“Well, he finally decided to clean the attic. Almost had the job done, too…”

“...Yeah, until he tried to lift me. It sure put his back out of whack. His doctor’s got a real job to do — trying to ease both the pain and the strain.”
When stress results in muscle strain and pain

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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.

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COVER: Symmetrical arrangement of surface subunits of a virus, magnified over one million diameters (design by Raymond A. Geary). See page 185.

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RESPONSIBILITIES OF SCIENTISTS—
A CLOSER LOOK

From a reading of today’s lay and scientific press, one must conclude that scientists are most important people. And, indeed, the signs of their beneficence are on every hand—rapid travel, instant communication, globe-encircling rockets, moon probes, food surpluses, longer life spans, and all manner of creature comforts which by now are taken for granted. Verily, ours is the age of scientific marvels and we are in the debt of those who have made it possible.

But let’s pause a minute. By our uncritical awe aren’t we perhaps forcing scientists to take themselves too seriously? And aren’t we letting science as a discipline off too easily? If we look beyond conveniences, what have the sciences—the social, the biological, the physical sciences—given us that is of broad significance or of lasting benefit? Really very little, so far.

The social sciences have as yet told us nothing as to the causes of man’s deceit, his greed, his lust for power, nor have they suggested any plausible remedies. Human nature may be immutable, but if we understood it better we might find ways by which man could learn to live decently with himself.

The biological sciences have made our lot a more comfortable one but not significantly longer or more productive. Medicine still knows virtually nothing about the cause of man’s most disabling ailments—mental disease, atherosclerosis, and cancer—nor has it faced squarely such basic issues as birth control, euthanasia, and the continued breeding of those who are genetically defective.

The physical sciences have engineered the miracles of the space age but have not yet controlled atmospheric pollution. They have given us a source of enormous potential benefit, atomic power, but cannot control radioactive fallout—nor prevent the use of atomic power to destroy us all.

The sciences have produced benefits that are largely superficial or external or potential; they have given man increasing control over nature but not over himself. They have not enabled him to expand his internal dimensions, to control his passions, to build a world where he can live without starvation, or segregation or slaughter. The scientists have failed to accomplish these things, partly because man himself is not wholly reasonable, partly because they have not applied in the larger sense scientific knowledge to society’s needs. They have left this responsibility to the politicians who often do not understand what should be applied, or who do not always find the application expedient.

And so the scientists find their technological offspring being raised by political fathers whose parental abilities are sharply limited by a lack of scientific training. Without such training no man can fully appreciate the implications, present and future, of a rapidly advancing technology. We cannot afford to have decisions on such problems as the health of the nation or atomic control or atmospheric pollution forced by the default of scientists into the hands of laymen. Scientists must take an active role in formulating policies which relate their discoveries to the public welfare—not, as in the past, fight a rear guard action against policies that they have been unwilling to help develop. This active role will be possible only if scientists learn to understand the impact of a rapidly advancing technology on society.

Buchan* said: “Statesmanship demands two gifts—the conception of wise ends and the perception of adequate means.” The scientist has never been at a loss to perceive adequate means; but in our present science-dominated society, he must also take an active role in the conception of wise ends.

—FG

The Patient Has the Floor*

ALISTAIR COOKE

Chief United States Correspondent, The Guardian

I'm sure that Dr. Johnson—your Dr. Johnson—gave me an unintended opening when he wrote to invite me to be your speaker and added: "You may discuss any topic of your choice; although all of us in Rochester who are involved in this program are primarily in some branch of medicine, we do not necessarily expect an address related to medicine. Any topic of broad general interest would be suitable."

This is, I imagine, the usual courtesy offered to pacify the fears of some statesman, lawyer or other magnifico who never appears before a doctor except to have his chest tapped, his knees jerked, his tongue depressed, his innards photographed, his rectum proctoscoped and all his juices filtered, measured and pronounced upon. It is, though you may not know it, a permanently humiliating relationship: I mean the relationship between doctors and the rest of mankind. And it is because most people do not care to bring it up in public that I believe it might be useful for me to do so.

In fact, I think it is my duty as a journalist to speak for the patients to you. Because a journalist has always been the social link between the expert and the layman, between the public and the private man. At his worst he can become the publisher's disciple, the politician's yes-man, the tycoon's sycophant, the actor's press agent. But at his best he reports the world not as it ought to be but as his eyes and ears tell him it is. He is a fox, in the sense used by Prof. Isaiah Berlin, when he divided all mankind, writers especially, into hedgehogs and foxes: a hedgehog being one who relates everything he sees and feels to a central vision of what he believes life ought to be; a fox being, at the other end of the pole, a man who "seizes upon a variety of experiences and objects for what they are in themselves without seeking to fit them into any . . . unitary inner vision."

The fox, wrote the Greek poet Archilochus, "knows many things, but the hedgehog knows one big thing."

So here am I, a fox before a convention of hedgehogs. And I am here not to represent the foxes but the rest of the animal kingdom. For while we are dividing the world up so grandly into two sorts of people let us admit that the medical profession is the only one on earth that divides mankind into doctors and their raw material. It is this obvious, but seldom mentioned, fact that makes doctors arrange to be treated everywhere with special respect; and which makes the mass of mankind blind themselves to the fact that there are just as many mediocre or incompetent doctors as there are incompetent tailors, waiters or jockeys. Because our only relation with our doctor occurs when we need him badly we must all, for our self-respect, adopt in a mild form the delusion which every young mother hugs to her person: the belief that her obstetrician is the only man who has ever safely delivered a baby.

So I speak up for the patient, because the patient, when you see him, is usually too terrified to speak up for himself—I mean too terrified to speak about doctors. The raw material rarely answers back, which is what makes laboratory research so satisfying. But if the Mediterranean fruit fly could talk it would doubtless acquaint the farmer with some of his misapprehensions. The dolphin, whose whistles and grunts constitute a pretty sophisticated language, is already beginning to make us look silly. It is just possible that the layman, the patient tottering wide-eyed into this strange jungle of viruses and cultures and men in white, may see a few simple things which you do not see.

May I give you an instance, which happened the only other time that I dared to appear, so to speak, as a lay preacher before the College of Cardinals?

A few years ago, I was invited to Boston to speak at the annual dinner of the Massachusetts Heart Fund. I was expected, as I understood it, to launch the drive and supply, if possible, a slogan. I tell you, I would not have accepted this scholarly assignment if I hadn't learned that the year before it had been done by Dr. Ed Sullivan. When I arrived I found, to my embarrassed astonishment, that all my dinner companions were eminent heart specialists, including Dr. Paul Dudley White, who—you may recall—preserved General Eisenhower.

My qualifications for addressing a distinguished body of heart surgeons and probers were hardly less pathetic than they are for facing you today, although my two closest friends at Yale were medical stu-
ventive medicine. I myself, after a tomies down to an inflamed appendix, and the hospitals to have appendectomy. Healthy families were retiring to the States, in theories of education, in cocktails, sex, architecture—in teaching piano, in bathroom gadgets, or bridge-building, or planting, or a proxy fight or a launching pad at Cape Kennedy. So I shuffled in front of the doctors samples of their own jargon. I don't suppose I fooled any of the formidable men present. But even the most disinterested specialist in any country takes on the prejudices of his own land. And my own peculiar history—that of an Englishman born and bred, and an American tamed and naturalized—had forced me by accident into a peculiar specialty of my own, which is the continuous observation of what is British about Britain and American about America.

So facing these tolerant, though solemn, medical men, I took the risk of recalling that the United States is at all times a country with a passion for fashion. By which I don't mean it has a fetish for women's clothes (which country does not?)—I mean its ears are alertly tuned for the last cry in every kind of process: the latest trick in book-binding, or tree-planting, or bridge-building, or teaching piano, in bathroom gadgets, in theories of education, in cocktails, sex, architecture—in ideas.

All I could offer the doctors was the reminder that this trait extends also to the learned practice of medicine. For I had noticed that when I first arrived in the United States every bellyache and strained muscle on the right side was put down to an inflamed appendix, and healthy families were retiring to the hospitals to have appendectomies en masse as a form of preventive medicine. I myself, after a bout with bathtub gin (it was then the twilight—thank God—of the Noble Experiment), was seized by the university butchers and to this day I bear the scar of that particular fashion. A little later, every rash or sneeze was attributed to an allergy and a roaring business was done by manufacturers of flockless pillows and proprietors of Canadian resorts above the ragweed line. And so it went—down to that memorable evening before the heart specialists, which I dwell on because it explains why I am here and some of its lessons may apply to us.

At that time, the word "cholesterol" gibbered through the land as the word "Unclean" used to herald the approach of a leper. There was a tremendous to-do about the lethal snags created in the bloodstream by carbohydrates and animal fats, either separately or in combination. Four or five years ago it was established, at least to the satisfaction of a panicky populace and the makers of anticoagulant pills, that cholesterol was as fatal as silt along a river bed and was responsible for most of the seizures and strokes of what are called successful men (that is, men who decide to take a first trip around the world and then keel over at their desks).

I gather that this precious discovery is now not only in doubt but is looked on by some specialists as a naive superstition, a hangover from the Dark Ages of medicine (namely, the 1950's). The rush to consume only soybean and vegetable fats was declared to be premature. But carbohydrates are now more suspicious than ever. So there is a national retreat from pastries and a grateful stampede back to beef, and lately, a learned pamphlet advises me, back to alcohol.

All I could say to this medical gathering was that if the cholesterol theory was true, and if animal fats and carbohydrates were certain prescriptions for heart attacks, then they would have to explain the miracle whereby fifty-five million Britons were still alive. For of all known civilized communities the British are the connoisseurs of animal fats and the compulsive addicts of carbohydrates—with their morning toast and eggs bubbling in bacon fat, their biscuits at 11 o'clock, their lunch of more meat and potatoes and (worse) suet, then tea and more biscuits and cake, and dinner and meat and bread again, and potatoes and pudding—and perhaps an emergency snack of cheese and biscuits to guarantee coming safely through the night. How to explain the endurance, the ignorant but cheerful survival, of the British?

I saw that the doctors were now tensed and puzzled, which is always a sign that you have a specialist by the tail. I was bold enough to offer an answer. Britain, I had noticed, maintains rights of way across fields and meadows and builds footpaths alongside highways, and uses the phrase "Let's go for a walk" almost as an idiom. In America you cannot walk across fields except in pursuit of a ball with a liquid center—and there are no footpaths once the town ends. The British walk, and cycle and walk, even in the rain. Let us face it gentlemen, I said—"they function!" Could it be, I wondered—like Harvey groping towards the theory of the circulation of the blood—could it be that lumps of cholesterol could be shaken loose from the walls of the arteries by a lively bloodstream, as rocks and weeds are carried away by a river in flood? Perhaps the secret of avoiding blood clots lay in the humble admonition of the London bobby: "Keep Moving!"

After this barefaced performance I sat down in some embarrassment until Dr. White told me that I had spoken words of the profoundest wisdom, and that he wished the slogan "Keep Moving" might be taken over and plastered on billboards throughout the United...
States. I told him it was not copyright but the trick would be to get the American population to learn, as a novelty, the very old process of walking to work, or simply upstairs.

The vainglory of this occasion came back to me when you flattered me with the invitation to be here today. I don't expect, and you shouldn't, any similar moments of clairvoyance. But sometimes the patient who doesn't know what ails him can help the doctor find out by merely reciting his gripes and grievances.

I have two. And they are the minor and the major themes of this talk.

The first is the subtle tyranny of fashion, even in the sciences, even in medicine. I've already suggested that it is worth any doctor's while to pause from time to time and ask himself whether he's really pursuing a new and fruitful line or whether he's running with the herd; whether he's falling back on a well-won conviction or whether he's falling back on a national prejudice, or even a prejudice of the school he was trained in. Edward Rist, in his essay, "What Is Medicine?", noticed that "in every country our colleagues have their phantoms and their ghosts. For the Englishman it is uric acid, for the German the exudative diathesis, for the American focal infection."

It is simpler even than that. I have noticed in knocking around the world, and getting the same (the traveler's) complaint in several countries, that doctors, however circumspect, tend to take on the folk prejudices or habits of their country. Thus in France, every stomach upset is at once attributed to a malfunction in that old debbil liver, which all Frenchmen alike regard as the most vulnerable of all human organs. They consequently soothe the stomach with bowls of vegetable soup and a glass of wine three times a day. In Germany, they administer first a black draught and then having tapped the belly with a wooden hammer to see if it gives off a tremulous hollow echo, they put you on black bread, chicken broth and charcoal. In England, they instantaneously prescribe a bland (not to wander around in search of a finer word) a bland diet of tea, blanc-mange and bread soaked in hot milk. In Scotland, I am glad to say, even eminent gastroenterologists order up a soothing draught of milk and whisky, the milk (a rather toxic fluid) being cut down and cut off as the patient improves. In America, the patient is abandoned at once to bouillon and jello; and to ice water—to which, by the way, the British ascribe all American afflictions from peptic ulcer and coronary thrombosis to shortness of breath, sinusitis and the existence of the Republican party.

Now let us go to the main theme, which is about the dangers and the dullness of professional jargon: the use you make of the language that we—the doctors and the patients—have in common. What I want to do this evening is to make a plea to you as professional men whose main business is to restore men and women to their normal place in society (that is to say, whose professional aim is—as old Adolf Meyer said about psychiatrists—to bow out of the lives of your patients as soon as possible), I want to ask you to come half way to the patient and society in explaining to him health and disease. In other words, this is to be a little lecture on jargon, offered to a profession that is more prone to it than most. Why this should be so I have been unable to work out. In my boyhood the most practical aim of learning Latin was to help you employ as little Latin as possible in the use of English. But doctors, with their passionate love of Latin (and Greek) and their hearty dislike of the English language, behave as if the whole idea was to help people use four syllables for things that English describes in one. If you know the roots of a word like "circumlocution" it is then easy to see that the English word is "roundabout." I am amazed that doctors still talk about "bright red blood" when they could talk about a "hemoroidal fluid of high-intensity rosy hue." However, give them time.

A few years ago I had a lively argument with a French journalist who started reciting to me all the English and American writers he had decided wrote badly. I couldn't guess his criterion until he mentioned that none of them "wrote like Dickens." I told him there was no compulsion to do that. He was astonished. He explained at elegant if laborious length that in France there was really only one acceptable prose style, outside of the argot and vernacular of farm and city life. The style had been established in the Eighteenth Century, if not earlier. Molière wrote it, Flaubert wrote it, so did Victor Hugo and so did President De Gaulle. I am happy to say that he was even more astonished when I told him that the beauty of English was its resilience, its great variety, the fact that it could embrace—and rejoice in—the styles of Dr. Johnson and Art Buchwald, of Chaucer and Henry James, of Dryden and H. L. Mencken, of John Milton and James Thurber, of Hemingway and S. J. Perelman, of Bernard Shaw and John O'Hara, of Mark Twain and the King James Bible.

You may say that you are not in the business of style. May I say that you are in the business of describing as precisely as possible what is happening to a man, woman or child that seemed to be healthy and is now certainly sick. I truly believe that the best doctors are trying with all they have to practice and vindicate the scientific method, which I take to be the effort to find a generalization that covers all the known facts. There could be no nobler aim in science or in writing. You are, in fact, faced with the central problem of style: which is to say as cogently
as possible what a given audience can understand. When it is brilliantly done in medicine you have, by your own admission, the classic descriptions of disease—Buerger, Osler, Freud on the central nervous system, a mere journalist (I am proud to say), Defoe, on the signs and symptoms of the plague.

It is always a hard task but I'd like to elaborate on the fact that it is not peculiar to medicine. When something is exactly analyzed, and the definition is stripped to the bone, it is always memorable; which may be why centuries of students have memorized the propositions of Euclid. For when Euclid says “the angles at the base of an isosceles triangle are equal,” it stays said; just as Will Rogers’ definition of a holding company has outlived all others: “the people you give your money to while you’re being searched.” Very often the thing defined is something that’s been noticed for generations but never said so well. Aristotle was the first man to notice that “a play tends to have a beginning, a middle and an end.” This sentence guaranteed his immortality for over two thousand years, until the last few Broadway seasons gave him the lie.

I think one thing that holds good medical men back from the attempt to translate their jargon into Anglo-Saxon is the fear that they will lose their academic standing and become known as a popularizer, which among American scientists is a horrid word implying a degradation of truth in the interests of fat royalties, public popularity or an invitation to appear on television. God knows we have as many of these fakers among the hyperthyroid members of the clergy. But because something is done badly is no reason why it should not be done well. A Frenchman has told the history of the world more lucidly in a hundred pages than Sandberg can tell the history of Abraham Lincoln in four verbose volumes. We are short, and in an age of mass communications, pathetically short of good let alone great popularizers. I am sorry to have to say that I think the British have been in our time, and before our time, more concerned with the effort to reduce their professional longhand into the universal shorthand of the common speech. For classic examples we need go no further than one family and read T. H. Huxley on the habits of the ant or the butterfly and Julian Huxley on the biology of the penguin.

I know that most of you have not the time to say in two hundred words what the Journal of the American Medical Association manages to say in two thousand. I respect the scruple of any professional man who refuses to fall into slap-happy generalizations for the sake of simplicity. Where it is a matter of life and death, or even of pain and discomfort, it is better to be accurate than lucid. But what I am saying is that, given a simple fundamental change in medical education, rather a fundamental supplement in the early days, it would be possible for many more doctors to be both lucid and accurate. Suppose—that a first-rate teacher of the English language gave regular courses to medical students during their internship—or, better, that there was always someone on hand to translate into English the parts and functions of the body at the moment a student was learning them, so that he discovers why fingerbones are called phalanges, because he is reminded of the array of a Greek phalanx; and he learns also that lumbar is simply a “loin”; then the day might even come when doctors would talk to patients about collarbones instead of clavicles, and treatment instead of therapy, and admit to a scared patient that an edema is nothing more or less than a swelling.

If this happened, who—you may ask—would be the gainer? The answer is, you and the patient and medicine; because the more you tried to talk in sensible monosyllables, the more—I think—you’d find yourselves getting to the root of what was wrong and what was right. I certainly believe that if medical students were compelled to spend some time of every week translating passages from the Journal of the American Medical Association into English, they’d be surprised to discover how much of the professional jargon simply said the same thing over and over (or in a complicated way said nothing at all), how many of these learned men had the gift which Winston Churchill attributed to Ramsay Macdonald: “... of compressing the smallest possible amount of thought into the greatest possible number of words.” I think, if you try out these little translation experiments for yourself, you will find that your work will be quickened by a directness and informed with a healing humanity, for which none will be more grateful than the patients. And let us not get too solemn about what is meant by humanity: it ought always to mean compassion, but it might also include humor, which dignifies both the giver and the receiver and is an excellent medicine in itself.

Before I started a trip around the world a doctor said to me that I ought—and I quote him—“to equip yourself with appropriate cathartics and also with some handy provision against dysentery.” He was really not saying any more than a friend of mine, a layman, who only a few days later gave me the essential advice for all travelers in distant lands. “You’ve got,” he said, “to load up with stoppers and starters.” If I may say so, I am often struck, more often in America than anywhere else, with the contrast between the vivid and honest accuracy of the vernacular we all use and the often elephantine jargon of the specialist.

Jargon, too, is often a cagey, noncommittal attempt to walk all
around the description. I mean this with all respect to anyone sweating to work his way through to fundamentals. When you really are unsure about a function or a process, you tend to get lost in a maze of protective adjectives and in many abstractions, which are the linguistic elements of cloudiness and fog. But abstractions breed abstractions, as swirling vapors build fog. Soon the jargon, if repeated often enough, is doing the thinking for you. As a man who works at a bench with words, I sometimes look back over my daily pieces to try and spot words or expressions that I am using too often; for of course there is as much jargon in politics as in anything else. On the New Frontier, nobody decided anything; they made "a determination" or "a judgment." "Task forces" were called on to prepare "position papers," until it was seen that a task force was no more than a committee trying to see where we stood. In the Great Society, wars are no longer extended or spread but "escalated," causing the British cartoonist, Osbert Lancaster, to show a gentlemen of the old school hoping that "since the Costa Brava is becoming so crowded in July, I hope the movement will not escalate to Frinton-On-Sea."

I am not saying you should drastically reform the Journal. It's your playground and you should be allowed to have fun in it. I am not saying that you should not use ilium and tibia among yourselves, but the patient will probably feel more relieved to know that all he has is a pain in the groin or the shinbone. Of course, the impulse towards jargon is very much a matter of character; and it's likely that you can no more cure a naturally pompous person than you can relower a virgin. So that you won't think I'm attributing indigenous pomp to the medical profession, let me give you some melancholy proof that the jargoneer appears in all walks of life.

In Hawaii, I noticed a couple of weeks ago, the natural prospect is so pleasing that I suppose it would hurt to hint that it could hide sickness or mental disturbance. So the signposts to the state hospitals point to "correctional facility."

Road builders, you would think, would be more down to earth than other men. But in California a low bridge is not marked as a low bridge. It is "impaired vertical clearance."

The gerontologists are in league with the real estate men to disguise, among other facts of life, the unavoidable one that we all grow old. So that an Englishman arriving in Phoenix, Arizona, and asking for the famous old folks' home is met by stony looks and directed to the "senior citizen's retirement community." In the United Nations, there are no longer the rich and the poor; though the most menacing social fact of our time is that the rich countries are getting richer while the poor countries are getting poorer. But the poor will not be called poor; after a few years they resented being called "underdeveloped"—they are now known as "developing."

I should like to suggest to the airlines that anybody who is approaching Chicago is approaching Chicago. But no. You are approaching "the Chicago area." The military are as bad as anybody. There is a type of unfortunate who used to be called a wounded soldier. No more. He is now an I.C.P. —"impaired combatant personnel"!

Shall we now take a look at your own beloved profession? Briefly, for it is a painful experience, and this should be a joyous occasion. I am looking at a piece in a recent issue of your favorite journal about which jobs produce anxiety in the young. At one point the author reveals "the finding that occupation-related emotional stress may play a more significant role in the causation of coronary attacks in young persons than heredity." I take this to mean that the stresses of particular jobs may cause more heart attacks in the young than heredity. Next the author says: "To determine whether or not such a gradient in coronary heart disease prevalence does indeed exist." This can be accurately translated as, "To find out whether this is so . . . ." What did he do? He, as he says, "conducted a survey in selected types of employment which differ significantly with respect to tensions created by routine demands of the job." In other words, he decided to look into certain jobs that seemed to induce more or less tension in the young.

He had his troubles, especially with the questionnaire: "It is recognized," he says, "that certain weaknesses are inherent in the questionnaire method of survey, chief of which is the unknown prevalence of disease among nonrespondents." (You can never know how sick are the absent.) Finally, he produces this pearl: "Moreover, this method does not provide data on deceased subjects." This great man has discovered not only that dead men tell no lies—they also answer no questions.

Once, just before the floating bridge was to be built that was to be used for the invasion of Normandy, the Admiralty officials sent a note to the Prime Minister asking permission to start building the bridge at once. First they explained the job (pardon, the project) in elaborate language, and then wrote: "Permission is urgently requested for the immediate implementation of this directive." Mr. Churchill sent the request back with a note in the margin: "If you mean should you build the bridge, build it—do it—carry on!"

Ladies and gentlemen, do not equip yourselves with appropriate cathartics. Get some starters. Do not contrast living humanoids with "deceased subjects." Study rather the quick and the dead. Do not implement a directive, ever. Carry on.
Virus Infections—What of the Future?

ROBERT J. HUEBNER

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The rate of progress in virus research today is so rapid that for many the present is the future. Consequently, in order to discuss the future in comprehensible fashion, I must talk about the present state of affairs—to discuss current knowledge which furnishes the basis for contemporary enormous interest in viruses. It is precisely those considerations which will furnish the springboard for future progress in understanding and controlling the effects of virus infections.

Virology has developed into a special, rather new, bioscience which has implications for all branches of biology and medicine. In addition, previously unimagined new viruses have been found infecting virtually all living things, including plants, insects, fish, fowl, and mammals. It is now clear that viruses are major sources of economic loss in agronomy, in animal husbandry, and work loss in all the industries and businesses on which America's 90 million industrial and agricultural work force depends. Billions are now known to be lost for lack of sufficient understanding to control what should be controllable infectious diseases, and millions are spent in attempts to control them.

This is a relatively recent development. No more than 15 years ago the number of viruses known specifically to infect men could be counted on the fingers and toes, and virus research laboratories devoted to virus diseases were uncommon; today there are over 100 different known viruses in one generic group alone—the total number of human viruses is well over 200. A large proportion of these were discovered and characterized only very recently. Each month and year witnesses the discovery in many new virus laboratories scattered over the earth of additional new viruses, and the problem of identifying and classifying so many of them has produced a taxonomic crisis which appears to be insoluble, except by national and international collaboration on the production and use of standard viral reagents.

Fortunately the newer technologies which opened this Pandora's box have also provided considerable understanding of the nature and behavior of many new viruses. Methods for quick identification and classification have permitted extensive long term follow-up studies of virus infections of selected human populations. To this end, virologists, clinicians and epidemiologists are now observing virus infection in certain human populations. For instance, my associates and I have observed multiple and repeated infections with more than 80 different kinds of viruses in infants housed in a District of Columbia nursery; this group has been under constant surveillance for more than seven years. In the course of these studies, thousands of separate virus isolations were made, and all of them had to be identified. Such an eventuality was technologically impossible less than a decade ago, and the actual observations on viruses and illnesses which were made could not have been imagined then. Many of the virus infections caused only mild or severe illnesses; many other infections, however, were silent, i.e.,
not associated with any measurable contemporary illnesses. In still other studies and under other circumstances, those same viruses apparently caused severe and occasionally fatal illnesses, differing conditions apparently playing a decisive role in whether illness was absent, mild, severe, or fatal.

Thus, while these viruses most frequently cause uncountable numbers of mild to moderate illnesses in infants and children, many of them are now known, under certain conditions, to be able to cause:

1. paralytic disease in humans (polioviruses and certain other enteroviruses);
2. aseptic meningitis and encephalitis (Echo and Coxsackie viruses);
3. heart disease (myocarditis), frequently fatal in infants (Coxsackie B virus);
4. central nervous system disease—hydrocephalus (salivary gland virus);
5. eye diseases—corneal lesions and blindness (herpes, adenovirus 3);
6. fatal pneumonias and group illnesses (respiratory syncytial virus, influenzas, para-influenzas, adenoviruses);
7. fatal cancer in hamsters, rats and mice (adenoviruses 7, 12, 18).

**Effect of Large and Crowded Populations**

Other groups, as well as my associates and I, have also studied virus infections in different military cadres, especially in recruit camps, schools, and colleges, where those susceptible to infection are brought together in large groups. At the same time, studies were done on naturally occurring virus infections in man's nearest animal associates, domestic and commensal animals (table 1), revealing a remarkable similarity in viral flora in all these species to those observed in man, a flora not generally observed in wild animals or in commensal animals living in the "wild" state.

The results of all these studies serve to re-emphasize the enormous importance of crowding—the effects of large and dense populations—on the prevalence, spread, persistence, and evolution of numerous different virus infections.

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**TABLE 1**

Comparative Virus Experiences* of Man and His Domestic Animals (Mice, Cattle, and Chickens)

<table>
<thead>
<tr>
<th>Virus Category</th>
<th>Man</th>
<th>Monkey</th>
<th>Mice</th>
<th>Cattle</th>
<th>Chickens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myxoviruses</td>
<td>3 influenzas</td>
<td>Para-influenza 3</td>
<td>Para-influenza 1</td>
<td>Para-influenza 3</td>
<td>Newcastle Fowl plague SV 5</td>
</tr>
<tr>
<td></td>
<td>4 para-influenzas</td>
<td>SV 5</td>
<td>(Sendai)</td>
<td></td>
<td>SV 5</td>
</tr>
<tr>
<td>Measles virus</td>
<td>Measles</td>
<td>Monkey measles</td>
<td>Not known</td>
<td>Rinderpest</td>
<td>Not known</td>
</tr>
<tr>
<td>Picorna viruses</td>
<td>3 polio</td>
<td>Many serotypes</td>
<td>Theiler's EMC</td>
<td>Foot and mouth disease</td>
<td>Several serotypes reported</td>
</tr>
<tr>
<td></td>
<td>28 Echo's</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>29 Coxsackie's</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 rhinoviruses</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reoviruses</td>
<td>Types 1, 2, 3</td>
<td>Types 1, 3</td>
<td>Types 2, 3</td>
<td>Types 1, 2, 3</td>
<td>?</td>
</tr>
<tr>
<td>Adenoviruses</td>
<td>28 serotypes</td>
<td>Many serotypes</td>
<td>1 serotype to date</td>
<td>2 serotypes</td>
<td>Gal virus</td>
</tr>
<tr>
<td>Papovaviruses</td>
<td>+ SV 40</td>
<td>Polyoma</td>
<td>+</td>
<td>Not known</td>
<td></td>
</tr>
<tr>
<td>Herpes virus</td>
<td>Herpes simplex</td>
<td>B virus</td>
<td>Not known</td>
<td>IBR</td>
<td>Not known</td>
</tr>
<tr>
<td>Salivary gland virus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Pox virus</td>
<td>Smallpox</td>
<td>Monkey pox</td>
<td>Ectromelia</td>
<td>Vaccinia</td>
<td>Fowlpox</td>
</tr>
<tr>
<td>Ornithosis</td>
<td>Psittacosis LGV</td>
<td>Probable</td>
<td>MPV</td>
<td>Encephalomyelitis of calves</td>
<td>Several acute and chronic respiratory disease agents</td>
</tr>
</tbody>
</table>

Other domestic animals—hogs, horses, dogs, and cats—also share similar viruses with man.

*These have almost never been studied in the same ecological environment.
Indeed, since viruses, like all other species, are products of evolution, the “virus explosion” we are currently witnessing must in great part result from the “population explosion” itself.

Importance of Viruses

What are viruses? They are definable biophysical and biochemical entities that can be taken apart—nucleic acid and protein can be separated and put together again. There is no question that some day soon chemists will be able to synthesize some of the simpler viruses. If this is so, can we think of viruses as being really alive? I think we must decide that they are. Viruses are the smallest entities capable of independent existence in the sense that they carry their own duplicating machines, genetic information (DNA or RNA) which orders their replication within susceptible cells. Although completely dependent on sympathetic living plant or animal cells for reproduction and continuous existence, they are probably no more dependent than the cell itself, or for that matter, the organ or host which also depends upon sympathetic milieu for persistent existence. These arrangements are apparently reflections of different orders of adaptive mechanisms which guarantee the survival of species and subspecies.

What, in a more specific sense, is so important about viruses that they command some of our best scientific minds and millions of dollars of research funds? Primarily, of course, because they are known to be responsible for much disease, and are suspected to cause even more. Most modern virus disease investigations are currently focused on one of four important virus disease problems:

1. delineation and prevention of acute respiratory diseases, including the common cold—man’s most frequent illness;
2. delineation of the role of viruses in the cause of cancer—man’s most dreaded illness;
3. definition and prevention of viral causes of birth defects—perhaps man’s most devastating and irrevocable illness;
4. determination of the possible role of viruses in chronic and degenerative diseases.

The recognition, by scientists and laymen alike, of the enormous importance of these problems to human health has led to unprecedented nationally financed efforts to solve them. However, the overriding importance of viruses may turn out to be something much more fundamental, namely, their possible role in the evolution or natural selection of higher species. Animal viruses, as well as bacterial and plant viruses, are now known to carry genetic information which frequently alters host cells, and effects fundamental changes in inheritable characteristics of cells. Thus, numerous viruses are now known to transform normal cells into cancer cells whose progeny continue to reflect specific virus effects and carry specific virus markers indefinitely. Although in many cases the changes may be largely functional, other changes are almost certainly genotypic.

As I pointed out above, viruses are unique biochemical, biophysical and biological entities, and have different meanings for the biochemist and biophysicist, the biologist and the medical virologist. While they apparently do not contain enzymes, hormones and other synthesizing materials which enable them to achieve independent existence, i.e., to multiply and mature through feeding on non-living substrates, they do contain genetic materials in the form of DNA’s or RNA’s which are capable of ordering cells to reproduce their own prototypes. Although viruses usually require more than nucleic acid, namely a protein coat, in order to fully mature and survive as nomadic living particles in the natural order (and it is this protein coat which allows us at present to see them in the electron microscope and more easily identify them in laboratory systems), it is now clear that the naked nucleic acids (DNA or RNA) of viruses can, by themselves, carry out the total functions of intact viruses once they gain a foothold inside cells. In other words, the essential part of a virus is its nucleic acid core, which, like the genetic apparatus (chromosomes) of the male sperm once it gains entry to a susceptible cell, is fully competent. Indeed, some viruses (bacterial viruses) insert their internal genetic material into their host cells without themselves entering the cell. However, like spermatozoa, viruses in order to continue in their nomadic existence, i.e., to leave one host and discover another, require ancillary equipment—namely, specific protein coats.

Molecular Biology, Molecular Disease, and Virology

The twin concepts of molecular disease and molecular biology did not appear in any electric or immediate sense; they are the products of a unifying idea which has been growing in the minds of biomedical investigators for a long time. They are not based on a single theory and cannot be simply stated; they are often called the “molecular basis for disease” and depend primarily on biochemical, biophysical and genetic concepts of how living cells come into being, operate and respond to outside influences. These theories recognize many identifiable particles involved in the great adventure expressed in the term “life”—particles which are smaller than living cells, most frequently sub-units and products of living cells, such as genes, enzymes, and hormones. What are they? The first are made up of nucleic acids, which apparently order the others around. Important scientific disciplines are now devoted to the study of enzyme, hormone and nucleic acid chemistry. However, it seems there
are also nomadic particles that are sinister as well as interesting—viruses which frequently gain entry to cells, and which actually depend entirely for their existence on their ability, once inside the cell, to create a revolution, to establish an entirely new order for the cell, which results sometimes in death of the cell. This happens when poliovirus affects a motor nerve cell. More often, death may not be the outcome of virus infection; the cell remains alive but vitally changed, something as radical and as devastating as a cancer cell. Or, in other circumstances, viruses can produce defective cells and organs, resulting in something as pitiful as a deformed baby, a crippled child, and perhaps even a chronically ill or demented adult. It is obvious, therefore, at this comparatively advanced stage of medical knowledge, that it will prove difficult to avoid the now well known “microbial” concept of disease in looking for causes of so-called “non-infectious diseases.” While frustrating to many scientists, the prospects for progress in prevention of infectious illnesses, including viral diseases, are immeasurably greater than for diseases having causes that are less well understood. After all, the techniques for treatment and prevention of infectious diseases were developed, tried, sharpened and proven over a period of nearly 100 years—ever since Pasteur managed to diagnose and cure the infectious illnesses of French wines and devised a preventive vaccine for rabies. These techniques range from administration of wonder pills stemming from Erlich’s magic bullet, of vaccines stemming from Jenner’s early observations on the unblemished complexions of milkmaids, to simple but community and nationwide application of the principles of sanitation and hygiene.

What Does the Future Hold?

Perhaps final answers to the questions and problems discussed in the foregoing; perhaps the prevention and cure of some types of chronic diseases and cancer. We can look for newly conceived and highly sophisticated research approaches to the prevention or cure of virus infection.

Taking a long view of scientific progress, the first half of the 20th century doubtless will be most remembered as the age of the conquest of the atom; but it might better be regarded as the period when fatal infectious diseases were finally conquered, and man’s life expectancy greatly increased as a result. It is likely that the second half of the 20th century will be heralded as the age of the conquest of space, but equally possible, and perhaps more importantly for human destiny, it may become noted as the period during which the “molecular” basis for life was first defined, the age during which the mysteries of subcellular dysfunctions responsible for cancer, chronic disease, genetic defects, mental disease, and perhaps even aging, were finally understood. The latter part of the 20th century could therefore also become famous as the period in which the microscopic world of living cells, constantly beset by ultramicroscopic particles (viruses) which behave as if they were also living, was discovered to have more meaning, and in a certain sense, larger dimensions, than the cosmos itself.
The Pathogenesis of Atherosclerosis and Coronary Heart Disease*

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Fifty-five percent of all deaths in the United States are ascribed to cardiovascular disease. The vast majority of these deaths are due directly or indirectly to atherosclerosis and its ischemic complications (table 1, Am. Heart Assoc., 1965; U.S. President's Commission on Heart Disease, Cancer and Stroke, 1964).

Atherosclerosis is defined in Dorland's dictionary (24th edition) as "a lesion of large and medium-sized arteries with deposits in the intima of yellowish plaques containing cholesterol, lipid material and lipophagocytes." Typical atherosclerotic lesions of the coronary arteries are shown in figure 1. Even a casual inspection of these lesions shows the incompleteness of this conventional definition. It is obvious that thrombosis accounts for the occlusive terminal phase of atherosclerosis and, indeed, is supposed by some to initiate atherosclerosis.

The pathogenesis of the atherosclerotic plaque and of the overlying occluding thrombus remains disputed (Katz and Stamler, 1953; Moses, 1963; Sandler and Bourne, 1963; Thomas et al., 1965). The classical theory of atherogenesis propounded by Anitschkow is probably the most widely accepted. This theory holds that there is a continuous percolation of lipids from the blood stream through the endothelium and media of large and middle-sized arteries. Presumably above some threshold value, the presence of lipids, notably cholesterol, in the inner vascular coats induces the proliferation of primitive smooth muscle cells and disruption of the internal elastic membrane. These new cells appear in the subendothelial space and, along with increasing amounts of amorphous and fibrillar material, raise a fibrous plaque. These lesions are characteristically segmental, occur primarily at points of shearing stress, and are probably universal in all humans. While, unlike the simple antecedent fatty streak, the fibrous plaque is irreversible, it does not impede blood flow and is of no clinical consequence (Thomas et al., 1965). Progression of the proliferative lesion frequently leads to necrosis and the accumulation of more lipid and debris, forming the typical atheroma. Ultimately the atheromatous plaque may rupture, discharge its pultaceous contents into the arterial lumen, and form an atheromatous ulcer. A clot promptly forms over this devitalized and denuded surface, which may be large enough to occlude the already narrowed lumen. This sequence of events seems plausible, but it is curiously rare to find an occluding clot in the coronary arterial system when death has occurred immediately (Weinberg and Helpern, 1959). It is quite possible that fibrinolytic activity persisting after death could account for this phenomenon. If the victim has survived a few hours before death, however, a clot is regularly found. This has led to the paradoxical suggestion that myocardial necrosis may cause the thrombosis (Warren, 1965; Myasnikov, 1964). Occlusion of the arterial inflow to the brain, to a kidney, to a leg, or other organ evokes appropriate signs and symptoms of acute ischemia. These complicated atherosclerotic lesions give rise to no symptoms before occlusion, although they can be visualized by angiogram. The chronic atheroma tends to become cicatricial and calcified.

The major competing theory of atherogenesis is the thrombotic theory originally suggested by Rokitansky and recently revived and extended by Duguid (1949; Katz and

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>Rate per 100,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary heart disease</td>
<td>284</td>
</tr>
<tr>
<td>Stroke</td>
<td>106</td>
</tr>
<tr>
<td>Hypertension; hypertensive heart disease</td>
<td>40</td>
</tr>
<tr>
<td>Myocardial degeneration</td>
<td>27</td>
</tr>
<tr>
<td>General arteriosclerosis</td>
<td>20</td>
</tr>
<tr>
<td>Rheumatic fever; rheumatic heart disease</td>
<td>10</td>
</tr>
<tr>
<td>All other cardiovascular disease</td>
<td>28</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>515</td>
</tr>
</tbody>
</table>


*Presented at the American College of Cardiology program on cardiorespiratory physiology, November 16, 1965, Richmond, Virginia.
Stamler, 1963; Morgan, 1956; Moses, 1963). Proponents of this theory maintain that the initiating event is the deposition of a clot, perhaps first a platelet clump, but ultimately a fibrin clot. For some reason fibrinolytic mechanisms do not promptly dispose of the clot, which becomes organized, imbibes fat, and finally presents a picture indistinguishable from that of the atheromatous plaque already described. The arterial blood pressure flattens the clot into a lamellar structure; repeated episodes of thrombosis literally silt up the vessel. The thrombotic theory of atherogenesis is especially attractive since it supplies a common explanation for the atheroma and the occluding clot. Although not disproved, neither has it been shown that in patients with clinically manifest atherosclerosis are clotting tendencies enhanced or fibrinolytic activity diminished. Acute alterations in platelet stickiness may be importantly involved in atherogenesis (Slack et al., 1964).

Whatever the ultimate nature of the atherogenic process, it is clear that numerous ancillary factors are involved such as the physical characteristics and composition of the vessel wall; its mobility and the thickness of the tunics; rheological forces; and the amount and the suspension stability of the lipids in the blood. The most frequent and the most lethal manifestation of atherogenesis is coronary heart disease (CHD), which becomes increasingly common after early middle life when its socioeconomic impact is most devastating. The sequence of events culminating in a heart attack usually evolves over several decades. The diagnosis of CHD prior to this late and often immediately fatal complication is impossible by ordinary clinical methods. Compared with an acute infectious disease, the study of CHD is fraught with many and possibly insuperable problems, as illustrated in table 2. A short time course and specific causation typify acute infectious diseases, while chronicity and multifactorial causation are the rule with degenerative diseases. It is well to remember that chronic infectious diseases tend to be protean in their manifestations which may evolve only over many years and are often misdiagnosed despite the ready availability of specific diagnostic tests. Tuberculosis and syphilis need only be cited. Historically, many infectious diseases yielded to empirical modes of control before it was known that they were transmitted by specific biological agents, for example malaria, puerperal fever, and typhoid fever. Such interventions were made possible by accurate observation of host and agent and environment, in short, the epidemiological method. Analogously, it is hoped that the careful longitudinal study of degenerative diseases such as CHD may provide clues to effective, even if empirical, modes of treatment and prevention. Epidemiology is no more than the application of clinical methods to large groups of individuals who can be defined biologically, chemically, culturally, occupationally, and so forth. The interaction of disease and host is observed over time, as modified by endogenous and environmental factors: the ecology of disease. From systematic observation it becomes possible to identify certain etiological factors, that is, characteristics of both host and environment, which appear to be associated with high and low incidence rates of disease. Some of these etiological or risk factors are in theory susceptible to deliberate prophylactic and therapeutic modification. In the case of CHD it is hoped, but far from proved, that such intervention may lessen morbidity and mortality rates. Several of the presently recognized risk factors will now be enumerated and briefly discussed.

**Age.** It is by now clear that coronary heart disease is not an inevitable consequence of ageing, for there are populations in which the disease is rare even in old age.
Fig. 2-The increasing death rate from coronary heart disease (CHD) with increasing age as a percentage of all deaths.
occupations remain in this country which demand sustained heavy energy expenditure. Diet has received great attention. In both world wars, but more accurately documented in the second, there was a striking drop in cardiovascular deaths as well as in thromboembolic disease in those countries in which fat consumption was greatly restricted due to drastic food rationing. The specificity of such an association is, of course, highly questionable (Katz and Stamler, 1953; Moses, 1963). There is, nonetheless, a great intuitive appeal to the proposition that dietary fat intake is closely related to blood lipid levels and to atherosclerosis. The limited observations of Keys, first in southern Italy and subsequently extended to several European countries, have seemed to offer persuasive support to this theory (Keys and Anderson, 1954). The grave difficulties of obtaining reliable information on the amount and type of food actually consumed and differences in physical activity, as well as in other variables, demand extreme caution in the acceptance of this simple triangular hypothesis (Yerushalmy and Hilleboe, 1957). In the United States substantial variations in fat intake in carefully studied populations show no correlation with blood lipid values or with the incidence of new events of CHD (J. H. Browe et al., unpublished data). Recent appreciation of the important influence of carbohydrate intake on blood lipid values further complicates the picture. Toxins of all conceivable varieties could in theory play a role in atheroma formation. Trace elements, the mineralization of water, infections and smoke have all been suggested. Only tobacco smoking, as will be shown subsequently, has been found unequivocally to be associated with increased rates of CHD.

These, then, are the major factors which are thought to have more or less important associations with CHD. This information originally derived from many sources: clinicians, hospital wards, the autopsy room, death certificates. A major limitation of such information is that it is retrospective. It has been gathered after the event of CHD and hence from a highly selected population. Little if anything is known of the characteristics of the entire population or universe from which the patients come. Numerous selective factors operate which either bring the patient to attention or cause his being overlooked. Observations are unstandardized and, more often than not, incomplete. The alternative to these types of studies is the prospective study, which directs its attention to a defined population from a known universe and employs standardized criteria and observational methods to all participants. Such studies are not particularly glamorous; are laborious in that painstaking, repeated observations must be made for many years; are tremendously expensive; and are, to some extent, inflexible since the experimental design cannot be much altered. Furthermore, unpredictable variations in the characteristics of the population may occur which could influence prognosis: they might, conceivably, all start exercising or stop smoking, or drinking, or overeating. It is scarcely surprising that few such studies have been undertaken. These are, notably, the United States Public Health Service study of a random sample of adult men and women residing in Framingham, Massachusetts, which began in 1949; and the Albany Cardiovascular Health Center study of a large group of middle-aged men working for the State of New York, sponsored by the Health Department, which began in 1953 (Doyle et al., 1957b; Dawber, Moore, and Mann, 1957). Despite various methodological differences, it is gratifying that information on the prevalence and incidence of CHD in these two studies is remarkably similar and is, indeed, very similar to information gathered in other areas of the United States.

Selected findings from the Albany study will now be presented with brief comment on their significance and limitations. The mode of presentation is in the form of a modified life table. This technique permits the amalgamation of observations made for varying lengths of time on individuals of differing age. It must necessarily be assumed that the intensity of the disease process is constant over time and within chronological age (Kinch, Gittelsohn, and Doyle, 1964).

On admission to the study, approximately 3% of the men, then between the ages of 39 and 55 years, had some manifestation of coronary heart disease. Figure 3 shows that the average annual incidence of CHD in the Albany study is about 1%. This basic statistic in the subsequent figures is related to various characteristics.
thought to influence risk. Incidence rates are related to characteristics measured at the time of entry into the study.

In figure 4 risk of CHD is related to ideal body weight according to the standards of the Metropolitan Life Insurance Company. Little positive or negative association is apparent here, in the Framingham study, or in other studies. It seems likely that the hapless overweight patient has been made a whipping boy on little other than esthetic grounds.

In figure 5 risk of CHD is related to blood pressure standards promulgated some years ago at the Princeton Conference (Doyle, 1960). Once again there is little relationship between blood pressure level and the incidence of CHD. More recently, however, we have reviewed the experience of individuals whose diastolic blood pressure was 100 mm Hg or more on at least half the occasions they were examined. A substantially higher incidence rate of CHD occurs in the consistently and definitely hypertensive population. This observation illustrates particularly well a serious methodological difficulty of the prospective studies, viz. the unavailability of statistical technique to evaluate the influence on prognosis of a risk factor of varying intensity. The arterial blood pressure is notoriously variable. While by arbitrary criteria about 20% of our group are at any one time hypertensive, the composition of this group varies greatly and unpredictably from year to year.

In figure 6 the serum total cholesterol concentration at the time of entry into the study is related to the subsequent experience of CHD. It is clear that those whose cholesterol was below the mean fared better than average while those with only moderate hypercholesterolemia or higher had a rate at least four times higher than the favored group. There is a gradient of risk: the higher the cholesterol level the greater the likelihood of CHD. Unlike the arterial blood pressure, the serum total cholesterol concentration remains remarkably constant so long as it is measured in the same season of the year (Doyle, Kinch, and Brown, 1965). It has been suggested that the serum triglyceride level might be better correlated with susceptibility to CHD than cholesterol. That this is not true is shown in figure 7, in which the population has been divided into thirds according to cholesterol and triglyceride levels (Brown, Kinch, and Doyle, 1965). The geometric relationship of serum total cholesterol and of triglyceride concentrations with the incidence of CHD are different, but neither shows a superior predictive value and both are nearly identical in the higher ranges.

The association between the use of tobacco and the incidence of CHD has been of particular interest since the first major studies of lung cancer. These surveys showed, of course, a twenty-fold greater likelihood of bronchiogenic carcinoma in heavy cigarette smokers than in non-smokers. They also showed a great excess of deaths from CHD (Doll and Hill, 1956; Hammond and Horn, 1958a and b; Dorn, 1959). The early observations made in the Framingham and Albany studies failed to show a significant relationship between tobacco habit and the prevalence and incidence of CHD. Since it appeared likely that this was a statistical artefact due to insufficient numbers, it was decided to pool information on men exposed to risk. This collaborative effort, based on observations made over six to eight years on 4,120 men between the ages of 30 and 62 years, provided convincing evidence that the consumption of 20 or more cigarettes daily is associated with an incidence of sudden death or of myocardial infarction over three times greater than in non-smokers (fig. 8). Ex-smokers and pipe and cigar smokers appeared to be at about the same risk as non-smokers. An anomalous finding was that there is no association between smoking habit and angina pectoris (Doyle et al., 1962; 1964). Similar observations had been made years previously by White and Sharber (1938). This apparent discrepancy may, however, be one of several pieces of collateral evidence that the anatomic and physiologic bases of angina may be different from other manifestations of CHD.

It is only natural to query whether combinations of these risk factors are associated with a risk of CHD equal to or greater than the sum of their individual contributions. Figure 9 is an attempt to answer this question. As might intuitively be assumed, hypertensive, hypercholesterolemic heavy smokers have a risk of CHD five times as great as individuals without these characteristics. Since, however, there are fewer than 8% as...
many individuals in the high risk group, it cannot be asserted with statistical confidence that these differences are real. The solution of the problem of accumulating adequate numbers of reliable observations on the influence of multiple risk factors on susceptibility to CHD is extraordinarily difficult. In the past year we have done cardiovascular screening studies on 6,000 men and women between the ages of 21 and 71 years and have found the combination of risk factors considered in figure 11 in only a very small number of men and women.

Cardiovascular disease, especially CHD, at present accounts for more than half of all deaths in the United States. The anatomic basis of coronary heart disease is narrowing of the coronary arteries by atherosclerosis. In the majority of instances where death occurs more than a few hours after a heart attack, a fibrin clot is found to have occluded the diseased artery and caused ischemic necrosis of the myocardium nourished by that artery. The pathogenesis of atherosclerosis and of thrombosis is unknown; it is possible that they represent different stages of the same process. Epidemiological studies of the relationship between measurable biological characteristics of defined individuals at risk have permitted identification of what seem to be important epidemiological factors in CHD. These include the relative immunity of women from the ischemic complications of atherosclerosis; sustained diastolic hypertension; an elevated serum total cholesterol concentration; and consumption of 20 or more cigarettes daily. It is probable but unproved that diet, specifically the ingestion of large amounts of saturated fats, may cause hypercholesterolemia, activate clotting mechanisms, and thus lead to an increased risk of CHD. It is likewise unproved that habitually high levels of physical activity protect against CHD. There is no acceptable evidence that prolonged stress influences the frequency of CHD. Although it is probable that combinations of risk factors increase the risk of CHD, no prospective study has yet been able to provide sufficient numbers of cases to prove this point or to indicate whether risk is simple or compounded.

There is understandably tremendous popular pressure, despite our still inadequate knowledge of the pathogenesis and etiology of CHD, to plunge into widely advertised evangelistic programs for the prevention of CHD. In such an emotionally charged atmosphere it is almost impossible to evaluate such efforts in the required detached and objective fashion. Indeed, it may fairly be questioned whether practical and effective modes of intervention in the course of CHD are yet available. It is to be feared that public disenchantment with such premature interventions may gravely impede scientific investigation into the seats and causes of this major threat to health and longevity.

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PATHOGENESIS OF ATHEROSCLEROSIS


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Inappropriate Antidiuresis: Examples of an Hyponatremic Syndrome Resembling Exogenous Vasopressin Administration in Man

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Hyponatremia has been recognized as a clinical entity since the finding by Loeb in 1932 that significantly lowered serum sodium and chloride levels are a characteristic finding in Addison’s disease. It soon became apparent with the work of Winkler and Crankshaw in 1938, that hyponatremia was an accompaniment of diverse disease states as repeatedly confirmed since the introduction and clinical application of flame photometry for sodium analyses (Berry, Chappell, and Barnes, 1946; Fox and Baer, 1947; Hald, 1947; Bowman and Berliner, 1949; Wallace et al., 1951). Early papers emphasized the occurrence of hyponatremia in the two broad categories of cerebral and pulmonary disease (Peters et al., 1950; Sims et al., 1950; Welt et al., 1952; Cort, 1954); additional reports indicated a frequency of occurrence in tuberculous meningitis (Rapoport, West, and Brodsky, 1951; Harrison, Finger, and Fleischman, 1952; Cheek, 1956; Arblaster and Whitehead, 1957). Despite the renal sodium loss in these patients under certain circumstances, evidence indicates that primary water retention is present upon one or another bases in most cases of hyponatremia (Fuisz, 1963). In recent years cases have been described in which this clearly appears to be mediated by continuous antidiuretic activity. Whether or not these cases for the most part represent failure of physiologic control or inactivation of pituitary antidiuretic hormone (vasopressin), with abnormal biosynthesis of substances possessing vasopressin activity being importantly implicated in cases of bronchogenic carcinoma, is an issue that needs clarification. This report deals with clinical illustrations of this syndrome, and with special physiological studies pertinent to the underlying mechanisms.

Case Histories

Case 1, C.G. A 49-year-old carpenter entered the MCV hospital because of a left hilar density seen on a chest roentgenogram. Following an episode of pneumonia 13 months before admission he had frequent upper respiratory infections and was aware of progressive malaise with 17-pound weight loss. Sputum was bloodstreaked on a few occasions. He had smoked one pack of cigarettes per day for more than 25 years. On physical examination he was without complaint and appeared to be well-nourished; his weight was 59 kg and his height, 167 cm. Breath sounds were diminished posteriorly on the left from the inferior border of the scapula to the base. The liver was not enlarged. The pulse was 88 per minute and the blood pressure 155/80 mm Hg. Neurologic examination was normal. Chest film showed a nodular left hilar density 4 cm in diameter. Hemoglobin was 13 g/100 ml; white cell count was 10,200/mm³ with a normal differential count. Serum sodium concentration was 125 mEq/L, K, 3.8; Cl, 88 and bicarbonate 23 mEq/L. Blood urea nitrogen (BUN) was 8 mg/100 ml. Alkaline phosphatase was 3.41 Bessey-Lowry units. Other blood studies including calcium, phosphorous, total protein, albumin, GO transaminase, bilirubin and Bromsulphalein were normal. Admission urine specific gravity was 1.012 and was not otherwise remarkable. Skull roentgenogram and bone survey for metastatic disease showed no pathologic changes. Bilateral scalene node biopsies revealed chronic lymphadenitis without tumor; bronchoscopy failed to reveal a lesion. Arterial hemoglobin-oxygen saturation was 92%.

Five days after admission serum sodium was 128 mM/L and two days later, it was 118 mM/L. By then the patient complained of constant severe headache. Serum osmolality was 236 mosm/kg, while urine osmolality was 316 mosm/kg. Twenty-four-hour urine 17-hydroxy- and 17 keto steroid values were normal, with a normal rise after 50 units ACTH on two successive days. Urea N was less than 7 mg/100ml. Urine culture was negative. At this point the procedures detailed under “Special Studies” were begun. During the course of this study...
the patient exhibited bizarre behavior associated with serum sodium values below 110 mEq/L, and with values approaching 100 mEq/L, he developed vomiting and pain in the abdomen. These symptoms subsided with rise of sodium values toward normal associated with moderate water restriction and a 9-a-fluorohydrocortisone administration. Right carotid arteriogram, performed because of the persistent headache, failed to show filling of the right middle cerebral artery; electroencephalogram revealed only mild generalized slowing. Coincident with improvement of the patient’s cerebral symptoms, his liver became palpable. Repeat bronchoscopy revealed a lesion of the left main stem bronchus which was biopsied. Open biopsy of the liver was carried out to determine the advisability of thoracotomy for definitive removal of the tumor. Biopsies from each site showed poorly differentiated bronchogenic carcinoma of the oat cell type. Subsequently the patient developed a left pleural effusion in addition to suspected obstruction of the left main bronchus. The patient died three weeks later.

Autopsy revealed extensive neoplastic involvement of the lower lobe of the left lung with occlusion of the left main bronchus and distal atelectasis. The tumor was identified as poorly differentiated bronchogenic carcinoma, oat cell type, with extensive metastases involving lung tissue; several small nodules of metastatic tumor were found in the adrenals, and on the left there was a small encapsulated cortical adenoma. The kidneys were normal. Gross and microscopic examination of the brain revealed no abnormality. A tiny focus of fibrous tissue containing dilated vessels was seen in the anterior pituitary, but comprised only a small portion of the parenchyma. The posterior pituitary was normal.

Case 2, W.M. The patient, aged 50, entered the V.A. Hospital on October 18, 1962, complaining of intermittent fever (101° to 102°) for five months, associated with severe bilateral ocipital headaches, recurrent vomiting, diffuse myalgias, and progresse generalized weakness. He had been treated by his local physician with penicillin and tetracycline with remission of these symptoms, except that emesis had progressed to a frequency of four to five times daily. He had had no hematemesis, no alteration of bowel habits, and there had been no nuchal rigidity, scotomata, diplopia, or dysphagia. He had had one episode of mental confusion several weeks before admission and had recently noted episodic dizziness and unsteadiness of gait. One year before admission he had had a tender swollen right knee, yielding purulent material on aspiration, and subsequently responding to penicillin without further aspiration.

On admission, temperature was 101.4 F, pulse, 90, respiration, 24, and blood pressure, 155/100. He was a normally developed, muscular male who was well oriented, but who exhibited peculiar ideation. He was lethargic, but easily arousable, and his gait was unsteady. There was diminished hearing bilaterally, especially for higher tones. The remaining cranial nerves revealed no deficit. Chaddocks, Oppenheim, and Babinski’s signs were present on the right, and the left ankle jerk was diminished. Hemoglobin was 14.8 g/100 ml, hematocrit 44%, WBC 8,100, 77% neutrophils. Urine specific gravity was 1.021. Admission serum Na was 135; Cl, 88; K, 3.1 and bicarbonate, 29 mEq/L. Serum Ca, PO, GO transaminase, albumin, and alkaline phosphatase, albumin, globulin, cephalin flocculation, blood and spinal fluid serology, routine febrile and heterophil agglutinations were normal or negative. Cultures of sputum, urine, blood and cerebrospinal fluid were negative for ordinary pathogens, M. tuberculosis, and fungi. India ink preparations, and complement fixation and hemagglutination tests on acute and chronic serum showed no abnormality. Skull films showed an upward shift of the pineal. Lumbar puncture revealed an opening pressure of 330 mm saline and a low glucose (25 mg/100 ml with concomitant blood glucose of 93 mg/100 ml). Spinal fluid protein was 142 mg/100 ml with 35 cells (8 neutrophils and 26 lymphocytes/mm³).

Although acid-fast organisms were not seen on direct smear, isoniazid, streptomycin and p-amino salicylic acid therapy was begun on the third hospital day. The patient was afibrile by the 11th day, but was increasingly lethargic and confused. At the same time serum sodium and potassium had fallen. It was found that fluid administration had to be limited to 1,000 ml/day. Urine osmolalities were 593 and 505 mOs/m at times when serum osmolality was 252 mOsm/kg. With fluid restriction of 1,000 ml/day the patient complained of no thirst, his sensorium cleared, and serum electrolyte values returned to normal. Audiogram prior to streptomycin therapy confirmed a bilateral auditory deficit and 6th and 7th nerve deficits were subsequently noted. Upward shift of the pineal on plain skull x-rays, together with cranial nerve involvement, were interpreted as brain stem arachnoiditis consistent with presumptive tuberculous meningitis. These findings showed marked remission between

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20th and 30th days. By the 55th hospital day the patient could tolerate daily fluid intakes of 3,000 ml without a reduction in serum sodium.

Case 4, W.M.W. This patient was a 43-year-old woman who noted midline low back pain without radiation three years before admission. Initially, the pain could be relieved by symptomatic measures, but more recently it had increased in severity, had begun to radiate into the left hip and inguinal area, and was now made worse by coughing and sneezing. She was admitted to the orthopedic service at MCV on March 6, 1963. On admission the temperature was 98.4 F, the pulse, 86 and respirations, 18 per minute. There was joint tenderness at L4-L5, associated with moderately severe paravertebral muscle spasm. Straight leg raising produced pain at 45°. No neurologic deficit was demonstrable. The pain was not noticeably benefited by continuous traction, and exploration for a herniated nucleus pulposus was carried out on the fifth hospital day. Post-operatively the patient had an elevated temperature, leucocytosis and a urine culture positive for E. coli. Despite treatment for urinary tract infection, daily temperature spikes continued as high as 102.6 F. She complained of weakness and numbness of both lower limbs, and showed objective weakness of the right foot and ankle and diminished sensation over both feet. Mental confusion, nuchal rigidity and divergent strabismus were noted two days later. Left patellar and both triceps reflexes were absent and the right patellar reflex was weak. The patient was lethargic and would reply only to direct questioning. Lumbar spinal fluid pressure was 410 mm Hg with periodic fusion rate was accurately calibrated for use in the calculation. After 90 minutes (six clearance periods), infusion of 5% NaCl at the rate of 0.125 ml/kg/min was given over a period of 45 minutes. Clearance periods were continued for an additional 60 minutes after completion of this infusion.

Varying Sodium Intake

During a 37-day period, dietary sodium was 10 mEq/kg with periodic supplementation by oral sodium chloride tablets to increase the intake to 45 to 80 mEq/kg/day. On the indicated days, additional sodium was given in the form of hypertonic saline intravenously in the following amounts: 10th and 11th (410 mEq); 21st, 22nd, 26th (425 mEq). Water was restricted on the 13th to the 17th day; water intake was 1.0 to 2.4 L from the 18th to the 30th day; and was 0.3 to 0.8 L from the 31st to the 41st day. Thereafter water intake was from 0.5 to 2.5 L as regulated by the patient’s desires. 9-α-fluorohydrocortisone was given on the 21st, and continued to the 41st day. Sodium chloride in tablet form was omitted; and the diet was changed to contain approximately 100 mEq/kg/day on the 38th day until the end of the study. On four occasions analyses of the diet revealed 85 to 104 mEq sodium content. During the period of 9-α-fluorohydrocortisone administration, the patient received an additional 60 units of corticotropin on days 44 through 50.

Case 2. Sequential Water Loading-Salt Loading

These studies were carried out on February 7th and 27th, 1962 (138 days and 158 post-injury), on May 10th and 14th, 1962 (213 days and 220 days post-injury), and repeated one year later. Details of the test were as follows. Control weight, and blood and urine specimens for sodium content and total solute content, were obtained before water loading. The patient then ingested 20 ml water/kg over a one-hour period. We used an indwelling multiholed catheter to collect urine, and an inlying thin-walled 20 gauge needle with stylet to collect blood samples. Cumulative urine volume was recorded and replaced by additional ingested water. Inulin and sodium p-aminohippurate priming injections and sustaining infusions were begun, and after 30 minutes' equilibration time, urine was collected for clearance periods every 15 minutes, with midpoint blood collections. In the experiment where inulin space was measured, priming injection was omitted, and the infusion rate was accurately calibrated for use in the calculation. After 90 minutes (six clearance periods), infusion of 5% NaCl at the rate of 0.125 ml/kg/min was given over a period of 45 minutes. Clearance periods were continued for an additional 60 minutes after completion of this infusion.

Varying Sodium Intake

This was studied for a period of 24 days (days 118 through 142 post-injury) beginning with the patient’s transfer to the medical service on January 16, 1962. He was placed upon a 10 mEq/kg/day sodium diet for eight days and was allowed to drink as much fluid as desired, which was 2 to 3 L/day. On the fifth day an additional 300 mEq sodium chloride was added in saline solution intravenously, and blood and urine specimens for sodium content and total solute content, were obtained before water loading. The patient then ingested 20 ml water/kg over a one-hour period. We used an indwelling multiholed catheter to collect urine, and an inlying thin-walled 20 gauge needle with stylet to collect blood samples. Cumulative urine volume was recorded and replaced by additional ingested water. Inulin and sodium p-aminohippurate priming injections and sustaining infusions were begun, and after 30 minutes' equilibration time, urine was collected for clearance periods every 15 minutes, with midpoint blood collections. In the experiment where inulin space was measured, priming injection was omitted, and the infusion rate was accurately calibrated for use in the calculation. After 90 minutes (six clearance periods), infusion of 5% NaCl at the rate of 0.125 ml/kg/min was given over a period of 45 minutes. Clearance periods were continued for an additional 60 minutes after completion of this infusion.

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sodium was given as 5% NaCl solution. On the ninth day dietary sodium was increased to 153 mM/day and an additional 500 mM sodium, as a 5% solution intravenously, was again administered. On day 15, fluid restriction to less than 1,000 ml a day was begun and sodium intake was lowered to 118 mM/day. These studies have been reported in more detail elsewhere (Haden and Knox, 1965).

Results

Case 1. Findings in a 43-day period of study are depicted in fig. 1. Serum sodium concentration fell from 121 to 110 mEq/L during sodium intake of 40 to 80 mEq/day, and water intake of 1.2 to 2.9 L/day in the initial nine days of the study. On days 9 and 10, the administration of a total of 1,120 mEq sodium caused a brief rise in serum sodium concentration from 108 to 120 mEq/L, but most of the administered sodium (913 mEq) appeared in the urine. The remainder was accountable by a slight increase in weight; and serum sodium promptly fell again to 106 mEq/L. An attempt to restitute serum sodium by water restriction alone was abandoned when serum sodium value fell to 99 mEq/L, although with maintenance of blood pressure at 140/80 mm Hg. A second large sodium load (980 mEq) was given as intravenous hypertonic saline and oral sodium chloride tablets on days 20 and 21, with a rise in serum sodium concentration to 122 mEq/L on day 22. On 80 mEq sodium/day plus 9α-fluorohydrocortisone but without water restriction, serum sodium rose to 128 mEq/L but no higher; on the addition of water restriction it rose to 143 mEq/L on day 43. Thereafter it fluctuated between 128 and 140 mEq/L, the lower values being associated with water intake over 1.5 L/day.
During this 43-day period and an additional 13 days of observation, urine total solute activity always exceeded that of serum by 70 mOsm/kg or more; minimal osmolal U/P ratio observed was 1.3. The range of urine osmolality was 288 to 866 mOsm/kg and 24-hour urine volume, 602 to 3,130 ml/day.

In an acute study (fig. 2) water loading failed to produce a dilute urine and revealed a maximal urine flow rate of 3.6 ml/min (minimal osmolality, 442 mOsm/kg). Urine flow rate increased to 5.4 ml/min during the first 10 minutes following alcohol ingestion but the urine remained hypertonic (minimal osmolality, 410 mOsm/kg). Serum sodium was 236 mOsm/kg, before and 224 mOsm/kg after; sodium excretion remained at 200 µEq/min throughout. Creatinine clearance, which was 202 ml/min/1.73m² (three periods) before water loading, fell to 173 ml/min/1.73m² (four periods) after alcohol ingestion. Inulin and PAH clearances were also measured when the patient was comparably hyponatremic (serum sodium 116 mEq/L, serum osmolality 236, mOsm/kg). Inulin clearance was 238 ml/min/1.73m², PAH clearance was 1,109 ml/min/1.73m², and filtration fraction was 0.30. The volume of distribution of inulin was 14.1 L (24% of body weight).

Case 2. During eight days on sodium restriction to 10 mEq/day, and ad libitum water ingestion (2,930 to 3,190 ml), minimal sodium excretion was 20 mEq/day and serum sodium fell to 107 mEq/L, rising only to 111 mEq/L with infusion of 300 mEq NaCl as 5% solution intravenously. Beginning with day nine, water intake was limited to less than 1,800 ml/day (870 to 1,800), and dietary sodium increased to 153 mEq/day, with rise in serum sodium to 125 mEq/L. Beginning with day 15, dietary sodium was decreased to 118 mEq/day and water limited to less than 1,000 ml/day (840 to 920) for three days, with rise of serum sodium to 138 mEq/L, following which water intake was again liberalized.

A sequential water loading and salt loading test (table 4) was done on the 22nd study day (four months post-injury) at which time serum sodium was 115 mEq/L and serum osmolality 242 mOsm/kg. Urine osmolality was 599 mOsm/kg water prior to water load, and urine sodium excretion rate 280 µEq/min (2.43% of filtered load). After the water load, serum sodium fell to 113 mEq/L and serum osmolality to 295 mOsm/kg. Urine osmolality fell from 599 to 301 mOsm/kg following water load, and urine sodium excretion rate rose to 730 mEq/min (6.34% of filtered load). After completion of infusion of 425 mEq sodium as hypertonie NaCl, serum sodium was essentially unchanged at 115 mEq/L with a serum osmolality of 265 mOsm/kg. Urine osmolality fell slightly more to 281 mOsm/kg, with urine sodium excretion rate rising to 1,240 µEq/min (10.8% of filtered load). Net reabsorption of solute free water was present throughout (1.9 — 2.5 ml/min.) Inulin clearance was 116 ml/min/1.73m² (for his single kidney) and inulin space 16.0 L (26% of body weight).

This patient was restudied in May 1962, 10 weeks later (eight months post-injury). It was now noted from random specific gravities that he occasionally had dilute urine. After water loading, he had a free water clearance of only 0.48 ml/min, with a minimum urine osmolality of 233 mOsm/kg (serum osmolality being 264 initially, falling to 253). On salt loading, free water clearance doubled (to 1.09 ml/min), with no fall in minimum urine osmolality (the latter rose to 266 mOsm/kg). Minute sodium excretion rose from 468 µM/min initially to 614 at termination of the water load, and did not exceed 1.3% of filtered load. Inulin clearance was 69 ml/min/1.73m².

On the final study, one year later (May, 1963), serum sodium was 133 and serum osmolality 244. On water loading, serum sodium fell to 129, and serum osmolality to 240. Free water clearance was 1.5 ml/min and minimum urine osmolality, 200 mOsm/kg. On salt loading, free water clearance rose to 4.0 ml/min but minimum urine osmolality remained over 200 mOsm/kg. Urine sodium excretion rate rose from 214 to 1,644 µEq/min (from 1.5% to 11.8% of filtered load) and he again had a high inulin clearance (97ml/min/1.73m²).

Discussion

These cases illustrate the typical findings in a naturally occurring

### TABLE 1

A Classification of Hyponatremic Syndromes

<table>
<thead>
<tr>
<th>Hyponatremic Syndrome</th>
<th>Low Filtration</th>
<th>High Filtration</th>
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<tbody>
<tr>
<td>I. (A) Edematous (Na⁺ retaining)</td>
<td>Circulatory failure</td>
<td>Primary Na⁺ retention:</td>
</tr>
<tr>
<td>(B) Non-edematous (Na⁺ wasting)</td>
<td>Renal saline volume loss:</td>
<td>1) Cirrhosis</td>
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<td></td>
<td>1) Mineralocorticoid failure</td>
<td>2) Nephrosis</td>
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<tr>
<td>II. Non-edematous (Na⁺ retaining)</td>
<td>Non-renal saline volume loss (appropriate vasopressin excess)</td>
<td>Primary H₂O retention:</td>
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</table>
syndrome simulating the effects of prolonged exogenous vasopressin administration in man (Leaf et al., 1953; Levisky, Davidson, and Berliner, 1959; Stormant and Waterhouse, 1961; Jaenicke and Waterhouse, 1961), to which Schwartz and co-workers (1957) have given the designation “syndrome of renal sodium loss and hyponatremia probably resulting from inappropriate secretion of antidiuretic hormone.” Since it is still not possible to measure serum ADH activity directly with ease and precision, the diagnosis of this entity remains a presumptive one. The criteria presently accepted as warranting its presumption are:

Diagnostic Features of the Syndrome

1. Hyponatremia, either asymptomatic or associated with frank water intoxication, is the usual reason for suspecting the syndrome.

2. Hypertonic urine is excreted in the presence of hypotonicity of body fluids. The latter may be confidently inferred from hyponatremia if this is unassociated with hyperglycemia or hyperlipemia. It is not actually necessary that the toxicity of urine exceed that of serum water, merely that it exceed that of the intake, if solute and water intake can be readily calculated. In practice, urine usually is markedly dilute (osmolal urine to serum water ratio as low as 1:5) in the absence of vasopressin, which would be the expected response to true body fluid hypotonicity.

3. Renal sodium conservation, as evidenced by low urine sodium concentrations, does not occur in association with hyponatremia in these patients, nor do they develop edema. Edema is absent despite the fact that extracellular fluid space and total body water are expanded.

4. Absence of azotemia, hypotension, or stigmata of so-called dehydration distinguish this syndrome from adrenocortical failure and from the salt-wasting forms of primary renal disease. Although the salt-wasting is to a certain extent reversible by mineralocorticoid hormone administration, this may be less efficient than water restriction alone.

5. The response to hypertonic salt infusion is augmented urinary salt loss; hence this method of therapy is usually ineffectual in increasing serum sodium concentration.

Differential Diagnosis of Hyponatremic Syndromes

In table 1, the relation of this particular form of hyponatremia to other hyponatremic syndromes is considered. Usually the edematous hyponatremic syndromes may be identified by the presence of edema although recent therapy or sodium restriction may obscure the tendency. With or without edema these patients are distinguishable by their sodium retaining tendency, manifesting a) a low urine sodium excretion regardless of its sufficiency in the diet, and b) a tendency to reaccumulate edema if its intake is liberalized. In both of these respects such patients differ from the patient who has a genuine saline volume deficit due to losses by an extrarenal route, in the face of unimpaired and appropriately invoked renal mechanisms for conserving both sodium and water. The latter patient tends to maintain circulatory filling at the cost of some dilution of body fluids (appropriate vasopressin excess). Because of the importance of a low filtration rate in the genesis of cardiac edema, patients with hyponatremia may be classified, frequently on the basis of serum urea nitrogen concentration alone, into low and high filtration states. Edematous patients with cirrhosis and the nephrotic syndrome frequently have supernormal filtration rates and a serum urea nitrogen concentration that is either normal or distinctly low. In the non-edematous hyponatremic syndromes, serum urea nitrogen again is helpful in distinguishing low filtration states, such as Addison’s disease or the salt-wasting forms of renal disease, from the entity under discussion, where filtration rate may be high and serum urea nitrogen low.

In table 1 the high filtration variety of non-edematous hyponatremia is referred to as “Primary Water Retention” in deference to

| TABLE 2 |
|---------------------|-----------------|---------------------|
| **Distinguishing Features of Three Varieties of Hyponatremia** |
| | Hypoadrenal | Hypopituitary | Inappropriate ADH |
| I. Pathophysiology | | | |
| 1) Defect | Mineralocorticoid and glucocorticoid deficiency | Glucocorticoid deficiency | Vasopressin excess |
| 2) Extracellular fluid volume | | | |
| II. Signs | | | |
| 1) Urine Na⁺ | May be low | May be low |
| 2) Urea N | 0 | 0 |
| 3) Hypotension | | | |
| III. Symptoms | + | 0 | + |
| IV. Response to Rx | | | |
| 1) Saline | + | + | 0 |
| 2) Cortisol | + | + | 0 |
| 3) H₂O restriction | 0 | 0 | + |
the fact that two possibly separable defects may account for the failure of urinary dilution inherent in such a syndrome. In the one circumstance, exemplified by the cases discussed here, a normal tubular mechanism for diluting the isotonic filtrate may be present, only to have its effect vitiated by the presence of vasopressin suppression of vasopressin, though not of mineralocorticoids, upon the renal tubule. The absence of the effects of glucocorticoids, upon the renal tubule. The osmolality (Aubry et al., 1963).

Vasopressin, though not of mineralocorticoids, is a permissive effect of cortisol, though it has been postulated that there is a permissive effect of cortisol upon water excretion, residing in its property of "inhibiting back diffusion or reabsorption of water in the loop of Henle, distal tubule, the concentrating segment," in the absence of vasopressin.

In table 2 the features of hyponatremia in Addison’s disease (high urea N) are contrasted with those of hypopituitarism and of vasopressin excess (both characterized by a low urea N). The low urea N in hypopituitarism may in part note less urea production, and hence may not infer a supernormal filtration rate to the same degree as would an equally low urea N in vasopressin excess. The distinction between the latter two entities, rather than simply lumping them as “Primary Water Retention,” is of some importance in that treatment with cortisol is efficacious in hypopituitarism, while less efficacious in vasopressin excess (both characterized by a low urea N). The low urea N in hypopituitarism may in part note less urea production, and hence may not infer a supernormal filtration rate to the same degree as would an equally low urea N in vasopressin excess. The distinction between the latter two entities, rather than simply lumping them as "Primary Water Retention," is of some importance in that treatment with cortisol is efficacious in hypopituitarism, while less efficacious in vasopressin excess (both characterized by a low urea N). The low urea N in hypopituitarism may in part note less urea production, and hence may not infer a supernormal filtration rate to the same degree as would an equally low urea N in vasopressin excess. The distinction between the latter two entities, rather than simply lumping them as "Primary Water Retention," is of some importance in that treatment with cortisol is efficacious in hypopituitarism, while less efficacious in vasopressin excess (both characterized by a low urea N).

In patients with carcinoma of the lung, as in Case 1, it has been proposed that the liberation of a humoral substance from the tumor itself (Roberts, 1959; Dossetor et al., 1961) might account for sustained hypertonicity of the urine. Thus, reports of the presence in such patients of an antiuretic substance in urine (Thorn and Transbol, 1963) and antiuretic material extracted from specimens of tumor are of considerable interest (Amatruda et al., 1963; Bower and Mason, 1964). In two of these reports (Amatruda et al., 1963; Thorn and Transbol, 1963), employing incubation with thioglycollate, the antiuretic material was indistinguishable from arginine vasopressin.

**Carcinoma of the Lung**

Previous reports indicate that carcinoma of the lung, particularly the oat cell type, which constitutes

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**TABLE 3**

<table>
<thead>
<tr>
<th>Etiology</th>
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<td>herpes simplex</td>
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<tr>
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<td>Carter et al., 1959; 1961</td>
</tr>
<tr>
<td></td>
<td>Vogel, 1963</td>
</tr>
<tr>
<td></td>
<td>Haden, 1963</td>
</tr>
<tr>
<td>Head Injury</td>
<td>Rapoport et al., 1951</td>
</tr>
<tr>
<td></td>
<td>Harrison et al., 1952</td>
</tr>
<tr>
<td></td>
<td>Cheek, 1956</td>
</tr>
<tr>
<td>Meningitis, tuberculous</td>
<td>Arblaster, 1957</td>
</tr>
<tr>
<td>Ascending paralysis</td>
<td>Fourman and Lesson, 1958</td>
</tr>
<tr>
<td>Paroxysmal cerebral dysfunction</td>
<td>Epstein and Levitin, 1959</td>
</tr>
<tr>
<td>Congenital cerebral malformation</td>
<td>Epstein et al., 1961</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>McCray and Macouley, 1957</td>
</tr>
<tr>
<td>Porphyria</td>
<td>Grumer et al., 1962</td>
</tr>
<tr>
<td></td>
<td>Ludwig and Goldberg, 1962; 1963</td>
</tr>
<tr>
<td></td>
<td>Hellman et al., 1962</td>
</tr>
<tr>
<td></td>
<td>Recant and Lacy, 1963</td>
</tr>
</tbody>
</table>
the majority of tumors associated with inappropriate antidiuresis, may produce other biologically active materials with manifestations of adrenal cortical hyperfunction (Huth, 1961) and hypercalcemia (Lee, Jones, and Barreclough, 1964). These observations seem to lend credence to the possibility that many malignant lung tumors associated with inappropriate antidiuresis may themselves produce an antidiuretic hormone.

In Case 1 the lack of dilution following a water load and the administration of ethyl alcohol, a known inhibitor of vasopressin release, suggests that the mechanism for release of antidiuretic substance was not responsive to these suppressive stimuli and that the source of the hormone maintained a degree of autonomy. Similarly, other instances in which alcohol was administered under comparable circumstances in patients with carcinoma of the lung (Amatruda et al., 1963; Thorn and Transbol, 1963; Bower and Mason, 1964), central nervous system disease (Epstein et al., 1961; Goldberg and Handler, 1960), and intermittent porphyria (Hellman, Tschudy, and Bartter, 1962) failed to result in a dilute urine. A patient with carcinoma of the lung (Thorn and Transbol, 1963) and another with hypothyroidism (Goldberg and Reivich, 1962) demonstrated transient dilution of the urine with alcohol, although dilution was submaximal, indicating a defective mechanism for suppression of vasopressin.

The sustained production of a physiologic excess of antidiuretic substance by tumors of the lung is indicated by the absence of further concentration of the urine with administration of exogenous vasopressin (Amatruda et al., 1963; Bower and Mason, 1964). This is further suggested in a less direct manner by the observation in Cases 1 and 2 that over a long period of time there was a highly significant direct linear correlation (p. < .001) between the total solute concentration of the 24-hour urine collections and the daily serum sodium, irrespective of other considerations known to influence concentrating ability. Such a relationship has been described with prolonged administration of vasopressin and water (Jaenicke and Waterhouse, 1961) and the same tendency was noted in a patient with carcinoma of the lung (Schwartz et al., 1957). Thus, with continuous secretion of antidiuretic substance in maximal amounts, this correlation of serum sodium over a wide range with urine solute concentrations could, according to current concepts of the concentrating mechanism (Gottschalk, 1964), reflect primarily the state of tissue hydration, specifically that of the renal medulla.

**“Cerebral” Hyponatremia**

Case 2 is representative of the occurrence of hyponatremia in a wide variety of disorders affecting the central nervous system (Peters et al., 1950; Sims et al., 1950; Rapoport et al., 1951; Harrison et al., 1952; Welt et al., 1952; Cort, 1954; Cheek, 1956; Arblaster and Whitehead, 1957). More recently reported cases (McCorry and Macauley, 1957; Fourman and Lesson, 1958; Carter et al., 1959, 1961;

### TABLE 4
Sequential Water Loading-Saline Loading Tests in Case 2

<table>
<thead>
<tr>
<th>Date</th>
<th>Plasma Osmolality</th>
<th>Urine Osmolality</th>
<th>Urine to Plasma Concentration Rate</th>
<th>Urine Flow Ratio</th>
<th>Urine-Sodium Concentration</th>
<th>Urine-Sodium Excretion Rate</th>
<th>Free Water Clearance</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/7/62</td>
<td>242</td>
<td>599</td>
<td>2.48</td>
<td>1.6</td>
<td>175</td>
<td>280</td>
<td>-2.37</td>
<td>Baseline Water Load</td>
</tr>
<tr>
<td></td>
<td>235</td>
<td>301</td>
<td>1.28</td>
<td>7.8</td>
<td>107</td>
<td>203</td>
<td>-1.90</td>
<td>Saline Load</td>
</tr>
<tr>
<td></td>
<td>245</td>
<td>320</td>
<td>1.31</td>
<td>12.4</td>
<td>131</td>
<td>1624</td>
<td>-2.80</td>
<td></td>
</tr>
<tr>
<td>2/27/62</td>
<td>260</td>
<td>729</td>
<td>2.80</td>
<td>0.7</td>
<td>152</td>
<td>106</td>
<td>-1.26</td>
<td>Baseline Water Load</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>539</td>
<td>2.16</td>
<td>2.2</td>
<td>156</td>
<td>343</td>
<td>-2.55</td>
<td>Saline Load</td>
</tr>
<tr>
<td></td>
<td>288</td>
<td>514</td>
<td>1.78</td>
<td>2.0</td>
<td>218</td>
<td>436</td>
<td>-1.98</td>
<td></td>
</tr>
<tr>
<td>5/14/62</td>
<td>264</td>
<td>480</td>
<td>1.82</td>
<td>1.6</td>
<td>108</td>
<td>173</td>
<td>-1.32</td>
<td>Baseline Water Load</td>
</tr>
<tr>
<td></td>
<td>252</td>
<td>243</td>
<td>0.96</td>
<td>6.4</td>
<td>84</td>
<td>538</td>
<td>+0.48</td>
<td>Saline Load</td>
</tr>
<tr>
<td></td>
<td>234</td>
<td>275</td>
<td>1.18</td>
<td>7.3</td>
<td>99</td>
<td>723</td>
<td>+1.09</td>
<td></td>
</tr>
<tr>
<td>5/7/63</td>
<td>244</td>
<td>510</td>
<td>2.09</td>
<td>1.6</td>
<td>110</td>
<td>176</td>
<td>-1.70</td>
<td>Baseline Water Load</td>
</tr>
<tr>
<td></td>
<td>256</td>
<td>200</td>
<td>0.78</td>
<td>10.0</td>
<td>84</td>
<td>840</td>
<td>+1.20</td>
<td>Saline Load</td>
</tr>
<tr>
<td></td>
<td>280</td>
<td>236</td>
<td>0.84</td>
<td>24.4</td>
<td>99</td>
<td>2416</td>
<td>+4.10</td>
<td></td>
</tr>
</tbody>
</table>
of Carter et al. and in our Case 2. However, after some months, it became apparent that the patient could, sporadically, though by no means appropriately, produce a dilute urine, with a continued, though less severe, tendency to plasma hypotonicity, if excess fluid intake was not scrupulously avoided. As in the patient of Carter et al. we found that the patient could now (table 4) partially suppress vasopressin activity under the stimulus of plasma volume expansion (following hypertonic saline infusion), producing a urine slightly hypotonic to plasma. The patient still did not, however, dilute his urine normally in response to dilution (following water loading).

Summary

We have reviewed some of the features of hyponatremic syndromes, unassociated with sodium retention and edema, but associated with primary water retention. The syndromes were probably caused by excessive vasopressin activity, in the presence of normal circulatory, renal and adreno-cortical function. Underlying diseases, including bronchogenic carcinoma, head injury, and tuberculous meningitis, illustrated the diverse etiologic bases of this condition.

References


Some Malpractices in Medical Statistics

S. JAMES KILPATRICK, JR.

Department of Biometry, Medical College of Virginia, Richmond

Since it is now fashionable for papers in medical journals to contain statistical notations, it also follows that in a certain fraction of these the statistical content is wrongly applied. These malpractices may be classified as numerical, statistical, and methodological. To illustrate some of the most commonly occurring errors the following examples are given.

Numerical Conventions

The number of decimals given in an observation should show the accuracy of the measurement. For example, blood pressure is measured to the nearest mm Hg. However, if one comes across a systolic blood pressure of 123.2, one is entitled to expect that the person used a sensitive manometer which could read to tenths of a mm Hg. Another example, which is especially relevant at Medical College of Virginia, is the question of how many decimals should be shown in students' standard scores.

It is generally assumed that, when repeated observations are made, the person collecting the data is measuring to the same degree of accuracy throughout. The same number of decimal points should therefore be given. Thus, if one were describing the elevation of systolic blood pressure, where this is calculated as "after treatment minus before treatment," one might measure to one decimal, e.g., 0.4 mm Hg. In this case one would expect that a zero reading be given as 0.0. This would mean that no detectable difference was observed to the nearest tenth mm Hg. An anomaly arises when measurements made to the nearest fraction are converted into decimal notations. Thus, if body weight was measured to the nearest quarter pound because the quarter pound was the smallest weight on a scale, one might want to record a weight of 170¼ as 170.25 lb. This figure, however, suggests that weight was measured to the nearest hundredth of a pound, which is not the case. There is no easy solution to this anomaly except to work in the basic units, in this case quarter pounds. In this way decimals are eliminated: 170¼ would then be given as 641 quarter pounds.

Another useful convention regarding the numerical presentation of data is that comparative statistics should be expressed to the same base. In a recent article on cystic fibrosis of the pancreas, an author used on the same page odds, fractions, and percentages as are exemplified in the following hypothetical extracts:

"From an earlier paper we showed that the odds of having a further affected child were 1/13. In this study the proportion of affected children (excluding the propositus) was 8/57." The difficulty of comparing 1 in 13 with 8 in 57 can be alleviated by writing "From an earlier paper we showed that the proportion of having a further affected child was 0.1 or 1/14. In this study the proportion of affected children (excluding the propositus) was 0.14 or 8/57." It is now apparent that the current study revealed a slightly higher frequency of affected children than the older study, but that this was unreliable because of the small number on which the first estimate was based, as is revealed by its expression to only one decimal point.

"The pH of sweat in 11 of the normal sibs was measured, and this was found to be elevated in 5 (45.45%)." This might better be written as "The pH of sweat in all of the normal sibs was measured and this was found to be elevated in about 0.5 (5/11)." The well-known tendency to express everything in terms of a percentage leads to a spurious degree of sensitivity if the base is much less than a hundred. Moreover, percentages are often grossly misleading especially when no denominator is given. Consider the statement "43% of patients in the current study with regional ileitis had blood group O. This was lower than the 56% reported by our earlier study." On the face of it this would suggest that regional ileitis is changing its relationship to blood group O. However, when one realizes that the 43% is based on 3 patients out of a total of 7 having blood group O and the 56% based on 5 out of 9, the difference is immediately seen to be unimportant.

Wrong Denominators

Percentages may also be misleading because they are expressed in terms of the wrong denominator. Mainland (1964) quotes an example from the British Medical Journal in which 139 members of the Woman's Royal Air Force who showed temporary amenorrhea...
used. Perhaps the most serious criticism of the use of the mean is that it covers up what was actually done. A mean may not reveal, for example, that three observations of a given response were made and the figure reported represents the average of the two of these which were closest together. Here the investigator is essentially throwing away one-third of his information. Again, an average says nothing about the underlying distribution which is too often assumed to be normal.

Estimations of Accuracy

A convention has become established of reporting statistics plus or minus a small number. This convention may have been borrowed from engineering or laboratory sciences in which the number following the plus or minus sign is an estimate of the degree of accuracy of the foregoing figure. However, nowadays this small figure usually represents a statistical estimate of variability. The question immediately arises of what estimate this is. Thus, the mean hemoglobin of four aliquots of blood may be given as 10.5 ± 0.2 grams per 100 ml. This figure of 0.2 could either represent a measure of variability (the standard deviation) of the four observations around the mean, or it might refer to an estimation of the variability of this mean and others, based on four estimations around the true value of hemoglobin for this pool of blood (the standard error). One can only discriminate between these two alternatives in the light of other information given in the report or in the context of the use of the 10.5 ± 0.2. There have been occasions in which statistic comparable to 0.2 was calculated as the standard error of the mean, that is, it represented the accuracy of the mean about the true value, but this figure was subsequently used as though it described the variability of the original observations about the sample mean, giving thus, a spurious degree of reproducibility to the technique.

Assumptions of Normality

Parametric statistical techniques are based largely on the assumption of an underlying normal distribution. In a large sample, the assumption of normality can be tested directly. Most medical applications of parametric statistical methods are made, however, to small samples in which the normal assumption cannot properly be tested. Many practitioners of statistics today prefer not to have to rely on an assumption as the cornerstone of their analytical methods. Hence, the increasing tendency to use non-parametric or distribution-free procedures. The results of this new approach are reflected in statistical tables. For example, *Documenta Geigy* (1962) gives exact confidence limits for a sample proportion and exact $\chi^2$ values for $2 \times 2$ contingency tables for sample sizes up to 60.

Inappropriate $P$ Values

The use of $P$ in medical journals has become so widespread that it is perhaps useful here to redefine how this is used and what it means. In terms of a comparative trial such as the comparison of two drugs, one may assume generally, for example,
1) that the two treatments have in reality no different effect,
2) that patients or subjects are allocated strictly at random to one or other of these treatments,
3) that the distribution of the response to therapy follows a normal distribution,
4) that the variability of responses is the same in the groups compared.

The statement "$P < 0.05$" then means that the probability is less than 5% of finding a difference as great or greater than that observed due to random sampling variation. Such a low probability is interpreted as spuriously low because one of the four assumptions is not warranted. If the first assumption is wrong, then in fact there is a difference between the effects of treatment.

The statement "$P < 0.05$" clearly then does not prove the reality of treatment differences. Other interpretations are always possible unless the other three assumptions are known to hold. Even then, we always have the possibility (even though this is unlikely) that the observed difference was due to random sampling variation and that no real treatment difference exists.

Probability values should not then be calculated or quoted when the four assumptions given above or some other set of assumptions previously specified are unrealistic. An example of the inappropriate use of $P$ (unhappily this is a real example) occurred in a teaching handout to medical students in a British university. In this handout, statistics were given on the differential death rates from leukemia in males and females. This was followed by the statement, "These sex differences are clearly significant ($P < 10^{-19}$)." Such a statement is wrong since the equivalent assumptions to 2), 3), and 4) in this situation are not warranted. Moreover, the statement is superfluous. There is clearly a difference in these population-based sex-specific death rates from leukemia.

Significance of Repeated Tests

Not only are tests of significance inappropriately applied to surveys, but often by the multifactor nature of the data and the lack of specific hypotheses, batteries of significance tests are run rather than the appropriate multivariate analogue. Example: Suppose there are 15 different items in a survey. An investigator (especially one who has ready access to a computer and a suitable program) might ask for correlations between every pair of variates.
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all, he has asked for \((15 \times 14)/2 = 105\) values. If the 5\% level of significance is applied throughout, approximately five of the 105 values will be sufficiently large to be judged technically significant even though the 15 items are uncorrelated in the population.

Inclusion of Pilot Data in a Subsequent Experiment

This is another malpractice somewhat similar to the above. The experimenter, by inclusion of pilot data, tends to prejudice the result of the experiment in terms of a favorable result. If a full-scale experiment is done, this is often because the results in the pilot have been encouraging. By adding pilot data, the experiment is already halfway towards technical significance. The analysis of a fullscale experiment should not, therefore, incorporate the pilot data except after deep consideration on the effects of such inclusion on the results.

Indices and Ratios

There are 125 indices listed in Dorland's Illustrated Medical Dictionary. This shows how fashionable it is to construct an index to report results. Statistically, there are a number of reasons why the use of indices should be avoided where possible. Consider a situation in which there are \(p\) different responses and \(q\) concomitant factors. Let the responses be \(y_1, y_2, \ldots, y_r\) where \(r \geq 1\), and the concomitant factors be \(x_1, x_2, \ldots, x_q\) where \(q \geq 0\). According to the number of \(y's\) and \(x's\), the research worker tends to use a simple ratio of \(y/x\), or a weighted sum \(\Sigma wy\) of the \(y's\), or a combination \(\Sigma wy/x\) or \(\Sigma wy/\Sigma wx\).

Ratios of \(y/x\) of a response \(y\) to a concomitant variate \(x\) are used extremely frequently, especially in therapeutic experiments, e.g., dose per kg body weight, or in the response to treatment of a part to the whole, e.g., change in weight of an organ/change in body weight. Two statistical criticisms of the use of ratios are that the ratio of two normal variates is not necessarily normal, and that the use of a ratio assumes a linear relationship. Rather than assuming a proportional relationship, it is better to estimate the relationship from the raw data. The original analysis of total acid output (T.A.O.) in rats on different doses of thyroxine (Blair et al., 1965) used T.A.O. mg/100g body weight. This was, however, unnecessary since further analysis showed body weights did not differ significantly among tested groups. If they had differed, the estimated regression of T.A.O. on body weight could have been used.

Weighted sums occur when there are a number of responses to be summarized and are of the form \(\Sigma wy\) where \(w\) represents the relative weight. Examples occur in diagnostic indices, e.g., in the clinical diagnosis of thyrotoxicosis (Crooks, Murray, and Wayne, 1959), in the diagnosis of rheumatoid arthritis (Mainland, 1964). Another example is the combination of standard scores of medical students. There is a dangerous tendency today to arbitrarily score subjective impressions. This leads to pseudo-quantification. In adding these scores, much information is lost in the process. Moreover, an additive combination is not necessarily the best because of the non-independence of different signs or symptoms. The determination of weights may also be made on an extremely ad hoc basis. Thus, it is often better to use a multiple classification or, if the responses are measured, to use multivariate techniques which, with the advent of fast digital computers, are becoming increasingly practicable.

Indices of the form \(\Sigma wy/y/x\) or \(\Sigma wy/\Sigma wx\), i.e., a weighted sum of ratios or the ratio of two weighted sums. An example of the first occurs in a retrospective study of births (Gruenwald and Minth, 1961).

The authors quote a mean ratio of placenta weight (PW) to a birth weight (BW). This might be expressed as \((1/n)\Sigma(PW/BW)\) where \(n\) is the number of births. Fortunately, the authors mistakenly calculate the mean placenta weight divided by the mean body weight.) The index formed from the ratio of two weighted sums is best exemplified by the Standardized Mortality Ratio (S.M.R.). This compares the mortality in an occupational or other group relative to a standard population. The most common misuse of this index is to form the ratio of two S.M.R.'s which has little meaning or justification since the weighting systems in two S.M.R.'s are different (Kilpatrick, 1963).

The use of indices and index numbers is not then recommended since no single figure can summarize all the relevant information in a comparison and since an index may be misleading because the tacit assumptions underlying its use may be wrong.

Design of the Investigation

All appearances to the contrary, most of the above malpractices are not serious in that they can be remedied by recourse to the original data if this is still available. Much more serious are those errors which affect the basic data recorded. Statisticians prefer to be consulted before the study is initiated in order to guard against this type of irremedial error.

The first objective of good design is to provide estimates of important effects which are independent of (not confounded with) other effects or influences. This is achieved by orthogonality in experimental design. In general, in a balanced design, one can estimate the effects of a factor averaged over different levels of other factors. The most frequent criticism made today by N.I.H. reviewers of proposals for medical research projects is that the proposed data will not unambigu-
In a hypothetical experiment to determine the effect of different diets, A, B, C, and the amount of water drunk on weight changes in rats, two different experimental strategies might be as follows in tables 2 and 3, where the numbers indicate the number of animals allocated to different treatment modalities of diet and water. A typical reaction is that the research worker would not do the experiment this way. He might use the single-factor design shown in table 4 to find which diet (say B) has the greatest effect on weight when there is no limitation on water and then repeat as follows in table 5. The above procedure implies that he is interested in the combination of diet and water which most increases body weight. If this is so, then the “one factor at a time” approach is inefficient, (more animals, more time) and may even be misleading because of interaction (Diet C with moderate water may give best results). Many efficient experimental designs are now available for use in medical research.

Recently, Box (1954) and others have developed designs for industrial multifactorial experiments with the objective of estimating that combination of treatment levels which maximises the response. There is every reason to believe that factorial and response surface designs could be usefuly applied in bio-medical research.

References


Laboratory analyses of biological materials are ranked in order of magnitude and summed across materials to give a list of laboratory scores. Under the assumed hypothesis that there is in fact no difference between laboratories, Monte-Carlo techniques are used to establish two-tailed 5% rejection limits for various combinations of laboratories and materials. The hypothesis that there is no difference between laboratories is rejected if any laboratory’s score lies outside the 5% limits.

Suppose that one needs to run a group of tests on a particular set of materials (chemical or biological), using a number of different laboratories, and wishes to insure before starting that the laboratories are reliable, i.e., that (a) they run the test according to required specifications or directions and (b) if they run the same test twice, they will get, within some tolerated instrument variation, the same results.

I shall develop a statistical test here based on the ranked laboratory results which does not assume that the data have any particular distribution. The basis for this work was done by Dr. W. J. Youden of the National Bureau of Standards (1963), but his work is done primarily with a view to industrial applications.

I have endeavored here to simplify the statistical procedures and to stress biological applications by way of examples.

Experimental

Suppose that we have a number of different materials, A, B, C, . . . which we want to be analyzed by a number of different laboratories, 1, 2, 3, . . . Consider material A which will be analyzed quantitatively by all the laboratories; rank the results as follows: give the rank 1 to that laboratory with the highest numerical result. Give the rank 2 to the laboratory with the next highest result, and so on until the laboratory with the lowest result gets the highest rank. Repeat this same procedure for all the materials and record the results in a format similar to table 1.

Example 1: There are seven protein materials to be analyzed by 15 different laboratories. Each material is analyzed quantitatively, and the laboratory with the highest result is given the rank 1, the next 2, and so on. The results are shown in figure 2.

If a tie exists, e.g. two laboratories tie for third place, assign the rank of 3.5 to each. If three are tied for fourth place, assign the middle rank (here equal to 5) to all three. The ranks are then added across (i.e., a sum of ranks, or a score, is found for each laboratory).

It is clear that the minimum possible score is 7 (highest every time) and the maximum score is 105 (lowest every time). The average score (just halfway between the maximum and minimum) is 56.

The obvious question that one should ask of the data is, “On the basis of the data shown, how can I judge which laboratories are consistently too high or too low in their analysis?” It is clear that laboratory 4 has a higher score than the others, and laboratory 13 has the lowest score, but are these differences attributable to physical reasons, i.e., faulty analysis, or are they merely due to natural (or random) variation? After all, one laboratory must be first and one must be last.

Statistical Analysis

The development of the theory is as follows. If no real reason exists for one laboratory to be higher or lower than the others, then the ranks of the laboratories for a particular material are random variables, and each laboratory is equally likely to get a particular rank. Furthermore, the scores of the laboratories will cluster around some central point (the mean). To find what kind of result might be obtained if in fact the laboratories are not really different, I will simulate the scoring procedure with a method known to be random, viz. take 15 cards numbered 1 through 15, shuffle them into a random order. Taking the top card, write its number against laboratory 1, write the next card's number against laboratory 2, and so on until all 15 cards have been viewed. Repeat this until seven sets of ranks have

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>A</th>
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been entered for each laboratory, then sum the ranks and obtain a score for each laboratory. If this process is repeated a large number of times, one will have an idea of how the scores are distributed.

With the aid of a computer, this process was repeated 1,000 times, giving 1,000 sets of 15 laboratory scores, or 15,000 scores. Examination of these scores showed that there were 26 scores of 23 or less and 24 scores of 89 or more, making a total of 50 lying outside the limits 23 to 89. These 50 make about one-third of 1% of the 15,000. There are 15 scores (laboratories) in any particular test, so that the chance of a given test having one of these extreme scores is 15 times 1/3%, or 5%.

Remember that the basic assumption was that no laboratory was different from any other, and that differences in scores were due to random variation. Thus, we can say that if there is no underlying reason for laboratories to differ, then 5% of the time we will have scores outside the range 23 to 89. Thus if one uses the limits 23 and 89 as a criterion for judging the laboratories, and there is no difference between laboratories, one will record a difference (i.e., make a mistake) one time in twenty.

Using these criteria, from table 2 one can conclude that laboratory 4 has results which are not due to random variation, i.e., there is some physical reason for laboratory 4’s high score.

The numbers 23 and 89 are called the 5% limits in the case of 15 laboratories and seven materials. It would be convenient to have similar limits for various combinations of materials and laboratories (table 3).

If, more generally, we have L laboratories and M materials, then the sum of the ranks for any laboratory is \((1 + 2 + 3 + \ldots + L)\), or \(L(L + 1)/2\), and the mean sum of the rank for each laboratory is the sum of the ranks divided by \(L\), i.e., \((L + 1)/2\); thus, the mean

\[
M^2 = \frac{L(L + 1)^2}{12} - \frac{L(L + 1) - L^2}{12}
\]

\[
S^2 = M \sum_{i=1}^{L} \left( i - \frac{L + 1}{2} \right)^2
\]

Denote by \(S^2\) the actual sum of squares about the mean of the scores for each laboratory. Friedman (1937) has shown that

\[
\chi^2_{L-1} = \frac{S^2}{\frac{S^2}{L-1}} - (L - 1)
\]

is distributed approximately as a \(\chi^2\) variate with \(L - 1\) degrees of freedom.

If there are no differences between laboratories, then \(S''/S^2\) should be close to unity, and if differences do exist, \(S''/S^2\) would be greater than unity. Thus, we compare \(\chi^2_{L-1}\) to the \(\chi^2\) variate with \(L - 1\) degrees of freedom and reject the hypothesis that no real differences exist for large values of \(\chi^2_{L-1}\).

From the figures in table 2, \(S'' = 1960, S^2 = 3030, \chi^2_{L-1} = \chi^2_{14} = 21.56.\) Since the 90% limit for a \(\chi^2\) variate with 14 degrees of freedom is 21.06, we reject the hypothesis of all laboratories being the same at the 10% level.

In summary, formula (2) provides a quick method for evaluating the data to see whether differences between laboratories exist. To find which is the offending laboratory, use of table 3 is required.

Example 2: Suppose that a number of volunteers is required for a breathing experiment, and that for some reason or other it is necessary to accept only those whose duration of apnea is average; neither too long nor too short.

If there are 14 volunteers, one might check the duration of apnea three times each. Thus measure the duration of apnea for each of the 14, rank them giving the one with the longest duration the rank 1, the next 2, and so on until the volunteer with the shortest duration is given the rank 14. Repeat this three times and sum the ranks for each volunteer. Table 3, with the vertical column at 14, and the horizontal at 3, gives the 5% limits, 4 and

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Average = 56
41. Thus if any volunteer's score falls outside of these limits, reject him as being non-average.

**Example 3:** If, instead of testing for a difference between laboratories, one wishes to test for a difference between for example 10 chemical analyzing machines using six different substances, then the routine is as before. Rank each substance by machine and add the totals for each machine. From table 3, the 5% limits are 14 and 52.

**Discussion**

It would appear more profitable at first glance, to leave the laboratory analysis results in their raw state, rather than ranking them, and to perform a straightforward ANOVA. However, such an analysis would have to assume the underlying normality of the data and would at the same time not have the advantages of simplicity inherent in this design. This ranking test appears to be a useful tool for the statistically unsophisticated to determine departures from "averageness."

**References**


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Effects of Salmonella Vaccination on Metabolism and Resistance to Infection of Rabbit Peritoneal Cells*

MARVIN J. ALLISON, ENRIQUE GERSZTEN, AND BLANCA SANCHEZ

Department of Pathology, Medical College of Virginia, Richmond

Earlier work by one of us (Allison, Zappasodi, and Lurie, 1962b) demonstrated that, after BCG vaccination in the rabbit, there was a period of depression in the metabolism of mononuclear cells from the peritoneal cavity. This period of depression in metabolism was associated with a depression in resistance to tuberculosis (Allison et al., 1962a). Later, the metabolism of the mononuclear cells of the host rose to a level considerably above normal, and during this period the host displayed an increased resistance to tuberculosis. Since other studies (Howard et al., 1959; Shaedler and Dubos, 1957) have shown that BCG vaccination also raises the resistance against a variety of heterologous infections, it was reasonable to assume that this alteration of resistance by BCG might be non-specific. Vaccination by other organisms might possibly produce the same effects on metabolism of mononuclear cells as well as host resistance. This study was designed to show the effect of another vaccine (Salmonella) on the depression of cellular metabolism and its corresponding depression of the host's resistance to an acute infectious process.

Materials and Methods

The rabbits used in these experiments were New Zealand white, purchased from local breeders, housed under standard conditions of temperature and environment, and fed on Purina rabbit chow. Water was available ad lib. The rabbits were divided into six groups of four to six animals. Group 1 served as controls for metabolic studies and received no vaccine. Group 2 received subcutaneously 1 ml of killed Salmonella vaccine containing 1,000 million typhoid, 250 million paratyphoid A, and 250 million paratyphoid B. Four days post-vaccination, this group was killed, and the peritoneal exudate was used for metabolic studies. Group 3 received two subcutaneous injections of the same standard Salmonella vaccine 30 days apart. The animals were killed four days after the second injection; the peritoneal exudate cells were used for metabolic studies. Group 4 received three injections of the standard Salmonella vaccine, 30 days apart. The animals were killed four days after the last injection; the peritoneal exudate cells were used for metabolic studies. Group 5 consisted of normal non-vaccinated animals injected intravenously with a suspension of Candida albicans. Group 6 was vaccinated with three injections of standard Salmonella vaccine spaced 30 days apart, and four days after the last injection these animals were infected with C. albicans. The animals infected with C. albicans were permitted to live for a maximum period of 10 days. At the 10-day period, the surviving animals were killed. The exudates were obtained using mineral oil as previously described (Allison et al., 1961) and all animals were killed by air embolism. The first four groups were used for
METABOLISM OF PHAGOCYTES AND RESISTANCE TO INFECTION

Metabolic measurements; the last two for resistance studies.

The enzyme studies were done with the Thunberg technique as described before (Allison et al., 1961). The following substrates were used: lactic acid, sodium succinate, sodium glycerophosphate, DL-β-hydroxybutyric acid, malic acid, glycerol, and α-keto-glutaric acid. Simultaneous measurements of endogenous respiration were also performed. Additional metabolic studies were performed, in some cases, using Smithies' vertical starch-gel method (Smithies, 1959) to separate the enzyme lactic dehydrogenase into its various isozyme components. The enzymatic activity was measured by a modified Nachlas substrate and quantitated with a microscope photometer scanner previously described (Allison, Gerszten and Sanchez, 1963).

The culture used to test the resistance of the animals was a strain of C. albicans recently isolated from a human case of candidiasis. The animals received a dose of 60 million organisms from a four-day growth of the Candida culture. When the animals died, a complete autopsy was done, and sections were taken for microscopic examination. Gomori-methanimine silver nitrate stain was used to visualize the Candida organisms in the tissue; hematoxylin and eosin stains were also done.

Results

The peritoneal exudates from the various groups of rabbits showed essentially the same numbers of total cells recovered and the same type of differential pattern. Approximately $600 \times 10^6$ cells were recovered for each rabbit; 97% of these cells were mononuclears.

Table I presents the values for the metabolic studies done on the peritoneal exudate cells. These values were expressed as the slope of the line for the reduction of methylene blue per 10 million exudate cells. It is evident from this table that a single injection of Salmonella vaccine causes a drastic reduction in the general metabolic activity, of a number of dehydrogenases, four days post-vaccination. The administration of a second injection of Salmonella vaccine approximately 30 days later caused a further drop in activity with most of the substrates studied. This period seemed to be the lowest point in metabolic activity, for a

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Group 1—Non-vaccinated.
Group 2—1 dose Salmonella vaccine. 4 days post-vaccination.
Group 3—2 doses Salmonella vaccine. 4 days post-vaccination.
Group 4—3 doses Salmonella vaccine. 4 days post-vaccination.
third injection given 30 days after the second did not cause any further decline in the metabolic activity of these cells.

In an attempt to determine whether this reduction in enzyme activity after vaccination with *Salmonella* was due to loss of enzyme or simply loss of some co-factor, extracts of the peritoneal exudate cells in vertical starch-gel were electrophoresed. They were then exposed to a lactic acid substrate using phenazine methosulfate and diphosphopyridine nucleotide as the electron accepter. An average value of 492 units was found for the control and 423 for the experimental, a difference which was not significant. Thus the reduction in activity must be due to a reduction or loss of co-factors rather than a loss of enzyme itself.

Since this experiment served to strengthen further the correlation of resistance to infectious disease with metabolic activity of mononuclear cells, evaluation was needed of the resistance of these animals whose peritoneal exudate cells were at a very low metabolic level.

The experimental rabbits vaccinated with *Salmonella* all died earlier than 60 hours post-infection with *C. albicans*. The non-vaccinated control animals lived for periods over 60 hours after infection, some surviving for as long as 10 days.

The vaccinated rabbits infected with *C. albicans* died of an acute generalized pneumonic process characterized by massive edema, severe congestion, and a moderate amount of macrophage infiltration. The control rabbits, on the other hand, exhibited a moderate interstitial pneumonia, but the principal lesions were located in the kidneys. These consisted of multiple, pinpoint abscesses. These lesions were not visible grossly in the experimental animals, although a small number were identified microscopically. Typical lesions found in the lungs and kidneys are illustrated in figures 1 and 2.

Fig. 1—Pulmonary findings (140X) in the rabbit. (A) Control, with minimal interstitial pneumonitis. (B) Experimental, with severe pulmonary edema, congestion and early bronchopneumonia.

Fig. 2—Candidiasis of the kidney (140X) in the rabbit. (A) Control, with extensive abscesses. (B) Experimental, with minimal infection. (C) The growth of the organisms in the control kidney is brought out by the G.M.S. stain.
The main pathological findings in the control animals were in the kidneys; in the vaccinated animals the major changes were in the lungs.

**Discussion**

Previous studies (Allison et al., 1962a and b) have demonstrated that in tuberculosis there is a close correlation between resistance of the animal and the level of metabolic activity of peritoneal mononuclear exudate cells. This study was concerned with the reduction in metabolism associated with a reduction in resistance to an acute disease such as candidiasis. The observations on the metabolism of mononuclear peritoneal exudate cells following *Salmonella* vaccination would indicate that a reduction in metabolism occurs at about the same time as that following vaccination with BCG. This reduction in metabolism is also associated with a reduction in resistance of the host to a heterologous acute infection caused by *C. albicans*.

The candidiasis in the experimental animals was primarily a pulmonary disease, whereas in the controls it was a severe kidney disease. Histopathological studies tend to support the findings of Evans and Winner (1954) with regard to the kidney lesions, but not with regard to the pulmonary lesions. The severe pulmonary edema that was a constant feature in the experimental animals at the time of death was an integral part of the disease process and could not be attributed to outside influences such as chloroform (as suggested by Evans and Winner). The presence of extensive pulmonary edema can be associated with the increased susceptibility of these animals, and this pulmonary disease was the primary cause of death. It would appear that the kidney disease as the main focus of the infection would depend on the host’s living for longer periods of time. Studies in mice by Hurley and Winner (1963) would also tend to support this hypothesis. The lengthened life span could be attributed to a greater resistance of the host, as in our case, or a reduction in the number of organisms injected, as in the studies of Hurley and Winner.

In previous studies, one of us (Allison et al., 1962b) noted that this reduction in metabolism was not due to a loss of enzyme, since reactivation of the enzymatic activity was possible by the addition of heated mononuclear cells from normal animals. In this present study we demonstrated that loss of enzyme was not the factor responsible for the reduction of the metabolic activity. If phenazine methosulfate was used as the hydrogen accepter, no difference in activity of the enzymes was noted between control and experimental animals. This work, and previous work using BCG as the vaccine, would tend to support a clinical impression among some physicians that patients are often more susceptible to colds, influenza, and other infectious diseases shortly following vaccination or immunization procedures. The mechanism of this reduction in resistance may be related to a depression in certain enzymes of the host due to the action of the vaccine, and a study of human leukocytes following immunization may be of value.

**Summary**

*Salmonella* vaccine caused a depression of metabolism of peritoneal exudate cells from rabbits. This effect was associated with a depression in resistance of rabbits to infection with *C. albicans*. This depression in metabolism is similar to the one previously noted following BCG vaccine and associated with a depression in resistance to tuberculosis.

**References**


A Simple Rapid Method for Determining Oxyhemoglobin Affinity: Illustration Using Blood from the Rhesus Monkey*

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The oxyhemoglobin affinity curve, also known as the "oxygen dissociation curve," "oxyhemoglobin equilibrium curve," or "oxygen-hemoglobin dissociation curve," describes the qualitative relation between oxygen pressure and oxygen uptake or release by hemoglobin. If oxygen pressure (P_o2) is measured in mm Hg, and oxygen uptake is measured as percent saturation of hemoglobin with oxygen, an oxyhemoglobin affinity curve can be constructed by varying only the P_o2. Of course, other factors which affect the affinity of hemoglobin for oxygen, such as salt ionic strength, pH, carbon dioxide tension, and temperature, must be kept constant. The curve is obtained by plotting the percent saturation of hemoglobin with oxygen (ordinate) against the oxygen pressure (abscissa), and drawing a best-fit line through the points measured. A preliminary report on the spectrophotometric method to be described was made by Hall (1935), Riggs (1951), Redmond (1955), Rossi-Fanelli and Antonini (1958), and in personal communication with Dr. Clyde Manwell at the University of Illinois, Urbana. As many as eight points on the curve can be determined in less than two hours.

Description of Apparatus

The set-up (see fig. 1) is as follows: a three-way stopcock is connected to a one-meter manometer (filled with mercury to the 500 mm mark), and a vacuum pump with a five-gallon carboy is placed between the pump and stopcock as a compensator-trap. Optical density readings are made in specially constructed tonometers in a spectrophotometer such as the Coleman, Jr. The tonometer is a 125 ml separatory funnel connected to a cuvette by a rubber stopper. When in use the stopper is affixed to the open end of the funnel which has a light coating of high-pressure vacuum grease on the outer rim only. An epoxy resin is used to seal the glass cuvette to the rubber stopper. With the stopcock on the funnel closed and the tonometer in place on one opening of the three-way stopcock, a partial vacuum can be pulled on the tonometer, and the oxygen pressure can be measured by reading the manometer. With the apparatus in position, an oxyhemoglobin affinity can now be determined.

Procedure

Preparation of Hemoglobin Solution

1. Centrifuge 5 to 10 ml of heparinized blood in a 15 ml centrifuge tube for five minutes at 3,000 rpm. Aspirate the plasma without disturbing the cells. Now add cold, physiological saline solution to the 15 ml mark on the tube, and mix gently. Place the tube in the centrifuge, and spin it for five minutes at 3,000 rpm. Repeat washing twice.

2. Hemolyze the washed cells by adding two parts cold distilled water, and 1 ml of toluene. Stopper the tube, and mix gently for several minutes. Now place the tube with the hemolysate in it in the centrifuge and spin at 5,000 rpm for five minutes. Aspirate the clear anduffy layers above the red hemolysate, and filter the hemolysate. Centrifuge the filtered hemolysate for one hour in a refrigerated centrifuge at 20,000 X g. If a refrigerated centrifuge is not available, use the highest speed possible on the available centrifuge; do not centrifuge for a prolonged period, however, or the hemoglobin will be denatured. Keep the hemolysate refrigerated until it is used. Hemoglobin prepared in this manner may also be used to "spot" in electrophoresis as well as in determining an oxyhemoglobin affinity curve.

* Supported in part by NIH grant H-8774.
SIMPLE METHOD FOR DETERMINING OXYHEMOGLOBIN AFFINITY

Oxyhemoglobin Affinity Curve

1. Place 1.0 ml of the hemoglobin (Hb) hemolysate (1 to 2 g% Hb) in a 15 ml centrifuge tube. Add 3.0 ml of phosphate buffer:
   a. Buffered sample of 6.8 pH and ionic strength (u) of 0.3:
      Add 24.98 g potassium phosphate, monobasic \((\text{KH}_2\text{HPO}_4)\), to 12.56 g potassium phosphate, dibasic \((\text{K}_2\text{HPO}_4)\), and make up to one liter with water. Mix hemolysate 1:3 (v/v) with buffer.
   b. Buffered sample of 7.4 pH and ionic strength (u) of 0.3:
      Add 9.58 g potassium phosphate, monobasic, to 19.13 g potassium phosphate, dibasic, and make up to one liter with water. Mix hemolysate 1:3 (v/v) with buffer.

2. Pipette 4.0 ml of the buffered hemolysate into a 125 ml separatory funnel (with stopcock closed). Attach the stopper which was affixed to the spectrophotometric cuvette to the open end of the funnel.

3. Open the stopcock on the tonometer to room air, or 100% oxygen (water-washed). Equilibrate the buffered hemoglobin solution for five minutes with gentle shaking in a water bath at the desired temperature, or use a platform shaker without a water bath for room temperature determinations. Allow the solution to flow back carefully into the cuvette, close the stopcock, and read the optical density (absorbency = \(A\)) on the spectrophotometer at a wave-length of 640 m\(\mu\). (This wave-length setting is obtained by previously making an absorbency plot for oxyhemoglobin, and one for reduced hemoglobin solution, and then determining by inspection where a large difference occurs between the two hemoglobin plots at a point on the graph. For mammal blood this is usually at a wave-length of 640 m\(\mu\)). The first reading is taken as 100% oxygenation and is represented as \(A_0\). The tonometer must be oriented in the holder of the spectrophotometer in the same manner for each subsequent reading; e.g., if the stopcock was aligned to the right, it should be read each time in that position. Do not leave the tonometer in the spectrophotometer any longer than it takes to record a reading, since heat too will denature hemoglobin. In taking readings, it may be more convenient to read percent transmittance (\(\%T\)) and convert it to optical density in the calculations.

4. Evacuation of the tonometer is done with the pump and mercury manometer apparatus described above. Connect the manometer to the tonometer with the stopcock closed; slowly open the stopcock on the tonometer until the left side of the manometer reads about 650 mm Hg. This corresponds to a \(P_{100}\) approximately 85 to 90 mm Hg. Close the stopcock of the tonometer, place it on the platform shaker and equilibrate it for five minutes. Now put it in the spectrophotometer and read the optical density as \(A_s\). Remove the tonometer, open the stopcock to room air, and then connect it to the manometer for...
Fig. 2—Oxyhemoglobin affinity at pH 7.4 and 6.8 for the Rhesus monkey (*Macaca mulatta*). Data for both curves were obtained at 23 C. The $P_o$ is 15 mm Hg at pH 7.4, and 23 mm Hg at pH 6.8.

5. Repeat the above procedure for $P_o$'s of approximately one-half for each successive reading, e.g., 40, 20, 10, 5, and 2 mm Hg in order that the points may be spread over the curve.

6. After the last $P_o$ value has been obtained, the pigment should be deoxygenated (reduced) completely. Close the three-way stopcock, and evacuate the manometer to the full extent of the pump. Now connect the tonometer to the three-way stopcock, and evacuate the tonometer. Be cautious here because prolonged evacuation might lead to loss of water with consequent increase in Hb concentration. Disconnect the tonometer, equilibrate for five minutes, and read the optical density as $Ar$.

7. The percent saturation of hemoglobin with oxygen ($S$) is calculated as: $S/100 = (Ar - As)/(Ar - Ao)$, where $Ar$, $As$, and $Ao$ represent, respectively, deoxygenated (reduced), partially oxygenated, and fully oxygenated hemoglobin.

8. The $P_o$ (oxygen pressure in mm Hg) is calculated as: $P_o = (barometer reading - manometer reading - vapor pressure of water) \times 0.2094$. (Note: the manometer reading is made by subtracting the reading of the right arm from the left arm.)

Data are presented in fig. 2 for oxyhemoglobin affinity of the Rhesus monkey (*Macaca mulatta*) as an example of applying the procedure described above. The curves were derived from determinations made at pH 7.4 and 6.8 at 23 C. The oxygen pressure at 50% saturation of hemoglobin with oxygen ($P_o$) was 15 mm Hg at the higher pH, and 23 mm Hg at the lower pH. Samples were run simultaneously in two different tonometers. The total time for making the determinations and plotting the curves was less than three hours.

**Summary**

Blood was obtained by cardiac puncture from the Rhesus monkey (*Macaca mulatta*). Hemolysates were prepared by filtration and centrifugation at 20,000 $\times$ g at 4 C. Oxyhemoglobin affinity curves were determined by a simplified spectrophotometric procedure on hemolysates diluted with phosphate buffers at pH of 7.4 and 6.8, and at an ionic strength of 0.3. The spectrophotometric readings were made at a wavelength of 640 m$\mu$ and at a temperature of 23 C. The oxygen pressure at one-half saturation ($P_o$) for Rhesus homoglobin at a pH of 7.4 was 15 mm Hg, and at the more acid pH of 6.8, 23 mm Hg. Using this spectrophotometric method, as many as eight points can be obtained and plotted, so that an oxyhemoglobin affinity curve can be constructed, in less than three hours.

**References**


A Tissue Culture Perfusion Chamber with a Substratum of Reconstituted Collagen*

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Since the earliest days of tissue culture research, one of its primary objectives has been the development of an in vitro system that would enable complex adult tissues to be cultured for extended periods of time. A number of different approaches to this problem have been devised over the years (Fell and Robison, 1929; Wolff and Haffen, 1952; Chen, 1954; Trowell, 1954, 1959; Grobstein, 1956; Schaffer, 1956; Jensen et al., 1964). According to Trowell (1954) the inability to supply sufficient oxygen to the deeper cells within the tissue is the main limiting factor in long-term tissue culture. Consequently, the basic approach in all of the systems offered to date has been to support the tissue as close as possible to the air-medium interface in an attempt to provide the tissue with the maximum amount of oxygen. Most variations in the different proposed techniques concern the means of supporting the tissue at the air-medium interface and the nature of the supporting material.

The long range objective of this study was to develop a culture chamber in which the tissue can be exposed not only to the air-medium interface above, but also perfused with oxygen rich medium from beneath. The immediate problem then became the designing of a system in which the tissue could be supported within a “perfusion-type” chamber. It was decided that the basic compatibility of the various components of the experimental system could be evaluated by the chamber’s performance in conventional cell culture. The results of these initial studies suggested a potential immediate value of the system in cell culture research and as such form the basis for this report.

Materials and Methods

The experimental system consisted of a modified Sykes-Moore (1960) perfusion chamber in which a collagen-coated stainless steel screen could be mounted. The coated screen divided the interior chamber into a shallow upper and deep lower portion. The tissue to be grown was explanted onto the collagen membrane in the upper chamber. Following assembly, the entire chamber, upper and lower portions, was filled with culture medium (fig. 1).

Perfusion Chamber

Ten Sykes-Moore tissue culture chambers were modified by the supplier (Bellco Glass Company, Vineland, N. J.) to 10 mm depth rather than the standard 5 mm. Ten standard Sykes-Moore chambers served as controls.

Collagen-coated Screens

Reconstituted rat tail collagen was prepared according to the techniques of Ehrmann and Gey (1956). The firm collagen gel produced by long dialysis (5 to 8 days) was preferred to the liquid variety.

* Supported by American Cancer Society In59E-3486 and Gen. Res. grant FR 5345-04.
The gel was cut into slices with a "kitchen-type" egg slicer and placed on thoroughly cleaned discs cut from stainless steel screen. The coated discs were supported on slender glass rods in the bottom of open petri dishes which were in turn placed in a chemical dessicator. The gel dried in approximately three days to a thin, transparent membrane a fraction of a millimeter thick that adhered tightly to the screen. The screen discs were cut from sheets of 24 mesh "Tensibolt" stainless steel bolting cloth (Newark Wire Cloth Company, Newark, N. J.). The collagen-coated screens were sterilized before use by either soaking for several minutes in 70% ETOH or exposing them at approximately three feet for 24 hours to ultraviolet light from a G15T8, 15-watt germicidal lamp. There was no detectable adverse reaction of the membranes to either treatment and both gave satisfactory results. Before use, the coated screens were equilibrated with double strength physiological saline (Hank's balanced salt solution) for 24 hours and rinsed one hour in the final culture medium. All tissue culture reagents used were stock items obtained from Grand Island Biological Company, Grand Island, N. Y.

Assembly of Chambers

Before the introduction of the tissue onto the membranes, the chambers were assembled completely except for the upper coverslip and the retainer ring (fig. 1). Three to five explants of either hamster kidney or cheek pouch tissue, each approximately 1 mm³ in size, were then placed on the collagen surface. The explants were arranged in a circle a short distance from the center of each screen. The top coverslip and retainer ring were put in place and partially tightened. A "vent" needle placed in the chamber before final tightening of the retainer ring virtually eliminated breakage of coverslips which was initially a major problem. Likewise, the introduction of a vent prior to disassembly prevented the disturbance of the growing tissue by the sudden release of pressure.

Following final assembly, the chambers were filled with culture medium consisting of "Eagle MEM" medium plus 15% fetal calf serum to which penicillin and streptomycin (100 units of each per ml of medium) were added. Uniform filling of both the upper and lower chambers was facilitated by perforating the collagen membrane in several spots before completing the assembly. Plasma clots on the bottom coverslip of the control chambers were prepared by mixing equal portions of cockerel plasma and chick embryo extract.

Microscopic observations were made with a Zeiss Phase Contrast Microscope with a long focal length condenser and a long focal length, water immersion 40× objective. This optical system permitted viewing the cultures without inverting the chambers. Observations were made at daily intervals for three to four days.

Determination of Chamber Efficiency

The speed with which an explant exhibited proliferation, and the number of proliferating explants in a particular chamber, were taken as criteria for the efficiency of the chamber. Proliferation was considered synonymous with growth and was assumed to have occurred when a minimum of six distinct cells with their nuclei could be observed beyond the edge of the primary explant. Doubtful cases were assumed negative.

Results

A total of six individual experiments were run. In the first four, various techniques and media were tried and modified before the methods reported here were adopted. Experiments five and six were run under essentially standardized conditions and are thought to reflect accurately the relative performance of the test systems. In each series, those cultures represented as growing on plasma were grown in
standard Sykes-Moore chambers (hereafter designated as small chambers) while those grown on collagen were grown in the experimental chambers (hereafter designated as large chambers).

**Experiment Five**

**A. Cheek Pouch:** Within 72 hours all of the chambers, both small and large, contained one or more explants that exhibited growth. This indicated that both types of chambers were capable of supporting growth. There was, however, considerable difference in the response of the individual explants to the two different culture chambers. Only 55% of the explants in the small chambers had exhibited growth within 72 hours as compared to 75% of those in the large chambers. Furthermore, of the successful explants in the large chambers, 45% of them were established within 24 hours (figs. 2, 3, and 4) and all of them were established by 48 hours. The cultures in the small chambers, on the other hand, showed no growth during the first 24 hours, and 67% required 72 hours.

**B. Kidney:** The kidney explants were more erratic in their behavior than those of cheek pouch. As can be seen in table 1, none of the cultures became established until 72 hours when 72% of the large chamber cultures appeared, as opposed to only one explant in the small chamber. Although no attempt was made to classify the various outgrowths morphologically, there was a striking difference between many of the kidney cultures and those of cheek pouch. The difference was particularly evident at the leading edge of the culture where the kidney cultures frequently exhibited a serrated boundary preceding narrow, spindle-like cells (figs. 5 and 6). The cheek pouch cells showed a typical epithelial pattern with broad “fan-like” pseudopodia usually preceding the sheet.

**Experiment Six**

**A. Small Chamber (Plasma Substratum):** Some growth occurred in six of the eight chambers. But with one exception which yielded two explants, only a single explant became established in each chamber by 96 hours of incubation. Of the 31 initial explants, only seven (23%) exhibited growth during the period of observation, and none of the explants became established within the first 36 hours of incubation.

**B. Large Chamber (Collagen Substratum):** 68% (22 of 32) of the successful explants were established within 24 hours. These explants were distributed among eight of the 10 chambers. Ten additional explants exhibited growth by the end of 36 hours for a total of 32 successful explants in nine of the ten chambers. Three of the chambers had 100% success in the establishment of the initial explants and in three others, four out of the initial five explants were successful.

**Discussion**

The broad field of tissue culture research is generally considered to encompass two rather well-defined specialties, cell culture and organ culture (Dawe, 1963). As mentioned earlier in this paper, a variety of organ culture techniques have been proposed which attempted to improve the availability of oxygen to tissues and organs growing in vitro. None of them, however, considered perfusing an oxygen-enriched medium through the system. On the other hand, a wide variety of “perfusion-type”
tissue culture chambers have been utilized in "cell-culture" research. (For an extensive review of perfusion chamber literature, see Dawson, 1963.) Analysis of the many proposed chambers indicated that with a few minor exceptions, the "Rose chamber" (Rose et al., 1954) and the "Sykes-Moore chamber" (Sykes and Moore, 1960), between them, incorporate all the basic advances of the earlier chambers. In turn, the Rose and Sykes-Moore chambers are themselves quite similar in design. The Sykes-Moore chamber was chosen as the basic unit in the present study because it more nearly satisfied the preliminary requirements developed in the planning stages of this project, and it was available commercially (even in modified form) at considerably less cost than others.

As in the matter of culture chambers, there has also been considerable variation between the two tissue-culture specialties in the types of substrata used to support the growing tissue. Among organ culturists, Fell and Robison (1929) used plasma clots; Wolff and Haffen (1952) used gelled agar; Chen (1954) used rafts of floating lens paper, while Shaffer (1956) employed rafts of cellulose acetate mesh. Trowell (1954) introduced a fixed platform of tantalum or stainless-steel mesh which Jensen et al. (1964) covered with an open mesh "tea-bag" paper. The most successful cell culture studies, on the other hand, have utilized such substratum materials as clotted whole lymph (Harrison, 1907), sheets of perforated cellophane in a single layer (Evans and Earle, 1947) or in a bilaminate "sandwich" (Rose et al., 1958; Sandström, 1965). The bare glass surface of the culture vessel has also been widely used (Earle et al., 1951).

Recognizing the unphysiological nature of all the foregoing materials, Ehrmann and Gey (1956) introduced reconstituted hydrolyzed collagen as a substratum for cell culture. They demonstrated a marked improvement in the growth of a variety of cell strains cultured on collagen as compared to similar cells cultured directly on glass. Despite the seemingly logical choice of collagen as a substratum and the relative ease with which it can be prepared, this technique has not received the acceptance that would seem indicated.

A number of possible substratum materials other than collagen were considered in the initial planning of this project. Several of the materials given preliminary tests were Millipore filters, Silastic membranes, Teflon grids, and bare stainless steel mesh of various gauges from 16 × 16 to 200 × 200 mesh. None of these materials offered the combined advantages of the reconstituted collagen. An unexpected dividend of the use of collagen was its exceptional optical qualities which permitted high-resolution phase contrast microscopy.

The success of a culture system is measured both by the speed with which proliferation is initiated and its ability to sustain the resulting growth. It has been suggested that the lag between the time of explanting and the initiation of growth is a consequence of the "shock" to the explanted tissue from the mechanical trauma of surgery and the subsequent exposure of the tissue to the in vitro environment (Earle et al., 1954; Puck and Marcus, 1955). Thus, the more compatible the in vitro conditions, the more rapidly the tissue should adapt and begin to grow. The performance of the standard Sykes-Moore chamber including its ability to support active growth for extended periods has been well documented (Sykes and Moore, 1960). It was, therefore, felt that a valid test of the experimental chamber in this study would be a comparison of its ability to promote early growth of the explants relative to this same factor in the standard chambers. If this criterion is accepted, then the performance of the experimental chambers with the collagen membranes was far superior to that of the standard chambers with plasma-clot substrata. No attempt was made to substantiate these conclusions by statistical analysis since in many cases the number of samples was too small. Particular attention is called, however, to the total performance of the cheek pouch cultures. Not a single explant cultured on plasma clot exhibited proliferation within the first 24 hours while
63% (26 of 41) of the successful explants on collagen exhibited growth within that time. Likewise, the total number of successful explants on the two different substrata is interesting; 68% (41 of 60) for the collagen, 31% (13 of 42) for the plasma clot.

The performance of the experimental chamber in these studies verifies that the system is compatible with good cell growth and, in addition to its potential applications to cell culture research, should meet the basic requirements of the proposed organ culture system. The necessary modifications have already been worked out that permit eliminating the upper coverslip from this present assembly thereby converting it to an organ-culture chamber. Experiments are currently being designed that will utilize this chamber to test the hypothesis that improved growth of complex adult tissues will result when such tissues are perfused with oxygen-enriched medium.

### Summary

A tissue culture chamber is described that introduces the concept of a fixed platform within a “perfusion type” chamber. The platform is made of stainless steel mesh covered with a membrane of reconstituted collagen. The collagen membrane serves as the supporting substratum for the cultured tissue and is of such an optical quality as to permit phase-contrast microscopic observation of the tissue. The per-

### TABLE 1

The Performance of Large Chambers with Collagen Substratum Compared to Small Chambers with Plasma-clot Substratum.

<table>
<thead>
<tr>
<th>Experiment Five</th>
<th>Chamber</th>
<th>% Explants/Chamber</th>
<th>24 Hrs.</th>
<th>48 Hrs.</th>
<th>72 Hrs.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Cheek Pouch</td>
<td>Plasma</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>(Small chamber)</td>
<td></td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>2</td>
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<tr>
<td></td>
<td>Total</td>
<td>11</td>
<td>-</td>
<td>2</td>
<td>4</td>
<td>6 (55%)</td>
</tr>
<tr>
<td></td>
<td>Collagen</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>(Large chamber)</td>
<td></td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>12</td>
<td>4</td>
<td>5</td>
<td>-</td>
<td>9 (75%)</td>
</tr>
<tr>
<td>B. Kidney</td>
<td>Plasma</td>
<td>9</td>
<td>5</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>(Small chamber)</td>
<td></td>
<td>10</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>Total</td>
<td>15</td>
<td>-</td>
<td>-</td>
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<td></td>
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<td>5</td>
<td>-</td>
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</tr>
<tr>
<td></td>
<td>Total</td>
<td>11</td>
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<td>8</td>
<td>8</td>
<td>8 (73%)</td>
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<th>Experiment Six</th>
<th>Chamber</th>
<th>% Explants/Chamber</th>
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<th>36 Hrs.</th>
<th>96 Hrs.</th>
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<td>Plasma</td>
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<td>3</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>(Small chamber)</td>
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<td>2</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>1</td>
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<td></td>
<td>Total</td>
<td>31</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>7 (23%)</td>
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<tr>
<td>B. Collagen</td>
<td>Collagen</td>
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<td>5</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>(Large chamber)</td>
<td></td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>48</td>
<td>22</td>
<td>10</td>
<td>-</td>
<td>32 (67%)</td>
</tr>
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fusion chamber is a Sykes-Moore tissue culture chamber modified to twice its normal depth to receive the screen. Cell growth in the experimental chamber is superior to growth in the control chamber both in speed of proliferation and in the percentage of successful explants. The experimental chamber has immediate applications in cell culture and potential value as an organ culture chamber.

Acknowledgment

The excellent technical assistance of Mr. C. N. Christian III, a first-year dental student and a summer fellow in this laboratory, is gratefully acknowledged.

References


Marvin J. Allison (Effects of Salmonella Vaccination on Metabolism and Resistance to Injection of Rabbit Peritoneal Cells), a graduate of the College of William and Mary, holds a Ph.D. in microbiology and experimental pathology from the University of Pennsylvania. He is associate professor of clinical pathology at the Medical College of Virginia, where he has taught since 1961.

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Jack D. Burke (A Simple Rapid Method for Determining Oxyhemoglobin Affinity: Illustration Using Blood from the Rhesus Monkey) is professor of anatomy at the Medical College of Virginia and chairman of the section on cell biology in the new curriculum of the medical school. Dr. Burke was a fellow of the American Physiological Society at Duke University School of Medicine and taught at the University of Florida, Longwood College, and the University of Richmond before joining the MCV faculty. He earned his B.A. at the University of Tennessee, M.S. at West Virginia University, and Ph.D. (in physiology) at the University of Florida.

Alistair Cooke (The Patient Has the Floor) is a native of Manchester, England. He was educated in England, receiving his A.B. degree from Cambridge University in 1934. Subsequently, Mr. Cooke received fellowships to further his study in dramatic research at Yale and Harvard. On his return to England, he joined the staff of the British Broadcasting Company for whom he later came to the United States. Since 1945, he has been chief United States correspondent for the Manchester Guardian, while continuing his broadcasts for the BBC. Many Americans will remember Mr. Cooke's weekly "Omnibus" series as one of the most enjoyable and instructive programs.
Joseph T. Doyle (The Pathogenesis of Atherosclerosis and Coronary Heart Disease) was born in Providence, Rhode Island. He is a graduate of Harvard College and Medical School. His postgraduate clinical training on the Harvard Medical Unit at the Boston City Hospital was completed in 1949, after a tour of military service. He then moved to Atlanta, Georgia, where for several years he was engaged in the study of the human pulmonary circulation at the Emory University School of Medicine. He was briefly at Duke University School of Medicine before moving to the Albany Medical College in 1953, where he is professor of medicine and director of the Cardiovascular Health Center.

Enrique Gerszten (Effects of Salmonella Vaccination on Metabolism and Resistance to Infection of Rabbit Peritoneal Cells) is assistant professor of pathology at MCV where he has been since 1960. Dr. Gerszten was born in Buenos Aires, Argentina, where he received his medical degree in 1958. He took his training in pathology at Goldwater Memorial Hospital, New York, and MCV.

Carl Goldsmith (Inappropriate Antidiuresis) is assistant professor of medicine at Georgetown University Medical School and chief of the renal service of the Georgetown University Medical Division of the D. C. General Hospital. He was born in Massachusetts, received his A.B. degree at Harvard, and his M.D. at the University of Virginia. His major field of interest is sodium metabolism.

Thomas M. Harris (A Tissue Culture Perfusion Chamber with a Substratum of Reconstituted Collagen), a native of Louisville, Kentucky, is a graduate of Emory University (B.A.) and the University of North Carolina (Ph.D.). From 1960 to 1961, he was a research fellow at the National Institutes of Health. Dr. Harris taught at the University of Richmond before coming to MCV in 1964, as assistant professor of anatomy.
Robert J. Huebner (Virus Infections—What of the Future?) is a graduate of the University of Cincinnati and St. Louis University School of Medicine. Early in his research career, Dr. Huebner joined the U. S. Public Health Service in the Laboratory of Infectious Diseases, National Microbiological Institute. He served as chief of the Section on Virus and Rickettsial Diseases in the Laboratory of Infectious Diseases, and is now chief of that laboratory at the National Institute of Allergy and Infectious Diseases. Dr. Huebner is recipient of the Pasteur Medal from the Pasteur Institute, the Distinguished Service Medal from the U. S. Public Health Service, and an honorary L.L.D. from the University of Cincinnati.

S. James Kilpatrick, Jr. (Some Malpractices in Medical Statistics) is chairman of the recently formed department of biometry at MCV. He graduated from the Queen's University of Belfast, Northern Ireland and undertook postdoctoral training at Iowa State University. Before joining the faculty at MCV, Dr. Kilpatrick was a joint lecturer in the departments of statistics and social medicine at Aberdeen University, Scotland.

Richard H. Kirkland (Inappropriate Antidiuresis), associate professor of medicine at the Medical College of Virginia, is a graduate of the Schools of Pharmacy and Medicine at MCV. Dr. Kirkland took his hospital training in internal medicine at MCV and spent one year at the University of California in experimental radiology.

Eugene L. Klingler, Jr. (Inappropriate Antidiuresis) joined the department of medicine at the University of Mew Mexico School of Medicine in 1964, as instructor in medicine and clinical investigator at the Albuquerque Veterans Administration Hospital. A graduate of Tufts University School of Medicine, Klingler was a fellow of the Virginia Heart Association under Dr. Solomon Papper, when a portion of the studies reported in this issue was carried out.
Ruben G. Lancestremere (*Inappropriate Antidiuresis*) is clinical investigator at the Hospital de Clinicas (Teaching Hospital) of the University of Buenos Aires Medical School, Argentina. A graduate of the same university, Dr. Lancestremere was in the U. S. between 1959–1962 as fellow of the Massachusetts Heart Association at Tufts University and the Boston Hospital, and later as research fellow of the American Heart Association and the Roanoke Valley Heart Association at the renal and metabolic division of the department of medicine at MCV. From 1962 to 1965 he was Established Investigator of the National Research Council of Argentina. His primary field of interest is water and electrolyte metabolism.

Joseph H. Magee (*Inappropriate Antidiuresis*) is a graduate of the University of Virginia Medical School. He interned at the Johns Hopkins Hospital and was medical resident at the Philadelphia General Hospital and the Veterans Administration Hospital, Washington, D. C. He joined the Medical College of Virginia staff in 1957 as associate in medicine, and is now assistant professor at the Jefferson Medical College, Philadelphia.

Kenneth Mullen (*A Ranking Test in the Biological Sciences*) was born in London, England in 1939, received his B.A. from Western Reserve University, and his Ph.D. in mathematical statistics from Virginia Polytechnic Institute. He is presently assistant professor in the department of biometry at MCV and interested in the application of mathematical principles to biomedical problems.

Blanca Secades Sanchez (*Effects of Salmonella Vaccination on Metabolism and Resistance to Infection of Rabbit Peritoneal Cells*), a native of Cuba, received her M.D. from the University of Havana in 1951, and took her hospital training at the University of Havana Hospital Outpatient Clinic of Pediatrics. Dr. Sanchez came to the U. S. in 1962, and since then has been research assistant in the department of clinical pathology at MCV.
the “spasm reactor” in your practice
The Machine Age man still possesses a Stone Age stomach; sometimes the job of merely coping with today's environmental stress may prove too much. For some (the "spasm reactors" in your practice), tension, anxiety and worry may find expression through the voice of gastrointestinal or other smooth muscle spasm. To treat these patients with antispasmodics alone is often to miss the point of origin of their disturbance; to rely solely on tranquilizers often proves discouragingly slow or ineffective in relieving spasm and pain.

To quiet and quell Donnatal can promptly and effectively quell the spasm and quiet the tensions that trigger it. Prescribed by more physicians than any other antispasmodic-sedative, Donnatal continues to provide the classic answer.

The "Donnatal Effect" The characteristic, over-all effect of Donnatal has been observed in many thousands of children and adults, clearly establishing its value as a versatile sedative-antispasmodic. Outstanding in effectiveness, safety, economy, uniformity of composition and dosage convenience, Donnatal continues to be desired and prescribed by a majority of physicians.

In a multiplicity of indications Particularly useful when anxiety and tension accompany, aggravate or account for smooth muscle spasm, Donnatal is indicated for the symptomatic relief of recurring, persistent or chronic visceral spasm. More than two dozen distinct and separate indications for Donnatal are listed on page 869 in the current PDR.

Brief summary Blurring of vision, dry mouth, difficult urination, and flushing or dryness of the skin may occur on higher dosage levels, rarely on usual dosage. Administer with caution to patients with incipient glaucoma or urinary bladder neck obstruction. Contraindicated in acute glaucoma, advanced renal or hepatic disease or a hypersensitivity to any of the ingredients.

when the “gut issues” get to him he deserves

"the Donnatal Effect"

QUIETS THE STRESS/QUELLS THE SPASM

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Each Tablet, Capsule or 5cc. of Elixir</th>
<th>Extentab®</th>
</tr>
</thead>
<tbody>
<tr>
<td>hyoscyamine sulfate</td>
<td>0.1037 mg.</td>
<td>0.3111 mg.</td>
</tr>
<tr>
<td>atropine sulfate</td>
<td>0.0194 mg.</td>
<td>0.0582 mg.</td>
</tr>
<tr>
<td>hyoscine hydrobromide</td>
<td>0.0065 mg.</td>
<td>0.0195 mg.</td>
</tr>
<tr>
<td>phenobarbital</td>
<td>(¼ gr.) 16.2 mg.</td>
<td>(¼ gr.) 48.6 mg.</td>
</tr>
</tbody>
</table>

(warning: may be habit forming)
“Heart symptoms”—chest pain, tachycardia, arrhythmia—invariably alarm and preoccupy the patient, though they may be completely without organic basis. Such symptoms often are somatic masks of psychic tension, arising from constant encounters with stressful situations.

When the problem is diagnosed as emotionally produced, consider Valium (diazepam) as adjunctive therapy. Valium (diazepam) acts rapidly to calm the patient, to reduce his psychic tension and relieve associated cardiovascular complaints.

Neurotic Fatigue—the chronic tiredness resulting from emotional strain which so often accompanies psychogenic “heart” symptoms—also can be alleviated by this highly useful agent. Valium (diazepam) often achieves results where other psychotherapeutic agents have failed.

Valium (diazepam) is generally well tolerated, and usually does not impair mental acuity or ability to function. If side effects such as ataxia and drowsiness occur, they usually disappear with dosage adjustment.

Contraindications: Infants, patients with history of convulsive disorders or glaucoma.

Warning: Not of value in the treatment of psychotic patients, and should not be employed in lieu of appropriate treatment.

Precautions: Limit dosage to smallest effective amount in elderly patients (not more than 1 mg, one or two times daily) to preclude ataxia or oversedation. Advise patients against possibly hazardous procedures until correct maintenance dosage is established; driving during therapy not recommended. In general, concurrent use with other psychotropic agents is not recommended. Warn patients of possible combined effects with alcohol. Safe use in pregnancy not established. Observe usual precautions in impaired renal or hepatic function and in patients who may be suicidal; periodic blood counts and liver function tests advisable in long-term use. Cease therapy gradually.

Side Effects: Side effects (usually dose-related) are fatigue, drowsiness and ataxia. Also reported: mild nausea, dizziness, blurred vision, diplopia, headache, incontinence, slurred speech, tremor and skin rash; paradoxical reactions (excitement, depression, stimulation, sleep disturbances and hallucinations) and changes in EEG patterns. Abrupt cessation after prolonged overdose may produce withdrawal symptoms similar to those seen with barbiturates, meprobamate and chlordiazepoxide HCl.

Dosage—Adults: Mild to moderate psychoneurotic reactions, 2 to 5 mg b.i.d. or t.i.d.; severe psychoneurotic reactions, 5 to 10 mg t.i.d. or q.i.d.; alcoholism, 10 mg t.i.d. or q.i.d. in first 24 hrs, then 5 mg t.i.d. or q.i.d. as needed; muscle spasm with cerebral palsy or athetosis, 2 to 10 mg t.i.d. or q.i.d. Geriatric patients: 1 or 2 mg/day initially, increase gradually as needed.

Supplied: Tablets, 2 mg, 5 mg and 10 mg; bottles of 50 for convenience and economy in prescribing.

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