klose (1920) reviewed all brains of the so-called elite and of such individuals who had exhibited certain one-sided gifts. To this material Klose himself added the description of the brain of a musical prodigy. The brain of Ernst Haeckel has been the subject of a detailed investigation by Friedrich Maurer (1924). The latest investigations were devoted to the brain of Pilsudski, described by M. Rose (1939), and the brain of C. von Monakow, described by R. Anthony (1935). The Moskow Brain Institute reported on the brains of Gorky, Pavlov and other Russian scientists and artists (Blinkov and Poliakov, 1938). We have not been able to obtain any details.

What has been the result of these investigations? In the first place, the number of negative cases is far less than that of the positive ones. Only Sernow (1879) and Stieda (1908) were not able to find any peculiarities in the cerebral surface of brains of distinguished people.

The investigations of Retzius appear to have shown such peculiarities, especially a particularly rich development of the fissures. Thus, Retzius writes about the brain of the histologist and physiologist Christian Lovén

> . . . that the external morphology of his brain is quite in accord with his well recognized intellectual gifts. We are, however, not yet able by studying the differences in the behavior of the gyri to draw conclusions from these differences concerning the special gifts of a particular individual.

It is to be expected that the peculiarities of the fissural pattern become clearer the more distinct and the more localizable are the peculiarities of the individual whose brain is examined. In other words, as certain special characters emerge within the total pattern, it should become possible to recognize their morphological expression in the brain. This has been demonstrated particularly well in the brains of outstanding musicians examined by Auerbach (1906, 1908, 1911) in the Frankfurt Neurological Institute. The acoustic sphere was indeed particularly well developed in these brains. Auerbach was inclined to consider the superior temporal gyri as important for the understanding of music. Those of the left hemisphere are probably more important than those of the right one, although their dominance may not be quite as pronounced as in the case of language. In principle, Klose (1920) corroborated this assumption. He found in the brain of the pianist Goswin Soekeland an exceedingly good development of the superior temporal gyrus, including Flechsig's acoustic gyrus and also the central gyri, particularly the precentral one, predominantly their middle third. The special development of the temporal gyrus was regarded as an expression of outstanding sensory gifts, that of the precentral gyrus as an expression of outstanding technical abilities. Moreover, Klose found a well pronounced supramarginal gyrus, considered as a substrate of an ability for musical invention and composition. Spitzka (1907) reported a particular development of the occipital lobe in visually gifted people. It seems to be in accord with these findings that the brain of Ernst Haeckel showed abundant convolutions of the outer aspect of the occipital lobe and a large development of the calcarine fissure. Haeckel was an outspoken "visual type."

It is widely believed that for creative acts not only the inferior parietal lobule but also the frontal lobe is important. Spitzka pointed to its high development in philosophizing persons. Maurer found the frontal lobe and the angular gyrus especially well developed in Haeckel's brain. In the brain of C. von Monakow, Anthony (1935) found a general increase in volume and weight of the left hemisphere and a much more complicated fissural pattern on the left than on the right side. Particularly remarkable was the doubling of the left second frontal gyrus. The left insula, too, was extremely complex.

I was given the opportunity and the honor of studying the morphology of the brains of two prominent scientists. The brain of Ludwig Edinger, a foremost representative of comparative neurology, was studied with Kurt Goldstein (Riese and Goldstein, 1950).

Ludwig Edinger was decidedly an original thinker as is proved by his classification of cerebral structures according to their probable phylogenetic age, as well as by his theory of nervous diseases due to wear and tear (*Aufbrauchskrankheiten*). It was his wealth of ideas

which made him so unusually attractive to his students and which continually caused him to attack new problems. But he was not concerned with abstract ideas. Purely conceptual thinking was foreign to him. As a true type of a modern biologist, he focussed his attention on observables. His life's work brought into strong relief two of his unusual gifts: namely, his visual abilities, and his manual skill. In the light of these personal traits, the configuration of Edinger's brain is very remarkable. Considerable asymmetries, increase in the surface of the right frontal, parietal and occipital lobes were found. There can hardly be any doubt that this asymmetry is correlated with Edinger's left-handedness. Our findings agreed remarkably with those which Hansemann (1907) reported on the brain of the left-handed painter Adolph Menzel. It is tempting to look upon the special development of the *precentral* as well as of the postcentral *gyrus* as important instrumentalities, although by no means as the only condition for Edinger's manual skill, and to look upon the high development of the *occipital lobe* as a morphological correlate of his visual gifts, although it should not be forgotten that Edinger's gifts were far larger than what goes under the title of physiological optics in the stricter sense of the word. In contrast to the morphological behavior of those parts which mirror Edinger's natural gifts, the *temporal* lobe is structurally much less outstanding. Edinger was indeed acoustically poorly endowed. Although with some reservation, one can bring the enlarged *frontal lobe* and the enlarged *parietal lobe* (particularly its inferior part) in connection with Edinger's creative gifts (fig. 1).

Of course, neither the mass of the entire brain nor one of its parts is a reliable criterion for the *quality* of the so-called higher mental functions. Moreover, most of our knowledge about the frontal lobe is based on animal experimentation, on neurosurgery and on neuropathology. The functional disorders which have actually been observed in these conditions throw no direct light on the role this or any other part of the brain may play within the framework of the always total action in an intact organism.

The second brain of a prominent scientist (Riese, 1953) which I was able to study was that of an American scholar, physician and author known for his numerous books and papers. He, too, was an

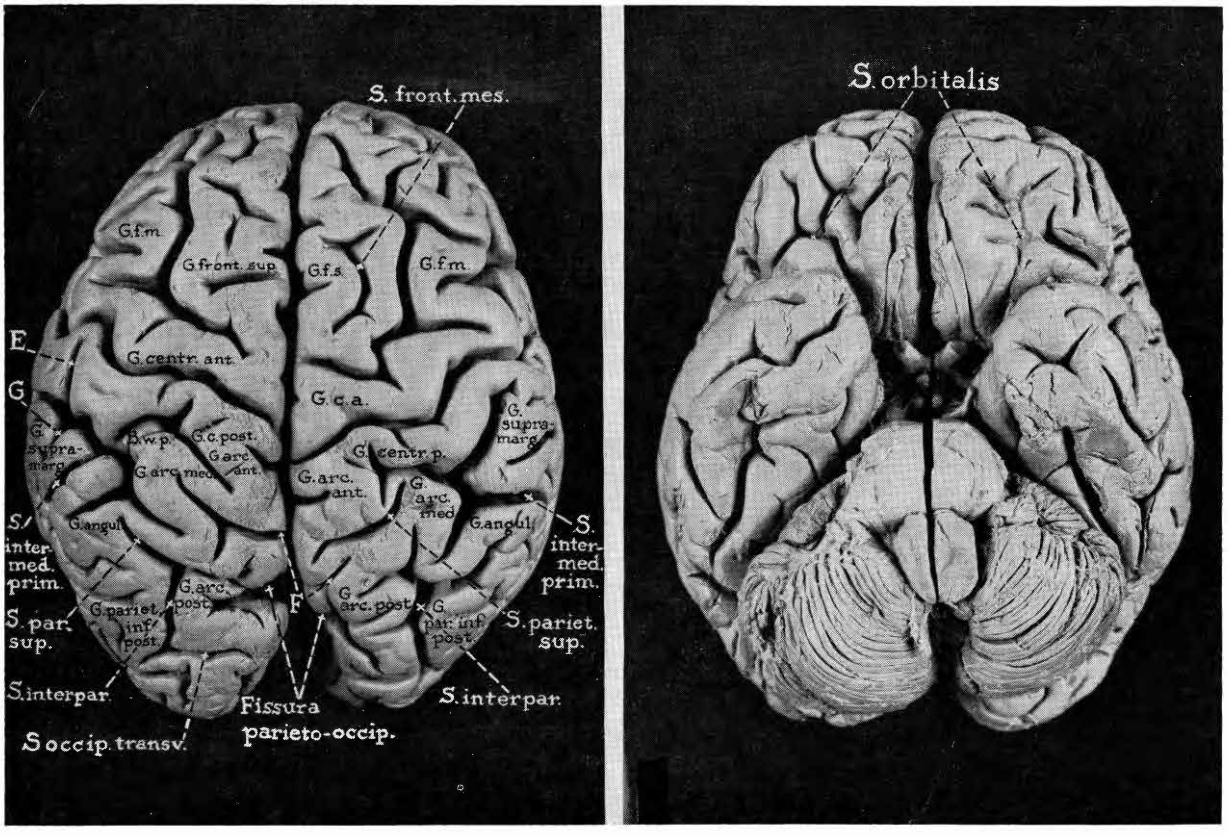

Fig. 1—The brain of Ludwig Edinger. Strong asymmetries in the frontal and parietal areas. Edinger was decidedly left-handed (figs. 2 and 3, W. Riese and K. Goldstein, *J. Comp. Neur.* 92: 162).

original thinker, somewhat esoteric and speculative, though also experimentally minded.

The study of the *convolutional pattern* revealed regional over-developments determining, in their turn, considerable asymmetries of the fissural pattern. Some of these overdevelopments were found on the left, others on the right hemisphere. This individual was decidedly right-handed. In this respect the more massive aspect and more tortuous course of the left *sensory-motor area,* including both pre- and post-central gyri, cannot be overemphasized. As a rule, the overdevelopments did not affect a given lobe as a whole; nor were the parts affected within the same lobe always on the same side. Thus, in the *frontal* lobe, the gyrus frontalis medius was much richer in convolutions on the right, while the gyrus frontalis inferior and the precentral gyrus were overdeveloped on the left hemisphere. Four gyri fronto-polares could be identified on the right hemisphere. In the *parietal* lobe, the post-central gyrus, especially its lower part, was more massive and more tortuous on the left, while the convolutions of the superior (gyri arcuati med. and post.) and, above all, those of the inferior parietal lobes (gyri supramarginalis and angularis) were much better developed on the right hemisphere. In the *temporal* lobe the left supra-temporal plane showed three instead of two Heschl convolutions. The *island of Reil* was more massive on the right hemisphere. While in the *occipital* lobe cuneus and praecuneus were more massive, more fissurated and more convoluted on the left hemisphere, a very marked operculum occipitale was found on the right hemisphere. The visual areas (areae striatae) were found to be morphologically as well as cytoarchitecturally very extensive on both sides, reaching beyond the occipital pole and cutting far into the lateral aspect of the two hemispheres.

There is no doubt that these overdevelopments must be considered as *cerebral gifts* with which this person was endowed from the beginning. However, any attempt of correlating the unusual intellectual capacities with regional structures must be made with utmost caution though the morphological gifts in the *frontal and parietal areas* of this brain cannot be overlooked.

The overdevelopment of the left and so-called dominant sensori motor area of this strongly right-handed individual, the presence of an additional Heschl gyrus or acoustic area (area 42) on the same side and the unusual extent of the visual areas (area 17) on both hemispheres stand out as a precious and an undebatable body of information obtained from the study of this brain made in correlation with the basic structure of this personality.*

CONCLUSIONS

In conclusion, in the brains of outstanding right-handed people (Haeckel, Monakow, Pilsudski) the more complicated convolutional and fissural patterns were found on the left hemisphere; in those of outstanding left-handed people (Menzel, Edinger) on the right one. The second brain reported here revealed some overdevelopments on the right, others on the left hemisphere.

Strangely enough, very little is known about side-differences in the average brain. It seems that the middle frontal convolution and the inferior parietal lobe are more developed on the right hemisphere, the inferior frontal convolution on the left one. Nothing is known about the handedness of the bearers of brains showing these side-differences.

Should one cling to the specific functional significance of regional areas of the cerebral cortex, one may be more willing to admit this significance for those relatively elementary functions (movements and sensations) susceptible of expression in terms of space than for those (thought, creative activities) resisting the latter. On this ground, the study of the brains of prominent people as well as the recently obtained knowledge of the side-differences in the anonymous brain (Connolly) would lead to the following conclusions: judging from the cortical morphology, the human brain is built asymmetrically. This asymmetry is due to regional overdevelopments. Though the latter attained an impressive degree in the brains of outstanding people, they were also found in average or anonymous brains. It seems certain that more or less extensive overdevelopments of the sensori-motor cortex are to be encountered constantly on the left hemisphere in right-handed people and vice versa. So far, no brain has ever been described in which the sensori-motor area or parts of it were more developed on the right hemisphere in a right-handed individual or on the left hemisphere in a left-handed individual. But overdevelopments of frontal and parietal areas readily related to the more elaborated and so-called higher psychic functions (semantics, language) appear on the left hemisphere of outstanding right-handed people, but also on the right hemisphere in the anonymous brain. It is tempting to conclude that many an anoymous brain belonged to an individual who, due to circumstances, was not allowed to use his cerebral gifts. This is but a brain-anthropological expression of the well known fact that many gifted people remain undetected and undeveloped.

Structures are only *instruments* which do not decide whether they may be used, to what extent and for what purpose. Moreover, the question raised by the cortical over-

* This brain is the first so-called elite brain in which a comprehensive *cytoarchitectural* examination was carried out, though only according to qualitative needs, namely for the purpose of identification, particularly of some critical or over-developed areas.

developments found in gifted individuals cannot be answered in a dogmatic manner. It depends on strictly individual constellations whether the frontal instruments of human intelligence are used, for what intellectual activities and tasks, in combination with which other cerebral instruments, by which type of individual, at which stage of his life and intellectual growth or decline, and at which stage of the history of mankind and the intellectual style proper to each period. No structure carries in itself the necessity of its permanent and exclusive use for a given performance. As far as human intelligence or any of its departments are concerned, these are ever changing functional wholes, whose anatomical counterparts or representations cannot be compressed into small compartments of our brain.

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Observations on Chromaffin Tissue

HERMANN K. F. BLASCHKO

Department of Pharmacology, University of Oxford, England

My friendship with Ernst Fischer and my interest in the chromaffin tissue both date from the same period. In 1923 I went to Göttingen, where Ernst and I spent our internship together in the Medical Clinic of the University, under Erich Meyer. Ernst left Göttingen to join Albrecht Bethe in Frankfurt, and there he stayed until he emigrated from Germany. I left Göttingen to start work with Otto Meyerhof, and in 1933 I too came to England, where I met Ernst again, when he worked at the Marine Biological Laboratory in Plymouth. In the nineteen twenties we had both been in England, at University College, London, in A. V. Hill's laboratory, but at different times.

I made my first experimental acquaintance with chromaffin tissue when, in February 1924, a male patient aged 36 years was transferred from the Göttingen Neurological Clinic to the ward to which I was then attached, with the diagnosis diabetes mellitus. He excreted large but variable amounts of sugar in the urine. Blood pressure readings varied from 208/128 to 225/125 mm Hg. I take these and some of the following details from a paper by Biebl and Wichels (1925). The patient died of a cerebral hemorrhage, and tumours of the suprarenal gland were found on both sides. Biebl and Wichels describe the positive chromaffin reaction given by the tumour tissue. They do not mention that they gave us, my friend Rudolph E. Siegel (M.D., later physician and historian of Medicine, first in Frankfurt and now in Buffalo, N.Y.) and me, a small amount of fresh tissue. Siegel was at the time demonstrator in the Pharmacology Department under Wolfgang Heubner; he collected the heads of frogs that had been decapitated for use in the student's class. In the evening we prepared an extract of the tumour tissue, and we were thrilled to see that the pupils dilated when we instilled the extract, just as they did when we applied a solution of adrenaline. I am afraid we omitted to determine the adrenaline:noradrenaline ratio!

Soon after I came to Cambridge in 1934 I took up the study of adrenaline. My friends and I described the action of amine oxidase on adrenaline, noradrenaline and dopamine. This was followed, in 1939, by the suggestion of the main pathway of catecholamine biosynthesis, a pathway that has since been firmly established by the work of a great number of laboratories. However, even after the discovery of noradrenaline, both in adrenergic nerves and in the chromaffin tissue, the validity of the pathway suggested remained in doubt. So we read in 1952: "It is therefore hard to regard hydroxytyramine as a serious candidate for the synthesis of noradrenaline" (v. Euler, 1952).

Here in Oxford, we decided to make a more systematic study of the specific biochemical properties of chromaffin tissue. In 1950, a visitor to our laboratory from Switzerland, Dr. H. Langemann, described the presence of a very active L-dopa decarboxylase in the bovine adrenal medulla. Since Langemann's publication (1951), this observation has been amply confirmed and extended, not only to chromaffin tissue from other species but also to adrenergic neurones and to the brain. Today it is well established that chromaffin tissue is able to catalyse the various steps in the formation of the catecholamines from L-tyrosine.

Parallel with the work on the enzyme equipment of the chromaffin tissue, studies have been made of its cytology. The work of the Oxford laboratory started from a finding, made with A. D. Welch, that it is possible to prepare cell-free homogenates of chromaffin tissue in which the catecholamines were present in a sedimentable form (Blaschko and Welch, 1953). In the sediment the amines were present in a state in which they exerted only a small fraction of their biological activity, but on adding distilled water all the activity could be released in an instant (Blaschko, Hagen, and Welch, 1955).

Further analysis of these findings led to the characterization of the chromaffin granules, and these structures have been isolated from the mitochondria (Blaschko, et al., 1956; Blaschko, Hagen and Hagen,

1957) and from the lysosomes (Smith and Winkler, 1966). All these particulate elements have not only been separated by centrifugation techniques, but they have also been seen by the electron microscopists.

The cytologists have in recent years obtained evidence of the separate storage of adrenaline and noradrenaline. It has been possible to show that noradrenaline and adrenaline are differently distributed in a sucrose density gradient. The first indication of this was obtained by Eade (1956), who used the bovine adrenal medulla. Even better resolution of particle fractions containing adrenaline and noradrenaline respectively was obtained by Schümann (1957) who used homogenates of chicken adrenal gland. It might be mentioned here that Coupland and Hopwood (1966) have recently described an electron microscopic method that distinguishes between adrenaline- and noradrenaline-storing granules.

A biochemical study of the chromaffin granules began with the discovery by Hillarp, Högberg, and Nilson (1956) of large amounts of adenosine triphosphate (ATP) in the adrenal medulla. We were able to show that the bulk of the ATP was present in the chromaffin granules (Blaschko et al., 1957).

Lysis of the chromaffin granules releases not only the low-molecular-weight constituents, but also a considerable fraction of the granule protein (Blaschko et al., 1956). This protein has recently been studied in our laboratory. A preliminary purification of the soluble protein fraction was achieved by Mrs. Karen Helle (Helle, 1966a and b: *see also* Blaschko and Helle, 1963). This work has been continued by Smith and Winkler (1965) who described a method of obtaining the main soluble protein fraction of the bovine chromaffin granules in a pure form. Using this method, Mrs. Helle has been able to show in Bergen that this protein has antigenic properties (Helle, 1966). With the help of this specific antibody, Banks and Helle (1965) have recently shown that upon stimulation of the perfused adrenal gland by carbachol, the medulla releases into the perfusate not only catecholamines, but also the soluble granule protein. This finding has added a new fact to our knowledge of the physiology of the chromaffin cell. It is of particular interest in view of the recent finding by Douglas and Poisner (1966) that the ATP is released intact from the chromaffin tissue when the amines are released. It seems that catecholamines, ATP and soluble protein are released together when the chromaffin cell is stimulated.

Another constituent of the chromaffin granules is phospholipid. It has recently been found that the phospholipids of the chromaffin granules exhibit one distinctive feature: they are relatively rich in lysolecithin, a hydrophilic phospholipid usually present in the tissues in very small amounts (Blaschko et al., 1966). It is of interest that the presence of lysolecithin in the adrenal medulla has been known for some time (Hajdu, Weiss and Titus, 1957). It is interesting that another amine-storing cell, the mast cell, is rich in lysolecithin (Keller, 1962).

In 1924, the diagnosis phaeochromocytoma was not arrived at during the patient's lifetime. Today many of these tumours are removed. Forty-one years later, in 1965, we were able through the kind cooperation of Sir George Pickering, Regius Professor of Medicine at Oxford University, to obtain such a tumour that had just been removed by operation. The cytology of the tumour will be described elsewhere in collaboration with Dr. A. H. T. Robb-Smith. The tumour tissue contained 59% of adrenaline and 41% of noradrenaline. A homogenate of the tissue in isotonic sucrose was prepared, and on centrifugation about two thirds of the total catecholamines were found in the sediment; this is a figure very similar to that found in normal chromaffin tissue. Also the distribution of the catecholamines upon ultracentrifugation over a sucrose density gradient was normal, and the molar ratio, catecholamine:ATP of 5.1 was similar to that normally found in the fraction in which the amine-storing granules were principally recovered. The main difference between tumour tissue and normal chromaffin tissue was in the very high catecholamine content per unit of weight. In the electron micrographs, chromaffin granules appeared to be very numerous.

It is to be hoped that more of these tumours will be studied by these techniques, as this might help to lead to a correlation between clinical symptoms and amine storage.

An attempt has been made to give in this article a review of some of our activities since I first met Ernst Fischer in Göttingen many years ago. I have not related the work of our laboratory to that of others. Also, I have only described observations made on chromaffin tissue. The catecholamines have acquired a much wider importance with their discovery in adrenergic neurones and, more recently, in the central nervous system. However, the study of a more homogeneous tissue has the advantage of greater simplicity. Some of the findings made on the adrenal medulla, e.g., the enzyme studies, are of immediate relevance to nervous tissue. To what extent the new findings on amine storage and release have a counterpart in neurones, remains to be elucidated in the future.

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A Deadly Poison Becomes a Useful Tool

JOHN W. MOORE

Department of Psyhiology, Duke University Medical Center, Durham, North Carolina

JAPANESE ROULETTE

Japanese have long loved to eat "very delicious" puffer fish (also known as "blowfish" or "globefish") which they call "fugu" although it is known to contain a deadly poison. The many deaths attributed to the eating of puffer fish have caused the poison to be of special interest to Japanese scientists. Recent laws have restricted the serving of fugu fish to licensed restaurants where the chefs have been trained to remove the organs where the poison is most concentrated. These organs (the poison is most concentrated in the ovaries) are collected by a chemical and drug concern and the poison extracted. It is called tetrodotoxin from the family name Tetraodontidae.

ELECTROPHYSIOLOGY OF TETRODOTOXIN

Although studies of the effects of this poison have been done for some years on whole animals, nerve trunks and muscles, it has been used in single cell electrophysiological studies only quite recently.

Narahashi et al. reported in 1960 that tetrodotoxin (which hereafter will be called TTX) blocked excitability in skeletal muscle membrane without changing its slow electrical rectifying properties. About the same time Furukawa et al. (1959) found that the response of the muscle end plate region retained its sensitivity to acetylcholine although the excitation process itself was blocked.

Dr. Narahashi joined me at Duke in 1962 and we tested his hypothesis that TTX selectively blocked the sodium conductance increase associated with excitable membranes. We used the voltage clamp technique (which separates sodium and potassium membrane currents into two distinct time courses) on single giant axons from lobsters. It was found that the poison did indeed block the sodium flow very selectively and at almost incredibly low concentrations (10^{-8} to 10^{-7} molar).

About this time another toxin, tarichatoxin, thought to be somewhat different but almost equally powerful in blocking nerve impulses (Kao & Fuhrman, 1963) was isolated from eggs of the California newt *Taricha forosa* (Brown & Mosher, 1963).

Dr. Takata and I with Kao and Fuhrman tested tarichatoxin by the same technique on lobster axons and found its effect to be the same as that of TTX. Shortly thereafter it was shown by a number of chemical and physical techniques that the two toxins were in fact identical (Buchwald et al., 1964). An excellent review of the symptoms, physiology and pharmacology of TTX poisoning up to 1964 was written by Mosher et al. (1964).

RECENT RESULTS

Similar selective blockage of the voltage-sensitive sodium conductance systems has been observed in squid axon, single nodes of frog nerves and in the eel electroplax (Nakamura et al., 1964). It does not appear to affect smooth muscle or barnacle muscle. The latter is thought to be made excitable by an increase in conductance to calcium ions (Hagiwara & Naka, 1964). It appears to affect cardiac muscle only in a very limited way (Hagiwara & Nakajima, 1965).

Studies with internally perfused squid axons have shown that the inside may be perfused with a relatively high concentration of TTX without effect on the axon's excitability (Narahashi et al., 1966). This would seem to clearly localize the site of excitation on the outside surface of the membrane rather than on the inside as had often been supposed.

TTX (OR DERIVATIVES) AS AN ANESTHETIC

Voltage Clamp studies on a number of axons have shown that the mechanism of the excitation block by TTX is distinctly different from that caused by procaine.

Concentration Ratio for Blocking

Procaine reduces the ionic conductance of squid and lobster axon membranes at a concentration of about 3 to 4 mM (Taylor, 1959; Blaustein and Goldman, 1965) and blockage is usually sure with a concentration of 10 mM. In contrast, a concentration of only 90 mM of

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TTX blocks within five minutes. The toxin is therefore more effective by a factor of 10^5 in terms of the concentration required.

Selectivity

The toxins have been found to block only the sodium entry, leaving the potassium current unaffected (the present results; Narahashi et al., 1965; Nakamura et al., 1964; Moore, 1965). On the other hand, procaine affects the magnitude of both ionic conductances (Taylor, 1959; Blaustein and Goldman, 1965).

Time Course of the Conductance Changes

Our results show that the toxins do not alter the kinetics of the sodium or potassium current increase. Taylor (1959), Blaustein and Goldman (1965), and unpublished experiments in our laboratory have shown that procaine causes a distinct increase in the time for the sodium current to reach its peak. There is also a very marked slowing in rise of the late potassium current (Taylor, 1959; unpublished experiments in our laboratory).

Location of Action

Procaine appears to be effective in blocking nerve excitation when internally perfused in the squid axon at a concentration which blocks externally (1 to 10 mM, Narahashi, et al., 1966). In contrast, TTX has been found to be ineffective when internally perfused at a high concentration (1,000 mM) for long times (30 minutes). Preliminary evidence for TTX being ineffective on the inside of the membrane was shown by Moore (1965).

Interaction with Calcium

Blaustein and Goldman (1965) report that procaine appears to act at the same site that calcium does. Both cause shifts of the conductance curve along the voltage axis and alter the time course of the conductance changes. Although our experiments with tarichatoxin were not designed to study this point, it is clear that high calcium gives some protection against the toxin and definitely enhances the ability of the nerve to recover from a strong toxin depression of conductance.

The very high potency of TTX and its highly selective block of nerve and skeletal muscle fibers has already shed a great deal of light on their excitation properties and should lead to the ability to specify an entirely new type of anesthetic agent even if the toxin itself does not also turn out to be useful agent in this respect.

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On the Nature of the Resting Frog Skin Potential*

PRISCILLA M. WINN, NANCY S. LaPRADE, WILLIAM R. TOLBERT AND ERNST G. HUF

Department of Physiology, Medical College of Virginia, Richmond, and Department of Physics, University of Richmond

I. SHORT CONTEMPORARY HISTORY

Investigations on the electrical properties of frog muscle, nerve, and skin belong to the oldest in the history of bioelectricity. Interest in the nature of the resting P.D. of frog skin was heightened by the discovery that there occurs in the epidermis of this tissue "active ion transport" (Huf, 1935; 1936; Ussing, 1949), suggesting a possible relationship between the electrical and the chemical events. There is, as yet, no completely satisfactory explanation of the frog skin potential. Many investigators assume that there are at least two electrogenic layers within the multilayer epidermis, and numerous speculations on the nature of the skin P.D. have been offered on the basis of the two (or more) layer concept (Steinbach, 1933; Greven, 1941; Fukuda, 1942; 1944; Meyer and Bernfeld, 1946; Koefoed-Johnsen and Ussing, 1956; 1958; and others. For earlier investigators, *see* Steinbach, l.c., and Greven, l.c.). A rather penetrating analysis of the electrochemical behavior of frog skin has been presented by Linderholm (1952; 1954). He came to the conclusion that a single-layer concept was adequate to explain the electrical and diffusion properties of skin. Fukuda's work is of particular significance. He showed that the electrogenic outer layer† requires the presence of Na^+, but not of K^+, whereas the inner layer depends on the presence of K^+ in the adjacent bath. Fukuda suggested that the nature of the skin P.D. is intimately related to the preferential Na^+ permeability of the outer layer, and the preferential K^+ permeability of the inner layer.

Greven (1941) and Linderholm (1952; 1953) have proposed physico-chemical models of skin which explain quite well the experimentally found relationship between change in NaCl concentration in the outside bath and skin P.D. Both investigators calculated and found a P.D. change approximately 35 mv for a tenfold concentration change, excluding measurements at relatively high ionic strength ($\mu = 0.1$). Greven and Linderholm have not studied the electrical response of the inside to changes in ionic concentrations. The model of the skin proposed by Koefoed-Johnsen and Ussing (1956; 1958) gives emphasis to the already mentioned preferential permeability of the outer and the inner layer for Na^+ and K^+, respectively. When anion penetration was experimentally circumvented (by replacing Cl^- by ½ SO_4^{2-}), these investigators found that the skin P.D. changed by nearly 59 mv when the outside Na^+ concentration, or the inside K^+ concentration, was changed by a factor of 10. Therefore, Koefoed-Johnsen and Ussing regarded the total skin P.D. as the sum of two Nernst diffusion potentials which are generated at the Na^+ permeable, and the K^+ permeable outer and inner layer, respectively. In other words, in their experiments, the outer layer behaved like a nearly perfect reversible Na^+ electrode, and the inner layer like a nearly perfect K^+ electrode. It is interesting to note that prior to this it was claimed that the inner layer behaved like a reversible H^+ electrode (Meyer and Bernfeld, 1946). Fleming (1957) has tried to confirm this without success. Subsequent work has only in part confirmed the observations of Koefoed-Johnsen and Ussing on sulfatet skins. Disagreement exists especially about the response of the outer layer to changes in Na^+ concentration. Lindley and Hoshiko (1964) and Cereijido and Curran (1965) have reported a P.D. change of approximately 35 mv for a tenfold concentration change. This agrees with our measurements given below.

The high degree of perfection of the technique of micro-puncture and micro-P.D. measurements has, of course, attracted numerous investigators to test the two-layer concept of the nature of the frog skin P.D., reference to which was

* Supported by Public Health Service grants GM 03545 and GM-K6-16,687.

† In this paper the expressions "outer layer" and "inner layer" are used rather loosely. Nothing can be said with certainty about their location. The assumption of their presence is made because certain observations make it likely that such layers (or barriers) exist in the epidermis.

made above. Ottoson et al. (1953) were the first to apply this method to frog skin. They were followed by Engbaeck and Hoshiko (1957). The latest report is by Chowdhury and Snell (1964) who may be consulted for additional references on this topic. So far, the results have not been in complete agreement with each other. When a slow, inward penetration of the epidermis is made with the microelectrode, one to four P.D. steps have been observed, but their exact location in the epidermis is not certain. The electrode becomes increasingly positive with advancement of the tip, relative to the outside bath if both sides of the skin are exposed to salt solutions. Chowdhury and Snell (1964) are the only investigators who have obtained a nearly continuous and smooth potential profile. They are inclined to interpret discrete P.D. steps as the result of some distortion of the cellular and tissue structure by the advancing microelectrode. A recent statement by a group of competent and experienced investigators (Leb et al., 1965) strikes a note of warning to use great caution in the interpretation of data: ". . . the application of microelectrode techniques to frog skin is beset with formidable technical difficulties from the standpoint of adequate control." In this paper, therefore, more confidence is placed in results which were obtained with the classical technique of P.D. measurement on intact skin using agar bridges and calomel half cells.

II. STATEMENT OF PROBLEMS. EXPERIMENTS

Upon closer inspection of each of the papers cited in section I, it becomes clear that the interpretation of the data rests upon a great number of explicit and implicit assumptions. This, of course, is in the accepted tradition of scientific writing, but it also explains why the nature of the skin P.D. still is in a state of considerable controversy. The review of the pertinent literature has led us to carry out the following experiments, some of which deal with the controversial quantitative aspects of the electrical responses of the skin to changes in Na^+ and K^+ concentration, and the effect that substitution of Na^+ by Mg^{2+} has on these responses. Measurements of Q_{O_2}, and of Na^+, K^+, and Cl^- content in skin were made to evaluate the extent of damage, if any, to the skins exposed for several hours to sulfate solutions of rather unphysiological composition. Studies were also undertaken on the electrical response of osmotically and metabolically damaged skins to find out whether the Na^+ response can be diminished or abolished, if only transiently, without affecting the K^+ response, or vice versa.

Methods

The experiments were performed during all seasons, except winter, on belly skin of large frogs (*R. pipiens*). The skin was mounted in a two chamber (each 18 ml) cell made of lucite. The skin area was 4.9 cm^2. Continuous mixing of the fluid (25 C) was achieved by using circulating pumps (20 ml per min). Skin P.D.'s were measured in the conventional way with calomel half cells, millivolt recorders (Varian Associates, Model G-10; Sargent, Model SR) and occasionally Keithley Electrometer, Model 600A. Careful attention was given to asymmetry and junction P.D.'s in the system. They were either absent or played only a minor role, and when used for corrections did not significantly alter the observations and conclusions drawn from the data. Measurements on skins were started about 1 hour after mounting of the skins while exposed to sulfate solutions, pH 8, containing Na = 100; K = 10; Tris (hydroxymethyl) amino methane = 10, μeq per ml. Keeping constant the composition of the solution at one side of the skin, the Na^+ and K^+ concentration of the solution at the other side was altered, lowering $[Na^+]$ and elevating $[K^+]$, but keeping $[Na^+] + [K^+]$ constant. Total osmolarity: 135 milliosmols per liter by the method of freezing point depression. $[Na^+] =$ 110 (no K) will be designated as Na_1; lower $[Na^+]$ will be designated as Na_2, and Na_2/Na_1 will be designated as r. $[Na^+]_0$ and $[Na^+]_1$ stand for sodium concentration in the solutions at the outside and at the inside of the skin, respectively. Solutions were changed at about 10 min intervals when fairly stable new P.D. levels were usually seen. The data on skins which gave less than 90% recovery in Na = 100, K = 10 were discarded. Usually the response of the outside was tested before testing the inside, but no differences in results were found due to the order of testing. *P.D.* will designate the potential difference across the whole skin (inside +). $\Delta V = (P.D.)_2 - (P.D.)_1$, *i.e.*, the difference in P.D.'s at Na_2 and Na_1. Oxygen uptake measurements (20 C) on fresh skin samples (120 mg) were carried out with the Warburg method. The belly skin was cut into several pieces which were randomly placed into Warburg flasks containing solutions of various compositions. Estimations of Na^+ and K^+ in skin were done as described earlier (Huf et al., 1955). For Cl^- estimations, the method of van Slyke and Sendroy (1923) was employed. A drop of picric acid was added to the standard solutions to simulate the yellow color of skin digests.

Electrical Response of the Outside (Outer Layer of the Epidermis; June 1963 through June 1964)

Studies on 19 skins (63 measurements) gave results (table 1, col. 3) which fitted the computer calculated regression equation:

$$P.D. = 35.7 \log [Na^+]_0 + 17.9 \quad (1)$$

TABLE 1

Dependence of frog skin P.D. on varying composition of salt solution at the epithelial side. Belly skin of Rana pipiens. $Na_1 = 110$; $K = 0$. Na_2 = lower Na^+ concentration, as given in column 1. Composition at the dermal side of the skin was kept constant: Na 110; K = 10. THAM 10; pH 8, 25 C. Common anion SO_4^{2-}.

1			2	3	4	5
Solution pH 8						
Na^+	K^+	THAM*	$r = Na_2/Na_1$	P.D. (inside +)	ΔV	$\alpha\dagger = P_K/P_{Na}$
μEq/ml				mv	mv	
110	0	10	1.000	92		0.410
75	35	10	0.682	84	−8	0.365
35	75	10	0.318	73	−19	0.280
10	100	10	0.091	53	−39	0.167
2	108	10	0.018	30	−62	0.077‡

* Tris (hydroxmethyl) amino methane.

† Calculated from $\alpha = (r^{0.59} - r)/(1 - r)$; see section IIIa.

‡ Comparable to the value given by Lindley and Hoshiko (1964); see introduction.

TABLE 2

Electrolyte composition of fresh skin and experimental skin (after use). Experimental skins were soaked for one hour in sulfate solutions containing, in μEq/ml, Na: 110; K 10. During the experiments the skins were exposed, in sequence, to sulfate solutions pH7 of decreasing Na concentration (110 → 0 μEq/ml), and increasing K concentration (10 → 60 μEq/ml). Once or twice in each experiment Mg SO_4 (50 μEq/ml) replaced Na or K. All solutions were isosmotic. Time of study, March and April 1963.

	Experiment No.	Testing inside (i) outside (o)	Duration of experiment (hrs)	μEq/gm dry wt. at end of experiment			% H_2O
				Na	K	Cl	
Fresh Skins (Huf et al., 1955)				254	164	215	74
Experimental Skins	2	i	3	321		7.2	79.8
	3	i	4		195	12.0	80.6
	4	i	22	471*		19.0*	83.1*
	5	i	6		196	21.8	82.2
	6	o, i	6		181	6.0	79.8
	7	i, o	4	324			80.3
	14	o, i	5	229	210		78.6
	15	o, i	6	366	210		81.0
Average				310	198	11.7	80.5

* Not included in the average value.

with confidence limits of about ± 4 mv. In 3 of the 19 cases, the salt solutions contained 2 mM per liter $CaSO_4$. The results were not different from those seen when Ca^{++}-free solutions were used. Another series on six skins (24 measurements) was performed with Mg^{++} containing solutions of the following composition (μosmols per ml): $Na^+:K^+:Mg^{++}$ = 75:10:25; 35:10:65; 10:10:90; 2:10:98. The regression line was:

$$P.D. = 23.3 \log [Na^+]_0 + 17.4 \quad (2)$$

Substituting Mg^{++} for Na^+ increased the ionic strength of the solutions by factors varying from 1.4 to 2.5. No significant difference in freezing point depression was found, however, between solutions with and without Mg^{++}. A Beckman sodium electrode 39278 in conjunction with a Beckman Model 76 Expanded Scale pH meter was used to check the Na^+ activities in the solutions. We consistently found that for a tenfold change in $[Na^+]$, the P.D. of the Na^+ electrode changed by 53 to 54 mv.

Electrical Response of the Inside (Inner Layer of the Epidermis; June through August 1963)

From experiments on nine skins (31 measurements) it was found that a tenfold change in $[K^+]_1$, changed the skin P.D. on the average by 57 mv. In five additional experiments (20 measurements) with solutions containing 10 K^+, and Na^+ and Mg^{++} in varying proportions, but totaling 110 milliosmols per liter (see under I), the skin P.D. was always 64 mv. In contrast to the response of the outside to Na^+, the response of the inside to K^+ was little if at all affected by Mg^{++}. There seems to be no objection, therefore, to the use of Mg^{++} as a substitute for Na^+ when testing the electrical properties of the inside of the skin.

TABLE 3

Oxygen consumption of frog skin in solutions of varying composition. All solutions were buffered with 5 mM/1 THAM. Each solution was tested on six to eight skins of different frogs. Q_{O_2} data are given as mean values ± one standard deviation of the mean.

Series	Time of Exp.	pH	Composition of Solutions						Milliosmoles/1 from ΔC°	Ionic Strength μ	Q_{O_2}
			Na	K	Mg	SO_4	$[Fe(CN)_6]$	Sucrose			
			Milliosmoles/liter								ml × mg dry wt.$^{-1}$ × hr^{-1}
H	August	6	100	10	0	55	0	0	135	0.165	0.54 ± 0.033
H	1963	7	100	10	0	55	0	0	135	0.165	0.53 ± 0.043
H		8	100	10	0	55	0	0	135	0.165	0.52 ± 0.040
1	April	8	100	10	0	55	0	0	135	0.165	0.55 ± 0.033
2	June	8	50	60	0	55	0	0	136	0.165	0.51 ± 0.039
3	1963	8	0	60	50	80	0	0	137	0.290	0.32 ± 0.028
4		8	0	60	0	30	0	55	135	0.090	0.34 ± 0.026
6	May	8	50	10	50	80	0	0	138	0.290	0.36 ± 0.047
7	1963	8	50	10	0	30	0	55	136	0.090	0.41 ± 0.019
9		8	0	110	0	55	0	0		0.165	0.28 ± 0.022
8	April	8	50	10	0	0	15	75	138	0.150	0.52 ± 0.037
5	1963	8	0	60	0	0	15	75	136	0.150	0.45 ± 0.036

Metabolic, Electrolyte Measurements on Skin (March and April 1963)

Seven skins which had been used in 3 to 6 hours experiments were analyzed for Na^+, K^+ and Cl^- (table 2). The average results were: Na^+, 310; K^+, 198; Cl^-, 11.7 μeq per gm dry wt, and H_2O, 80.5%. Except for the per cent water, which was about 9% above control values, (Huf et al., 1955) there was no significant alteration in the Na^+ and K^+ content of whole skin. It should be noted that about 5% of the Cl^- in fresh skin remained in skin which was kept for several hours in chloride-free sulfate solutions. Skins in $Na^+ + K^+$ sulfate solutions showed normal respiration rate 0.53 ± 0.04 ml O_2 per mg per hr (table 3). No significant decrease in Q_{O_2} was seen during a period of five hours. The lowest Q_{O_2} (0.28 ± 0.02) was seen in Na^+ free K_2SO_4 solution. This is in agreement with Zerahn's work (1956). Skins in solutions containing 50 Na, 10 K, and either 50 milliosmols per liter Mg^{++} or sucrose had the same Q_{O_2}: 0.36 ± 0.05 and 0.41 ± 0.02 for skins in Mg^{++} and sucrose solutions, respectively. In the foregoing, all errors are expressed as one standard error of the mean of eight measurements for each case. These observations suggest that skins in sulfate solutions do not suffer severe metabolic alterations which, in other media, are readily detectable by the method of whole skin analysis (Huf et al., 1955; 1957; Huf and Doss, 1959).

Electrical Response of Osmotically or Chemically Damaged Skin (March through May 1964)

Results obtained on 5 of 11 experiments with skins which were osmotically damaged by soaking for several hours in bicarbonated water (Winn et al., 1964) are shown in figure 1. The experimental conditions, other than those mentioned under *Methods,* are given in the legend. At the end of the experiments the epidermis could easily be separated from the corium. In some of these experiments the outside (normally negative relative to the inside) became slightly positive. This was the case when the K_2SO_4 concentration at the inside was high, giving rise, probably, to a K^+ diffusion potential. Similar results were obtained on 30 skins which were treated before and during the testing with 0.02 M diethyl malonate, or 0.02 M NaF, or 0.001 M quinone (Eubank et al., 1962). In all experiments both sides of the skin failed *concurrently* to respond in the manner typical for fresh skin.

III. DISCUSSION AND INTERPRETATIONS

Response of the Outer Layer

It has been found by Koefoed-Johnsen and Ussing (1956; 1958) that the epidermis of the brown frog (*R. temporaria*) behaves like an almost ideal Na^+ electrode over a wide range of concentrations (1 to 100 mM). Our experience and that of others (Lindley and Hoshiko, 1964; Cereijido and Curran, 1965) is that this is not the case

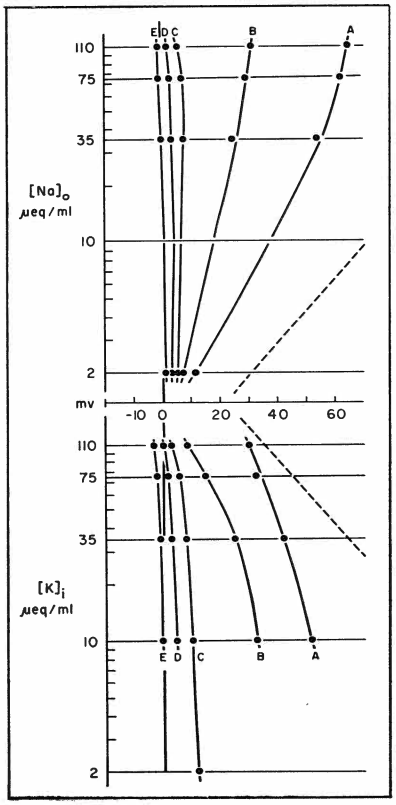

Fig. 1—Results obtained on five skins which were osmotically damaged by soaking for several hours in bicarbonated water. Semi-log plot of change in P.D. (abscissa) with changing outside $[Na^+]_o$ (keeping inside solution constant at 110 Na 10 K), and, following this, with changing inside $[K^+]_i$: (keeping outside solution constant at 110 Na 10K). Sum of $[Na^+] + [K^+]$ in the test solutions was kept constant 110. Soaking periods: *A* and *D*, 2 hours; *B* and *C*, 1½ hours; *E*, 4 hours.

for the skin of the leopard frog (*R. pipiens*) and the bullfrog (*R. catesbiana*). When placed in sulfate Ringer's solution, these skins gave a P.D. change of only about 36 mv (23 mv, if Mg^{2+} was present in the solutions) for a tenfold change in Na^+ concentration, instead of the expected P.D. change of 59 mv. The results of the experiments presented above suggest that this is typical for the normal fresh skin and is not related to a poor physiological condition of the skin membrane. Skins which deviated greatly from the ideal Na^+ electrode behavior (*see* above) performed quite well when the dermal side was tested for response to potassium, *i.e.*, a P.D. change of almost 59 mv for a tenfold change in K^+ concentration was obtained. In experimentally damaged skin, both sides failed *concurrently* to respond in the manner typical for fresh skin. This, it would seem, rules out the possibility that an increased "sulfate-shunt" (Ussing and Windhager, 1964) was the cause for the non-Nernstian behavior of the outer layer of fresh skin. One would expect that skins in poor physiological condition leading to increased anion permeability would fail in their Na^+ and K^+ responses, like the experimentally damaged skins. It should be mentioned here that, in the experiments with metabolic inhibitors, every stage and degree of damage was applied, reasoning that in mildly poisoned skins a transient isolation of the Na^+ from the K^+ response might occur. This, however, was never achieved. The skins exposed for many hours to solutions of rather unphysiological composition had a respiration rate and a Na^+ and K^+ content comparable to control skins. It must be granted that the method of whole skin analysis is not a very sensitive method to detect alterations in skin electrolytes. On the other hand, the same method permits a demonstration of gain in Na^+ and loss in K^+ in metabolically poisoned skins. It cannot completely be ruled out that the skin of *R. temporaria* behaves differently from the skin of the other species mentioned above. For instance, the skin of *R. temporaria* is thinner than the skin of *R. pipiens*. The same is true, however, for the skin of *R. pipiens* as compared to the skin of *R. catesbiana*, and yet, there is no difference in the Na^+ response in the skins of the last mentioned species. An entirely satisfactory explanation for the electrical behavior of the outer layer of frog skin when the outside Na^+ concentration is changed is not available at the present time. Any discussion of this topic should include the following thoughts: a) Significance of K^+ leakage from the epidermis; b) Greven's skin model; c) Linderholm's skin model; d) Koefoed-Johnsen and Ussing's skin model. The role of K^+ leakage is discussed first because this relates more immediately to the experiments described above.

a) *K^+ leakage from the epidermis.* It is well known that the epidermis shows leakiness to potassium (Steinbach, 1937; Huf et al., 1952; Huf and Wills, 1953; Bricker et al., 1963; Klahr and Bricker, 1964). A skin with K^+ leakage would be analogous to a glass Na^+ electrode with a "potassium error." It would explain qualitatively why a P.D. change of less than 59 mv per tenfold change in Na^+ concentration is to be expected. The following quantitative considerations will show that K^+ leakage seems to play some role, but it does not fully account for difference between the ideal 59 mv and the actual 36 mv P.D. change.

If one chooses the Goldman-Hodgkin-Katz equation for calculations of ratios of the permeability coefficients, $\alpha = P_K/P_{Na}$, one obtains from equation (3) below (Lindley and Hoshiko, 1964):

$$\Delta V = \frac{RT}{F} \ln \left[\frac{Na_2}{Na_1} (1 - \alpha) + \alpha \right]$$

$$= 59 \log [r(1 - \alpha) + \alpha] \quad (3)$$

Equating (3) with (1), applied to Na_1 and Na_2, one obtains

$$\alpha = \frac{r^{0.59} - r}{1 - r}$$

The limiting value of α for $r = 1$ can be obtained as:

$$\lim_{r \to 1} \frac{r^{0.59} - r}{1 - r} = \lim_{r \to 1} \frac{d(r^{0.59} - r)}{d(1 - r)}$$

$$= 1 - 0.59 = 0.41$$

The thin curves in figure 2 show the predicted relationships between ΔV and r, calculated from equation (3) by assuming several values for α. The heavy line is the regression line fitted to the experimental data (table 1, col. 4). The graph suggests that the linear semi-log relationship between $Na_2/Na_1 = r$, and ΔV may be the result of a decrease of α with decrease of r. Whereas this possibility cannot be excluded, it is, intuitively, a somewhat remote possibility. A better argument against this possibility comes from the following facts. From the studies of Cereijido et al. (1964) and those of Winn et al. (1964), a P_{Na} for the outer layer of the order of 8×10^{-6} cm per sec may be calculated, valid for $[Na^+]_o = 100$. P_{Na} increases rapidly with decreasing $[Na]_o$. From the data of Huf and Wills (1953) and those of Klahr and Bricker (1964), a rough estimation of P_K (corrected for skin P.D. where needed) shows that P_K is probably below 1×10^{-6} cm per sec. From these data, values for α may be calculated over a wide range of $[Na]_o$, all of which are far below the α values shown in table 1, col. 5. It is doubtful, therefore, that the high values for α shown in table 1 have any real meaning.

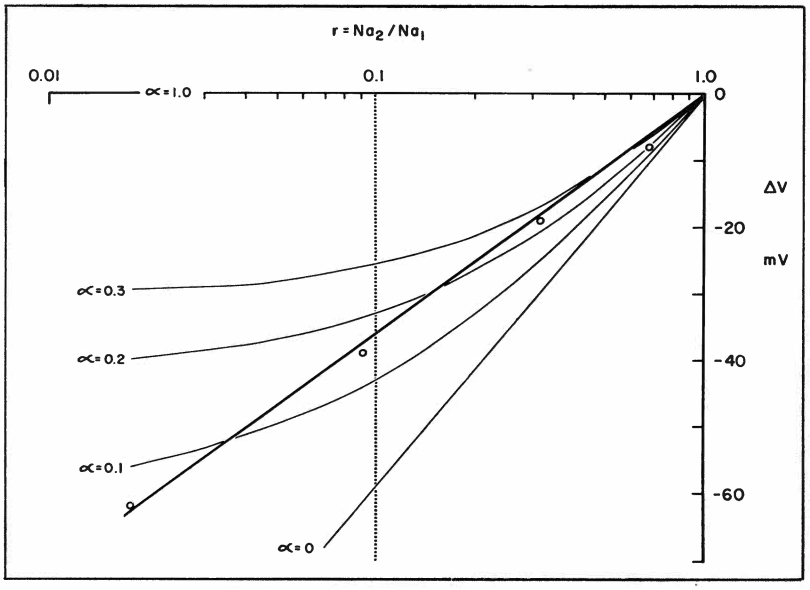

Fig. 2—Semi-long plot (heavy line) of the change of difference in skin P.D. (ΔV) with changing $r = Na_2/Na_1$ in the outside solution. Na_1 is the original Na^+ concentration = 110, K = 0; Na_2 is the Na^+ concentration of the subsequently used test solutions. The sum of $[Na^+] + [K^+]$ was kept constant at 110. Inside solution 110 Na, 10 K. $\alpha = P_K/P_{Na}$.

b) *Greven's skin model* (1941). Greven made the assumption that the outer layer of the frog skin contains "Festions," A, so that the "membrane" condition may be represented as $m^+A^+n^-$, where m^+ and n^- are the diffusible ions, *e.g.*, Na^+ and Cl^- in the skin. When a NaCl gradient is imposed upon the skin in the direction: outside → inside ($c_1 > c_2$), a P.D. (or E) is generated which can be expressed as the sum of a diffusion potential and two Donnan potentials (one on each side of the membrane). This P.D. can be calculated by applying the theory of Teorell-Meyer-Sievers. One obtains:

$$E = \frac{RT}{F}\left[u \ln \frac{x_2 + Au}{x_1 + Au} + \frac{1}{2} \ln \frac{(x_1 + A)(x_2 - A)}{(x_1 - A)(x_2 + A)}\right]$$

in which

$$u = \frac{U_K - U_A}{U_K + U_A};$$

$$x_1 = \sqrt{4C_1^2 + A^2};$$

$$x_2 = \sqrt{4C_2^2 + A^2}$$

To test the validity of this model, values for U_K and U_A were taken from physicochemical tables. c_2 was kept constant, 120 mM per liter NaCl; c_1 was varied from 120 to 0.47 mM per liter NaCl. Values for A were assumed, ranging from 60 to 0.001 meq per liter. All measured P.D. values were corrected for the asymmetry potential which existed when $c_1 = c_2 = 120$ mM NaCl per liter. It was found (*see* fig. 9 of Greven's paper) that the behavior of the model was in remarkably good agreement with the behavior of the skin. The model also predicted one maximum in the P.D./log c_1 curve. This maximum was also seen in experiments, if NaCl was used. It occurred in solutions of NaCl ≧ 30 mM per liter. Below this concentration, a tenfold change in c_1 gave a P.D. change of about 40 mv. It is known that maxima are not seen if sulfate Ringer's of comparable ionic strength solutions are used. An explanation for this has been given by Linderholm (1952; 1954; see below).

c) *Linderholm's skin model* (1952; 1954). Greven's assumption of fixed charges in frog skin has been criticized by Linderholm, who gives reasons which make it un-

likely that fixed charges are of significance (*see also* Linderholm, 1960). Considering the well-known specificity of the response of the outer layer to Na^+, Linderholm suggested that the form of the P.D./log c_1 curve may have something to do with the active transport of Na^+ ion involving a specific carrier. His skin model is described as follows: "The frog skin membrane is supposed to be inhomogeneous in so far as there are some parts of the membrane, where active transport does not take place but where both Na and other ions diffuse through the skin as passive ions, maybe through fine pores. . . . The other part of the membrane contains a sodium carrier, and here the active transport takes place. It may be thought of as a liquid membrane, essentially impermeable to other ions than those transported by the carrier." Applying principles of electrochemistry to this model, Linderholm could derive the following equation for the P.D. (or φ) of a "hypothetical frog skin" separating two NaCl solutions:

$$\varphi = \varphi_a^{Na} \frac{G_a^{Na}}{G_a^{Na} + G^{Cl}} - \frac{G_a^{Na} - G^{Cl}}{G_a^{Na} + G^{Cl}} \frac{RT}{F} \ln \frac{a_2}{a_1}$$

The meaning of the symbols is as follows: φ_a^{Na} = effective active transport potential; G's = partial ion conductances; a_1 and a_2 = activities of the NaCl solutions at the outside and the inside of the skin. The model expresses the P.D. as the algebraic sum of a fraction of φ_a^{Na}, and a diffusion potential. φ_a^{Na} is itself c_1 dependent; it decreases with increasing c_1, although not quite linearly with respect to log c_1. Linderholm has shown that the behavior of model and skin are in good agreement. The model also predicts a maximum in the φ/log c_1 curve (*see* previous section). Linderholm found that in skins with high total conductance the maximum was often at low c_1, and vice versa. The model has not yet been tested for the case $G^{Cl} \ll G_a^{Na}$, or $G^{SO_4} \ll G_a^{Na}$. Applying simple algebra, however, it can be seen that φ remains the algebraic sum of (c_1-dependent) φ_a^{Na} and a diffusion potential. This feature of the model makes it useful for a quantitative analysis of the electrical response of the outside of the skin to changes in the outside electrolyte concentration.

d) *The Koefoed-Johnsen and Ussing skin model* (1956; 1958) The attractiveness of this two-layer concept lies in the fact that it attempts to explain the skin P.D. in terms of two intra-epithelial Na^+ and K^+ diffusion potentials (*see also* Andersen and Zerahn, 1963; Hansen and Zerahn, 1964). The claim that skin in sulfate Ringer's solution gives a nearly 59 mv change for a tenfold change in Na^+ concentration of the outside bath has never been confirmed (see sections I and II). In other words, it still has to be shown that the φ/log c_1 relationship is quantitatively predictable from the model for skin in sulfate, or in chloride Ringer's.* All investigators seem to agree that the φ/log c_1 relationship for skin in sulfate Ringer's does not have a maximum in a solution approaching an ionic strength of $\mu = 0.1$. The reason for this may be found in the much higher total conductance of sulfated skins, as compared to skins in chloride Ringer's (Cereijido and Curran, 1965). This would shift a possible maximum to a higher c_1 (see Linderholm, 1952; 1954).

Response of the Inner Layer

When Linderholm (1952; 1954) proposed his version of the one-layer concept of the skin P.D., he did not consider the interesting work of Fukuda (1942). He showed that, upon removal of K^+ from chloride-Ringer solution at the inside of the skin, the total skin P.D. rose and, upon stepwise increase of the K^+ concentration, the P.D. stepwise decreased. This is perhaps better explained if one assumes the involvement of a second electrogenic layer in the generation of the total skin P.D. Koefoed-Johnsen and Ussing have extended Fukuda's work, using sulfate-Ringer's instead of chloride-Ringer's. They noticed that the inner layer behaved very nearly like a reversible K^+ electrode. A tenfold change in K^+ concentration at the inside of the skin gave a skin P.D. change of about 59 mv. This result was confirmed by Cereijido and Curran (1965); our own measurements reported in section II are also in agreement with those of Koefoed-Johnsen and Ussing. Any hypothesis on the nature of the electrical response of the inner layer must, of course, take into consideration the mechanism of active Na^+ transport, which may be located in this region. Studies on electrolyte distribution and active ion transport in frog skin under varying metabolic conditions (Huf et al., 1957) have suggested that a metabolically forced 1:1 $Na^+ \rightleftarrows K^+$ exchange may be an essential step in the mechanism of active sodium transport according to the following sequence of reactions:

$$\text{outside bath} \rightarrow Na^+ + RK_{m+1}Na_{n-1}$$
$$\rightarrow RK_mNa_n + K^+ \leftarrow \text{inside bath}$$
$$\text{leakage} \leftarrow K^+ + RK_mNa_n$$
$$\rightarrow RK_{m+1}Na_{n-1} + Na^+ \rightarrow$$

R is a "carrier," which may have a definite but as yet unknown chemical entity, or it is perhaps simply a special compartment where certain energy transformations take place which do not occur in ad-

* We have found that when the sodium concentration on the outside was lowered from Na_1 to Na_2, skins in sulfate-Ringer's almost followed the law $\Delta V = RT/F \ln \sqrt{Na_2/Na_1}$, and $\Delta V = RT/F \ln \sqrt[3]{Na_2/Na_1}$ in the presence of Mg^{2+} (equations (1) and (2) respectively), rather than the Nernst law $\Delta V = RT/F \ln(Na_2/Na_1)$.

jacent areas. The reasons for the assumption of attachment of several atoms of Na^+ and K^+ to R are given in the quoted paper. Operation of a carrier system involving the structure RK_mNa_n implies that no active transport takes place if either the K^+, or the Na^+ concentration, or both, are too low in the transport compartment. This is in agreement with the experimental data of Huf and Wills (1951), Ussing (1954), and Curran and Cereijido (1965). Figure 3*A* is a simplified version of the model shown in figure 4 of the paper of Huf et al. (1957). On the basis of these experimental results and assumptions, several speculative models describing the electrical response of the inner layer to changes in the K^+ concentration on the inside of the skin may be constructed.

a) Figure 3*B* shows the well-known model of Koefoed-Johnsen and Ussing (1958). It is assumed that the inner layer is the seat of the Na^+ pump, generating the force E_{Na} on Na^+ crossing this border. Because of the K^+ electrode behavior of the inner border and also because of the equivalence rule (short circuit current = net Na^+ flux, Ussing and Zerahn, 1951), Koefoed-Johnsen and Ussing have assumed that E_{Na} is kept electroneutral by means of a 1:1 $Na^+ \rightleftarrows K^+$ exchange across the inner border (cell membrane). For a sulfated skin in steady state, therefore, this model visualizes the existence of an electroneutral, K^+-coupled Na^+ pump and a Nernst-type K^+ diffusion potential across the inner border.

b) An alternative model, equally lacking unequivocal experimental support but preferred by us, is the following one.† Implicit in the

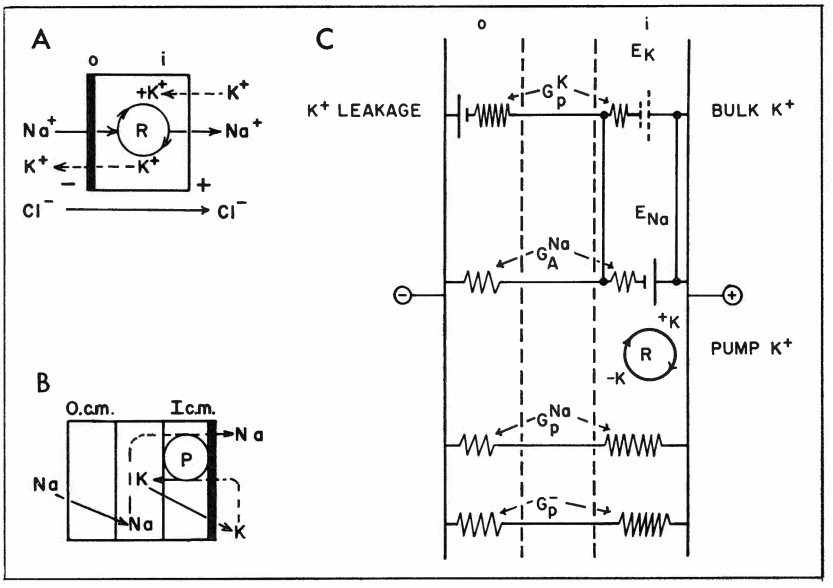

Fig. 3*A*—Active Na^+ transport model by Huf et al. (1957). R is a hypothetical polyvalent metabolically supported carrier forming a complex RK_mNa_n which can exchange one ion for the other when energy transfer occurs. 3*A* depicts an electrogenic Na^+ pump, since K^+ is assumed to recycle only within the transport compartment, and does not cross the "inner layer." See also the similar Klahr and Bricker model (1964). 3*B*) Frog skin model by Koefoed-Johnsen and Ussing (1958) depicting an electroneutral Na^+ pump. O.c.m. = outer cell membrane. I.c.m. = inner cell membrane. 3*C*) Hypothetical electrical equivalent circuit representing the open frog skin. The scheme is essentially a combination of the model proposed by Linderholm (1952; 1954), and the active Na transport model suggested by Huf (3*A*). The two-layer concept ("o" and "i"), rather than Linderholm's one-layer concept has been adopted. (Section I and III of this paper). Without the K^+ parameters, the scheme is identical with Linderholm's model (1954), with the main resistances (or conductances, G) located in both layers. The subscripts A and p indicate "active" and "passive" respectively. E_{Na} (Linderholm's ϕ_A^{Na}) is the true transport potential of the sodium pump. In accordance with the data of Huf et al. (1957), K^+ is treated as if it were present in two compartments: pump potassium, and bulk potassium. The K battery (E_K) is shown in dashed lines to indicate that for the skin in steady state (K^+ influx = K outflux) this battery is inoperative. A K^+ leakage system in the outer layer is also indicated.

† First presented on May 7, 1965 at the 43rd Meeting of the Virginia Academy of Sciences, Richmond, (*Va. J. Sci.* 16: 391, 1965). It is interesting to note that Cross et al. have presented similar arguments and supporting data on frog muscle (*J. Physiol.* 181: 865–880, 1965).

model discussed above seems to be the assumption that all cell potassium is functionally in one compartment. It has been shown, however, (Huf et al., 1957; 1959) that transcellular active Na^+ transport and cellular Na^+-K^+ balance are separable (*e.g.,* with fluoroacetate, or changing temperature), but not entirely separate mechanisms, suggesting the presence of K^+ in the cell in at least two functionally different compartments: *pump potassium* which need not cross the inner border, and *bulk potassium* which, if it crosses this border freely, may account for the K^+ electrode behavior of the inner border of the epidermis. For the normal skin in steady state, bulk K^+ may be kept in electrochemical balance by an *electrogenic* Na^+ *pump* in accordance with the Ussing-Teorell equation

$$\phi_{in}/\phi_{out}(K^+ \text{ flux ratio}) = \exp.\ (E - E_K)F/RT.$$

When the membrane potential

$$E = E_{Na} = E_K = 59 \log [K^+]_{cis}/[K^+]_{trans}$$

(bulk K^+ concentration across the inner border), the K^+ fluxes are equal. The P.D. drop seen at the inner border when $[K^+]_i$ is increased is a transient, not a steady state phenomenon, as the important experiments of Klahr and Bricker (1964) have shown. In their studies, using sulfate solutions, skins regained 40 to 120% of the original steady state P.D. within about 1 hour.‡ Huf et al. (1955) also have observed recovery and maintenance of the P.D. of skins in chloride Ringer's at elevated $[K^+]$. This was associated with elevation in K^+ accumulation in the non-chloride space. These observations are consistent with the interpretation of the electrical response of the inner layer to K^+ as follows: Steady state P.D.: $E_{Na} \pm 0$; P.D.: shortly after tenfold increase of $[K^+]_i$: E_{Na}-59, transiently, leading slowly to the original steady state P.D.: $E_{Na} \pm 0$ (ideal recovery). For steady state conditions, this model, therefore, visualizes the existence of an *electrogenic, K^+-coupled* Na^+ *pump* which operates with a fraction of the total cell K^+. In doing so, this mechanism effects transcellular active Na^+ transport and maintenance of cellular K balance without the appearance of a K^+ diffusion potential. Under non-steady state conditions a Nernst-type K^+ diffusion potential does appear, which, however, is transient in nature.

c) There is no proof that there exists any coupling, tight, ionic (a) or loose, electrical (b), between active transcellular Na^+ transport and cellular K^+ balance. Both processes may occur independently of each other. This view is supported by several facts, among them the observation (Huf et al., 1957; Curran and Cereijido, 1965) that certain drugs when applied in low concentration inhibit only Na^+ transport. When used in higher concentration, however, the skins loose K^+ and gain Na^+. This suggests active uptake of K^+ into the cells, independent of active transcellular Na^+ transport, to balance K^+ loss via a diffusion pathway. Steinbach (1937) over 30 years ago had already published data in favor of "potassium secretion" in the inside → outside direction. A model of the skin such as this would not be incompatible with the K^+ electrode behavior of the inner layer.

IV. SUMMARY

1. In this paper some of the highlights of research on the nature of the resting frog skin potential have been presented. Reviewing a period of about 30 years, it was the intention to show that several key problems have been recognized by a number of investigators who,

‡ The short circuit current also fell sharply and transiently when $[K^+]_i$ was raised.

through their experimental work, have tried to find unequivocal solutions to such problems as follows: a) The number and location of electrogenic layers (barriers) within the rather complex epidermis. b) The characterization of these barriers in terms of specific permeability properties. c) The electrical response of the two sides of the skin to changes in ionic concentrations in the solutions at the skin surfaces. d) The role of intra-epidermal active ion transport in the generation of the skin P.D. e) The correlation between active ion transport, skin P.D. and intra-epithelial (intracellular) electrolyte distribution.

Highly refined methods of study are now widely in use. This, of course, is unavoidable and necessary, to find conclusive answers to the problems mentioned. On the other hand, one must be on guard about possible pitfalls when applying such refined techniques. The study of the P.D. profile within the epidermis, using microelectrodes is beset with difficulties (*see* section I). Another example is the study of permeabilities of diffusion barriers within the epidermis by the method of applying radioisotopes to opposite surfaces of the skin. The analysis of data requires the consideration of such knotty problems as coupled flows and isotope interactions (Kedem and Essig, 1965). Although progress is made in these areas, it must be admitted that at this time no completely satisfactory explanation of the resting frog skin P.D. can be given.

A summary of viewpoints presented in this article is shown in Figure 3*C*. This tentative skin model is essentially a modification of the scheme suggested by Linderholm (1954). If the skin is in steady state, K^+ movement may not contribute to the skin P.D., except that the small outward K^+ leakage may be a factor. As has been pointed out in section III, the Linderholm skin model gives a reasonably good quantitative explanation for the electrical response of the outside of the skin to changes in the Na^+ concentration. The model shown in 3*C* raises the problem of the nature of the coupling between E_{Na} and E_K at the inside (inner layer) of the skin; it may vary from an electrical (in open skin) to an ionic (shorted skin) coupling if cellular K^+ is to be maintained in either case. The model describes the P.D. as a function of ion concentrations in the solutions at the two sides of the skin membrane. The model does not explain the skin P.D. in terms of intra-epidermal electrolyte gradients and the P.D. profile. The solution of this problem still lies in the future.

2. A reinvestigation was made on the electrical response of the outside and the inside of the skin to Na^+ and K^+ sulfate in the presence and absence of Mg^{++}. Fresh skins, metabolically poisoned skins, and osmotically damaged skins were used. Skin electrolytes and skin respiration were measured to evaluate possible tissue damage in skins kept for hours in sulfate solutions of rather unphysiological composition. The results are briefly summarized in section II.

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Rate of Diffusion of Radioactive Ions in Gels*

THOMAS C. SMITH, ADDISON D. CAMPBELL AND ERNST G. HUF

Department of Physiology, Medical College of Virginia, Richmond, and Department of Physics, University of Richmond

Gelatin, a protein (Meyer, 1942; Gustavson, 1956), and agar, a polygalactose, (Meyer, 1942; Tseng, 1946; Mori, 1953) very easily form aqueous gels, i.e., semisolids which have desirable properties in many types of chemical and physiological studies, including measurements of the diffusion coefficients of salts and non-electrolytes. Gels, reducing the effective volume of the solvent (water), somewhat eliminate the problem of thermal and mechanical mixing encountered when using solutions and free solvent. Sharp diffusion boundaries are usually seen when using gels. These advantages of gels have already been exploited by several investigators (Stiles and Adair, 1921; Friedman, 1930; Felicetta et al., 1949; Fujii and Thomas, 1958; Lauffer, 1961; Schantz and Lauffer, 1962; Newson and Gilbert, 1964; Winn et al., 1964), who have taken measurements of the rate of diffusion of substances using either the "solution-to-gel" or the "gel-to-gel" method.

In the following, a rather simple "gel-to-gel" procedure for the estimation of diffusion coefficients (D) is described. D values were obtained for the ions: Na^{22}; K^{42}; Cs^{134}; Cl^{36}; Br^{82}; I^{131}. These studies were done using agar gel that contained 0.11 M of inactive salts corresponding to the radioactive compounds which were under investigation. In other experiments D for Na^{22} was estimated, using NaCl-free and NaCl-containing gelatin gels at varying pH.

* Supported by Public Health Service Grants GM 03545 and GM-K6-16,687.

THEORY

As will be pointed out in detail below the method of estimating D is based on measurements on the distribution, $c(x, t)$, of a radioactive ion in a pair of semi-infinite gel cylinders, joined end to end, for which the boundary conditions are:

$$c = 0 \quad \text{for} \quad x < 0, \quad \text{at} \quad t = 0$$
$$c = c_0 \quad \text{for} \quad x > 0, \quad \text{at} \quad t = 0$$

t is the time, and x is the distance into the agar cylinders measured from the plane of junction ($x = 0$).

Following the deductions presented by Shewmon (1963) and applying the "thin film solution" of Fick's second law to the problem of the distribution, $c(-x, t)$ of the radioactive ion in the originally "cold" gel cylinder, one has

$$c(-x, t) \simeq \frac{c_0}{2\sqrt{\pi\ Dt}} \sum_{i=1}^{n} \Delta a_i \cdot \exp\left[-\frac{(-x - a_i)^2}{4\ Dt}\right] \qquad (1)$$

In this equation, a_i is the distance of the ith agar slice (of the originally "hot" agar) to $x = 0$. For n going to infinity, $\Delta\ a_i$ goes to zero and one has

$$c(-x, t) = \frac{c_0}{2\sqrt{\pi\ Dt}} \cdot \int_0^\infty \exp\left[-\frac{(-x - a)^2}{4\ Dt}\right] da \qquad (2)$$

The $c(-x, t)$ distribution can also be expressed in terms of an error function as follows:

$$c(-x, t) = \frac{c_0}{2}\left[1 - \operatorname{erf}\left(\frac{x}{2\sqrt{Dt}}\right)\right] \qquad (3)$$

or

$$\operatorname{erf}\left(\frac{x}{2\sqrt{Dt}}\right) = 1 - \frac{2c(-x, t)}{c_0}, \quad \text{for} \quad x < 0 \qquad (4)$$

Numerical values for *erf* corresponding to the measured $c(-x, t)/c_0$ data are obtainable from probability tables. A plot is then made of $-x$ vs $x/(2\sqrt{Dt})$ and D is calculated from the slope, $1/(2\sqrt{Dt})$, of the straight line which one obtains.

METHODS

Preparation of Gels

The agar gel was prepared by adding 3 g of Difco Bacto-Agar to 100 ml of de-ionized water. If the ion for which the diffusion coefficient was being determined was a cation, enough unlabeled chloride salt of the ion was added to the gel to give a salt concentration of 110 μ moles per ml of solution. If the ion was an anion, enough unlabeled sodium salt of the ion was added to give a salt concentration of 330 μ moles per ml. The salt concentrations were arrived at arbitrarily. The gel was then divided into two parts. To one of these parts was added the radioactive ion, contained

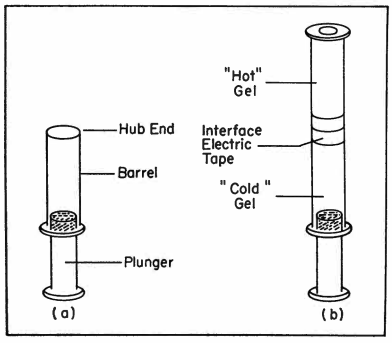

Fig. 1—Cylinder arrangement in the diffusion studies: *a,* modified 2 ml hypodermic syringe; *b,* two syringes joined together and held together by electrical tape.

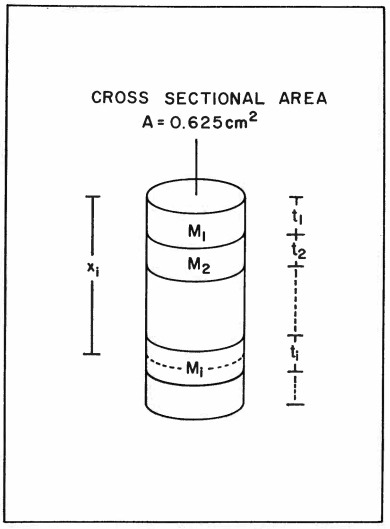

Fig. 2—Calculation of distances x_i, expressed in cm, from the weight of the agar slice (M_i), expressed in mg, and the factor $1000/0.625 \times 1.003 = 1.6 \times 10^{-3}$. 1.003 is the density of the gel.

in either the sodium or chloride salt of the ion of interest. The solution of the radioactive salt which was added had been previously neutralized and, in each case, the amount added to the gel did not significantly alter the salt concentration. Experiments in the agar gel were run for the ions of sodium[22], potassium[42], cesium[134], chloride[36], bromide[82], and iodide[131].

The gelatin gel was prepared in a similar manner. Sodium[22] was the only isotope used in this case. Three grams of Eastman purified calfskin gelatin and 3 g of agar were added to 100 ml of de-ionized water. The addition of agar was found necessary since the gelatin alone did not give a gel which would harden sufficiently to permit slicing. The isoelectric point of the gelatin was given by the manufacturer to be at pH 4.8. Three batches of the gelatin gel were prepared and the pH values were adjusted so that one was charged positively, pH 3.1 at 24 C, one was charged negatively, pH 6.5 at 24 C, and one was neutral, pH 4.8. This was done by reading the pH of the batch being adjusted on a Radiometer pH Meter and adding NaOH or HCl as was needed to obtain the proper pH value. After the pH adjustment the batch was divided into two, and to one of these was added the radioactive ion. Experimental determinations were made for the coefficients of diffusion for sodium at all three pH values in both salted (110 mM/liter NaCl) and unsalted gelatin. According to specifications by the manufacturers, both the agar and the gelatin contained small amounts of minerals. The precise minerals and amounts, however, are not known to the investigators.

Procedures of Measurements

The cylinders which were to contain the gel during the experiments were prepared by cutting off the needle ends of two 2-ml syringes. The end of the syringe barrel that had been cut was ground and fire

polished to form a smooth surface. For each experiment the plunger of one of the syringes was placed in the barrel, and friction was allowed to hold it in place near the end of the barrel (fig. 1*a*). The syringe was then placed in an upright position resting on the end of the plunger. Non-radioactive gel containing the ion being studied was poured into the syringe from a 10-ml pipette. Care was taken in the procedure to eliminate all bubbles from the gel. The gel was allowed to protrude above the syringe because the gels shrunk as they solidified. After the gel had hardened sufficiently, the protruding part was carefully sliced off. This slicing was done with a razor blade in the case of the agar gels. A thin, taut wire had to be used to slice the protein gel since the protein gel had a tendency to stick to the razor blade and consequently did not give a clean cut. A second syringe was mounted end to end atop the filled syringe and held firmly in place with electrical tape (fig. 1*b*). Gel containing the radioactive ion was introduced into this second syringe by an eyedropper which had been altered by drawing the end into a thin tip. Again care was taken not to introduce bubbles. The radioactive gel was always poured only after its temperature had fallen below 60°C to guard against melting the surface of the non-radioactive gel and thus mixing the two. The depth to which the radioactive gel was poured in the second syringe was adjusted so that it was equal in depth to the depth of the non-radioactive gel (about 3 cm). This was done so that equivalent boundary conditions would be obtained. After the gel in the second syringe had hardened, the plunger was carefully placed in the end. Undue pressure was avoided in this operation to prevent extruding the gel from between the syringes. These two syringes were placed in a plastic bag which was sealed and immersed in a water bath maintained at a temperature of 18.8 ± .1 C. After a period of from 5 to 10 hours they were removed from the bath and separated. The originally non-radioactive or "cold" gel was then sliced into thin sections by using the razor blade or taut thin wire. The slices were always taken from the cold side since it was found that a Gaussian distribution of concentration gradient was more closely obeyed on that side (Winn et al., 1964). The sectioning was done by exuding the gel from the syringe by applying pressure on the plunger. Ten slices were kept in a moist chamber to prevent evaporation while the weighing procedure was carried out. The slices were removed from the chamber one at a time and weighed on a Federal Pacific precision torsion balance to the nearest 10th of a mg. As the slices were weighed they were placed in individual test tubes. The relative radioactivity of each slice was then determined by counting with a Baird Atomic model 132 scaler using a shielded well in which the scintillating crystal and photocell were

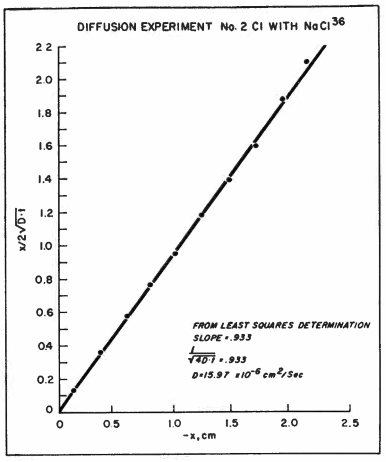

Fig. 3—Plot of $(-x)$ vs $x/2\sqrt{Dt}$ using data given in table 1. Calculation of D_{Cl} is also shown.

TABLE 1

Diffusion experiment No. 2 Cl with $NaCl^{36}$ in salted agar. Time of experiment: 5 hours. Original Cl^{36} concentration (C_0) in agar: 71.55 CPM/mg. Background: 690 CPM.

Agar Slice No.	Weight of Slice	Count Rate*	c_x Count Rate / Unit Weight	Cumulative Weight†	$-x_i$	c_x/c_0	*erf*	$x/2\sqrt{Dt}$
i	mg	CPM	CPM/mg	mg	cm			
1	137.5	4239	30.82	68.75	.1100	.4308	.138	.123
2	172.3	3744	21.72	223.65	.3578	.3036	.392	.363
3	113.8	1679	14.75	366.70	.5867	.2062	.587	.579
4	148.6	1468	9.87	497.90	.7966	.1380	.723	.769
5	137.2	871	6.34	640.80	1.0252	.0887	.822	.952
6	152.6	516	3.38	785.70	1.2571	.0472	.905	1.181
7	144.1	250	1.73	934.05	1.4944	.0242	.951	1.392
8	160.8	136	.84	1086.50	1.7384	.0118	.976	1.596
9	137.4	37	.26	1235.60	1.9769	.0037	.992	1.875
10	125.8	12	.09	1367.20	2.1875	.0013	.997	2.100

* Corrected for Background. † $\left(\frac{M_i}{2} + \sum_{i=0}^{i=(i-1)} M_i\right)$.

TABLE 2

Experimentally obtained diffusion coefficients (column 3) by the method described in this paper, of several ions in 3% salted agar at 18.8 ± 0.1° C. Diffusion rate of Br^{82} was measured in salted and unsalted (*) agar. The errors given are the standard errors of the mean values.

Ion Species Studied	Number of Experiments	Diffusion Coefficient, D, Found	Equivalent Ionic Conductance, λ_i, (18 C)	Diffusion Coefficient, D, Calculated
i		$(cm^2/sec) \times 10^6$	$cm^2/\Omega \times Eq$	$(cm^2/sec) \times 10^6$
$Sodium^{22}$	5	11.4 ± 0.3	42.8	11.1
$Potassium^{42}$	5	16.2 ± 0.4	64.2	16.7
$Cesium^{134}$	6	15.5 ± 0.2	67.1	17.4
$Chloride^{36}$	10	17.1 ± 0.4	64.3	16.7
$Bromide^{82}$	5	16.1 ± 0.1	66.3	17.2
*$Bromide^{82}$	10	14.3 ± 0.2	66.3	17.2
$Iodide^{131}$	5	18.0 ± 2.8	65.3	17.0

TABLE 3

Experimentally obtained diffusion coefficients, by the method described in this paper, of $sodium^{22}$ in 3%-gelatin-3% agar gel at several pH values. The isoelectric point of the gel is at pH 4.8. Salted gels contained 110 mM non-radioactive NaCl per liter (Kg) of gel.

Gelatin-agar Mixture	Number of Experiments	Diffusion Coefficient, D, Found
		$(cm^2$ per sec$) \times 10^6$
A. pH 3.1 unsalted	5	10.7 ± 0.5
B. pH 4.8 unsalted	6	7.2 ± 0.2
C. pH 6.5 unsalted	5	9.3 ± 0.2
D. pH 3.1 salted	4	11.7 ± 0.2
E. pH 4.8 salted	6	11.2 ± 0.4
F. pH 6.5 salted	5	11.3 ± 0.6

mounted. The original concentration of the radioactive ion was determined by a similar process. A single syringe was filled with radioactive gel and allowed to set for several hours. The gel was then sectioned and weighed. The slices were placed in test tubes and counted under the same conditions as were the experimental slices. No concentration gradient was found in this process indicating that the source was uniformly distributed throughout the syringe.

The distance, $-x_i$, into the plug from which each slice was taken was determined from the weight of the slice and the cummulative weight of all of the previous sections of the plug (fig. 2). Applying the equation

$$(-x_i) = \frac{t_i}{2} + \sum_{j=0}^{j=(i-1)} t_j$$

$$= 1.6 \cdot 10^{-3}\left(\frac{M_i}{2} + \sum_{j=0}^{j=(i-1)} M_j\right)$$

The data obtained from the weighing and counting were analyzed by use of the IBM model 1620 computer. By use of equation (4) and a plot of the distance into the plug versus the value of the limit of the error function, the value of the coefficient of diffusion was obtained.

RESULTS

Table 1 shows a typical data sheet. The value for the weight of the slice and the radioactive counts per minute were determined experimentally as previously described. All of the remainder of the data was rendered by the IBM 1620 with the exception of the value of the limit of the error function. This value was found from mathematical tables. Figure 3 explains how D was determined from data such as tabulated in table 1. The values of the slopes were obtained using the IBM 1620 and a least squares program. In most instances, only the first six experimental points ($-x \approx 1.4$ cm) were used to get

these values since it was found that the slope of the line changed somewhat at high values of the distance into the cylinder (Winn et al., 1964). Four to ten experimental determinations were made of the coefficient of diffusion for each ion of interest. The average results of these determinations are shown in tables 2 and 3. All values were rounded out at the first decimal. The errors resulting from weighing, counting, and temperature fluctuations were found to be much smaller than the statistical error introduced by averaging the values obtained for several determinations. The uncertainties tabulated are standard errors of means and were calculated from the formula

$$\sigma = \pm\left[\sum_{i=1}^{n} \frac{d_i^2}{n(n-1)}\right]^{1/2}$$

where d_i is the deviation of the ith value from the mean and n is the number of values used in the average.

DISCUSSION

Comparison of Results with Data of the Literature

When in a process of diffusion one isotope is replaced by another, one refers to this mode of mixing of particles as "self-diffusion." In all our experiments where salted gels were used, the isotopic ion diffused in this manner and hence the D values obtained under these conditions must be regarded as the self-diffusion coefficients of the ion species mentioned in tables 2 and 3. The experimental values agree reasonably well with D values calculated from the Nernst equation.

$$D_i = \frac{RT}{F^2}\lambda_i \qquad (5)$$

Numerical values for λ_i (column 4, table 2) were obtained from physico-chemical tables. The difference between D (found) and D (calculated by equation 5) are $\pm$ a few percent. ($RT/F^2 = 26.0 \times 10^{-8}$ Eq. Ω per sec).

In making these comparisons and calculations no consideration was given to the volume occupied by the gel particle. Schantz and Lauffer (1962) and others have pointed out the need for a volume correction in measurements on D when using the solution-to-gel method. This seems unnecessary in the gel-to-gel method used in the present study. Inspection of equation (4) shows that the ratio c_x/c_0 on which D depends remains the the same regardless of whether one expresses the concentrations in terms of CPM per total gel volume, or per free fluid volume; "cold" and "hot" gel plugs were prepared from the same batch of gel. It should also be pointed out that this is in agreement with the plot shown in figure 3 which shows that the regression line very nearly goes through the zero point. In some experiments, however, the regression line intersected the $x/2\sqrt{Dt}$ axis just above the zero point. The meaning of this was not further explored.

SALT AND pH EFFECTS

The term "unsalted gel" (or gel at relatively low ionic strength) is used to designate a gel to which no other than the radioactive salt was added. From analytical data available it is estimated that unsalted gels contained as impurity about 3 mM/liter NaCl. This was the condition under which the *bromide^{82} experiments (table 2) and the sodium^{22} experiments B (table 3) were carried out. In the latter case the gel prepared in distilled water had a pH of 4.8 which is the I.P. of the gelatin used. In the experiments with unsalted and salted gel at lower and higher pH some NaCl was introduced by making the pH adjustments. This amount is estimated to 10 to 20 mM/liter gel. Since no statistically significant differences were found among the $D_{Na^{22}}$ values (D, E, F, table 3) for salted gels at varying pH ($P > 0.3$), and since there were 10 to 20 mM/liter NaCl present in the "unsalted" gels at pH 3.1 and 6.5, it is assumed that the observed differences in the D's (A, B, C, table 3) are the result of salt rather than pH effects.

The diffusion coefficients for bromide^{82} and sodium^{22} in unsalted gels were significantly lower than in salted gels ($P = 0.01$ and $P < 0.01$, respectively). The possibility was considered that in the absence of added NaCl the isotopic elements diffused as $NaBr^{82}$ and $Na^{22}Cl$. From the equivalence conductances given in table 2 one can calculate for

$$D_{NaBr} = \frac{2RT}{F^2}\cdot\frac{\Lambda_{Na}\cdot\Lambda_{Br}}{\Lambda_{Na}+\Lambda_{Br}}$$

$$= 13.5 \times 10^{-6}\ \text{cm}^2/\text{sec}$$

This value is close to the one actually observed (14.3 ± 0.2 cm^2/sec, table 2). In the case of NaCl, however, the calculated D_{NaCl} value is 13.4 cm^2/sec, as compared to the observed value of 7.2 ± 0.2 cm^2/sec. The reasons for the observed low $D_{Na^{22}}$ value for unsalted gel at pH 4.8 remains unexplained.

SUMMARY

1. A simple gel-to-gel method is described for the estimation of diffusion coefficients (D) of radioisotopic chemicals. "Cold" and "hot" gel cylinders, enclosed in small hypodermic syringes with their needle ends cut off, were carefully joined together, and diffusion was allowed to proceed for several hours. Slices of gels were then obtained for counting of activities. Application of the classical theory of diffusion permitted calculations of D values. The method was applied to the ions of Na^{22}; K^{42}; Cs^{134}; Cl^{36}; Br^{82}; I^{131}. Both 3% agar and a mixture of 3% agar and 3% gelatin were used. Measurements were done on gels in water and on gels which contained "cold" salt (110 mM per liter, occasionally more); e.g., NaCl in Na^{22} and in Cl^{36} diffusion measurements; KCl in the case of studies

on K^{42}, and likewise in other cases.

2. The D values (self-diffusion coefficients) obtained when salted gels were used agreed well with data of the literature based on estimations of the equivalent ionic conductances in diluted aqueous solutions. No dependence of D_{Na} on pH of the gelatin-agar gel was seen.

3. $D_{Br^{82}}$ and $D_{Na^{22}}$ for unsalted gels (containing only 3 mM/liter NaCl) were significantly lower than the D's for salted gels containing added NaBr and NaCl, respectively. In the case of bromide this may be explained if one assumes that in the absence of added salt Br^{82} diffused as $NaBr^{82}$. In the case of Na^{22}, however, $D_{Na^{22}}$ (or $D_{Na^{22}Cl}$) $= 7.2 \pm 0.2$ cm^2/sec in unsalted gel at pH 4.8 is far below the calculated $D_{NaCl} = 13.4 \times 10^{-6}$ and $D_{Na^{22}} = 11.2$ to 11.7×10^{-6} cm^2/sec found in salted gel. The reason for the discrepancy remains unexplained.

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Self-Diffusion in Sodium Single Crystals

RAMA M. AIYAWAR* AND ADDISON D. CAMPBELL

Department of Physics, University of Richmond, Virginia

The objective of this investigation was the study of self-diffusion in single-crystal sodium using tracer techniques and the determination of the diffusion constant and activation energy. Precise values of these quantities can be obtained by using Fick's second law of diffusion and the relation $D = D_0 \exp(-Q/RT)$ provided the concentration of the diffusing isotope is kept very small. However, in the above relation, the quanties D_0 (diffusion constant) and Q (activation energy) may vary with the composition of the crystal and with impurity content but are independent of temperature.

MATERIALS AND METHODS

Sodium single crystals of 99.99% purity were grown by the Czochralski method at the Virginia Institute for Scientific Research, Richmond. The crystals were cylindrical in shape and ranged from ¾ inch to 1 inch in diameter. In all of these experiments we found that the technical preparation of crystals such as embedding, cutting, and polishing was very important.

The single sodium crystal was cleansed in methanol and isopropyl alcohol and then placed in an etching bath of xylene with about one part in fifty of isopropyl alcohol. After several minutes the crystal structure became clearly visible when the specimen was illuminated with directional white light. If the specimen were a single crystal, a slow rotation with respect to a fixed light source would reveal a continuous variation of reflecting blaze planes and less reflecting areas. The particular planes subject to preferential etching were not determined. Crystal orientation was not investigated due to limitations in experimental facilities. After the crystal was embedded in paraffin to prevent subsequent deformation, it was mounted in a microtome and a reference surface was cut with a string saw (private communication with R. Bowers, D. Pinnow, and S. Tallman, Laboratory of Atomic and Solid State Physics, Cornell University, Ithaca, New York). An electrolytic method of polishing the crystal surface was well suited for the final preparation of the sodium single crystal. The electrolyte used was cp anhydrous ethylenediamine (Putnam and Kobe, 1938), a highly basic ionising solvent which dissolved some of the alkali salts. Electro-polishing of the crystal surface and the deposition of the tracer isotope Na^{22} were carried out in an inert atmosphere.

After polishing, a thin film of Na^{22} was deposited on the surface of the specimen. Then specimens were subjected to diffusion anneal at a constant temperature for known lengths of time. The temperature of the annealing bath was controlled by a thermoregulator capable of maintaining temperatures to within ± 0.05 C. The annealing time on the average was 10^6 sec. After the diffusion anneal, the specimens were remounted in the microtome and sectioned into uniform slices of 50 μ thickness for radioactive assay. Three crystal samples were prepared for diffusion measurements by the previously described techniques.

RESULTS AND DISCUSSION

A summary of the diffusion data is shown in table 1. The diffusion coefficients D are obtained by plotting lnC Vs $(\overline{X})^2$. The lnC has been plotted as a function of the square of the average depth of each section and is shown in figures 1 and 2 for annealing temperatures at 293 K and 304 K respectively.

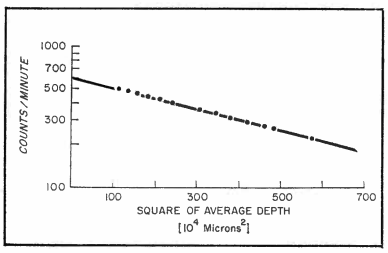

Fig. 1—Variation of concentration with square of average depth for annealing temperature at 293 K.

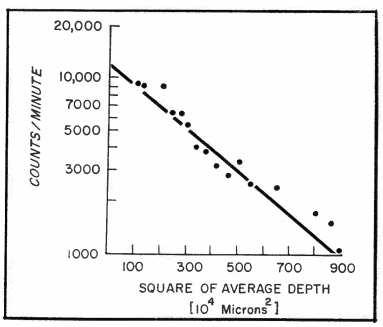

Fig. 2—Variation of concentration with square of average depth for annealing temperature at 304 K.

* Present address: Department of Physiology, Medical College of Virginia, Richmond.

The diffusion coefficients were obtained by analyzing the data on RPC 4000 computer. The logarithm of the diffusion coefficients obtained are plotted against the reciprocal of the absolute temperature as shown in figure 3. The slope of this curve gives the activation energy, and the intercept at $1/T = 0$ gives the diffusion constant. This plot yields the values $D_o = 0.313$ cm²/sec and $Q = 11{,}000$ Cal/mole.

The results of this investigation for sodium single crystals can be compared with the results obtained for polycrystalline sodium by Nachtrieb, Weil, and Catalano (1952). Their results have been reproduced in figure 3 and give 10,450 Cal/mole for the activation energy and 0.242 cm²/sec for the diffusion constant. From figure 3 it is observed that the values of D for single crystals lie consistently below the values obtained for polycrystals. Similarly, the values for the activation energy and diffusion constant in single crystals of sodium are greater than those for polycrystals; this may be due to traces of impurities in the crystals. However, the measured values of activation energy for single-crystal sodium are in good agreement with the values reported from the measurements of nuclear resonance line width in sodium by Gutowsky and McGarvey (1952; Gutowsky, 1951) are a little larger than those reported by Nachtrieb et al. (1952), and by Barr et al. (1951). No further comparison of the activation energies can be made since the kind, number, and effect of defects in the single-crystal sodium are not known. The ratio of the associated enthalpy to the melting point of solids, according to Zener (1952; Wert and Zener, 1949), and van Liempt (1935), applied to cubic metals should be around 32 Cal/mole, which is in good agreement with the value of 30 Cal/mole shown in table 1.

TABLE 1
Summary of Diffusion Data

Temperature on Absolute Scale ^oK	Diffusion Coefficient (D) cm²/sec	Activation Energy (Q) Cal/mole	Standard Error of Q	Diffusion Constant (D_0) cm²/sec	Standard Error of D_0 cm²/sec	$\frac{\Delta H^*}{Tm}$
293.0	2.01×10^{-9}					
304.0	4.07×10^{-9}	11,000.0	± 170.0	0.313	± 0.018	30.0
314.0	7.17×10^{-9}					

*Since Q, the activation energy, is the sum of two enthalpy terms, $Q = (\Delta H_f + \Delta H_m) = \Delta H$, it was replaced by ΔH. Associated enthalpy ΔH is expressed in Cal/mole. Tm is the melting point of sodium on Kelvin scale.

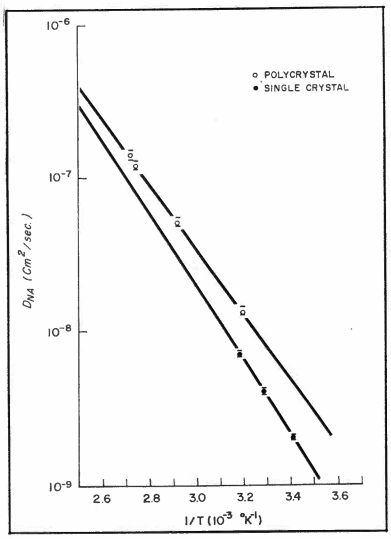

Fig. 3—Variation of D_{Na} with the reciprocal of absolute temperature. The polycrystal curve is reproduced from data of Nachtrieb, Catalano, and Weil (1952).

SUMMARY

In summary, no unique conclusion could be drawn concerning the mechanism of diffusion in sodium from diffusion rates and activation energies alone. Such a decision must be based upon the direct measurement of mean jump frequency.

Acknowledgements

We wish to express our sincere thanks to the Virginia Institute for Scientific Reasearch and the Scientific Computation Laboratory, Medical College of Virginia, for their invaluable coöperation and help.

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Cholinergic Responses of *Schistosoma mansoni*

ERNEST BUEDING

Department of Pathobiology, School of Hygiene and Public Health and Department of Pharmacology and Experimental Therapeutics, School of Medicine, The Johns Hopkins University, Baltimore

In higher animals, the role of acetylcholine as a transmitter of nerve impulses is well established. However, this substance makes its appearance already at an early stage in phylogeny. Bülbring et al. (1949) demonstrated the presence of acetylcholine in the motile protozoan parasite *Trypanosoma rhodesiense* and its absence from the non-motile erythrocytic forms of malarial parasites; this suggested a role of acetylcholine concerned with the motility of protozoa. Subsequently, it has been found that acetylcholine may be of considerable physiological significance to the regulation of the muscular activity of the metazoan parasite *Schistosoma mansoni*. The habitats of the adult forms of this trematode are the mesenteric-portal venous system and the liver sinuses. According to conservative estimates, 200 million human beings are infected with this or two other species of schistosomes (*S. hematobium, S. japonicum*). Accordingly, information about mechanisms essential for the functional integrity of these parasites may be pertinent to the rational development of chemotherapeutic agents effective in the treatment of schistosomiasis.

The presence of acetylcholine, and of the enzymes catalyzing its hydrolysis and its synthesis, acetylcholinesterase and choline acetylase, have been demonstrated in *S. mansoni* (Bueding, 1952; Barker et al., 1966). The concentration of acetylcholine and the activities of acetylcholinesterase and of choline acetylase in these worms are of a high order and equal those found in the gray matter of mammalian brain cortex. By the use of histochemical methods, it has been found that acetylcholinesterase is localized primarily in the nervous system of *S. mansoni* (Bueding et al., 1963). These observations raise the question about the physiological role of acetylcholine in *S. mansoni*. This problem has been studied by determining the effect of cholinomimetic and of cholinergic blocking agents on the motor activity of schistosomes.

The choline ester carbaminoylcholine (carbachol) has the same physiological actions as acetylcholine, but it is not hydrolyzed by acetylcholinesterase. This choline ester markedly depresses the muscular activity of schistosomes. Cholinesterase inhibitors, e.g., physostigmine, prostigmine, and di-isopropylfluorophosphate, have the same effect. Therefore, inhibition of cholinesterase appears to result in an accumulation of endogenous acetyl-

TABLE 1
Effects of Cholinomimetic Agents on Muscular Activity of *S. mansoni*

Compound	Minimal molar concentration reducing muscular activity of *S. mansoni*	Lack of effect on motor activity of *S. mansoni* at molar concentration range
Carbachol	2×10^{-5}	
Arecoline	2×10^{-7}	
Pilocarpine		1×10^{-7} to 1×10^{-2}
Muscarine		1×10^{-7} to 1×10^{-2}
Physostigmine	2×10^{-6}	
Prostigmine	5×10^{-5}	
Diisopropylfluorophosphate (DFP)	1×10^{-4}	

choline; this, in turn, produces decreased muscular activity and paralysis of the worm in the same manner as exogenous carbachol.

The three alkaloids, muscarine, arecoline, and pilocarpine, have the same actions on parasympathetic effector organs as acetylcholine. By contrast, only arecoline is a potent depressant of the muscular activity of *S. mansoni,* while muscarine and pilocarpine are inactive in this respect.

Reduction of the motor activity and paralysis of schistosomes produced by carbachol, cholinesterase inhibitors and arecoline, are abolished by atropine and two nonquaternary ganglion blocking agents, mecamylamine and pempi-

TABLE 2
Effects of Cholinergic Blocking Agents on *S. mansoni*

Blocking Agent	Site of blockade in mammalian host	Minimal molar concentration required to reverse cholinergic paralysis of *S. mansoni*	Lack of cholinergic blockade in *S. mansoni* at molar concentration range
Atropine	Parasympathetic effector organ at low concentrations (approx. 1×10^{-7} molar) and autonomic ganglia at higher concentrations	5×10^{-5}	
Mecamylamine	Autonomic ganglia	5×10^{-5}	
Pempidine		2×10^{-4}	
Hexamethonium			5×10^{-5} to 2×10^{-2}
Pentolinium			2×10^{-4} to 2×10^{-2}
Chlorisondamine			1×10^{-4} to 1×10^{-2}
Nicotine			1×10^{-7} to 1×10^{-3}
d-Tubocurarine	Neuromuscular junction		5×10^{-5} to 1×10^{-2}
Decamethonium			5×10^{-5} to 1×10^{-2}
Succinylcholine			1×10^{-4} to 1×10^{-2}

dine. Exposure of schistosomes to these compounds alone (i.e., in the absence of cholinergic agents) results in a marked motor hyperactivity of the parasite. This stimulatory effect can be accounted for by a block of an interaction of the worm's cholinergic receptors with endogenous acetylcholine, resulting in the failure of this humoral transmitter to exert its inhibitory effect on motor activity. In contrast to these secondary and tertiary amine blocking agents, even high concentrations of nicotine, of quaternary ganglion blocking agents, such as hexamethonium, chlorisondamine, and pentolinium, or of neuromuscular blocking agents (e.g., tubocurarine, decamethonium, and succinylcholine, are completely devoid of cholinergic blocking activity in schistosomes.

These observations, summarized in tables 1 and 2, indicate that cholinergic receptors of schistosomes have some similarities with cholinergic receptors of their mammalian hosts, but nevertheless are distinguishable from those of vertebrate autonomic cholinergic effectors organs and of autonomic ganglia because the muscular activity of the worms is affected neither by muscarine or pilocarpine, on the one hand, nor by quaternary ammonium ganglion blocking agents or nicotine, on the other.

One of the first effects of the administration of the antischistosomal drug, *p*-rosaniline (tris (*p*-aminophenyl) carbonium) to mice infected with *S. mansoni* (Elslager et al., 1961; Thompson et al., 1962) is a localized paralysis of two muscular organs of the worm, the oral sucker and the acetabulum, with which the parasite attaches itself to the mesenteric veins. In vitro, this paralysis is reversed within less than two minutes by atropine or by mecamylamine. This suggests that the paralysis is due to an accumulation of acetylcholine, whose action in depressing muscular activity of schistosomes is abolished by cholinergic blocking agents. This interpretation is confirmed by a histochemically demonstrable inhibition of acetylcholinesterase activity of the two muscular organs and of the central nervous system of the parasite following the administration of *p*-rosaniline (Bueding et al., 1963).

It is concluded that the use of pharmacological agents can provide opportunities to recognize and define similarities and differences in the mechanisms of transmission of nerve impulses in the parasite and the mammalian host. Such studies contribute to better understanding of comparative physiology and of the mode of action of antiparasitic drugs.

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Comments on Intracellular Studies of Presynaptic Inhibition*

ALFRED J. SZUMSKI

Department of Physiology, Medical College of Virginia, Richmond

The use of intracellular electrodes, e.g., (micropipettes), in electrophysiological studies of the central nervous system, has enhanced our understanding of the basic function of the nervous system. The purpose of this paper is to review a study in which this microtechnique was successfully employed in the spinal cord.

INTRODUCTION

When the tip of a microelectrode impales a motoneurone innervating a dissected muscle under study, certain responses can be recorded which indicate that the neurone has been "engaged," and agonist and antagonist influence on the neurone may be investigated. Criteria for evaluation of the response of the cell are: (1) shifts of the average level of the membrane potential in a hyperpolarizing (inhibitory) or depolarizing (facilitory) direction, (2) monosynaptic postsynaptic potential changes during neurone excitability tests, (3) synaptic activation "noise" (low voltage miniature potentials) which may spike in a hyperpolarizing or depolarizing direction and (4) alteration of the firing rate of a motoneurone stimulated by transmembrane currents passed through the tip of the impaling microelectrode. The first two criteria have been described by Eccles and co-workers (1964) in their intracellular studies; the latter two were first described for this type of investigation by Granit, Kellerth, and Williams (1964 a and b).

An evaluation of the above criteria by Granit and co-workers (Granit, Kellerth, and Williams 1964a and b) has shown that the recorded response of the neurone to stretch may be variable in relation to membrane potential and monosynaptic test response, but characteristic synaptic activation noise apparently depended on how near the microelectrode tip was to the source, i.e., the activated portion of the cell membrane. However, when the motoneurone was fired by transmembrane current and its muscle was stretched, the cell fired at an increased frequency. When an antagonist muscle was stretched, criteria (1) and (2) could be variable as is seen for the agonist, but criterion (4), in this case, showed a dramatic inhibition of the firing of the neurone. Since the cell membrane is directly affected by the "injected" transmembrane current, the response (facilitation or inhibition) is postsynaptic. Therefore, Dr. Kellerth and I decided to use the above criteria, especially criteria (3) and (4), to determine if these responses are relevant in identifying a type of synaptic inhibition described by Eccles (1964) as presynaptic inhibition. For this type of inhibition, these authors proposed that the synapse is located on an excitatory synaptic terminal (endbulb), and that these axo-axonal synapses act by depolarizing the synaptic terminals, thereby diminishing their release of excitatory transmitter substance and, in turn, diminishing the size of the postsynaptic impulses (the excitatory postsynaptic potential), and resulting in an inhibitory effect. Since this proposed inhibitory action occurs primarily on the presynaptic terminals, it has been called presynaptic inhibition.

The argument for the occurrence of the presynaptic-type of inhibition is based mainly on studies where the afferent inflow to the neurone has been induced by electrical stimulation of the peripheral nerves and the effects of these synchronous volleys were recorded intracellularly. The criteria for inhibition were: (1) decreased monosynaptic excitability as measured by a diminution of the excitatory postsynaptic potential (EPSP) or (2) hyperpolarization of the postsynaptic membrane, or both. A reduction in the size of the EPSP without or with only a negligible shift of the membrane potential in a hyperpolarizing direction sug-

* The work discussed here was conducted in the laboratory of Professor Ragner Granit at the Nobel Institute for Neurophysiology, Karolinska Institute, Stockholm, Sweden, with the collaboration of Swedish Medical Candidate Jan-Olof Kellerth. The research was done during my tenure of a Postdoctoral Fellowship from the Vocational Rehabilitation Administration, H.E.W. (1964-1965). This experience was possible through the efforts of Drs. Ernst Fischer and Robert Ramsey. I am grateful for their support and encouragement during the academic years and the postdoctoral program.

gested a presynaptic mechanism.

Further elucidation of the possible mechanism of presynaptic inhibition was provided by results from pharmacological investigations which suggested that presynaptic and postsynaptic inhibitions differ in their response to certain convulsive drugs: strychnine was found to eliminate postsynaptic inhibition, while the presynaptic component was left intact or even slightly enhanced, and picrotoxin had no effect on postsynaptic inhibition although it reduced presynaptic inhibition (Eccles, Schmidt, and Willis, 1963).

Granit, Kellerth, and Williams (1964a and b) showed that when a natural (asychronous) stimulus was used to initiate the afferent volley to the neurone, criterion (4), i.e., transmembrane current stimulation, was the only reliable method of revealing excitability changes. We therefore decided to further examine the validity of using strychnine and picrotoxin to differentiate between presynaptic and postsynaptic spinal cord inhibitions.

METHODS

The effects of afferent impulses from peripheral muscle receptors on spinal cord motoneurones in response to natural stimulation (muscle stretch) were studied in anesthetized cats (pentobarbitone, 35 mg/kg). Certain flexor and extensor hindlimb muscles were dissected so that their nerves were kept intact, and the muscle insertions could be attached to a strain gauge which recorded stretch or contraction.

Stimulating electrodes were placed on the nerves supplying these muscles and their cut ventral roots. Lumbosacral motoneurones were impaled with glass microelectrodes filled with 2M-potassium citrate, and a bridge circuit was so arranged that simultaneous records of transmembrane voltage changes could be recorded, and polarizing currents could be "injected" into the cell through the microelectrode. These methods are more completely described by Granit, Kellerth, and Williams (1964a and b). Postsynaptic inhibitions were identified using the criteria described above, and the effects of intravenously administered strychnine, picrotoxin or both were evaluated. Overt convulsive movements were controlled with Flaxedil (Kellerth and Szumski, 1966a and b).

RESULTS AND COMMENTS

Motoneurones were selected which responded with maintained discharge frequency during a long-lasting (14 to 40 sec) injection of depolarizing current. The unequivocal effect of muscle stretch on repetitive firing was first tested since it was the most sensitive criterion for inhibition. However, synaptic activation noise, shifts of the average level of the membrane potential, and monosynaptic EPSP size also were recorded and were generally found to respond in the same direction; in a hyperpolarizing direction during an inhibitory response and in a depolarizing direction during facilitation. In some instances, the synaptic activation noise wavelets were not recorded in a predominantly hyperpolarizing or depolarizing direction, and membrane potential shifts were minimal. This result indicated that the tip of the microelectrode was not always near enough to the source of the event.

With microelectrodes definitely recording postsynaptic events within a neurone during muscle stretch, a convulsive dose of strychnine abolished postsynaptic inhibition in some neurones, as was evaluated mainly by an increase in repetitive neurone firing (criterion [4]) during stretch of its antagonist muscle. This was the strychnine-sensitive postsynaptic inhibition described by Eccles and co-workers. A majority of the motoneurones in this study, however, showed exactly the opposite response to strychnine that is, an inhibition of the repetitive neurone firing to stretch of its antagonist muscle. This then was another response to strychnine, the strychnine-resistant postsynaptic inhibition. Therefore, a repetitively firing gastrocnemius motoneurone normally shows an inhibition of firing on stretch of the anterior tibialis muscle. In a strychninized cat, this same motoneurone sometimes fired through the period of anterior tibialis stretch (strychnine-sensitive postsynaptic inhibitions), but more often, there were postsynaptic inhibitions which were not sensitive to convulsive doses of strychnine (strychnine-resistant postsynaptic inhibitions).

The experimental results with picrotoxin also showed that the inhibitory mechanisms in the spinal cord cannot be readily categorized. The current view is that picrotoxin blocks presynaptic inhibition, but has no effect on postsynaptic inhibition. Using an experimental approach identical to that used with strychnine, records were obtained from motoneurones in cats injected with convulsive doses of picrotoxin in which repetitive firing persisted during the stretch of their antagonist muscles (picrotoxin-sensitive postsynaptic inhibitions), and from motoneurones in which repetitive firing was inhibited on stretch of the antagonist muscle (the described picrotoxin-resistant postsynaptic inhibitions).

A further indication of the complexity of inhibitory influences on a motoneurone was the response to picrotoxin during muscle stretch in a strychninized cat. After a convulsive dose of strychnine, a majority of neurones showed a strychnine-resistant postsynaptic inhibition. If then a convulsive dose of picrotoxin was injected, this inhibition was converted into an activation during the stretch period, demonstrating a reversal at the postsynaptic membrane not only to strychnine but also to picrotoxin.

Finally, if a motoneurone was impaled with a microelectrode filled with potassium chloride rather than

potassium citrate, chloride ions could be injected into the neurone by applying a hyperpolarizing current through the microelectrode (Coombs, Eccles, and Fatt, 1955; Eccles, Eccles, and Ito, 1964). The effect of this electrophoretic technique is to alter the intracellular chloride concentration, and thereby abolish or reduce the inhibitory postsynaptic potential (IPSP) in strychnine-resistant and picrotoxin-resistant postsynaptic inhibitions. This result is a further indication of the postsynaptic nature of these inhibitions.

CONCLUSIONS

No attempts were made during this study to determine the actual site of action of strychnine or picrotoxin, or the occurrence and distribution of axo-axonal synapses. However, based on the evaluation criteria used, the results suggest that the inhibitory influences on a naturally stimulated spinal cord motoneurone are quite complex and are predominantly postsynaptic in nature. At this time, the influence of presynaptic inhibition on naturally stimulated spinal cord motoneurones is unclear, since EPSP and membrane potential changes have proved to be unreliable as evaluating criteria. Based on more reliable criteria introduced by Granit and co-workers, and in contrast to current views holding that strychnine and picrotoxin could differentiate between presynaptic and postsynaptic inhibition, the present results indicate that no such strict pharmacological differentiation of these inhibitions is possible.

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Recollections of Professor Otto Meyerhof

CHALMERS L. GEMMILL

Department of Pharmacology, University of Virginia School of Medicine, Charlottesville

With Dr. Fischer's long interest in muscle physiology and with his background in Germany, I thought that my personal experiences in Professor Otto Meyerhof's laboratory in Heidelberg, 1931–1932, might be of interest.

This laboratory was at that time one of the outstanding centers for research in muscle physiology and chemistry. The laboratory was called the Kaiser Wilhelm-Institute für Medizinische Forschung. There were several such institutes for research in Germany established before World War I and they retained the name of the Kaiser for some time after this war. The Institute in Heidelberg was divided into four subdivisions; Biochemistry, Physiology, Pathology and Biophysics. The Institute of Biochemistry under Professor Kuhn and Physiology under Professor Meyerhof were very active, the other two were relatively quiet. The Institutes were housed in a modern well-equipped building situated close to the river. Professor Meyerhof's Institute was on two floors of one wing with animal quarters and shops in the basement. At the time when I was there, there were four full time members on the staff, Professor Otto Meyerhof, Professor Karl Lohmann, Dr. Herman Blaschko and Dr. H. Laser. Professor Lohmann was a well trained biochemist noted for his work on adenosinetriphosphate, who later went to Berlin. Dr. Blaschko is now a professor of Pharmacology at Oxford and a Fellow of the Royal Society. Dr. Laser went to England where he continued his work in Dr. Keilin's laboratory in Cambridge. There were numerous research workers coming to the laboratory for various periods of time. Dr. Eric Boyland, known for his work on the metabolism of carcinogenic agents, now connected with Royal Cancer Hospital in London, Dr. Eric Jacobson, known for his work on Antabuse, now Professor of Pharmacology, Royal Danish School of Pharmacy, Copenhagen, Denmark, Professor D. Nachmansohn, known for his work on the transmission of the nervous impulse now Professor of Biochemistry at Columbia, Dr. Donald MacEachern who worked on the metabolism of the brain but, unfortunately died early, Dr. Arthur Grollman, known for his work on cardiac output and pharmacological agents used in hypertension, now Chairman of the Department of Experimental Medicine at the University of Texas in Dallas, Texas. Also, prior to 1931–32 Professor Meyerhof had numerous students who now occupy important positions in medical research, for example, Ralph Gerard, Harold Himwich, Francis O. Schmitt, Fritz Lipmann, Severo Ochoa and E. Lundsgaard. Mention must be made of Mr. W. Schulz, a well trained technician, who carried out the chemical determinations for Professor Meyerhof and whose name appears on numerous papers with Profssor Meyerhof. He was also expert in the construction and use of laboratory apparatus. There were also two well trained technicians in the laboratory who were able to construct excellent equipment.

Professor Meyerhof ran what the Navy would call a "tight ship." His associates would await his arrival in the morning and as he was seen approaching on bicycle the cry would go through the laboratory "He comes". There would be much attention paid to reading thermometers and the pouring of solutions from one beaker to another. The laboratory was divided into two floors, with the visiting research workers in a large room equipped with chemical benches on the first floor and the private laboratories and offices for Professors Meyerhof and Lohmann on the second floor. On entering the laboratory, Professor Meyerhof would make the rounds on the first floor, asking each worker in turn the same two questions every day, "What did you do yesterday?" and "What will you do today?" He would then discuss the results of yesterday's work in light of the general problem and would outline the work of the day. If some one was absent, he would inquire about the missing worker. After he had completed his rounds he would go upstairs where he would work for one-half the day in his laboratory and the other half in his office writing papers for publication. If, on his rounds, he was satisfied that a worker had sufficient material for publication, he would take the data and later an article would ap-

pear in the Biochemische Zeitschrift with the worker's name and with or without the name of Meyerhof. He did not like to be disturbed after he had made his rounds. It was well to hold any questions until the following day when he was again making his rounds.

His laboratory at that time was designed around experiments with the Warburg apparatus, phosphate and lactate determinations. Each new worker was given a lactate sample to analyze, and, if the analyses checked, he was then given a problem. If it did not check he was told to continue to do lactate analyses until he acquired sufficient skill. Professor Meyerhof was in close touch with Professor A. V. Hill in London and they correlated their work very closely. For example, Professor Hill had published a theoretical paper on the diffusion of oxygen into a muscle (1928) and had speculated on the possibility of exhausting the carbohydrate supply of muscle by long continued slow stimulation of muscle. Professor Meyerhof gave me this problem to do experimentally. The problem involved stimulating the sartorius muscle of a frog in oxygenated Ringer's solution for periods of sixteen to twenty-four hours. Close correlation (Gemmill, 1932) was found between the experimental results and Professor Hill's theoretical calculations. Later, he gave me the problem of measuring the change in oxygen consumption with variation in initial tension in muscle. His mechanic had constructed a delicate platinum muscle lever mounted in a Warburg vessel. It was so arranged that the tension on the muscle could be varied by a micrometer screw. I had to return to Baltimore before the experiments were completed. Professor Meyerhof gave this problem later to Dr. G. Benetato from Rumania. The results (Meyerhof, Gemmill, and Benetato, 1933) were published with our three names on the paper. It was not until several years later that I met my coworker in Leningrad, Russia. He now holds a responsible scientific position in Rumania and I have seen Professor Benetato at several International Meetings.

Heidelberg at that time was a quiet country town with not many other interests beside the laboratory. The country side was good for bicycle trips, the beer halls were comfortable and food and rooms were reasonable. In fact, I had room and breakfast for the equivalent of 25 cents a night and meals at a restaurant called the Kaiser Hof were available at 20 cents a meal. They served a Kaiser Hof Special which consisted of a big slice of rye bread, covered with ham, with onions and pickles around the plate which was sufficient for a lunch.

Professor Meyerhof was not given to lighter moments. The nearest thing to a light remark was that he said of a certain man working on a biochemical problem involving eggs, "You always know where he works, there is egg on the floor." He did invite us to his home for a dinner and talked on philosophy in which he had a deep interest. I remember his daughter, Bettina, coming around the corner into the dining room and saying "Now don't eat up all the ice cream." This daughter came to America, received her M.D. at Johns Hopkins and is now in practice in Bellevue, Washington. At the International Physiological Congress in Rome in 1932, he invited his workers to a delightful meal at Alfredo's.

Professor Meyerhof had strong personal likes and dislikes. Embden was one of his dislikes when I was in Heidelberg. It is of interest that their two names are linked together in the Embden-Meyerhof pathway. C. F. Cori and A. B. Hastings were the two scientists that he admired in the United States. Professor Meyerhof used to mark the papers that he read in the journals. Only the papers of Cori and Hastings were marked in the American journals.

Professor Meyerhof's laboratory represented the Institute approach with all of the workers devoting their attention to the several aspects of the single problem of muscle metabolism. The number of papers (Meyerhof, 1930) and the great contributions from this laboratory represent the results of the dominant personality of one man. The tributes (Nachmansohn, 1950) paid to him in 1950 were published in *Metabolism and Function.* Comparable tributes are now being given to Dr. Fischer upon his retirement.

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Ambulatory Services in Teaching Hospitals

GABRIEL HILKOVITZ

Department of Medicine, Medical College of Virginia, Richmond

In recent months, care of the sick in the U. S. has received considerable attention from the Government, Congress, and various professional organizations, and has sometimes come under strong fire in the press.

We publish the following reports, without necessarily endorsing any of them, in the belief that physicians are interested first and foremost in providing the best medical care, and that, rather than fearing or resenting honest criticism, they Welcome it.—Ed.

The outpatient clinics and emergency rooms of metropolitan teaching hospitals have been criticized severely in recent years for providing poor patient care. In most teaching hospitals the ratio between outpatients and inpatients is three to one; therefore, if the critics are right, 75% of the patients who obtain medical care at the teaching hospital, are not getting the best that medicine has to offer today.

PROBLEMS OF OUTPATIENT CARE

To support their allegations the critics offer these comments: patients are herded into austere facilities where each step in medical care is preceded by hours of waiting; the atmosphere is impersonal; hospital employees are unsympathetic, discourteous, and condescending. Patients are subjected to many indignities, and many of the individual's needs are overlooked because there is too much emphasis on pathology and not enough understanding of the person who has the disease. The patient is passed from one specialist and clinic to another, and at each visit he receives attention to a single facet of his total problem. There is little direct communication between the specialists and consultants so that their opinions and treatments often clash and leave the patient confused. There is no continuity of care because the patient sees a different physician at each visit and cannot establish effective rapport with them. The supporting clerical, diagnostic, and ancillary medical services are disorganized so that delays, mistakes and missing reports or records are common. Expensive tranquillizers have replaced common-sense psychotherapy and physician-patient rapport. Laboratory tests and x-rays have increased in number as histories and physical examinations have grown more superficial. Physicians spend less time with the patient than they do reading or writing about him in his chart.

Medical educators are concerned because students are exposed to such poor examples and methods of medical practice. Sociologists point to the teaching hospital's preoccupation with research, education and the horizontal inpatient with florid disease, while the indigent, ambulant patient is neglected. They complain that the hospital remains aloof from many community needs and the problems of patients with chronic disease, psycho-physiologic ailments, emotional difficulties, and socioeconomic hardships.

Hospital administrators have become uneasy about the cost of providing outpatient services and are reluctant to expand or renovate the ambulatory departments where ill-feeling, emotional steam, and financial deficits are generated so often.

Physicians themselves are very critical of the conditions which prevail in the outpatient services. The part-time faculty clinician, who must toil there to preserve his privileges, enjoys almost no intellectual rewards and experiences a

great deal of frustration. He feels like a second-class citizen in an environment where outpatient care is considered relatively unimportant and where his clinical skills are overshadowed almost completely by the ingenuity of the research-oriented, full-time faculty member. Discouraged by this, he needs little excuse to forsake the clinic as often as possible and to seek refuge in his own practice.

RESEARCH IN PATIENT CARE

A decade of research and top-level discussion has left little doubt that there is much truth in these allegations. In one study, patients who had been attending a university hospital's clinics for two years were re-evaluated (Lashof and Turner, 1964); a surprising number were found to have undiagnosed diseases—diabetes mellitus, hypertension, urinary tract infections, anemia, and visual loss due to cataracts. The patients' charts contained abnormal laboratory reports which had been ignored; perhaps the physician who had ordered a test failed to record his clinical suspicions or did not see the patient again, but, whatever the reason, the unread laboratory report represents time and money wasted, to say nothing of the serious consequences for the patient.

Other studies have shown that the average outpatient makes contact with twelve different persons, employed by six or eight separate hospital departments, at each visit (Deitrick, 1966). This may indicate that the ambulatory services have adopted assembly-line techniques to cope with the overwhelming volume of work. Twenty-five percent or more patients break their appointments and, while this is possibly their own fault, the uncooperative attitudes among them may result from their past disappointments with outpatient medical care.

Work in the U.S.A. and England showed that less than one percent of the sick persons in a community seek medical care in the teaching hospital and then usually because of advanced or uncommon diseases (White, Williams, and Greenberg, 1961). The university hospital's patient population is not only a highly selected one from the socioeconomic standpoint, but also one which does not represent the true picture of illness in the community. Perhaps it is true, then, that the medical school's educational programs and the faculty's interests are not geared to community problems and needs, and that the medical student obtains a distorted view of medical practice which will leave him unprepared to recognize early symptoms and to treat minor ailments, chronic disease, or psychophysiologic problems.

It has also been discovered that many so-called indigent patients, who frequent the teaching hospital's outpatient departments, also use private physicians and other hospitals (Solon, Sheps, and Lee, 1960). In fact, the teaching hospital may be neither the sole nor the central source of medical care for many of them. It is therefore unwise to assume that a given patient has adhered to the treatment which was prescribed at an earlier visit to the clinic, and that his progress reflects the influence of that treatment upon his illness. It is tempting to wonder how much elegant research work has been based on the false or incomplete clinical information recorded in hospital charts.

COMPARISONS WITH PRIVATE MEDICAL CARE

All of these criticisms may be summarized by saying that the practice of medicine in the outpatient clinics and emergency rooms lacks many of the elements which physicians value so highly in their private practices—comfortable and attractive surroundings, personal and individual attention for each patient, continuity of care, well-organized supporting diagnostic and clerical services, well-coordinated methods of referral and consultation with effective communication between physicians, comprehensive evaluation of all the factors playing a part in disease and recovery, and treatment which is tailored to each patient's individual needs and circumstances.

Obviously, the conditions which prevail in the out-patient setting are vastly different from those in private practice: the patients belong to a different socioeconomic group and lack the educational or cultural background for excellent cooperation and understanding between physician and patient. However, the most important difference is that the patient load in most teaching hospitals has grown beyond manageable proportions, and the teaching hospital plays a role in the health-care of a community which differs from that of the private practitioner not only in size but also in the breadth of responsibility and obligation.

SUPPLY AND DEMAND

It is precisely because of these differences that the teaching hospital has evolved existing methods of patient care; faced with a tremendous consumer demand, the teaching hospital was almost forced to adopt assembly-line methods. Since it could not turn patients away, it distributed the available supply among all the consumers and had to be content with giving to each half a loaf rather than the whole. Medicine is a service, not an end-product, and medical care cannot be distributed by the industrialist's mass production methods. The fundamental problem lies in finding methods by which medical care can be improved and distributed widely without sacrificing those qualities which make it a personal and individual service. It is rather easy to say glibly that consumer demand has outgrown the supply, but it is very necessary to analyze the reasons for this before the answers to

the problem can be found. To be sure, the population explosion accounts for a large part of the problem, but medicine itself can also be blamed for some of the trends.

HISTORICAL DEVELOPMENT OF OUTPATIENT DEPARTMENTS

In this connection the views of the historian and sociologist are most interesting and bring out the irony of the situation. The short-term general hospital emerged relatively recently—in the mid 1800's approximately—after antisepsis and anesthesia had arrived on the scene. Before that time hospitals were charitable institutions caring mainly for the crippled, blind, insane, and the destitute. When hospitalization became relatively safe and practical, physicians began to congregate patients in hospitals but, even then, hospitals cared mainly for the poor; it was late in the 1800's before private patients were accommodated. With the growth of hospital-based medical schools, charity patients became "teaching material"; outpatient clinics provided after-care for discharged patients, and found new cases for the teaching program. This arrangement was mutually beneficial, the teaching hospital enjoying a ready supply of teaching material, while the indigent accepted gladly the free services of reknowned physician-teachers. It was in the outpatient clinics that many famous physicians made classical observations about the course, natural history, sequelae, chronicity, and prognoses of disease. They used well both the inpatient and the outpatient services to instruct their students and to sharpen their own abilities; fame and respect were their rewards, and the honorary appointment to a teaching hospital was a prize to be sought and enjoyed.

PRESSURES OF THE 20TH CENTURY

The 20th century brought many new pressures: population growth and the economic depression swelled the ranks of those who sought care in the clinics. Meanwhile, the criteria by which persons were judged indigent were relaxed; the new concept of "medical indigency" allowed many to qualify for free or cheap outpatient services even though they had jobs, property, and many material comforts. At the same time, physicians were advocating prevention and early diagnosis, proclaiming their new scientific successes, and inviting the public to avail itself of the offerings. Ironically, the same scientific progress that spurred consumer demand thinned the ranks of the physicians who were to deliver the service: scientific medicine sired specialization; the generalist all but disappeared; general practitioners retreated from academic halls, and their numbers declined; medical education became increasingly exacting and expensive; teachers of medicine focussed sharply on the inpatient and the laboratory; and the part-time clinical teacher gave way to the full-time clinical specialist and medical research scientist.

Soon there were not enough beds to accommodate the sick, and not enough physicians to treat them at home. More patients could be, and had to be, treated as hospital outpatients. They spent less and less time in bed, more and more time in the clinics; now that death could be averted more often, more diseases entered their chronic phases, and more old people arrived on the scene. Meanwhile, the full-time physician-teacher had to withdraw from the outpatient arena to devote all his time to the horizontal inpatient, his research projects, and that small specialty clinic which he had to protect jealously from too heavy a patient load lest this interfere with teaching.

THE EMERGENCY ROOM PROBLEM

Realizing that a breakdown had occurred in the supply lines, patients flocked to the emergency rooms where, at first, they could expect reasonably quick attention without the customary financial inquisition, a fairly complete evaluation with all consulting services readily available, and the convenience of unlimited credit because no one dared mention money when a life was at stake. In just a few years visits to emergency rooms multiplied 400% or more, half the patients having no urgent problem and enjoying the luxury of medical attention when the day's work was done or the weekend had arrived. Only the keenest eye could now distinguish between an emergency room and a clinic; both appeared to give identical care to the same patients, both underwent physical decay, and neither achieved its goals of good patient care or teaching.

Even at this point, some physicians could not agree that conditions were bad. There were differences also between clinical departments in the medical school: by and large the surgically-oriented specialties had been able to cope with the increasing volume of work because they spent less time, appropriately, in "work-ups;" dealt more often with short-term, curable disease; discharged many of their patients completely from medical supervision; had insatiable needs for operable cases; and generally avoided extensive involvement with problems which were not related closely to the presenting or major disease. Internists, pediatricians, and psychiatrists, on the other hand, could not adjust effectively to the load. Saddled with more chronic disease in older patients and more complexities in their patients' lives, to mention but two outstanding problems, they found themselves totally swamped.

The future holds evidence of even greater activity in the whole field of health care: more patients, more children, more aged patients, more chronic disease, greater public interest in early diagnosis and

treatment, more attention to minor illnesses and injuries, expanding medical insurance programs, and increased public purchasing power through economic growth and financial assistance. Little is known about the prevention of the degenerative and malignant diseases which account for so much morbidity and mortality and, even if preventive medical programs were expanded, these would increase rather than reduce the personnel required for vaccination, disease detection, and prophylactic treatment projects.

ROLE OF THE TEACHING HOSPITAL

These are indeed critical times for teaching hospitals and the situation demands early solutions, realistic reappraisal of the teaching hospital's role in health services, and the application of imaginative plans in the outpatient departments. There is one fundamental issue, however, which clouds all others and obstructs a clear view into the future: it relates to the teaching hospital's view of its obligations to the public. At one extreme one finds people who feel that teaching and research are the primary goals of the university hospital. To them patient care is a necessary but secondary objective which they would limit quantitatively to those patients who are needed for the educational and research programs. At the other extreme there are physicians who regard patient care as the primary purpose of any hospital, with teaching and research as important by-products of patient care. Between these two extremes lie shades of opinion and compromise, each containing elements of false reasoning.

It therefore becomes necessary to restate medicine's purpose—to restore health. Neither medical education nor research is an end in itself; their ultimate purpose lies in their application to patient care and so the latter should still be the primary goal of physicians and hospitals. The other functions of a teaching hospital—research and teaching—are additional goals, not substitutes for patient care. Using this as a basis for their reasoning, some have said that the teaching hospital has an inescapable obligation to provide medical service for its community, and that it cannot limit its patient population, particularly when it is supported wholly or in large part by appropriated tax revenues. Others refute this by saying that the teaching hospital in these circumstances represents the public's investment in a health care facility; therefore, it is the extent of the investment which determines how much medical service can be distributed and how well it can be done. The public, through its legislative representatives, has placed its own limits upon the teaching hospital's effectiveness. It appropriates a certain fixed, and usually inadequate, sum of money to the hospital saying, in effect, that it can afford only as much medical care as the money will buy. The hospital therefore has ample reason to place limits on the availability of its services. The teaching hospital must divide its grant from the public between patient care, teaching, and research, but here again the fundamental issues are misunderstood.

FACULTY MANPOWER

Government appropriations for the purposes of teaching are often estimated according to the numbers of faculty members needed to teach a given number of students, and it is not always understood why there have to be as many faculty members as students. The answer is very simple: faculty members spend most of their time caring for several hundred thousand patients while they teach. This basic difference between medical schools and most other schools needs to be emphasized much more than it has been. Teachers of law are not required to fight cases in court all day long; professors of engineering are not expected to build bridges or machines; teachers of architecture are not expected to design buildings; schools of fine art are not required to turn out paintings, musical compositions and plays—but teachers of medicine are required to heal the sick and to teach while they do so. Therefore, the size of a medical school's faculty should be based upon the numbers of patients served, not upon the number of students taught. Until the medical school is permitted to double or even triple its faculty and to employ more general clinicians, there will be no solution to the dilemma in the outpatient services.

LIMITS TO PATIENT CARE

When the problems of the outpatient clinics and emergency rooms are viewed in this light, it is clear that the public itself is setting limits on the quality of patient care by denying the teaching hospital sufficient funds to operate well. How unrealistic it seems, then to expect an already over-extended faculty to become community oriented and to worry about the sick who do not seek medical care as well as those who do. Every physician subscribes to this idealism but, in the face of the policies governing appropriations to teaching hospitals, the achievement of these ideals is remote. Not only is there insufficient money to give adequate patient care, but there are never any additional funds to expand or improve existing facilities, to study problems, to start pilot trails of new ideas, to replace or purchase new equipment, and to hire sufficient numbers of well-trained clerks, nurses, and ancillary medical personnel. Business and industry owe much of their success to their continual re-appraisal of services and products, and invest large sums of money in these self-evaluation procedures. The teaching hospital, however, is asked to render superb

service, but the money needed to institute new techniques, methods, and services and to experiment with new ideas is never available.

LONG-TERM SOLUTIONS

The long-term solution to the national problem of expanding consumer demand for medical services lies in the education of more doctors, nurses, and ancillary medical personnel, in the enlargement and proliferation of schools for this purpose, in the building of more hospitals and the renovation of existing ones, in greater recruitment of personnel for the health professions, in the training of more doctors specifically for family and general practice, and in the expenditure of huge sums to achieve these aims.

But the teaching hospital cannot wait for these long-term solutions to meet the present crisis in its ambulatory services and, for that matter, in nearly every aspect of its activities. It knows now that quality and quantity are compatible objectives only to a certain point; it should know that its obligations to the community are limited and the limit is determined by its operating budget. It should not try to calculate how many patients it needs for its educational programs (different departments will come up with different answers anyway), but it can calculate very easily that x number of dollars will purchase y number of patient visits. Having determined the patient load which its budget can support, it has every right to tell local government to assume responsibility for the patients who cannot be accommodated. When such a step is contemplated, a method must be devised whereby the patients themselves will not suffer unduly from the teaching hospital's refusal to treat anyone and everyone.

SHORT-TERM SOLUTIONS

The most obvious problem facing the teaching hospital's outpatient department is that it has had to assume responsibility for the general and specialized medical care of a large medically-indigent section of the metropolitan population. Yet, despite this new and expanded role in the area of general practice, the teaching hospital has failed or refused to organize within its walls a facility for general practice. Instead, it has allowed its emergency rooms and specialized clinics to become swamped and misused.

The attack upon the outpatient problem must therefore begin with the organization of a system of medical care at the general practice level which will protect the emergency rooms and specialized clinics from misuse. This can be accomplished in three steps:

(a) *By establishing a clinic for screening, primary evaluations, and general practice:* Ideally this clinic should have the capacity to deal with a hundred or more patients per day. All patients who present themselves for treatment without prearranged appointments should be seen by a screening physician with considerable experience. True emergencies should be allowed direct access to the emergency rooms, and the definition of an "emergency" can be broad enough to include minor cases of trauma or poisoning as well as illnesses characterized by chest pain, abdominal pain, bleeding, dyspnea, shock, disorders of consciousness, high fever, convulsions, paralysis of body functions and so on.

All patients with non-emergency problems should be interviewed and examined in the screening area; x-ray and laboratory facilities should be available there to complete a primary evaluation equivalent to that made by a competent general practitioner. This clinic should function also like a group practice so that the generalists who staff the area, and their interns and residents, have direct access to consultants from the various specialties. The consultants should come to the patients, give their advice, teach the generalists and housestaff while they do so, and plan with them an orderly program of management for the individual patient. The consultants will have the opportunity to identify patients who require specialized care and to prevent unnecessary referrals to their own clinics. If this general practice or primary evaluation and screening clinic were headed up by a competent general practitioner, he could develop a department or division of general practice with responsibilities and jurisdiction confined to the outpatient setting. He could also develop his own internship and residency programs for family and general practice, and make available for the school's continuing education program an area where general practitioners can receive refresher courses and training.

The patient would benefit enormously from this arrangement since his evaluation by the generalist and consultants is completed in one or two visits. Moreover, the physicians would be in direct contact with one another, thereby obviating the need for much writing and repeated review of the history. If this clinic also had the services of dieticians, social workers, rehabilitation experts, family counsellors and public health nurses, the medical services would be truly comprehensive and oriented toward individual, family and community needs.

Competent residents and trainee follows could function effectively as consultants at this level of medical care, and a clinic such as this could easily become a good teaching model offering excellent experience in ambulatory medicine and consulting practice.

Since the teaching hospital, through the medium of this clinic, would be making a sizeable contribution to community health, it could ask local government to finance the project. Funds should be sufficient to build the facility initially and then to equip, staff and

maintain it properly. This facility must remain open all the time, with a skeleton staff during nights and weekends when patients would receive only interim care to tide them over until the next full working session on a weekday.

(b) *By establishing or reorganizing a general service or routine after-care clinic:* After the primary evaluation has been completed and definitive management has been instituted, patients could be referred to a general service or follow-up clinic for routine after-care. This, also, should be financed by local government. A facility like this exists already at the Medical College of Virginia and at other teaching hospitals, but it functions poorly for several reasons. It is closed during the day and on certain weekdays; it lacks adequate laboratory, x-ray, and ancillary medical support. Patients have to make another visit to receive certain tests and a third visit to learn the results of those tests. Many patients are afraid or unable to attend the clinic in the evenings. A different physician sees the patient each time he attends, and far too many patients are crowded into each session.

If its hours of operation were expanded, and if each physician were assigned to a group of patients for whom he remains the central medical figure, the general service clinic would improve greatly. Experience has shown that the general service clinic is located best near the outpatient department of the teaching hospital so that patients' records can be obtained easily and quickly from the central record room.

Since local government cannot recruit enough physicians to staff a general service clinic, it has employed the teaching hospital's residents. As long as the service clinic remains a poor after-care facility, serious objections can be raised to this arrangement because it encourages or allows the residents to practice an inferior brand of medicine while they are being trained toward excellence. If, however, the service clinic were organized properly it could be argued strongly that it provides the housestaff with a learning opportunity in long-term medical care.

(c) *By establishing satellite clinics:* Local public health departments should establish satellite clinics in heavily-populated and economically-depressed areas for the primary evaluation and care of minor illnesses, well-baby care, preventive innoculation programs, routine ante-natal and post-natal care, and home care programs. These clinics could be operated jointly by local health departments and the medical school's department of preventive medicine and public health. Medical students could work in these satellite clinics with public health nurses, social workers, welfare officers, and specially trained medical orderlies like those which the armed forces have trained for the Special Forces' projects in Viet Nam.

The satellite clinics would help to decentralize routine health services, thereby preventing congestion in the teaching hospital's outpatient clinics and emergency rooms. They would also prepare the way for the establishment of a regional health center program such as that envisioned by the federal government. It is not unlikely that federal funds could be obtained for this purpose.

In essence, then, three steps can be taken to protect the teaching hospital's emergency rooms and specialty clinics from indiscriminate overuse. A system of medical care is established which reaches out into the community from the teaching hospital and fills the gap which now exists in general medical care. By this mechanism the emergency room resumes its former role as a precious, life-saving facility which stands ready to handle any major disasters in the community.

Much has been written and said also about the emergency room's role in comprehensive medical care. To illustrate this, Dr. George James, former Commissioner of Health for the City of New York, now dean of the new Mount Sinai School of Medicine, questioned the value of treating an old lady's cut finger while ignoring her poor eyesight and a carcinoma of the cervix (1965). His point was that medical care in the emergency rooms is so oriented toward the presenting complaint that diseases are ignored which have far greater significance for the patient's life. This situation has arisen because of the unprecedented misuse of the emergency rooms. If the routine, minor, and non-emergency work were removed to a more appropriate setting, every patient who truly had an emergency could be evaluated thoroughly. The emergency room simply cannot do everything for everybody, nor should it do something for everybody. It is much more logical to protect it so that it can do everything for some people, those who really need emergency care. Furthermore, the days of the general emergency room are numbered. It makes no sense at all to drain absesses where clean wounds are sutured, to treat pregnant women where D.O.A.'s are pronounced dead, to examine children where belligerent psychotics and alcoholics are seen, and to expose psychiatric patients to the sights, sounds and tensions of the general emergency room. The time has arrived to split the emergency service into several sub-parts—one for pediatrics, one for obstetrics and gynecology, one for psychiatry, one for the management of shock and trauma, and another for the treatment of acute illnesses in adults (a joint medical-surgical facility). In this way patients go directly to the physicians who are most competent in a particular area, with great benefit for the patients and much saving of time and unnecessary toil. Moreover, those emergency rooms should be located conveniently near each other so that they can be served by a central, special laboratory and x-ray unit.

SUMMARY

Space does not permit the exploration of other possible short-term solutions; only those which appear to offer dramatic improvements have been discussed. It is important to recognize that local government must be induced to make much larger contributions for health care and to assist the teaching hospital in establishing a pattern of medical care in the community which is oriented toward the public's needs and the teaching hospital's purposes. If local government cannot be induced to make these contributions, the teaching hospital will be forced to restrict its services. This, in turn, may mean a much larger expenditure by local government authorities to establish separate health-care facilities without the cooperation of the teaching hospital.

Having established a system for general and routine medical care in the community, the teaching hospital is then in a position to resume its special function as a referral center. Its specialized clinics could then concentrate more effectively upon complicated or difficult medical problems and improve both teaching and research in those areas.

The medical school, meanwhile, must impress upon government bodies its realistic needs in terms of physician manpower. The imbalance between research-oriented specialists and clinical generalists, which now exists in medical school faculties, should be corrected so that a larger section of the faculty will be available to participate in actual medical practice. This, in turn, would free the research scientist from patient-care duties which he now performs somewhat reluctantly. The time has arrived to distinguish clearly between those full-time faculty physicians who must do high-powered research, specialized teaching, and specialized patient care and those who must do general patient care, general teaching, and research into methods of patient care.

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The Changing Pattern of General Practice and Its Educational Implications*

FREDERICK J. SPENCER

Department of Preventive Medicine, Medical College of Virginia, Richmond

One of the many aspects of society which has changed during this century is the pattern of medical care. Home visits by physicians have decreased from 40% of their total visits in 1931 (personal communication with Dr. Odin W. Anderson, Center for Health Administration Studies, University of Chicago, using data from Falk, et al., 1933) to 5.4% in 1964 (U. S. Public Health Service, 1965). The changed pattern of home visits has, of course, been mirrored by a rise in office visits which constituted 50% of all visits in 1931 (personal communication with Dr. O. W. Anderson) and 69.8% in 1964 (U. S. Public Health Service, 1965). A similar trend is seen in hospital clinic and emergency room visits which contributed 11.9% of the total visits in 1964, an increase of 3.1% since 1959. These figures undeniably point to the fact that the ambulant patient today receives care in two main locations, i.e. the office and the hospital clinic-emergency room complex. Consultations in these locations comprised 81.7% of the total patient visits to physicians in 1964 (U. S. Public Health Service, 1965).

This change in the location of patient consultation has been accompanied by the rise of specialism in medicine. In 1931, 14% of physicians in private practice were full time specialists; in 1962 this figure had changed to 38.4%. During the same time, the classification of part time specialists and general practitioners dropped from 70.8% to 27.2% (Peterson and Pennell, 1962). This decrease was made greater by the increase in number of physicians entering other full time practice, e.g., teaching, government service, administration, and research, and also by the influx of doctors into internship and residency programs. These changes have resulted in the patient being cared for by a multitude of specialists in office and hospital instead of a family physician in office and home.

The general practitioner at the turn of the century was the only doctor available to most persons and was therefore required to practice all branches of medicine. He was able to do this because of the limited diagnostic and therapeutic measures available which, with the lack of hospitals, necessitated the bulk of his practice being conducted in the office and home. Today, however, the specialist and the hospital have assumed pride of place in medicine and consequently the general practitioner has seen his practice change from domiciliary general medicine to a type suited to the demands of his more medically sophisticated patients. Conversely, many specialists indulge in general medicine, at least until their practice is established. The assumption is, therefore, that the general practitioner is being forced to limit his practice in the specialties to some extent, particularly in the urban centers which now house 70% of the United States population and two thirds of the physicians in full time specialty practice.

SURVEY METHOD

The survey described below was made preliminary to a meeting on general practice which was convened by the Dean of the School of Medicine, Medical College of Virginia in October 1964. The aim of the survey was to define the content of general practice in urban and rural areas in the State of Virginia. A questionnaire was sent to all members of the Virginia Academy of General Practice with an accompanying letter signed by the Dean of the School of Medicine and the special consultant in general practice to the College, himself a general practitioner. The information requested was confined to two items:

1. The population of the town or city in which the practitioner was located.

2. The percentage of time spent in each of the specialties of internal medicine, pediatrics, surgery, obstetrics, and gynecology.

Three hundred and ninety-two (82%) of the 478 questionnaires sent out were returned. Of the 392, 20 could not be used because of incomplete information. A total of 372 (78%) questionnaires were

* Supported in part by NIH grant 5 P07 FR00016-04.

TABLE 1
Respondents by Population Size

Population Size	Number and Percentage of Respondents
Under 5,000	115 (30.9)
5,000 to 9,999	41 (11.0)
10,000 to 49,999	62 (16.7)
50,000 to 99,999	31 (8.3)
Over 100,000	123 (33.1)
Total	372 (100.0)

TABLE 2
Percentage Time Spent in Specialties in Areas of Under 5,000 and Over 100,000 Population

Specialty	Population	Percentage Time						
		0	1-19	20-39	40-59	60-79	80-99	100
Internal Medicine	<5,000	4.3	0.9	25.2	43.5	18.3	7.9	0
	>100,000	0	2.4	18.7	28.4	37.4	10.4	2.4
Obstetrics	<5,000	27.8	54.7	14.8	2.6			
	>100,000	52.8	33.3	13.8				
Pediatrics	<5,000	6.1	22.5	60.8	10.4			
	>100,000	8.9	26.0	57.7	7.2			
Surgery	<5,000	28.7	63.4	7.9				
	>100,000	31.7	61.0	6.5	0.8			
Gynecology	<5,000	23.5	65.1	11.3				
	>100,000	18.7	66.6	14.6				

TABLE 3
Respondents in Populations Above and Below 10,000 Reporting "No Time Spent in Specialty"

Population	Internal Medicine		Obstetrics		Pediatrics		Surgery		Gynecology	
	Total	%	Total	%	Total	%	Total	%	Total	%
<10,000	6	3.8	43	27.6	9	5.8	49	31.4	36	23.1
>10,000	1	0.46	104	48.1	19	8.8	62	28.7	39	18.1

therefore used for the evaluation. Respondents were classified according to population divisions (table 1).

RESULTS

There were marked differences in the percentages of time spent in two of the five specialties when towns of under 5,000 and over 100,000 were compared. A greater percentage of time was spent in internal medicine in the large cities, whereas in obstetrics the opposite was true (table 2). Half the practitioners in areas with a population of 100,000 or more were spending at least 60% of their time in internal medicine, almost double the percentage in the same category in the small towns. In the large cities, half the practitioners completely excluded obstetrics from their practice while this was true of only one fourth of the doctors reporting from rural practice. Differences in the other three specialties within this population distribution were not remarkable.

The percentage of practitioners reporting "no time spent in specialty" in towns with populations above and below 10,000 again showed that in the large cities about one half the practitioners practiced no obstetrics compared to 28% in the smaller towns (fig. 1). The opposite was true in internal medicine; practically all large town practitioners practiced some internal medicine, while some 4% of small town physicians said they had no internal medicine practice. This statement must, however, be viewed with suspicion because of the small sizes of the samples (table 3). In addition, small town practitioners saw a lower percentage of surgical and gynecological patients, while their urban colleagues practiced less pediatrics. The differences in these three specialties, however, did not have the magnitude of those in internal medicine and obstetrics. These data confirm the proposition already suggested, i.e. that there is more obstetrics practiced in small town, and more internal medicine in large town, general practice.

CONCLUSION

The survey confirms an assumption based on changes in population distribution, and in the medical profession, i.e. that the traditional pattern of family practice is more common in small towns and rural areas than in the conurbations which now contain almost three fourths of the United States population. The urban practitioner is becoming less of a general practitioner in the old sense of the term and spending more time in the practice of internal medicine. No attempt was made in this survey to find the percentage of time spent in psychiatry. It is probable, however, that a considerable amount of time spent with patients may be categorized as office psychiatry in all types of practice. There is, of course, no indication of the number of specialists who are practicing family medicine from this survey.

The implications of these interpretations have a considerable bearing upon the content of training for general practice. The inclusion of the traditional specialties, i.e. internal medicine, pediatrics, surgery, obstetrics, and gynecology is no longer valid for all general practice residencies. Where possible, a resident intending to enter a rural practice should still have the opportunity to work in these specialties. All general practice training should, of course, contain the fundamentals of psychiatry and social medicine. The latter term is used as a synonym for the more usual "preventive medicine" and applies to the understanding of the patient as a member of society and of the role of society in medicine. It may well be difficult for a general practitioner trainee to decide upon his future practice location before he begins his residency, but this would be most desirable if he is to derive the maximum benefit from his training program. Another way around the dilemma of residency content may be that of having certain requirements, e.g. psychiatry and social medicine, met during the first half of the residency and electives permitted before completion.

SUMMARY

The results of a mail questionnaire sent to members of the Virginia Academy of General Practice demonstrate the changes occurring in the content of family practice. Practitioners in small towns practice more obstetrics and less internal medicine than those in large cities, and conversely. The implications for training in general practice are discussed.

Acknowledgement

I wish to thank the members and staff of the Virginia Academy of General Practice who willingly cooperated in this survey.

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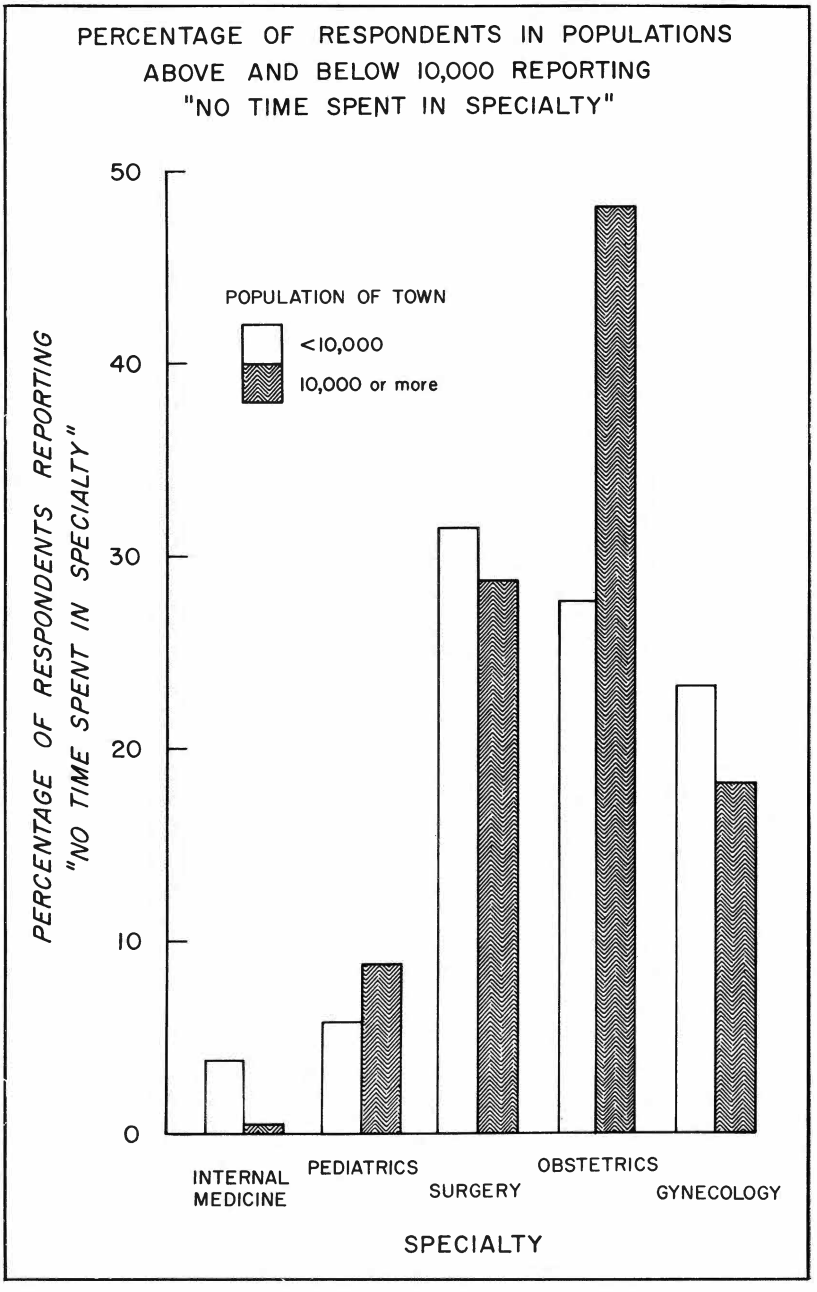
PERCENTAGE OF RESPONDENTS IN POPULATIONS
ABOVE AND BELOW 10,000 REPORTING
"NO TIME SPENT IN SPECIALTY"
PERCENTAGE OF RESPONDENTS REPORTING
"NO TIME SPENT IN SPECIALTY"
50
40
30
20
10
0
POPULATION OF TOWN
<10,000
10,000 or more
INTERNAL MEDICINE
PEDIATRICS
SURGERY
OBSTETRICS
GYNECOLOGY
SPECIALTY

Fig. 1

The Shame of American Medicine*

ELINOR LANGER

The success of American medicine is often attributed to the profession's ability to serve the public on its own terms. Why should doctors care if, from the patient's point of view, the terms chosen—solo practice and emphasis on the "doctor-patient relationship"—mean that a doctor performs unsupervised services for unregulated fees? What does it matter to them that the poor are outside the system altogether, treated in charity wards or public hospitals which are the medical equivalent of Andrew Carnegie's libraries, a small concession to charity from an accelerating machine of wealth, power, and influence? In a country proud of its "pluralism" and fearful of "government interference," a monolithic self-regulating profession is taken as a sign of health. Few people are persuaded that medical care is a fit object of social planning: We have no national health policy and we are mostly proud of it.

It has left us in an extremely unfortunate mess. At its best American medicine may very well be the best in the world, as its practitioners claim, which is why retired English kings and Arabian sheiks turn up regularly in our hospitals. But though excellent treatment is usually available to the very rich, the rest of the population finds even adequate services hard to come by. The charge frequently made by critics that ten countries have lower rates of infant mortality and longer life expectancies does not mean that the Peter Bent Brigham Hospital, for instance, is somewhat slipshod; it means that most people will never set foot in any place half so good.

The situation of the poor is particularly appalling. In Boston, a health survey of a public housing project indicated that among individuals over 65, 25 per cent had chronic bronchitis, 20 per cent had chronic nervous disorders, 12 per cent were blind or had visual defects, and that 40 per cent of these were not receiving treatment. In New York, former Health Commissioner George James has estimated that 13,000 poor people died last year because adequate professional care was not available. The maternal mortality rate for U. S. whites (in 1961) was 2.5 per 10,000 live births. For Mississippi Negroes, it was 15.3, more than six times as high. In a South Carolina county, every tenth Negro child died in the first year of life.

The poor are not wholly without opportunities for medical care. But the public facilities that do exist perpetuate a grotesque circle of personal humiliation and medical lunacy. In many cities a mother cannot take a well baby for a checkup to the same place she must take a sick child for diagnosis or treatment. If she suffers from both migraine headaches and pains in her chest she may have to go to two different clinics herself. Clinics (and emergency rooms) are often far away, in a sometimes unfamiliar "downtown." For a suburban

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mother with a car and a maid such problems would be easy to surmount. For the poor mother it is different. Each clinic visit may take a separate trip. Each trip means, if she is working, a day's lost pay; or, if she customarily cares for her children, an arrangement with neighbors. It means costly taxi fares or time-consuming bus trips. After a long wait in a crowded room arranged like a bus terminal, she may be ordered to go elsewhere or to return another day. She may be asked to undress in the hallways and, thus stripped, to explain her problem to various impersonal functionaries, to what bureaucratic purpose she can hardly be expected to understand. If she sees a doctor at all (no certainty) he will not be the one she saw last time or the one she will see next time. Her medical records may be scattered about the city. She is apt to be submerged in an avalanche of prescriptions and regimens incompletely understood (for there is no one to explain them to her) and often mutually incompatible.

And so the poor, faced with a system that discourages them from seeking care, and beset with other crises that may seem to them more urgent than a nagging cough, have acquired a certain reputation among the professions: They "don't care" about their health, "don't keep appointments," "won't cooperate," "don't do what you tell them," and even "don't mind being sick." The hoariness of this mythology is clear from a recent study of English hospital development by Brian Abel-Smith. He reports that during a government inspection of English pauper hospitals in the 1860s:

> At Kensington and Paddington some of the sick were "found washing in their chamber pots." The inspector was told by one medical officer that the patients preferred to wash in this way but he later established that they did this "against their will and their former habits at home." Only a few [institutions] provided lavatory paper on the grounds that "a very large proportion of the poor" were not in the habit of using it. There were, however, "numberless instances" of closets being blocked with "old towels, dusters and dishcloths—and leaves of Holy Scripture . . . One or more Bibles, and sometimes a Prayer Book, were found in each ward, but in a more or less imperfect and dilapidated state—a circumstance connected with the subject just discussed."

Even the best of the organized health plans have sometimes had difficulty staffing their units in the ghettoes: Disgust is the other face of charity.

But the medical system has not only failed the poor: It is also cheating the middle class. There is a joke popular with medical students: "What are the indications for a hysterectomy?—Two children, a Blue Cross card, and a uterus." Unfortunately, it is no joke. Every review of the quality of medical care has found a high rate of unnecessary and incompetent surgery, of faulty and delayed diagnosis, of sins not only against medical science but against common sense. A famous study by Columbia University's School of Public Health and Administrative Medicine of the medical care of a group of Teamsters and their families in New York City a few years ago concluded that one fifth of the hospital admissions were unnecessary and one fifth of the surgery was "poor." (1)

More than a third of the hysterectomies and more than half the Caesareans were held unnecessary. A study sponsored by the Rockefeller Foundation and the University of North Carolina Division of Health Affairs of North Carolina general practitioners in the 1950s found that 44 per cent were failing to take medical histories, using unsterile instruments, conducting incomplete examinations without using laboratory aids and without having patients undress or lie down, or prescribing irrelevant drugs. "The physicians studied came from many medical schools and had exhibited all degrees of academic success," the report stated, "so there is no reason to assume an adverse selection. It can . . . be stated with considerable assurance that in terms of medical education and training the physicians who participated in this study are not evidently different from general practitioners at large." (2)

Ethical controls are as lax as the medical ones. Denunciations of fee-splitting issue periodically from the professional associations. But doctors combine to buy pharmacies in medical buildings; take payments for journal articles they have not written endorsing drugs they have not tested; conduct medical and surgical experiments on their patients without telling them; cheat on insurance; and, like the GE executive who went to jail, they retain an honored place among their colleagues and within their communities. (3)

Middle-class medicine is facing a crisis in costs as well as quality. Hospital rates now average over $40 per day and insurance rates have taken off like a rocket. To a certain extent this is the price of technological achievement: A heart-lung machine, for instance, and a cobalt machine for treating cancer may cost in the vicinity of $100,000 each, and each requires a small army of skilled technicians for its upkeep. It also reflects the inroads of unionization on hospital pay scales. Salaries have been so low that in New York, for example, some hospital employees were recently receiving public welfare while holding down full-time jobs. But to a large extent the doctors themselves are responsible for the inflation: An electrocardiograph standing idle for thirty-five hours a week in the private office of a Park Avenue internist is an exceedingly costly instrument, and the costs are reflected in his bills. The inflationary pattern of solo practice is reinforced by the pattern of insurance plans. Nearly 150 million Americans have some,

but it covers on the average only 30 per cent of a family's regular medical bills. Hospitalization insurance is easy enough to obtain, but it is hard to buy policies that cover office or home visits, drugs, outpatient diagnostic tests, or psychiatric or nursing care. The payment system common to insurance —so much for a hernia, so much for a tonsillectomy—supports the ideology of solo practice in another way. It encourages both doctors and patients to think of health negatively, as a series of episodic battles against discrete afflictions. In this system the concept of "comprehensive" or preventive care has little place.

The result is poor medicine and poor policy. It is poor policy because it leaves both doctors and patients dependent on hospitalization—the patient, in order to pay his bills, the doctor to collect his fees—and obstructs development of more rational and humane outpatient, home, and nursing services that could be more cheaply arranged. The present dilemma of the hospitals—shortages of services in some areas and underutilization in others—has additional causes: administrative rigidity, regional competition, desultory Federal supervision, and technological leapfrogging that has left many small institutions unable to perform modern services adequately. But hospital-oriented insurance has played a major role not only in overcrowding many hospitals but in deflecting attention from their defects. In addition, the system leads to poor medicine because it subsidizes the costs of catastrophe, not the preventive care that might minimize catastrophe, and it is flourishing at a time when medical victories over many acute diseases and the growing proportion of old people have made arrangements for preventive and long-term care all the more essential. Illness is simply more flexible than insurance. As Anne Somers pointed out in a recent paper:

> The corollary of this shift [to an aging population] is increasing need for long-term preventive, rehabilitative, semi-custodial, and medical social services. Most chronic diseases are months or years in developing and require early diagnosis if they are to be handled effectively. The period of treatment is, by definition, extensive. If "cure" is achieved, there is often required a long "post-cure" rehabilitation. Generally, the most optimistic solution is stabilization—for example, in diabetes or glaucoma—under continuous life-time medical supervision. With such changes in morbidity and disability patterns, the distinction between health and illness becomes blurred, and the concept of medical need increasingly difficult to pinpoint in space or time. Rather there is a continuous spectrum with varying degrees of emphasis. It begins before we are actually ill; it does not cease when we are discharged from the hospital. Continuity and comprehensiveness have become indispensable aspects of effective medical care. (4)

The failure of health insurance to deal with this situation is not just a coincidence. As the Somerses' study makes clear, Blue Cross and Blue Shield originated in doctors' efforts to protect their incomes. (5) Blue Shield plans are dominated by local medical societies; Blue Cross plans by hospital representatives. In neither is there much effective public representation. The commercial plans have broken little new ground. In theory, health insurance might have been developed by independent groups who preserved some power to supervise the hospitals and private practice: There is growing pressure for such supervision now from regulatory bodies (state insurance commissions) and organized consumers (business and unions). They have begun to feel that their soaring payments for member or employee health plans cannot be justified without questioning both the cost and the quality of the treatment they are buying. But until now the system has been manipulated by the doctors to prevent outside control. The doctors opposed medicare because they feared that their freedom from review would come to an end under a system of government insurance, and that rising costs would ultimately force the government to institute controls. Medicare is a conservative step, however, whatever the doctors think; for relieving the pressure of the aged (who are bad risks) on the voluntary insurance system will temporarily conceal some of the cracks the system contains. We continue to revolve in a circle of high costs and high rates that leaves millions of people unable to afford insurance at all, and those who have it stuck with unsatisfactory policies which hardly begin to pay their bills. The result has called forth the invention of a new category of social dependency known as "medical indigence": According to a recent study, 80 per cent of the patients in New York's municipal hospitals were people who are not on relief and who normally "manage to cover their ordinary expenses but lack the margin in income, savings, or health insurance to pay the hospital and the doctor when they get sick." (6) If the doctors continue to have their way, they are likely to make medical indigents of us all.

What is to be done? For about thirty years, the "progressive" elements in American medicine—and there are some—have been formulating plans for the reorganization of medical care. These reformers are not an organized group but individuals associated chiefly with medical schools and public health programs who have come together, over the years, in foundation-sponsored and government-sponsored committees and study groups to consider the organization of medicine. (7) Their prescription has three interrelated ingredients. First, they believe that solo practice should be replaced by teams of specialists mobilized into "group practice," thus both enlarging the intellectual and technological resources

of the doctors, and lowering costs. Second, they propose that inclusive prepayment plans (providing, among other things, regular salaries for doctors) should replace traditional fee-for-service compensation. Third, they urge that hospital services should be expanded and more efficiently organized both regionally (to avoid the inequitable and inefficient maldistribution of expensive, specialized equipment) and within the hospitals themselves (to offer patients a range of flexible services correlated with their needs as these change during hospitalization). There is no reason why the patient who is getting better should be imprisoned in a reign of nursing terror when he could be helping to take care of himself. Increasingly, a fourth design has been prominent: the fusion of now-fragmented health resources—medical schools, hospitals, public and private health agencies—into a coordinated "health industry team," whereby unified, community-oriented planning would replace competition among hospitals; facilities would be carefully reorganized to avoid overlapping and to make a complete range of services easily available in each part of the city.

Some remarkable evidence from a few pilot projects makes plain that medical and economic logic are on the side of these reforms. The Health Insurance plan of Greater New York (HIP), for example, the largest group practice in the U. S., enrolls about 700,000 New Yorkers, many of them city employees. They are served by one of thirty-one medical groups located throughout the city, which include both a "family physician" (a G. P.) and a variety of specialists. For $4.50 a month a person can obtain all regular outpatient medical services from eye checkups to physiotherapy. Hospitalization costs are not included (subscribers are encouraged to join Blue Cross) although full surgical costs are. Physical examinations and other preventive services are offered without cost and without limit. The availability of outpatient care seems to promote both health and economy. Studies have consistently demonstrated that the rate of hospitalization and the length of hospital stays of HIP patients are substantially lower than for patients treated and insured by conventional means. (8) More striking, the health record is better. The prenatal death rate among HIP subscribers, for instance, is lower than among patients seeing private doctors. (The lower rate holds among comparable groups of whites and non-whites; among families with comparable incomes; and among families where the wage-earners have comparable occupations.) HIP subscribers suffer fewer infant deaths in the first week after delivery; the average weight of infants at birth is higher; the prematurity rate is lower. The record of other group health plans is the same.

In a limited way, it is true, some "reform" has already begun. The influence of the medical schools and hospitals is rising and solo practice is, statistically, on the decline. Nonetheless, the number of people being served by the new arrangements is small. Lying between successful demonstrations of progressive ideas and their wide application are two things. The first is the unrelenting obstructionism of organized medicine. In 1943 the Group Health Association of Washington, D. C. successfully brought an anti-trust suit against the AMA and the local medical society for conspiring to restrain trade. But elsewhere, from then till now, physicians entering organized groups have found themselves subject to harassments ranging from social ostracism to suspension of medical society privileges. Twenty-three states still have laws prohibiting group practice except in a form approved by the medical societies. In only about a dozen cities is it even possible to enroll in a full-fledged group practice program. In the same way, the profession has bitterly resisted the trend toward including specialists' services as part of hospitalization, insisting that the radiologist who takes X-rays or the anesthesiologist who gives the injection are private, personal physicians, equally entitled to that "special relationship" with their patients that permits them to send a bill. (9) Their fear arises from a domino theory of medicine: As radiologists go, so will go the obstetricians, gynecologists, and internists. Group practice will have a beachhead in the hospitals and fee-for-service practice will come to a stop. The Communists will be at Waikiki.

Supporting the intransigence of the profession in the face of change has been the weak and neutral policies of the federal government. We spend billions of dollars on medical research (paying particular attention to the pet afflictions of the aging politicians who appropriate the money) and billions more on hospital and other construction programs. These have succeeded chiefly in proliferating the interests opposed to change. But aside from providing direct medical care to specialized portions of the population (mainly federal dependents), the government has left what is known in the trade as "the delivery of medical care" alone. There is one exception, the Heart Disease, Cancer, and Stroke legislation passed in the last session of Congress. Following the "progressive" model, this calls for regional cooperation among existing health agencies to advance the research, diagnosis, and treatment of the three diseases. But like the medicare bill which promises that no Federal official shall be permitted "to exercise any supervision or control over the practice of medicine or the manner in which the services are provided," the Heart, Cancer, and Stroke Bill promises to accomplish its ends "without interfering with the patterns, or the methods of financing, of patient care or professional practice, or with the administration of hospitals."

Medicare itself may ultimately be responsible for overturning that intention. Experts anticipate that the availability of payment after July 1 will lead to a sudden, crushing demand for medical services that the present disorganized system will be unable to supply. If they turn out to be right, medical care could become a major political issue, and pressure from angry consumers could force the government to play a stronger role. But that is not the way it was planned. When federal officials go up to Capitol Hill to testify that the programs they are endorsing will "save us from socialism," the trouble is that they mean it. They are committed to a timid reformism that masks their unwillingness to retrieve power from the very institutions which need to be reformed.

The idea that the government would take the lead in ending the chaos in medical care was subtly undermined last summer. The AMA convention in New York last June was perhaps the lowest point in the profession's recent history. There were hysterical discussions of medicare ("we would be zombies stepping into involuntary servitude if we accept such fascist control") and intense debate about a doctor's strike (". . . it is ethical, proper, desirable, moral and legal not to participate in such socialistic schemes"). Peripheral groups of doctors, formed out of concern with racial discrimination, or with foreign policy, or with the economics of medicine, were beginning to talk seriously about founding a rival association. In Washington, the influence of two potential competitors to the AMA—the American Hospital Association and the Association of American Medical Colleges—became increasingly apparent. An influential coalition of physicians centered around philanthropist Mary Lasker had been moving away from its initial preoccupation with medical research and into questions of medical care. The AMA was in a shaky position and its leaders knew it. After the confusion of the convention, they went to Washington, timorously, to say that they would, after all, cooperate in drawing up the regulations to implement medicare. And the government—in effect, the chief officials of the Department of Health, Education, and Welfare—took them back. They supplied the doctors with new prestige—a visit with President Johnson—and took some advice on medicare rules and the Heart, Cancer, and Stroke Bill. The new guard at the department might have demanded positive evidence of a change in attitude and definite commitments for AMA support of creative legislation. Instead they lost themselves in public celebration of a fuzzy and undependable "partnership." This same concept of "partnership"—solicitude for established interests—is also rapidly obliterating hope of rapid progress in critical areas of environmental health. We pass a bill requiring a mild cigarette-label warning, but prohibit any other warnings on packs or ads till mid-1969. We pass a strong water pollution bill but leave intact a Jeffersonian formula for distributing grants that actually discriminates against the crowded urban areas where pollution is most serious. We permit the poverty program to offer birth control but refuse to let it instruct the unwed mothers who need contraceptives most. We support research on traffic acccidents but permit researchers to withhold the names of auto manufacturers with the most treacherous designs. To celebrate partnership is, usually, to celebrate a deal.

In the case of medical care, there has been a deal, and all of us are the objects of it. The system, in which the government has acquiesced, is designed to keep the doctors well-nourished and the middle class quiet. Discontent over the organization of care is diverted into humble appreciation of scientific triumphs. Doubts about the treatment of the poor are smothered by periodic stories of dramatic recoveries on the wards and by the Robin Hood notion that "our" prices are high because the doctors are working charitably for "them." From the system that offers both a cure for our tuberculosis and a salve for our conscience, we will suffer both humiliation and extortion. The middle class does receive better care and consequently has a better chance for survival than the poor have, but in a subtler sense it is equally victimized. The agility of middle-class patients increases their ability to navigate in the system, obtaining supporting diagnoses or shopping around for more compatible, or lower priced, or more fancily equipped, doctors. But none of us can really change the attitudes we encounter, modify the orders we are given, avoid the charges we are told to pay, or look to anything outside the closed shop for comfort or support. It was precisely this condition of dependence that weakened the wariness some government officials harbored secretly during their reconciliation with the AMA last summer: The officials knew that, from a practical point of view, the AMA represents the only doctors we have. The exceptions, the clusters of independents and critics, are too few numerically and too concentrated geographically (in urban centers) to be the base of a reorganized system of medical care.

Nor would the subtler defects of the system be fundamentally afected if there were more renegades. We would be at the mercy of the good guys instead of the bad guys, but the good guys share with the bad an instinctive commitment to the idea of total professional control. There are some exceptions. The Tufts Medical School has set up a health center in a desolate housing project on the edge of Boston that in effect combines group practice with public control. The formula is the standard requirement of the Office of Economic Opportunity—a board composed of members of the local community. But it is working out with the seeming

difference that, unlike most mayors, the Tufts doctors enjoy working with the residents in a non-authoritarian fashion and are actually committed to the idea of "community participation" in the process of medical care. No welfare mother is about to start taking throat cultures, but the doctors are trying to share power with the community in a number of nonspecialized areas of policy: The residents influenced the design and furnishing of the health center facility for example; more important, they helped to define the conditions of service (including clinic hours, payment, and so forth) and will help in their execution. Tufts also plans to train Columbia Point residents for a range of subprofessional jobs at the medical center, something that may help to reduce the psychological gulf between doctors and patients. The school is planning a similar project in the rural South. In a few other cities, elements of the scheme—the training of the poor as health assistants or the development of neighborhood health centers—are being talked about and tried. But these projects are confined to the poor and far too restricted to be called a trend. For the most part there is reason to believe that as the progressive vision is implemented, the incapacity of the public to exercise control over the medical profession will be not lessened but exaggerated. In the Heart, Cancer, Stroke program, for example, power will reside in Olympian regional coalitions resting on medical schools, hospitals, and public and private health agencies; in New York's controversial "Trussel Plan" the city has in effect turned over the administration and control of municipal hospitals to the private hospitals and medical schools. (10) The progressive vision in medicine is a corporate one, a response to institutional inefficiency and waste, not to personal inhumanity and confusion. But that, in all probability, is where we are heading. If they oil us now and then, and shore up our outworn parts, will we ask for anything more?

1. Selig Greenberg, *The Troubled Calling: Crisis in the Medical Establishment,* Macmillan, 1965, pp. 208-210.

2. Herman M. Somers and Anne R. Somers, *Doctors, Patients, & Health Insurance,* pp. 31-32, notes 7, 8 (Brookings, 1961) and Selig Greenberg, "The Decline of the Healing Art" in *The Crisis in American Medicine,* Harpers, 1961, p. 22.

3. Documentation of links between physicians and pharmaceutical operations can be found in last year's hearings of the Senate Anti-Trust Committee on "Doctor-Owned Pharmacies" and in hearings of the House Government Operations Committee on "Drug Safety." *Science,* Feb. 11, 1966, p. 663, and *The Saturday Review,* Feb. 5, 1966, p. 61, discuss human experimentation. Doctors' abuse of insurance is reported in Greenberg, *The Troubled Calling,* p. 207 ff.

4. Anne R. Somers, "Some Basic Determinants of Medical Care and Health Policy: Trends and Issues." Paper prepared for Seminar on Health Policy, Institute for Policy Studies, Washington; January 25, 1966.

5. Somers and Somers *op. cit.,* chapters 15 and 16.

6. Nora K. Piore, "Metropolitan Medical Economics," in *Scientific American, January,* 1965, p. 19.

7. George Baehr, "Medical Care—Old Goals and New Horizons," 1965, Michael M. Davis Lecture, The University of Chicago, May 13, 1965. Among the most important groups was the Committee on the Costs of Medical Care, established with philanthropic support in 1928. Another major study was produced by the Presidentially appointed Commission on the Health Needs of the Nation in 1949.

8. Somers and Somers, *op. cit.,* p. 177.

9. *Science,* July 9, 1965, p. 164.

10. Robb K. Burlage, "Issues of a Changing Hospital System, with New York Case Study." Preliminary study, Institute for Policy Studies, Washington, 1965.

Notes on Bibliography:

By far the most valuable book on the organization and financing of medical care is the volume by Herman M. Somers and Anne R. Somers, *Doctors, Patients, & Health Insurance,* Brookings, 1961. The Somerses drew freely on disciplines of political science, economics, and public administration in analyzing factors affecting medical care, and they succeeded in producing a study both lucid and comprehensive. Selig Greenberg's *The Troubled Calling: Crisis in the Medical Establishment,* Macmillan, 1965, $6.95, is essentially a journalistic popularization of the same issues treated by the Somerses, but it tends to be repetitive and not always to the point. A more original popular inquiry is *The American Health Scandal* by Roul Tunley, Harper & Row, 1966, $4.95. In addition to an unpretentious and readable chronicle of the organization of medicine in the US, Tunley provides a helpful survey of how England, Sweden, Yugoslavia, Germany, and Canada organize their health services. *Hospitals, Doctors, and the Public Interest,* John Knowles, ed. (Harvard University Press, 1965, $8.50) is a collection of essays by experts, some of whom approach their subjects from a perspective somewhat obscure to a general reader. The politics of the drug industry are closely examined in Morton Mintz's *Therapeutic Nightmare.* Finally in a class by itself, is *The Hospitals in England and Wales, 1800-1948* by Brian Abel-Smith, Harvard University Press, 1964. It succeeds not only in illuminating unexplored corners of British social history but in documenting how English and American medicine grew to be so different.

The Shame of American Medicine—A Reply

In the long stream of vitriol which Miss Langer has poured over the heads of physicians, the following specific complaints are presented:

1. Medical care of the poor is poor.
   A. The fee-for-service scheme and the unpleasantness of clinics discourage preventive medicine and continuing care of chronic disease.
   B. Clinic facilities are inadequate.
   (1) Numerically
   (2) Attendance at clinics requires loss of time from work.
   (3) A patient is shuttled from clinic to clinic and from doctor to doctor.
   (4) Clinics are impersonal and insulting.

2. Physicians
   A. Operate unnecessarily
   B. Take inadequate histories
   C. Perform inadequate examinations
   D. Fail to use laboratory facilities
   E. Split fees
   F. Own pharmacies
   G. Cheat on insurance
   H. Experiment on patients without telling them
   I. Keep expensive equipment idle

Miss Langer's specific solutions include:

1. Replacement of solo practice with teams of specialists in groups.
2. Prepayment plans, including regular salaries for doctors, rather than fee-for-service.
3. Expansion and more efficient organization of hospital services. (This recommendation is not very specific.)
4. "Fusion of now-fragmented health resources—medical schools, hospitals, public and private health agencies—into a coordinated 'health industry team', whereby unified, community-oriented planning would replace competition among hospitals."

The basic problem with the medical profession, in Miss Langer's view, lies in its self-regulation; the public has no control over the practice of medicine. ". . . a doctor performs unsupervised services for unregulated fees."

After recovering from my initial ire at this unfriendly attitude, I have set down the following reactions.

I. Regulation of the profession:

A. *Control of the quality of medical care.* It seems irrational for nonphysicians to judge medical knowledge. The public could insist that physicians be repeatedly tested, by the National Board of Medical Examiners, for instance. There is no way, however, to ensure by testing, kindness or genuine interest in patients. Intangibles such as these are still as valuable in the healing of people as is pharmacologic or surgical therapy.

Perhaps dissemination of information about the efforts of physicians in continuing education would reassure the public. I am unable to devise any practical scheme for control of the excellence of an individual practitioner other than those in operation, namely careful selection and training of medical students, including constant exposure to teachers who stress loving care for the whole person.

B. *Control of the cost of medical care.* The threat of direct governmental control of physicians' salaries seems remote. Private enterprise, self-reliance, and the worth and responsibility of the individual are still American ideals. Physicians become understandably irritable at suggestions that they accept government salaries, when others upon whom life and happiness depend, e.g. automobile manufacturers and mechanics, lawyers, plumbers, continue unregulated.

Doctors nevertheless could well heed Miss Langer's expression of apparently widespread resentment (*see* Harris, R., Annals of Legislation: Medicare, *The New Yorker,* July, 1966, for a carefully written shellacking of the AMA), and respond with practical improvements of existing inadequacies.

The fee-for-service payment system does discourage the repeated visits required for optimal care of chronic conditions for which effective palliative therapy is available, for example hypertension, congestive heart failure, diabetes mellitus, chronic bronchitis and emphysema. Unfortunately, the physician's fee-for-service, $5.00, is an insignificant contribution to the cost of chronic illness. Hospital costs, drugs, x-rays, and laboratory tests represent relatively enormous expenses. Medicare and private medical insurance

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plans do not cover the cost of drugs, nor, usually, the cost of laboratory tests for outpatients. Comprehensive pre-payment plans whose cost is reasonable should be encouraged by physicians. A reasonable reimbursement for physicians' services for a year, say $85; plus drugs—reserpine, a thiazide, and guanethidine cost about $12 per month—$150; plus chest and renal x-rays, $75; plus four BUN's; three sets of electrolytes, two urinalyses with cultures, one blood sugar, $90; plus an administrative fee for office operations, $25; total cost—$415; or about $35 monthly. To make such coverage available for the non-wealthy would require insurance of a very large number of persons healthy during that year. The community must make such care available; the primary consideration is the most efficient method. Coverage of the entire population by government may be most efficient. Those of us who distrust extension of government must provide efficient schemes, or give reasons more practical than the independence of individual physicians, for avoiding governmental finance for medical care. Blue Cross and Blue Shield are theoretically controlled by physicians, and represent the best hope we have of providing adequate coverage of the cost of chronic illness without resort to government regulation.

Miss Langer's criticism of clinic facilities applies accurately to Richmond, where the city's only general medical clinic meets three nights weekly in the downtown area and is perpetually overcrowded. The appointment system in the outpatient clinics of MCV, where all patients are told to arrive at 8 a.m., noon, or 5 p.m. seems designed to ensure long waiting lines at appointment desk, laboratory, and pharmacy. Public, consumer participation in the planning of outpatient scheduling might well improve service to patients.

Physicians whom Miss Langer knows are a scurrilous group. She has selected examples of physician-failure which are (1) from time past (own pharmacies, experiment without informed consent, split fees, operate unnecessarily), (2) half-truths (almost all fall short of perfect histories and physicals, and I skimp on lab tests to save the patient's money), or (3) are not true, in my experience, (cheat on insurance).

"Replacement of solo practice by teams of specialists" contains an obvious fallacy, which I'm sure Miss Langer realizes, namely, patients cannot be cared for by a committee—one person has to be responsible, and authoritative. Any sensible group of physicians realizes this fact, and it is possible to design a group which is a team of expert consultants available to the one physician who is responsible for the patient. Group practice has such obvious advantages in education, quality control, vacations, and attractiveness to the customer, that one suspects there must be some poorly understood (by Miss Langer and me) truth to explain their infrequency. My guess is that physicians are unusually independent people who by dint of brains and hard work can achieve financial and psychologic success as individuals. They resent interference. By the same process, physicians tend to become supporters of the status quo, scornful of the unsuccessful as lazy, and perhaps even a tad indifferent to the public interest. Voluntary regulation of the profession by physicians genuinely concerned for the interests of the public seems to me far preferable to control by government, since physicians are far better informed about the problems of medical care than is any other segment of the community. It is my hope that physicians individually and collectively will stop senseless opposition and become leaders in providing expert medical care for all Americans.

David W. Richardson, M.D.
Associate Professor of Medicine
Medical College of Virginia

Clinicopathological Conference:* *Complications of Rheumatoid Arthritis*

Discussants:

P. FRANKLIN MULLINAX, M.D., *assistant professor of medicine, Medical College of Virginia, Richmond*

R. PAGE HUDSON, JR., M.D., *assistant professor of pathology, Medical College of Virginia, Richmond*

CLINICAL RECORD

This 61 year-old white male was admitted to the Medical College of Virginia Hospital on 10/29/64 for further treatment of rheumatoid arthritis.

History

He had had severe arthritis for approximately 14 years. Joint symptoms had begun in his right shoulder with pain, swelling, and redness, and had gradually progressed to involve numerous joints. He did not require medication initially, but approximately four years after the onset of symptoms, he needed medication for relief of pain and deformity. He was treated initially with salicylates and later, with prednisone and Butazolidin. He had been hospitalized in 1961 because of severe muscle pain in his left lower leg. Physical examination then showed a blood pressure of 180/110, pulse 80 per min, and temperature, 98 F. The chief positive physical findings were limited to the extremities and included arthritic changes in the carpal-metacarpal joints of both hands and stiffness in both knees. Dorsalis pedis pulses were good, and the lower extremities showed some hyperemia and warmth. There was loss of muscle strength in the upper and lower extremities, and a loss of vibratory sense in the toes. All tendon reflexes were present.

Laboratory work in 1961 showed hemoglobin 16.2 g/100 ml, white cell count 18,350/mm^3 (88% polymorphonuclears, and 12% lymphocytes). The latex test was reactive. Two LE cell preparations were negative. Total proteins were 6.3 g/100 ml (albumin 3.1, and globulin 3.2 g/100 ml). Serum transaminase was 18 units. Serum uric acid was 5.8 mg/100 ml. Sheep cell test for rheumatoid arthritis was positive with a titer 1:220, and a sensitized human cell test was positive, with a titer of 1:1280. The spinal fluid protein was 40 mg/100 ml, and a cell count revealed 1 lymphocyte; CSF pressure was normal. Muscle testing showed extensive loss of strength in the leg and foot, and an electromyogram showed almost complete denervation of all muscles supplied by the left common peroneal nerve. There was early evidence of degeneration of the right common peroneal nerve. An electrocardiogram was negative. It was felt at this time that the patient had an acute exacerbation of rheumatoid arthritis and an associated prednisone-induced neuropathy. The patient gradually responded to treatment with intramuscular injections of gold, salicylates, heavy sedation, and physical therapy.

He had been doing quite well until about four weeks before the present admission, when he had an

* Prepared and edited from transcripts by R. Page Hudson, M.D., department of pathology, and John H. Moon, M.D., department of medicine.

exacerbation of his arthritis and became bedridden with severe joint pain. He complained of poor appetite, weight loss and weakness. Two weeks before admission he had developed cough, fever, and sore throat, for which he was admitted to another hospital and treated with antibiotics, including chloramphenicol. He was subsequently transferred to Sheltering Arms Hospital.

Physical Examination

Blood pressure 124/80, pulse 80/min, temperature 98 F, and respiration, 20 breaths/min. He was malnourished and pale. He was lying rigidly in bed, unable to move without pain. There were numerous decubitus ulcers on the occipital protuberance. The fundi revealed an increase in the tortuosity of vessels, with some narrowing. The neck was quite rigid. The mucous membranes of the mouth were dry. The chest was clear to percussion, but breath sounds were diminished throughout and there were moist rales at both bases. The heart was not enlarged to palpation. There was venous distention over the abdominal wall. The liver edge was palpable at the costal margin. The extremities showed severe arthritic deformities. There were "swan neck" deformities of the fingers and ulnar deviation bilaterally. There were bilateral ulnar rheumatoid nodules. The wrists, knees and ankles were swollen bilaterally, and motion was limited in both shoulders. There were multiple small, skin erosions over the bony prominences and large decubitus ulcers over the heels, ankles, sarcrum, elbows, upper arms, wrists and back of the head.

Laboratory Data

Hemoglobin 9.0 g/100 ml, hematocrit 29%, MCV 97, MCH, 30; MCHC, 31; white cell count 4,900/mm (40% neutrophils, 1% eosinophils, 53% lymphocytes, and 6% monocytes). A urinalysis was normal. Blood sugar was 108 mg/100 ml and BUN was 114 mg/100 ml. Serum electrophoretic pattern showed a marked decrease in the albumin, but there was no evidence of an abnormal globulin spike. Repeated blood cultures were negative. Stools were negative for blood on one occasion.

Upon transfer to MCV for rehabilitation therapy, the physical examination was unchanged. Laboratory work showed a hemoglobin of 6.5 g/100 ml. Examination of the peripheral smear revealed normocytic, normochromic red cells with adequate platelets and slight toxic changes in the granulocytes. A bone marrow aspirate showed a) a marked erythroid hypoplasia; b) plentiful myeloid elements, with an increase in promyelocytes, a few myeloblasts, and many megakaryocytes; c) plasmacytosis, with a few binucleated, trinucleated, and multinucleated cells and a rare one showing flame cytoplasm, and d) moderate increase in R-E and mitotic cells. A urine culture on 12/2/64 grew Klebsiella-Aerobactor group in a concentration of over 1,000,000 organisms/ml. X-ray of the chest showed minimal degenerative changes in the dorsal spine but the lungs were clear bilaterally and the heart was thought to be of normal size. An electrocardiogram showed non-specific T-wave changes.

Physical therapy was instituted with passive and active range of motion exercises while in the Hubbard tank. Attempts were made to clear up the decubitus ulcers. The patient was unable to cooperate sufficiently in the performance of his exercises as the slightest movement caused him to cry out with pain. He received several transfusions and was placed on chloramphenicol, but continued to run a fever. Physical therapy had to be discontinued because of increasing pain which was only partially relieved by aspirin. He was unable to eat without choking, and fluids were necessary. His temperature rose to 103 F on 12/6/64. He grew progressively weaker, coughed more but was less and less able to expectorate. He expired on 12/7/64.

CLINICAL DISCUSSION

Dr. P. Franklin Mullinax: After suffering for many years with arthritis, this patient was hospitalized because of severe leg muscle pain and was found to have a peripheral neuropathy with both sensory and motor involvement. Laboratory studies revealed leukocytosis, hyperglobulinemia, and high titers of rheumatoid factors. Four years later, an exacerbation of arthritis left him bedridden. At the time of hospitalization, he was severely debilitated and had multiple decubitus ulcers, skin hemorrhages, pronounced deformities and rheumatoid nodules. Clearly, he had advanced rheumatoid arthritis with widespread articular and extra-articular involvement.

Fortunately, rheumatoid arthritis usually takes a milder, more reasonable course. Before commenting further on this patient's disease, I shall summarize certain features of the natural history of rheumatoid arthritis. Up to 20% of persons will, at some time in their life, have episodes of polyarthritis, probable rheumatoid arthritis. Most of these, because of the mildness of their disease, or because of psychic strength, will never consult a physician. In patients with disease severe enough to require hospitalization, the prognosis is not so sanguine. Follow-up studies of 250 patients hospitalized for treatment of rheumatoid arthritis revealed that 10 years later, 53% were improved, 13% were unchanged and 34% were worse (Short, Bauer and Reynolds, 1957). Patients evaluated 20 years after the initial hospitalization were graded as follows: 35% improved; 27% unchanged; 63% worse.

It is usually said that rheumatoid arthritis cripples but doesn't kill. Actually longevity is decreased in

patients with rheumatoid arthritis, particularly in those with onset before the age of 25. A 30-year follow-up showed that one-third of rheumatoid arthritis patients had died, whereas, in a control population, only 13% had died. Prominent causes of death were pneumonia, chronic pyelonephritis, and nephrolithiasis. Occasionally patients die from lesions more specifically associated with rheumatoid arthritis, particularly granulomas of the heart, generalized arteritis, and amyloidosis. I believe that the patient being discussed today had at least one of these more specific complications of rheumatoid arthritis.

We can, to a certain degree, identify the persons who are going to develop the severe lesions of rheumatoid arthritis. These are the patients who have rheumatoid nodules, high titers of rheumatoid factors, unremitting disease for over two years, and onset in the later decades.

Rheumatoid factors and their possible involvement in the vascular lesions of rheumatoid arthritis are worthy of note. You will recall that the rheumatoid factors are, usually, 19S macroglobulins which act like antibodies to normal 7S γ-globulin. The 19S rheumatoid factors can combine with the 7S γ-globulin and form a heavy (22S) complex. Recent experimental work emphasizes the role of antigen-antibody complexes in causing vascular lesions. It is possible that the 22S and heavier complexes in rheumatoid sera are similarly responsible for vascular lesions (Baum et al., 1964). Rheumatoid factors can be evoked experimentally in rabbits by repeated injections of killed *E. coli* (Abruzzo and Christian, 1961). How or why these rheumatoid factors appear is not known.

I have suggested that the rheumatoid factors may be involved in the vasculitis of rheumatoid arthritis. I believe that the vascular lesions are not an occasional occurrence found only in patients with severe disease, but rather that vasculitis may underlie all the lesions of rheumatoid arthritis, even the mildest of synovitis. Evidence for this concept was presented by Sokoloff and Bunim (1957). Putting these thoughts together, I have assembled a tentative concept (table 1) of the pathogenesis of rheumatoid arthritis. According to this view, chronic, but at present, inapparent, infection stimulates in an unknown way the formation of rheumatoid factors; these in turn combine with γ-globulin and produce complexes which precipitate, drop out of the circulation, and produce vasculitis. When the complexes are deposited in smaller vessels such as those in the synovium, local vasculitis and synovitis appear. With involvement of larger vessels, a picture reminiscent of polyarteritis nodosa ensues. This schema is based on some facts, as well as on speculation. The facts are that high titers of rheumatoid factors are associated with severe, unremitting disease, and that the heavier complexes are seen in the sera of patients with the more severe disease. The suggested interrelations are speculative.

Four years prior to his terminal admission, this patient had a severe motor neuropathy, as shown by electromyographic evidence of virtually complete denervation of muscles supplied by the left common peroneal nerve. From a diagnostic point of view, this is the central event in the patient's illness. The occurrence of motor neuropathy in a patient with severe arthritis who is receiving steroids is highly suggestive of active arteritis of a degree sufficiently great to be pathologically described as polyarteritis (Irby et al., 1958; Bleehen et al., 1963). Again, I would simply say that this represents an intensification of the usual arteritis which is the hallmark of rheumatoid arthritis. Once this widespread arterial involvement occurs, one can expect that extensive, including visceral, lesions will develop (Schmid et al., 1961).

I am suggesting that rheumatoid arthritis is one of the causes of polyarteritis and that this was the problem in the present case. Table 2 presents a classification of polyarteritis. Anatomically, Rose (1957) has simply classified cases into those with or without lung involvement. Polyarteritis with lung involvement is characterized by eosinophilia and granulomas, particularly along the respiratory tract, whereas cases without lung involvement are not.

For years we have heard much of hypersensitivity and arteritis. It is, therefore, reasonable to further classify polyarteritis into lesions caused by antigen-antibody interactions and complexes, and those that are not. Unfortunately we are not yet able to say in which patients with lesions of polyarteritis are the antigen-antibody complexes the responsible agent.

Table 3 shows the sites affected in cases of polyarteritis without lung involvement. The patient under discussion obviously had involvement of muscle, joint, nerves and skin. Inexplicably, rheumatoid polyarteritis usually does not involve renal vessels.

Our patient had many problems associated with splenic enlargement: rheumatoid arthritis with high rheumatoid factor titers, pronounced arteritis, and possibly amyloidosis. I wonder if the spleen was not enlarged.

Amyloidosis, secondary amyloid, is found in up to 40% of patients with rheumatoid arthritis. The amyloid deposits may not be responsible for significant clinical disease, but the deposits are frequently found around smaller vessels. This case presents several features which lead me to suspect amyloidosis, and I suggest that minor lesions of amyloid, possibly visible only with the crystal violet stain, will be found at autopsy. First, he had long-standing rheumatoid arthritis. Second, the bone marrow specimen revealed pronounced involvement or stimula-

tion of his reticuloendothelial system and marked plasmacytosis, together with the serum globulin abnormalities. Finally, decubitus ulcers with chronic infection are among the most common causes of amyloidosis.

Though there is a great deal of overlap, it is generally true that secondary amyloidosis involves the liver, spleen, and particularly the kidneys, whereas primary amyloidosis involves heart, tongue, peripheral nerves and gastrointestinal tract. There is usually rather marked proteinuria, which this patient did not have. Hypertension, usually not present with amyloid renal disease, can even disappear subsequently to amyloid involvement. Out patient's hypertension did go away.

I should spell out what I believe to be the essence of the logic in this particular case history. A patient with severe rheumatoid arthritis developed a profound motor neuropathy. This occurrence strongly suggests arteritis of the vessels supplying that nerve. When an arteritis occurs, it can be reasonably predicted that arteritis and granulomas will occur elsewhere. There is little in the case history that requires postulation of lesions other than those of rheumatoid arthritis.

In summary then, I suggest that this patient had malignant rheumatoid arthritis with arteritis and granulomas of that disease; and that he had clinically insignificant deposits of amyloid.

Dr. W. Robert Irby (associate professor of medicine, MCV): I first saw this patient in July, 1961. At that time I thought he had peripheral neuropathy associated with steroid-treated rheumatoid arthritis. I was able to get him off the steroids and he left the hospital on salicylates. I did not see him again until November, 1964, when he was in his terminal illness. The anemia, abnormal plasma cells, and severe bone pain, suggested multiple myeloma, but I could not substantiate that diagnosis.

TABLE 1
Pathogenesis of Rheumatoid Arthritis (? ? ?)

1. Microbial infection (viral or bacterial) → → rheumatoid factors
2. Rheumatoid factors (19S) + normal γ-globulin ⇌ complex (22S)
3. Complex → arteritis
4. Arteritis → synovitis, granulomas

TABLE 2
Classification of Polyarteritis

A. Anatomic
   1) With lung involvement
   2) Without lung involvement
B. Pathogenetic
   1) Caused by antigen-antibody complexes
   2) Not caused by antigen-antibody complexes

TABLE 3
Polyarteritis Without Lung Involvement: Incidence of Affection of Other Sites*

Organ or System	% of Cases
Gastrointestinal tract	70
Liver	54
Muscle	46
Joints	27
Spleen	12
Peripheral nerves	36
Skin	27
No lesions post mortem	15

*Rose, 1957

Dr. John H. Moon: Dr. Mullinax, you equated the rheumatoid factor with the production of arthritis, but I recall that Harris and Vaughan (1961) transfused sera with high titers of rheumatoid factor into normal individuals without demonstrable change.

Dr. Mullinax: There is a great argument not only in relation to diseases of hypersensitivity, but in immunology in general, as to the role of circulating anti-bodies in the production of delayed hypersensitivity (tuberculin-like) reactions. It was long thought that delayed sensitivity could not be transferred by serum and that, therefore, circulating antibodies are not involved. There is suggestive evidence now that one can effect such a transfer, but one needs a chronic perfusion. For instance, in the tuberculin reaction, one needs a perfusion of skin for two days. If Harris and Vaughan had perfused into the patient the equivalent of the amount of blood that would flow through in two days, then I would be convinced that they had done a conclusive experiment.

Dr. Elam C. Toone (professor of medicine, MCV): In reaching the opinion about the animals' receiving the *E. coli,* how was the rheumatoid factor determined? Did these animals develop a polyarthritis? Was the rheumatoid factor determined by the latex, the sensitized human cells, sheep cells, or electrophoretic pattern?

Dr. Mullinax: The rheumatoid factor determinations were done by several serologic techniques, including F 11 latex, sensitized sheep cells, and tanned sheep cells coated with a variety of γ-globulins, and γ-globulin precipitations and absorptions. All were positive. The reactive materials were exclusively macroglobulins. The lesions, which were not described, were said to be compatible with serum sickness. No arthritis was described.

Dr. Toone: I think chronic symmetrical polyarthritis is more germane to the diagnosis of rheumatoid arthritis than the presence of the rheumatoid factor. It is quite important to know how the factor was detected, because the tests vary in sensitivity and specificity. I feel that the rheumatoid factor probably appears after the polyarthritis, since we see patients with full-blown rheumatoid disease in whom the factor tests may remain negative for weeks or even years. A recent report by Dixon (1960) is pertinent to this point. In his summary, the following statements are made: "Sixty-one in-patients with severe, active polyarthritis associated, with a persistently negative sheep cell agglutination test (S.C.A.T.) for rheumatoid arthritis are reviewed. The average duration of follow-up was 5.4 years. Of the 61 patients reviewed, twelve were found at follow-up to have developed a positive S.C.A.T. All but two of these had distribution of arthritis typical of rheumatoid arthritis and six developed subcutaneous nodules. The course of their arthritis was not different from that seen in 23 patients in whom the arthritis was also typical but in whom the S.C.A.T. remained negative. Both mild and severe end-results were seen. A persistently negative S.C.A.T. was compatible with typical rheumatoid arthritis of progressive course and fatal outcome. Eleven patients had proven or probable diseases other than rheumatoid arthritis. . . . Eight patients showed a persistently negative S.C.A.T. (sheep cell test) and an atypical arthritis."

Other entities such as liver disease, bacterial endocarditis, etc., may produce the rheumatoid factor. Recently, we have found that of 20 patients who received kidney transplant, 14 (66%) developed evidence of rheumatoid factor, and only one developed a chronic polyarthritis which might have been of the rheumatoid type (Waller et al., 1965). Dr. Marion Waller has had a patient under observation for six or eight years who has had rheumatoid factor in high titer and in whom there has been no evidence of rheumatoid arthritis or any other disease. This is just to illustrate that we really do not know the relationship of rheumatoid factor to either the cause or effect of the disease.

Clinical Diagnosis

1. Rheumatoid arthritis
2. ? Multiple myeloma
3. ? Septicemia
4. ? Steroid-induced gastric ulcer
5. ? Amyloidosis

Dr. Mullinax's Diagnosis

1. Malignant rheumatoid arthritis
2. Amyloidosis, mild

PATHOLOGIC DISCUSSION

Dr. Page Hudson: Most of us certainly do not think of rheumatoid arthritis as a fatal disease. When the term is mentioned we consider it only as an entity that involves peoples' joints. But, as Dr. Mullinax has pointed out, it involves vessels and connective tissues. Thus, every tissue, every organ in the body can be involved. Still rheumatoid arthritis is not a "fatal" disease ordinarily.

When confronted with the problem of death or a sudden severe illness following a "non-fatal" disease, we should consider five possibilities;

1) development of a different and unrelated disease;
2) emergence of a natural complication;
3) predisposition to another disease;
4) complication of therapy; or
5) reaching the "end of the spectrum."

For specific examples, first, this patient may have developed multiple myeloma which, as far as we know, is unrelated to rheumatoid arthritis. After all there were, as Dr. Irby reminded us, severe bone pain, anemia, and abundant, abnormal plasma cells. Secondly, amyloid is a well-recognized com-

plication of rheumatoid arthritis. Thirdly, due to the severe debilitation, secondary bacterial or fungal infection may have supervened. Next, complications of therapy are becoming more generally recognized. There are distinct hazards in the use of steroids, gold, Butazolidin, and even aspirin. Physical therapy is not without some danger in patients with rheumatoid arthritis. Fat and bone marrow embolism from manipulative procedures have caused death (Rosenberg et al., 1944; Gleason and Aufderheide, 1953). The fifth possibility is that the patient may have had an extreme form of the recognized disease. He might have been at the end of that spectrum of degrees of severity that all illnesses display.

The latter situation was the most likely one to Dr. Mullinax. He was quite correct except that the patient did not have amyloidosis.

The gross findings at autopsy were striking. The pericardial sac looked like white cake-icing and was tenaciously adherent to the heart (fig. 1). It was thick and hard, but not calcified. Pleural adhesions were marked over both the emphysematous lungs. The muscles were exceedingly pale and atrophic. The bones were soft and osteoporotic. Fibrous plaques with soft centers were apparent in the pleura, peritoneum, and other cavity lining membranes, including synovia. The spleen and lymph nodes were enlarged.

Dissection of the heart revealed greatly thickened, milky-white mitral valve leaflets and chordae tendineae. The distal thirds of the papillary muscle were extremely scarred. Despite these changes, the mitral valve and size of the cardiac chambers suggested that there had not been prominent functional mitral disease.

Microscopically, there was perivascular fibrosis in the heart as is often seen in inactive rheumatic fever. In addition, there were large aggregates of plasma cells in the papillary muscles, perhaps stuffed

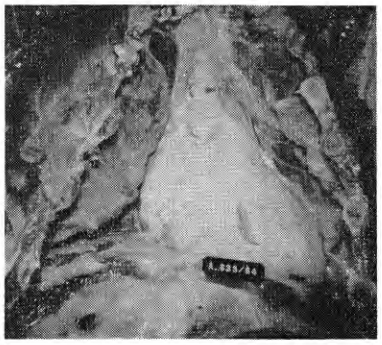

Fig. 1—The heart was encased in thick, white partly fibrous and partly gelatinous pericardial sac. The sac was totally adherent to the epicardium. There was no clinical evidence of constrictive heart disease.

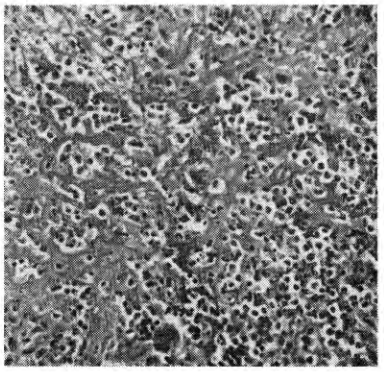

Fig. 3—Rheumatoid nodules from the epicardium; the radially oriented palisaded fibroblasts surround fibrin, inflammatory cells and necrotic debris (H&E, 100×).

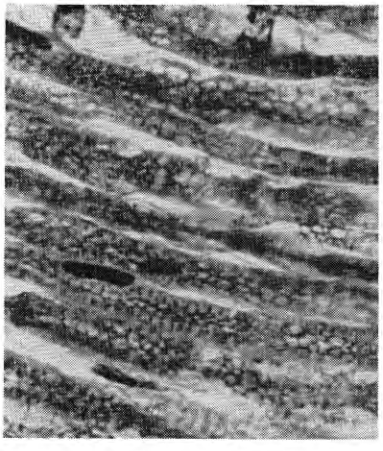

Fig. 2—Peripheral nerve showing myelin degeneration which gives the cytoplasm a foamy appearance. Multiple minute foci of lymphocytes were also seen about small vessels in nerve bundles (H&E, 450×).

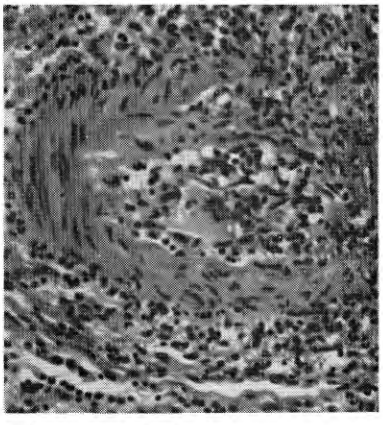

Fig. 4—Mesenteric artery showing partial destruction and almost complete occlusion. The intense inflammatory infiltrate consists of lymphocytes, eosinophils, polymorphonuclears, plasma cells and macrophages (H&E, 160×).

with Dr. Mullinax's "19S macroglobulin." Classical acute rheumatoid nodules were present in the epicardium, mitral valve, wall of the aorta, and in the right coronary artery which had been completely occluded by the reaction and superimposed thrombus. Recanalization of the coronary had occurred. No old infarct was seen. Cardiac lesions are relatively common in rheumatic arthritis patients, many of whom have no clinically discernible heart disease (Schoene and Risse, 1964). Some of the lesions are similar to those of rheumatic fever.

The terminal event was respiratory insufficiency due to a combination of chronic aspiration pneumonia and emphysema, with pulmonary fibrosis and mild interstitial pneumonia. Perhaps, too, limitation of pulmonary excursion by the massive adhesions was a factor. Recall that he was hospitalized elsewhere with pneumonia before his terminal admission here. A review of his last record at our hospital reveals, particularly from the nursing notes, the difficulty he must have had in swallowing. We can account for this by the myelin loss and inflammatory changes seen in many sections of nerve (fig. 2) including the glossopharyngeals and recurrent laryngeals. Also the muscles of deglutition revealed degenerative changes. In addition, sections of brain revealed occasional intense vasculitis and foci of encephalomalacia. Foreign body reaction around aspirated vegetable fibers was seen in all lung sections. Foci of acute and chronic inflammation within and about countless bronchioles were visible. Alveolar walls were thickened and fibrotic.

Pulmonary fibrosis and interstitial pneumonitis have frequently been described in cases of rheumatoid arthritis (Brannon et al., 1964). They do appear to be slightly more common in these patients than in patients with other chronic diseases. Possibly excepting "Caplan's nodules" seen in silicosis with rheumatoid arthritis, there is nothing really approaching a specific pulmonary parenchymal alteration in this disease. The classic rheumatoid nodules (fig. 3) may be seen on the pleural surfaces and in the interlobar fissures. This is a different matter and was marked in this case. These well-known nodules were widespread in the peritoneum, joints, and subcutaneous tissue as well as in the pleura, pericardium, and vocal cords.

Continuing the description of the generalized disease, there was a mild acute glomerulonephritis with swollen glomeruli, enlarged cells, and thickened basement membranes. That this condition was of recent onset was suggested by the lack of clinically detected renal disease. In addition to glomerulonephritis, renal lesions that have been noted in the other cases include amyloidosis, phenacetin-induced interstitial nephritis with or without papillary necrosis, and segmental parenchymal damage secondary to vasculitis (Allander et al., 1963).

The bloody diarrhea the patient suffered was due to ulcerations caused by focal mesenteric vasculitis affecting both arteries and veins but particularly the smaller arteries (fig. 4). This complication of rheumatoid disease was apparently only recently described (Adler et al., 1962).

In addition to the plasma cells taking part in cellular infiltrates in many tissues, the bone marrow contained vast numbers of those antibody and rheumatoid factor producing cells. This is seen in many severe chronic diseases, particularly the granulomatous and autoimmune diseases, both terms being applicable to rheumatoid arthritis. Bi- and tri-nucleate plasma cells and other atypical forms may be seen in conditions where there is a stimulant to plasma cell formation.

Despite the extent of the disease in this patient, he did not have scalp or meningeal lesions which are also occasionally seen. He was also spared scleromalacia perforans, another sometime complication.

In conclusion, our patient demonstrated the most extreme, diffuse, and malignant form or rheumatoid arthritis. At the "end of the spectrum" of clinical and morphologic disease, he expired with respiratory insufficiency.

Pathologic Diagnosis

1. Rheumatoid arthritis, severe, with involvement of multiple organs and tissues.
2. Pulmonary insufficiency, with aspiration pneumonia, interstitial fibrosis, emphysema, and massive chronic pleuritis.

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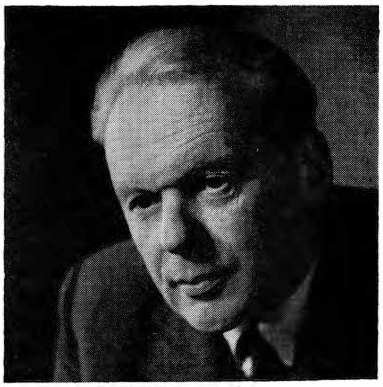
Contributors

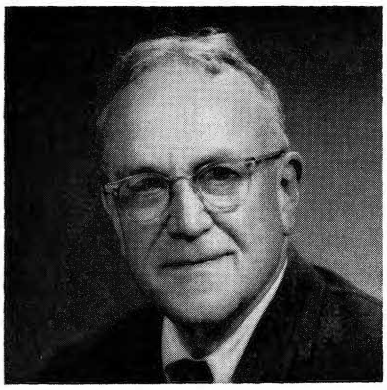


Rama M. Aiyawar (*Self-Diffusion in Sodium Single Crystals*), a native of Hyderabad, India, received his B.Sc. from Osmania University, India, and M.S. from the University of Richmond. He was recently a graduate student in the department of physiology at MCV, and is now a graduate student at M.I.T.

Hermann K. F. Blaschko (*Observations on Chromaffin Tissue*) took an M.D. degree at Freiburg e/Br., Germany, in 1925, and a Ph.D. at Cambridge in 1939. He worked in Germany with O. Meyerhof, and in England with A. V. Hill and Sir Joseph Barcroft. He went to the department of pharmacology at Oxford University in 1944, where he is now reader in biochemical pharmacology and a Fellow of Linacre College. He is a Fellow of the Royal Society and an honorary professor at Heidelberg University. He received a doctorate of Medicine *honoris causa* from the Free University of Berlin in 1966. He lectured at MCV in April, 1961.

Ernest Bueding (*Cholinergic Responses of Schistosoma mansoni*) was born in Frankfurt, Germany, and began his study of medicine at the University of Frankfurt, where he studied physiology under Dr. Ernst Fischer. He received his M.D. degree from the University of Paris, France, and has taught at the University of Istanbul, Turkey, New York University, Western Reserve, Oxford (on Fulbright and Guggenheim fellowships), and Louisiana State University. Dr. Bueding has been at the Johns Hopkins University since 1960, where he is professor of pathobiology in the School of Hygiene and Public Health and professor of pharmacology in the School of Medicine.

Addison D. Campbell (*Rate of Diffusion of Radioactive Ions in Gels* and *Self-Diffusion in Single Sodium Crystals*) is a graduate of Hampden-Sydney College, Virginia. Dr. Campbell was awarded the M.S. degree in physics from the University of Richmond, and the Ph.D. degree from the University of North Carolina. He is now associate professor of physics at the University of Richmond.



Chalmers L. Gemmill (*Recollections of Professor Otto Meyerhof*) is a graduate of Lafayette College and the Johns Hopkins University (M.D.). He did postgraduate work at the University of Chicago, at the Kaiser Wilhelm Institute in Heidelberg, in Lund, Sweden, and in Cambridge, England. Dr. Gemmill taught physiology at Johns Hopkins, and was director of research at the Naval School of Aviation Medicine before moving to the University of Virginia, where he is now professor of pharmacology.

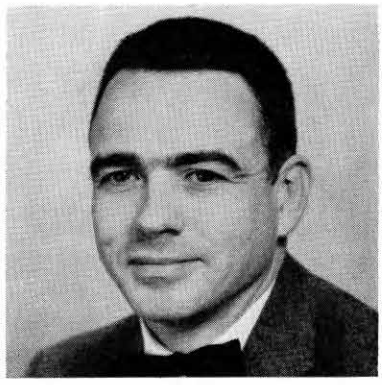
Ernest Gutmann (*"Slow" and "Fast" Muscle Fibers*) was born in Usti, Czechoslovakia and educated at the University of Prague (M.D.) and the University of Oxford (Ph.D.). He has worked at both institutions, and is currently head of the department of physiology and pathophysiology of the neuromuscular system at the Institute of Physiology of the Czechoslovak Academy of Sciences. His research interests include the nervous regulation of muscle metabolism, effect of use and disuse on neuromuscular function, and trophic function of the nerve cell. In 1963 he was a guest professor of physiology at MCV.

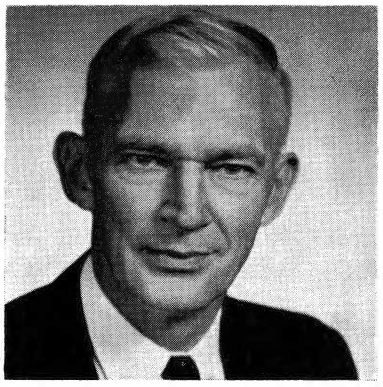
Gabriel Hilkovitz (*Ambulatory Services in Teaching Hospitals*) received his medical education at the University of the Witwatersrand, Johannesburg, South Africa. He interned and took part of his residency training at the Johannesburg General Hospital, and completed his hospital training in internal medicine at Queen Mary's, Greenwich, and University College hospitals in London, England. He came to the Medical College of Virginia in 1958 as a research fellow in hematology and is now assistant professor of medicine, curriculum coördinator, and director of ambulant patient services.

Ernst G. Huf (*On the Nature of the Resting Frog Skin Potential* and *Rate of Diffusion of Radioactive Ions in Gels*), a native of Metz, France, received his Ph.D. in chemistry and his M.D. from the University of Frankfurt, Germany. In 1946, Dr. Huf came to MCV where he is professor of physiology. He is also research professor of biophysics at the University of Richmond and an NIH Research Career Awardee. Dr. Huf's research has been chiefly in the field of active transport.

Elinor Langer (*The Shame of American Medicine*) graduated from Swarthmore College and attended graduate school at the Johns Hopkins University School of Advanced International Studies in Washington. Later she worked as a research assistant at the Washington center of Foreign Policy Research and spent a brief spell at the *Washington Post*. Since 1963, Miss Langer has been on the editorial staff of *Science,* for which she writes a column on science and public affairs.

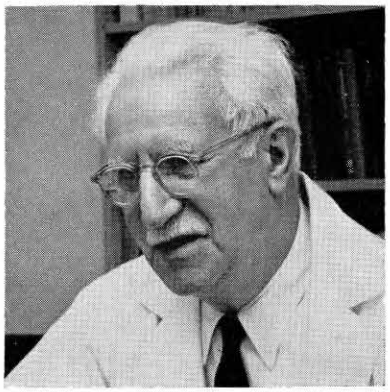
Nancy S. LaPrade (*On the Nature of the Resting Frog Skin Potential*) is a graduate of Mary Washington College, Fredericksburg, Virginia. During the year 1965-1966, she took graduate studies in physiology under Dr. Ernst G. Huf at MCV, as an NIH predoctoral fellow. Miss LaPrade (now Mrs. Thomas C. Smith), is currently employed as a chemist at the Philip Morris Research Center in Richmond.





John W. Moore (*A Deadly Poison Becomes a Useful Tool*) received a B.S. from Davidson College and a Ph.D. from the University of Virginia, both in physics. In the late forties he taught physics in the MCV School of Pharmacy. In 1950, he joined the biophysics division of the Naval Medical Research Institute, Bethesda, Maryland. He later moved with Dr. K. S. Cole to the National Institute of Neurological Disease and Blindness to form the laboratory of biophysics. Since 1961, Dr. Moore has been chief of the laboratory of cellular neurophysiology and Research Scientist of the National Neurological Research Foundation in the department of physiology and pharmacology at the Duke University Medical School.

Robert W. Ramsey (*Some Effects of Extreme Shortening on Frog Skeletal Muscle*) is professor of physiology at MCV and was chairman of the department from 1948 until 1963, when he gave up the chair for health reasons. He received the B.S., M.S., and Ph.D. degrees from New York University and taught there and at the University of Rochester before coming to MCV in 1944. Dr. Ramsey is also a member of the Marine Biological Laboratory, Woods Hole, Massachusetts, and of the board of administration of the Virginia Institute of Marine Science. His research interests include mechanics of single muscle fibers and physiology of human performance.



Walther Riese (*Brains of Prominent People: History, Facts and Significance*) was born in Berlin on June 30, 1890, graduating *Dr. med.* at the University of Königsberg in 1914. In the course of the next 20 years, he served chiefly at the Psychiatric Clinic and the Institute of Neurology of the University of Frankfurt a/M. In 1924, he qualified as *Privat-Dozent* for neurology there. From 1933 until 1940, W. Riese lived in France, working at the Psychiatric Clinic of the University of Lyons and at the Centre National de la Recherche Scientifique in Paris. Since 1941, he has been living in Richmond. He was chairman of the department of history of medicine, associate professor of psychiatry and neurology at MCV, and consulting neuropathologist to the Department of Mental Hygiene and Hospitals of the Commonwealth of Virginia. He also teaches at the School of Clinical and Applied Psychology of the Richmond Professional Institute. Walther Riese has twice been granted a Rockefeller Fellowship (1933-36 and 1941-43) and twice was awarded first prize in a *concours* organized by the Virginia State Hospital Board.

J. C. Rüegg (*The Contractile Fine Structure of Vertebrate Smooth Muscle*) was born in Zurich where he studied medicine and obtained his doctoral degree under the supervision of W. R. Hess. Later he worked in the laboratory of Kenneth Bailey (Cambridge) and H. H. Weber (Heidelberg) on the biochemistry of muscle, especially mammalian smooth muscle. In 1963, he became lecturer (Privat-Dozent) in biochemistry at Heidelberg University.

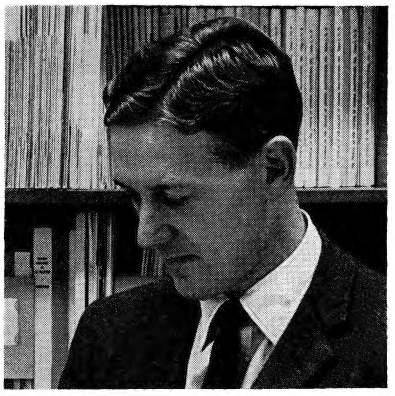
Alexander Sandow (*Latency Relaxation: A Brief Analytical Review*) obtained a B.Sc. from the Massachusetts Agricultural College (now the University of Massachusetts), and an M.A. from Columbia, both in bacteriology, and went on to get a Ph.D. in physics from New York University. His real interest, however, was in biophysics, and it is in this field that he has worked, mostly on problems of muscular contraction. Since 1959 he has been a member of the Institute for Muscle Disease and chief of its division of physiology. (Dr. Sandow is frequently asked if he is related to Eugen Sandow, the Strong Man, who was famous for his great strength some two generations ago. He is not, but he adds that he has always felt some kinship as he has tried to shed some light on the processes that make the muscles of men and mice, frogs and clams, perform their many wondrous functions.)

Michael N. Sheridan (*Some Effects of Extreme Shortening on Frog Skeletal Muscle*) is a graduate of Stephen F. Austin State College in Nacogdoches, Texas. He began graduate study in anatomy at Emory University and received the Ph.D. degree from MCV in 1963. Dr. Sheridan spent the following year at the Institute of Animal Physiology, Babraham, Cambridge, England, after which he joined the staff of the department of anatomy at MCV. He is now assistant professor of anatomy at the University of Rochester School of Medicine and Dentistry.

Archibald W. Sloan (*Dynamic Physical Fitness and Body Composition*) is head of the department of physiology and medical biochemistry at the University of Cape Town, South Africa. He was born in Glasgow, Scotland, and is a graduate of the University of Glasgow. Dr. Sloan served in the British army during World War II, then returned to the University of Glasgow to teach physiology until he left in 1955 to go to Cape Town. In 1965, he was visiting professor of physiology at MCV.

Thomas C. Smith (*Rate of Diffusion of Radioactive Ions in Gels*), a native of Charleston, West Virginia, received B.S. and M.S. degrees in physics from the University of Richmond. He is presently doing predoctoral work under Dr. Ernst G. Huf in the department of physiology at MCV.

Sidney Solomon (*Plasma Volume Expansion and Proximal Tubular Reabsorption of Salt and Water by Rat Kidney*) was born in Worcester, Massachusetts, in 1923. He received his B.S. degree from the University of Massachusetts, and his Ph.D. from the University of Chicago. His past professional affiliations included the Worcester State Hospital in 1947, and the Medical College of Virginia from 1952 to 1963. Since 1963, he has been professor and chairman of the department of physiology at the University of New Mexico School of Medicine, Albuquerque.

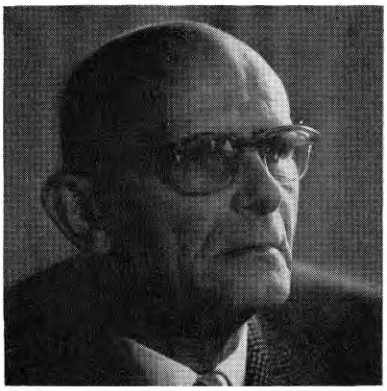


Harald Sonnenberg (*Plasma Volume Expansion and Proximal Tubular Reabsorption of Salt and Water by Rat Kidney*) was born in Danzig, Germany, in 1935. He received his B.S. and M.S. degrees at the University of Alberta, Canada, and his Ph.D. at the Free University of Berlin. He has been research technician at the University of Alberta, research associate in Göttingen and Berlin, and more recently, instructor and assistant professor in the department of physiology, University of New Mexico School of Medicine in Albuquerque.

Frederick J. Spencer (*The Changing Pattern of General Practice*), a native of Newcastle-on-Tyne, England, received his medical degree from the University of Durham, England, and took his hospital training at Dryburn Hospital. After serving in the British Army, Dr. Spencer did general practice in England, Canada, and the U. S., and then received a Master of Public Health degree from Harvard. Before coming to MCV in 1964, as professor and chairman of the department of preventive medicine, Dr. Spencer had been with the Virginia State Department of Health.

Sibyl F. Street *(Some Effects of Extreme Shortening on Frog Skeletal Muscle)* is research associate in the department of physiology at MCV. She received the A.B. degree from Vassar College and Ph.D. from the University of Chicago. Before coming to MCV, she taught at Vassar and the University of Rochester. In private life, she is Mrs. Robert Ramsey.

Alfred J. Szumski (*Comments on Intracellular Studies of Presynaptic Inhibition*) received a B.S. from Richmond Professional Institute and graduated from MCV's School of Physical Therapy before receiving an M.S. degree in physical therapy and a Ph.D. in physiology. At present, he is assistant professor in the department of physiology and in the School of Physical Therapy at MCV.



William R. Tolbert (*On the Nature of the Resting Frog Skin Potential*) is a native of Richmond, Virginia. He received his B.S. degree with majors in physics and mathematics from the University of Richmond in 1964. During his senior year and the following summer, he became interested in the field of biophysics through work with Dr. Ernst G. Huf. He is now working toward the Ph.D. degree in that field at the University of Wisconsin, where he received the M.S. degree in January, 1966.

Hans H. Weber (*The Contractile Fine Structure of Vertebrate Smooth Muscle*) was born in Berlin, Germany, and completed his medical studies in 1921, before working under Dr. Otto Meyerhof. Dr. Weber has held the chair of biochemistry at the University of Münster and the chair of physiology at the University of Königsberg and the University of Tübingen. Since 1954, he has been director of the Max Planck-Institute for Physiology, in Heidelberg. Dr. Weber's research interests have been in the molecular biology of muscle, and the electrochemistry of proteins. His connections with American scientific circles include honorary membership in the American Academy of Arts and Sciences, the American Physiological Society and the Harvey Society. He has been Harvey Lecturer and Dunham Lecturer.

Priscilla M. Winn (*On the Nature of the Resting Frog Skin Potential*) received B.Sc. degrees in zoology and chemistry from the University of Liverpool, England. In 1958 she was research assistant at the Bureau of Biological Research at Rutgers. She returned to England as research fellow in the department of zoology at the University of Sheffield, and obtained a Ph.D. in Science from the University of Liverpool. Between 1962 and 1964, she was on the faculty of MCV as instructor in physiology.

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