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CONTENTS

3  WALTER J. GEERAETS  THOMAS W. NOONEY  JOSEPH R. SVOBODA  FLORENCIO C. CHING
   Solar Retinopathy Following the Eclipse of March 7, 1970

8  DUNCAN S. OWEN, JR.  MARION WALLER  ELAM C. TOONE, JR.
   Rheumatoid Arthritis and Malignancy

11  IRA R. LEDERMAN  EDWARD R. BERRY  WALTER J. GEERAETS
   The Effect of Ruby Laser, Xenon-Light Coagulator and Diathermy on Vitreous Proteins

15  KEITH A. MANT
   Accidental Hypothermia in Medico-Legal Practice

19  ROGER A. GLOVER, JR.  CHAN H. PARK  FRED T. GIVEN, JR.
   The Effects of Hypothermia in Pregnant Rats: A Preliminary Report

23  Abstracts of Theses for Graduate Degrees
   MEDICAL COLLEGE OF VIRGINIA, JUNE 1969
Solar Retinopathy Following the Eclipse of March 7, 1970*

WALTER J. GEERAETS, THOMAS W. NOONEY, JOSEPH R.
SVOBODA AND FLORENCIO C. CHING

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In spite of extensive warnings by the news media prior to the solar eclipse on March 7, 1970, retinal burn injuries were expected to occur either in children too young to comprehend the dangers involved or in persons ignoring or disbelieving these warnings.

Eclipse burns have been described since ancient times, dating back to the fourth century BC. We read in Plato’s Phaedo of Rouse’s translation “I must be careful not to be affected like people who observe and watch an eclipse of the sun. What happens to them is that some lose their sight, unless they look at his reflection in water or something of that sort.” Probably the greatest number of retinal burns from eclipse watching have been reported in the 20th century. Several hundred cases of more or less severe ocular injuries were observed following the eclipse of April 17, 1912; as noted by Wendenburg (1914), Birch-Hirschfeld (1912), Böhm (1913), and others. Central scotomata of various degrees and severity were the usual findings; but, in addition, ring scotomata for colors were described for a series of cases by Jess (1913). The significance of metamorphopsia in patients with solar retinitis was discussed by Verhoeff, Bell, and Walker (1916). Work concerning intensities required for production of retinal lesions during sun watching had already been reported during the 19th and early 20th centuries [Czerny (1867); Deutschmann (1882); Herzog (1903); Verhoeff, Bell, and Walker (1916); and others]. More recent descriptions of solar retinitis have been reported by Rosen (1948), who listed a total of 23 cases.

About 40 per cent of the radiant solar energy reaching the earth is within the visible spectral range, and 55 per cent is in the nonvisible infrared region. Under normal conditions, the bright sunlight causes maximal constriction of the pupil; hence, the light intensity incident on the retina is greatly reduced. Moreover, painful photophobia experienced during direct observation of the sun elicits the blink-reflex (approximately 150 ms), thus providing further protection. The total amount of light entering the eye during observation of an eclipse is proportional to the percentage of the solar surface not obscured by the moon. However, the energy density per unit area on the retina remains the same; ie, although the image diameter of the sun on the retina is smaller, related to the portion of the sun hidden by the moon, the light remains equally intense over the image area the sun casts on the retina. Since during eclipse watching the pupil may not be constricted to the same degree as in looking at the sun under normal circumstances, the situation becomes even worse. This factor plays a particular role if inadequate filters are used, which, because of their darkness, may decrease the amount of visible light entering the eye and, thus, permit the pupil to dilate. At the same time, however, it would allow more nonvisible infrared radiation to enter the eye, possibly resulting in retinal burn injury.

Case Reports

Case 1

A 14-year-old Caucasian girl was examined for the first time five days after the eclipse on March 7, 1970. She gave a history of having watched the eclipse with her right eye in the Richmond, Virginia area shortly before 1:00 PM, estimating the time of viewing as having been about 15 to 30 seconds. No protective goggles or other filters were used. The patient initially noted a bright glare which after a few seconds became bluish and allowed for good and clear visualization of the eclipse. She discontinued her direct observation to answer a telephone call, at which time she noted that “Everything looked orange” (erythropsia) when viewed with the right eye. She then continued to watch the progress of the eclipse by using projection techniques. Following the eclipse the vision in her right eye was very hazy, and the next day she noted a dense black spot in the center of fixation. This spot increased somewhat in size over the next few days but had remained about the same for a period of 24 hours preceding consultation.

Ocular findings. On funduscopic examination, a small circular lesion was found, located in the center
of the right macula and covering the entire foveal region. The lesion appeared to be of a light orange color with some scarce pigmentation and a grayish center. The area immediately surrounding the lesion appeared somewhat "wet" and of darker coloration (Fig 1). No other pathology was noted.

Visual acuity. 20/200 OD improving to 20/70 by "scanning" single letters, and 20/20 OS. Normal pupil reaction. Pupillary diameter (daylight): 3.8 mm. Visual field plot using the Amsler Grid charts revealed a central scotoma 2 degrees in diameter with the aid of diagonal lines to maintain fixation (Fig 2). This size reduced to about 1 degree over the next six days. At this time her visual acuity for near at direct steady fixation was 20/200 distance equivalent, but equal to 20/20 in scanning individual digits.

Case 2

A Caucasian male, 30-years-old, was examined seven days after the eclipse on March 7, 1970. The patient stated that he had viewed the eclipse in Powhatan county near Richmond, Virginia intermittently with both eyes unprotected, about one minute at a time as the phenomenon developed. He continued observation to the completion of the eclipse. Estimated total time of viewing was three to five minutes. After total eclipse was reached he attempted to read but "could not make out the words exactly right." When looking at a person wearing a red garment from a distance of about 200 yards, he noticed fading of the red color to grey or even black. There was a sensation of slight film over his eyes.

Ocular findings. OD: Center of the macula showed a diffuse reflex near the nasal margin of the fovea with increased scattered pigmentation around the fovea, giving the small lesion a dark red appearance. All other ocular findings were normal. OS: A small circular lesion (about 1/10 disc diameter) covered the fovea, with some pigmentation, though less intense than in OD.

Visual acuity. 20/30 at distance, and 20/30 to 1 at near, for both OD and OS which improved to 20/25 over several more days. Pupil reactions were normal and the pupillary diameter (daylight) measured 3 mm in diameter OU. Visual field plots (Amsler Grid charts) indicated presence of dense central scotomata (diameter 1/2 degree) with a bluish outer edge surrounded by a whitish halo extending to 1 degree (Fig 3). Color vision was normal. There was some indication of metamorphopsia.

Case 3

A Caucasian male, 23-years-old, was examined seven days after the recent eclipse of March 7, 1970. The patient stated that he looked steadily at the eclipse with eyes unprotected, for a period of three to four minutes when about "¼ of the sun was left," avert-
ing his eyes then for awhile. Total viewing time was estimated at five to ten minutes. Beginning about 15 minutes after culmination of the eclipse, until late the same evening, the patient saw everything “red” (erythropsia). When reading he could only see the last few letters of words. The following morning he noted a black spot, about “the size of an orange” when looking toward the sky.

Ocular findings. Ophthalmoscopic examination revealed bilateral parafoveal hyperpigmentation and poor foveal reflex OU. All other ocular findings were normal.

Visual acuity. 20/50 OD for distance and 20/50−1 for near, improving to 20/30 over the next few days. OS was 20/30−1 for distance and 20/30−1 for near. Pupillary reactions were normal in both eyes. Pupillary diameter (daylight) measured 2.7 mm at the time of examination. Visual field plots (Amsler Grid charts) indicated presence of a dense black central scotoma (diameter 0.8 degree OD and 0.6 degree OS) with a gray outer ring extending to about 2 degrees (Fig 4). The adjacent black lines of the grid appeared somewhat fuzzy and distorted (metamorphopsia). No defect in color vision was noted.

Discussion

With an assumed irradiance of 71.7 mW/cm² at sea level, the energy density entering the eye with a pupil diameter of 3 mm can be calculated to be 5.1 mW/cm². This value would be 14.1 mW/cm² for a pupil diameter of 5 mm, increasing to 36.2 mW/cm² for a diameter of 7 mm. Based on calculations that 1.3 mW entering the eye will cause a 1°C temperature rise in the retina, the corresponding temperature elevations upon viewing the sun for the above given three pupil diameters would be approximately 3.9, 10.7, and 28°C respectively (Clarke, Geeraets and Ham, 1969). Expressed in power density on the retina for a given image diameter of the sun on the human retina of 158 μ, these values correspond to 21.8, 59.9, and 156.8 W/cm²; taking into account the ocular spectral characteristics for specific absorption in and reflection from the various structures (Geeraets and Berry, 1968) (Fig 5).

Eccles and Flynn (1944) gave a value above 50 cal/cm²/min for the production of a retinal solar burn in rabbits at an exposure time of 30 seconds, however, they used a telescope to enlarge the sun’s image on the retina about ten times. For the given retinal image size of the sun (158 μ in diameter) and an exposure time of 30 seconds, experimental data obtained in our own laboratories indicate that a power density of approximately 40 W/cm² is required for the production of retinal burn injury in the rabbit eye (Clarke, Ham, Geeraets, et al, 1969) (Fig 6). On the basis of these calculations and an estimated exposure time of
SOLAR RETINOPATHY

PERCENT ABSORPTION IN RETINAL PIGMENT EPITHELIUM AND CHOROID

![Graph of percent absorption in retina and choroid for light incident on the cornea.]

Fig 5—Per cent of spectral absorption in retina and choroid for light incident on the cornea.

30 sec during which the patient (Case 1) may have watched the eclipse with the unprotected eye, the pupil diameter should have been about 4 mm or larger to allow sufficient energy to be incident on the retina to result in a thermal lesion. The degree of ocular fundus pigmentation is, of course, another factor influencing the required energy for production of a retinal burn.

At the time the eclipse was observed by this patient (Case 1), approximately 60 per cent of the sun was still uncovered by the moon. However, as stated in the introduction, the energy density on the retina for the area on which the sun’s image impinged remained the same as if the entire sun had been observed. Only the size of the image was smaller, i.e., was proportional to the percentage of the unobscured portion of the sun.

The funduscopic examination and fundus photographs indicate that the lesion diameter is approximately 1.5 degrees or 400-450 µ. The larger lesion size in comparison to the calculated image size of the sun on the retina can be due, in part, to heat conduction from the site of exposure into the surrounding tissues, and to unsteady fixation. The central scotoma plotted by the patient (Case 1) at an observation distance of 28 cm on the Amsler Grid chart corresponds to a lesion size of approximately 540 µ in diameter. The difference between the first and second figure can be explained by reactive edema surrounding the lesion which was still present at the time of her first examination. This factor explains, as well, the decrease in visual acuity to 20/40 or better, provided a macular hole with concomitant central retinal detachment does not develop.

The relatively less severe macular lesions of the other two patients can be explained by the factor of pupillary diameter and possible variations in degree of pigmentation of the retina and choroid.

Summary

A case of unilateral retinal burn injury of the fovea and two cases of binocular injuries have been reported, in connection with the viewing of the eclipse of March 7, 1970. Some calculations of energy densities involved have also been presented.

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Rheumatoid Arthritis and Malignancy*

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In view of the fact that rheumatoid arthritis is considered to be a disturbance of the immune mechanism (Lawrence, 1965), we thought it seemed reasonable to direct a study of possible correlation with malignant diseases, which are also possibly related to immune mechanism disturbances. Calabro (1967), Lansbury (1953), Litman, et al (1966), and MacKenzie and Scherbel (1963) have reported the appearance of malignancy accompanying the development of polyarthritis. Calabro (1967) emphasized the importance of arthritis as a diagnostic warning of occult cancer.

Ragan and Synder (1955) noted that 2 per cent of 374 patients with rheumatoid arthritis, followed for more than five years, developed malignancy. However, he made no comparison to matched controls. Lansbury (1953) collected six cases of collagen disease which occurred in patients suffering from malignancy. MacKenzie and Scherbel (1963) studied the musculoskeletal symptoms of visceral malignancy in 77 patients. Only 18 of these had arthropathy, and the symptoms ranged from mild arthralgia to progressive polyarthritis. No rheumatoid nodules were present, and rheumatoid factor was present in only one patient.

Miller (1967) studied 17 patients with malignant lymphomas associated with a variety of so-called "immune diseases" such as rheumatoid arthritis, systemic lupus erythematosus (SLE), ataxia-telangiectasia, vasculitis, nephrotic syndrome, and dermatomyositis. He concluded that the same individual may be susceptible to both types of disease. In addition, Miller (1967) searched for cases of connective tissue disease in 1893 patients with solid malignant tumors and found 11. Eight were classified as rheumatoid arthritis, one as SLE, and one each as dermatomyositis and vasculitis. Among the 264 patients with lymphoproliferative neoplasms, only one case of rheumatoid arthritis was recognized among the five having diffuse connective tissue disease.

In none of these studies were the patients and the control population matched for age and socioeconomic status, nor was the possible influence of anti-inflammatory drug therapy on the incidence of malignancy taken into account.

The unique availability of private patient records covering a period of over 26 years with examination in all instances being done by either of two rheumatologists, plus long term follow-ups averaging over four years, provided an opportunity for a definitive study of the incidence of malignancy in patients with rheumatoid arthritis. These patients were controlled by carefully matched patients without rheumatoid arthritis, though suffering from a variety of arthritic disorders such as degenerative arthritis and gout.

Materials and Methods

A survey of the private patient files over a 26 year period showed 196 patients with rheumatoid arthritis whose ages were between 50 and 74 (mean age 60.3). These selected charts were carefully reviewed. There were 106 females and 90 males. The observation period ranged from one month to 24 years with a mean of 49.5 months. These patients had either classic or definite rheumatoid arthritis, according to the criteria of the American Rheumatism Association (Ropes, 1958). A rigorous selection resulted in loss of over 50 per cent of the patients originally thought to have rheumatoid arthritis. For comparison, charts of 125 patients with arthritis of a non-rheumatoid type—degenerative joint disease, primary gout, non-specific tendinitis, and psoriatic arthritis—and of the same age group (mean age 60.5) were reviewed. There were 72 females and 53 males. The observation period ranged from one month to 20 years with a mean of 56.8 months. An older age group was selected for both groups on the premise that the higher incidence of malignancies in this advanced age group would tend to make the results more valid in the relatively small number of patients. Cases of hypertrophic pulmonary osteoarthropathy were excluded from the study. All patients were, or had been, on the private service and in the middle or upper classes. Each patient had a complete history and physical examination performed, and those who were followed longer than one month had periodic complete examinations.

Laboratory studies performed on all patients included hemoglobin, white blood count and differential, erythrocyte sedimentation rate (Wintrobe), urinalysis, serum uric acid and rheumatoid pattern (latex flocculation, sensitized human cell, and sensitized sheep cell tests). Methods used include those previously described by Waller et al (1961), and the Venereal Disease Research Laboratory test (VDRL). If systemic lupus erythematosus was suspected, one or more LE cell tests were performed.† Appropriate X-ray studies were also performed on each patient.

* Supported by Public Health Service Research Grant AM 04549. This is publication number 30 from the Charles C. Thomas Arthritis Fund.

† Patients with positive LE cell tests were excluded from the study.
Results

A total of eight patients out of 196 with rheumatoid arthritis had systemic malignancies. Table 1 summarizes the main features of these cases. All cases had significant titers of rheumatoid factor. Six of the eight cases were receiving adrenocorticosteroid preparations at the time the malignancy was diagnosed, and all six had taken an equivalent of 5–10 mg of prednisone daily for over one year.

A total of five out of 125 patients with arthritis of a non-rheumatoid type were discovered to have systemic malignancies. Table 2 summarizes the main features of these cases. None had rheumatoid factor or were receiving adrenocorticosteroids.

Discussion

Correspondence with 12 clinicians throughout the nation, specializing in the treatment of connective tissue diseases, revealed that the majority felt that

---

### Table 1

Summary of 8 patients with systemic malignancies discovered in a study of 196 patients with rheumatoid arthritis in the 50-74 age group.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Malignancy</th>
<th>Length of RA prior to malignancy (years)</th>
<th>Rheumatoid Factor Latex SHC*</th>
<th>SSC**</th>
<th>Long term steroids (&gt;12 mos)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. A.M</td>
<td>74</td>
<td>F</td>
<td>Carcinoma of stomach</td>
<td>11 + 1:80</td>
<td>---</td>
<td>Yes</td>
<td>Metastatic disease</td>
<td></td>
</tr>
<tr>
<td>2. H.L.</td>
<td>63</td>
<td>M</td>
<td>Transitional cell carcinoma of urinary bladder</td>
<td>8 + 1:2048</td>
<td>1:2048</td>
<td>No</td>
<td>Metastatic disease</td>
<td></td>
</tr>
<tr>
<td>3. D.S.</td>
<td>68</td>
<td>F</td>
<td>Carcinoma of breast</td>
<td>4 + 1:1280</td>
<td>1:320</td>
<td>Yes</td>
<td>Metastatic disease</td>
<td></td>
</tr>
<tr>
<td>4. E.P.</td>
<td>68</td>
<td>M</td>
<td>Carcinoma of lung</td>
<td>17 + 1:640</td>
<td>1:160</td>
<td>No</td>
<td>Steroids—discontinued 6 yrs before development of malignancy</td>
<td></td>
</tr>
<tr>
<td>5. L.H.</td>
<td>65</td>
<td>F</td>
<td>Carcinoma of breast</td>
<td>10 + 1:1280</td>
<td>1:160</td>
<td>Yes</td>
<td>Peripheral neuropathy present</td>
<td></td>
</tr>
<tr>
<td>6. R.M.</td>
<td>51</td>
<td>M</td>
<td>Acute lymphatic leukemia</td>
<td>4 + 1:640</td>
<td>1:160</td>
<td>Yes</td>
<td>Pt. had not received phenylbutazone for 3 yrs</td>
<td></td>
</tr>
<tr>
<td>7. C.T.</td>
<td>74</td>
<td>M</td>
<td>Lymphosarcoma, reticulum cell type</td>
<td>4 + 1:640</td>
<td>1:40</td>
<td>Yes</td>
<td>Terminal development of stem cell leukemia</td>
<td></td>
</tr>
<tr>
<td>8. H.R.</td>
<td>50</td>
<td>M</td>
<td>Carcinoma of lung</td>
<td>4 + 1:320</td>
<td>1:160</td>
<td>Yes</td>
<td>Metastatic disease</td>
<td></td>
</tr>
</tbody>
</table>

* Sensitized Human Cell
** Sensitized Sheep Cell

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### Table 2

Summary of 5 patients with systemic malignancies discovered in a study of 125 patients with arthritis of a non-rheumatoid type in the 50-74 age group.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Malignancy</th>
<th>Type of Arthritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. M.B.</td>
<td>66</td>
<td>F</td>
<td>Carcinoma of colon</td>
<td>Degenerative joint disease</td>
</tr>
<tr>
<td>2. F.C.</td>
<td>55</td>
<td>M</td>
<td>Adenocarcinoma of kidney</td>
<td>Gout</td>
</tr>
<tr>
<td>3. J.G.</td>
<td>52</td>
<td>M</td>
<td>Carcinoma of prostate</td>
<td>Degenerative joint disease</td>
</tr>
<tr>
<td>4. E.H.</td>
<td>70</td>
<td>F</td>
<td>Carcinoma of breast</td>
<td>Degenerative joint disease</td>
</tr>
<tr>
<td>5. J.K.</td>
<td>71</td>
<td>M</td>
<td>Chronic granulocytic leukemia</td>
<td>Gout (onset 20 years prior to diagnosis of leukemia)</td>
</tr>
</tbody>
</table>
systemic malignancies were quite rare in cases of rheumatoid disease. Ragan and Snyder (1955), however, believed the incidence to be the same as in the general population.

Rheumatoid disease was diagnosed an average of 94 months prior to the onset of systemic malignancy in our eight cases. We did not observe the apparent malignancy-induced polyarthritis such as was described by MacKenzie and Scherbel (1963).

Essentially no difference (4 per cent of each group) was noted in the incidence of malignancies in the rheumatoid and non-rheumatoid groups. However, in the rheumatoid group a case of lymphosarcoma (reticulum cell type) with terminal state of stem-cell leukemia, and a case of acute lymphatic leukemia was noted among the eight cases of malignancy. The patient with lymphosarcoma (C. T.) had taken either phenylbutazone (Butazolidin®) or oxyphenbutazone (Tandearil®) on an intermittent basis for approximately 40 months after the onset of rheumatoid arthritis. The patient re-instituted phenylbutazone several weeks prior to the discovery of lymphosarcoma. He did not have Sjögren’s syndrome, an association noted by Talal (1966). The patient with the leukemia (R. M.) had not received phenylbutazone for three years prior to the discovery of the leukemia. A cause-effect relationship between these drugs and leukemia has been suggested by Fraumeni (1967).

Although Miller (1967) reported a statistically significant difference between the incidence of diffuse connective tissue disease in patients with solid tumors (0.58 per cent) and patients with malignant lymphomas (1.86 per cent), a review of his data shows no difference in incidence for the two groups of malignancies when the disease rheumatoid arthritis is considered by itself. Among his 254 patients with lymphoproliferative neoplasms there were five patients with connective tissue diseases, but only one had rheumatoid arthritis while two had dermatomyositis.

Hench (1962) found 22 cases of diffuse connective tissue disease in a study of 1000 cases of lymphoma (2.2 per cent), but the diffuse connective tissue diseases were not further divided into specific diseases. In a pension study in Great Britain, Lea (1964) noted a 6.5 per cent incidence of “rheumatic” disease in cases of lymphoproliferative disease, in contrast to 1.5 per cent in a control population. However, once again the rheumatic diseases were defined as all those conditions presently classified under this heading, with the exception of arthritis following trauma. The diagnoses were derived from hospital records. Lea (1964) concluded that there was a very definite association between the reticuloses and the “rheumatic” diseases. In Miller’s study of immune disease and malignant lymphoma (1967), there was no overall pattern to suggest a cause-effect relationship.

It is noted that six of our 13 patients (46.1 per cent) with malignancy had taken adrenocorticosteroids on a long-term basis. However, only 51 of the 308 patients (16.5 per cent) without malignancy had received this therapy. This is a significant difference (P is < 0.007).

This carefully directed study suggests that among patients in the age range of 50 to 74 years, malignancy may be more closely associated with steroid therapy than it is with the disease rheumatoid arthritis.

Summary

A retrospective study of the incidence of rheumatoid arthritis and systemic malignancy was performed using the records of 196 older age patients with classic or definite rheumatoid arthritis. The results were compared to 125 patients in the same age group who had arthritis of a non-rheumatoid type. There was no difference in the incidence of malignancy in the two groups of patients. However, there was a positive correlation between the incidence of malignancy and the use of long term adrenocorticosteroid therapy.

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The Effect of Ruby Laser, Xenon-Light Coagulator and Diathermy on Vitreous Proteins*

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Introduction

Vitreous haze had been noted ophthalmoscopically after moderate to heavy lesions had been produced on the retina of the rabbit, using a Hitex carbon arc at exposure times ranging from 0.5 to 1.0 sec (Ham, Wiesinger, Guerry, et al, 1957). Histopathological studies have shown the vitreous to be altered by coagulation (Okun and Collins, 1962; Geeraets, 1966), though this was not observed by Meyer-Schwickerath (1960) and Lavyel (1963). Since the ruby laser and Xenon photocoagulator have been introduced into clinical ophthalmology, and with the continued use of diathermy in certain pathological conditions of the eye, it was considered of importance to compare the three coagulation sources and evaluate the effects on the proteins of the vitreous. This study was limited to lesions of a clinically acceptable grade. The protein changes were evaluated by the technique of agar-gel electrophoresis (Wieme, 1959).

Methods

Dutch rabbits, 1 to 2 years old, were selected for a moderately pigmented fundus and no discernible eye pathology. The animals were separated into six groups of six rabbits each for the individual experiments. Exposure data are given in the Table. In each animal, six burns of equal intensity were produced in the equatorial region of one eye, while the contralateral eye served as the control to account for biochemical individuality (Berry, 1966). The photocoagulator and the ruby laser used were the same as described in previous studies (Ham, Williams, Schmidt, et al, 1963; Geeraets, Ham, Williams, et al, 1965). It should be reemphasized that in pulsing the Xenon high pressure lamp to achieve short exposures (4 ms), a shift in the spectral emission takes place, eliminating practically all infrared (Ham, Williams, Schmidt, et al, 1963). The

* This study was supported by Contract DA-49-193-MD-2241 from the US Army Medical Research and Development Command, Contract DA-49-146-XZ-416 from the Defense Atomic Support Agency, and NIH Training Grant 5 T1 NB 5176.
diathermy burns were produced by a 115 volt, 50/60 cycle unit, whose heat was generated by means of radio frequency power delivered to the surface electrode with a power output of approximately 18 watts.†

The animals were sacrificed immediately after exposure and their eyes were enucleated. The lesions were identified ophthalmoscopically and the tissues overlaying the burned area were removed by trephining. A syringe was used to aspirate 0.1 ml of the vitreous in juxtaposition to the lesion. The samples were transferred into micro-test tubes, lyophilized, and stored at −40 C until electrophoresis was performed. Each sample was reconstituted by adding 0.01 ml of distilled water, stirred gently to insure solubilization, and centrifuged at 900g for five minutes to clear any insoluble material. The supernatent was aspirated with a micro-pipette. The experimental sample and its control were then introduced into their respective slits in the agar on the microscope slide. Electrophoresis proceeded for 16 minutes under the following conditions: Veronal buffer, pH 8.5, 0.05M, Agarose 0.9 per cent, 140 volts, 4 C. The slide was then fixed, stained with Buffalo Black, and the pattern recorded by scanning with a photodensitometer (Williams, Ruffin, Berry, 1964).

The analysis of the electrophoretic patterns was designed to accommodate the complex changes produced by the various modalities using the simplest technique available to obtain maximum information. The Figure serves as a model for the description of the method.

† Manufactured by Medical Instruments, Research Associated, Inc., Boston, Mass.

### TABLE

<table>
<thead>
<tr>
<th>Exper. Group</th>
<th>Source</th>
<th>Exposure Time</th>
<th>Retinal Image Diameter (mm)</th>
<th>Infrared Filter</th>
<th>Average Retinal Dose (J/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Xenon Lamp</td>
<td>340 ms</td>
<td>0.9</td>
<td></td>
<td>26.6</td>
</tr>
<tr>
<td>2</td>
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<td>360 ms</td>
<td>0.9</td>
<td>KG III</td>
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<tr>
<td>3</td>
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<td>360 ms</td>
<td>0.9</td>
<td>(Schott)</td>
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<td></td>
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<tr>
<td>6</td>
<td>Diathermy</td>
<td>1 sec</td>
<td></td>
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</table>

The statistical model of the mean areas of the protein boundaries from the vitreous of normal rabbits.
and depicts the mean distribution of the areas obtained from all of the control samples in this study. Position 6 represents the slit made in the agar in which the sample was placed prior to electrophoresis, and as such, is an absolute marker for the pattern (Berry, Rosenfeld, Chanutin, 1956). The number of centimeters between boundary 1 and the slit is, therefore, the distance which boundary 1 migrated during the time of electrophoresis. The positions of 2, 3, 4, 5, and 7 are calculated as ratios, i.e., 0.800, 0.565, 0.319, 0.078, and −0.117 respectively. Lines from these points are erected perpendicularly from the base line. The front half of boundary 1 is then reflected about line 1 and drawn on the pattern (Berry and Chanutin, 1955). The area of the whole boundary is thus delineated and exposes the area due to boundaries 2 and 3. The area above the right side of boundary 1, included in the pattern between lines 1 and 2, is next measured by planimetry. This is the value of one-half of boundary 2. The value of one-half of boundary 3 is determined by subtracting from the total area between lines 1 and 3 the value of one-half of boundary 1 and the whole of boundary 2. This corrects for the small contribution of boundary 1 to the area between lines 2 and 3. This procedure is continued through the half boundary of 5. The area of one-half of boundary 7 is found from the right half of line 7. The area of the slit is the residual after the value of one-half of boundary 5 and one-half of boundary 7 are subtracted from the area between lines 5 and 7.

Since errors of dilution and concentration may enter into the preparation of the samples for electrophoresis, the error must be compensated for as previously described (Berry, Rosenfeld, Chanutin, 1956). The area as determined for boundary 1 is adjusted to a constant value; as is convenient, all other areas will be distributed proportionally. The difference of a given boundary may then be compared to that same boundary in the control sample, thus giving the percentage change over the control.

Duplicate analysis of ten samples revealed that the area of any boundary could be determined with an accuracy of 15 per cent, but this is illusory, since the control pattern of a given rabbit should not be compared directly to that of another. Each is distinct and distinguishable (Berry, 1966). Since the protein concentration of a boundary varied among rabbits, and there may have been variations in instrumentation and thermal history among the lesions, the data from each group of six experimental eyes were averaged in respect to the per cent change over the individual control eyes.

To avoid complexities which are not pertinent to this study, the areas of interest are designated by numbers and the label "fast." The range of mobility of the fast boundary is indicated by the dashed line with arrows, and the height of this line indicates the relative concentration. In some rabbits this boundary is lacking and in others it appears to consist of more than one component, but it does not reflect the influence of the energy delivered by the sources under the conditions of this study. The mobility of the large boundary (number 1) did not appear to be affected, nor was the shape altered except in respect to total area. The area designated by number 6 includes the slit, and is analytically important in regard to the effects of alterations in the size and shape of soluble proteins. The lip of each side of the slit is due to the mechanical tearing of the agar when the slit is made. The stained area around that artifact is due to protein molecules which, because of size and shape restrictions, were trapped (molecular seiving). The loss of area from a boundary indicates either a decrease in the amount present in the sample due to precipitation, aggregation, or polymerization; or a change in charge and/or shape, such that the protein species now migrates with a different mobility. A change in mobility due to conformational and/or charge differences may place the particular protein in a faster or slower classification. It should be noted that an increase of protein concentration in a particular area may be a low estimate of the true change, since protein could also have been removed from that area. This same probability exists for a negative change, i.e., a low estimate of true change if protein has been added to the area.

**Results and Discussion**

Due to the manifest complexity of the changes and the experimental design, it has not been feasible to correlate the changes in a particular boundary with the three modalities. On the other hand, the relative effects of a short pulse (250µ sec and 4 m sec), when compared to those produced by a relative long exposure (0.35 sec), reflect the same general picture as evidenced within the retinal proteins (Chan, Berry, Geeraets, 1963)—namely, the difference of thermal stability of the various proteins involved as to time-temperature history. Changes in boundary content in a positive or negative direction were present in all six experimental groups. The most obvious changes occurred in total area of all boundaries, with 8.9 and 14.9 per cent change for Xenon-light exposure at 0.35 sec exposure time, and 3.1 per cent change for Xenon-light exposure at 0.35 sec exposure time with 50 per cent reduction of retinal energy dose. There was a 4.4 per cent change for pulsed Xenon-light exposure (4 msec exposure time), and 0.3 per cent change for ruby laser exposure. The most pronounced changes occurred after diathermy, with 17.5 per cent over the control. Thus, reduction of the energy delivered per exposure appeared to be beneficial insofar as vitreous changes were reduced. Elimination of the infrared portion of the spectral distribution of the Xenon-light source by use of a filter (Schott KG III) did not seem to influence
the pattern of vitreous protein changes. On a comparative basis, in regard to the extent of alterations induced, surface diathermy applied to the sclera resulted in the greatest electrophoretic detectable protein changes, followed by long exposures to a Xenon-light source either with or without infrared filter, and finally by short exposures to the same Xenon source or ruby laser. From this observation one may postulate that exposure time is the main factor in the extent of vitreous protein changes, rather than the type of spectral source used—an observation described in earlier investigation (Geeraets, Ham, Williams, et al, 1965).

The described observations suggest that the changes observed in the vitreous proteins are secondary in nature. The energy of the light or laser beam incident on the retina is absorbed in part in the retinal pigment epithelium and choroid, with resulting heat generation. This thermal energy is then conducted to adjacent structures including the vitreous. Direct absorption of the light by the ocular media certainly takes place and is wavelength dependent, but plays only an insignificant role within the parameters pertinent to this experiment. Thus, exposure time and total energy absorbed in the retinal pigment epithelium and choroid seem to represent the only two important factors contributing to vitreous alterations with the type of radiant sources used in this investigation. This assumption is supported by the theoretical thermal profile calculated for similar experimental conditions (Geeraets, Ham, Williams, et al, 1965).

Summary

Vitreous haze produced by excessive energy delivered during clinical photocoagulation has been reported. The introduction of the ruby laser and the continued use of diathermy to produce therapeutic lesions lead to a comparison of the three modalities in respect to their possible effect on the proteins of the vitreous for an acceptable clinical retinal lesion.

Lesions of similar degree were produced in the eyes of rabbits by three sources of thermal energy. Vitreous in juxtaposition to the affected retina was aspirated, lyophylized, reconstituted, and then subjected to electrophoresis in order to evaluate the protein composition. The quantitation of the complex pattern of an individual rabbit was simplified to a system of seven boundaries. In each case, changes in the concentration of the soluble proteins were evaluated in respect to the control eye.

Each source of energy produced either precipitation, aggregation, conformational changes of the components, or combinations of these, as evidenced by electrophoretic analysis.

A shortened pulse length and a decrease in energy minimized the alterations observed. Diathermy produced the maximum deleterious effect, yet each source did produce a demonstrable change in the proteins of the vitreous at the level of a clinically acceptable lesion.

References


Williams RC, Ruffin RS, Berry ER: A broad-range recording microspectrophotodensitometer. Anal Biochem 8: 293, 1964
Accidental Hypothermia in Medico-Legal Practice*

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Accidental hypothermia has become a growing problem in Great Britain during the last 20 years and is a direct outcome of the increasing aged population. During a cold winter a few years ago it was estimated that some 10,000 old people were admitted to hospitals in a hypothermic state, that is, with body temperatures below 95 F.

The term accidental hypothermia is used in preference to the older description "exposure to cold" or "freezing to death," as a susceptible subject may develop hypothermia in an environment which is warm to the healthy individual. For example, during this last winter a lady was examined who was a patient in a geriatric ward, where the temperature was controlled at 72 F. The lady was a little obese and was confined to bed. It was noticed by the ward doctor during his morning round that she did not seem very well. He examined her and at once noticed that she felt cold. Her rectal temperature was 90 F.

Hypothermia may develop at the two extremes of age: in the premature or marasmic infant or in the senile adult. It may, of course, sometimes be present in middle age if the subject is suffering from certain degenerative conditions or from the effects of alcohol or drugs which act on the central nervous system.

A medico-legal pathologist is particularly interested in any specific changes he may elicit at post-mortem examination on patients dying in the hypothermic state. In many parts of Great Britain autopsies are carried out on all persons who die suddenly, even if the cause of death is believed to be a natural one, and on all those where some unnatural condition, such as hypothermia, may have hastened death. It is from the post-mortem examinations on known deaths in the hypothermic state that the criteria for the diagnosis of hypothermia at autopsy have been elicited.

The post-mortem appearances of fatal hypothermia are different in the neonatal and senile groups, so each group will be described separately; where pertinent, brief mention will be made of some of the ante-mortem symptoms.

* Talk given at the Medical College of Virginia, September 23, 1968.
ACCIDENTAL HYPOTHERMIA

The Neonatal Group

The hypothermic infant is usually premature. He has a characteristically pink face and extremities, the arms and legs are oedematous, the abdomen is distended due to paralytic ileus, and bullae are often present on pressure areas. The patient is vomiting as a result of the ileus, and invariably has hypoglycaemia. If the child is admitted to the hospital with a temperature below 90 F, his chances for survival are slight. X-ray examination of the abdomen of a hypothermic infant will show well-marked fluid levels.

Other conditions, besides prematurity, may predispose to the development of neonatal hypothermia. These include: congenital heart disease, congenital abnormalities of the central nervous system, neonatal asphyxia, respiratory distress syndrome in the newborn, intracranial birth injuries, and administration of analgesics to the mother.

It is clear that any condition which produces hypoxia predisposes to the development of the hypothermic state. It has been shown that the normal metabolic responses of the healthy newly born infant to cold can be inhibited by reducing the oxygen in the inspired air.

Post-Mortem Examination

Post-mortem examination, besides showing the external signs described above, will exhibit sclerema neonatorum. About one-third of the cases will show intrapulmonary haemorrhages apparently due to the inhalation of gastric contents; but the gastric and pancreatic changes which are such a feature of the adult case may be lacking, although Sly (1964) has reported a case of perforation of a gastric ulcer in a case of neonatal hypothermia. Peritoneal and pleural effusions are also found at autopsy.

The Senile Group

The senile hypothermic patient (Figure) is usually over 70 and often over 80 years old. In a number of cases the hypothermic state develops without there being any gross physical disease other than old age. The loss of temperature control almost appears to be part of the aging process. There is often a history of the patient falling about during the months preceding the onset of the hypothermic state, suggesting the presence of micro-degenerative lesions in the brain.

The fact that these patients have lost normal temperature control has been demonstrated by placing them, after recovery, in a cooler environment with other patients of the same age. The recently recovered hypothermic patients will start to cool, whereas the others will remain normothermic.

It will be seen from the Figure that there is a small group of persons aged from 40 to 70 years in this series of fatal hypothermic cases. All of these persons were suffering from either physical or mental disease. These conditions included rheumatoid arthritis, schizophrenia, and premature senility (Alzeimers disease). Some of these diseases may, of course, be significant in the senile or over 70 age group.

Unless there is a sudden exposure to cold, the hypothermic state would appear to develop at least over several days, although history is often lacking. The fully developed case of senile hypothermia is characteristic, and so resembles myxoedema that it is possible that, in the past, many primary cases of hypothermia have been diagnosed as being secondary to hypothyroidism. The blood chemistry, however, shows none of the changes associated with myxoedema, and after recovery from the hypothermic state by simple warming all the gross myxoedematous features disappear without the exhibition of thyroxin. As hypothermia lowers resistance to infection a number of patients die, after they have been rewarmed, from bronchopneumonia. However, many of the patients who are admitted to hospitals with temperatures over 80 F recover.

Autopsy Findings

The external features of a patient dying from hypothermia not only include oedema of the face and limbs, but also the presence of red erythematous areas on the limbs and sometimes on the face. Bullae may appear on the heels or other pressure areas.

The internal examination is usually conclusive even though the cooling may have been rapid.
Submucosal Haemorrhages and Ulceration of the Alimentary Tract

Gastrointestinal lesions were present in roughly 90 per cent of the 28 cases examined. The lesions are seen most frequently in the stomach, less commonly in the duodenum, and occasionally in the oesophagus. The lesions are initially numerous submucosal haemorrhages, black in color due to the presence of altered blood. These haemorrhages vary in size from 1 mm to 2 cm, but are usually in the range of 1-2 mm. They are most constant in the pyloric half of the stomach. If death is delayed, the mucosa over the haemorrhages sloughs leaving shallow ulcers. These ulcers may be difficult to see by direct vision but become readily apparent by transmitted light.

The duodenal haemorrhages tend to be larger and sparser. In one case in this series there was a perforation of an acute duodenal ulcer; minimal vital reaction was present. There appears to be no reason to separate the ulcer from other "Curling’s ulcers" of the duodenum.

Microscopy of the gastrointestinal lesions does not add much to the gross examination. The striking feature is the absence of vital reaction.

Pancreatic Changes

Gross pancreatic lesions were present in 82 per cent of the cases in this series. The changes varied from the occasional focus of fat necrosis to a frank haemorrhagic pancreatitis with fat necrosis, not only involving the whole pancreas, but also the tissues immediately adjacent. Fat necrosis away from the immediate vicinity of the pancreas was never observed. The localization of the changes would appear to be due to the inhibitory effect of cold upon the released enzymes (Nasbeth, Goodale and Reif, 1960). In cases which recover under treatment, the presence of pancreatic dysfunction is shown by the raised serum amylase.

The microscopical changes depend upon the severity and duration of the hypothermic state. In general, one finds areas of necrosis and haemorrhage with some leucocyte infiltration. If the hypothermia has been recurrent, areas of chronic pancreatitis may also be identified.

Myocardial Changes

Fatty changes in the myocardium appear to be invariable. Micro-infarcts may be found in a proportion of cases. Experimental hypothermia in dogs reveals similar changes and, in a proportion, severe fibrosis with heteroblastic bone formation may occur. It has been shown that, in hypothermia, ventricular fibrillation is likely at an early stage of the cooling if myocardial ischaemia is also present.

General Changes

Fat, or an increase in fat, is found in the kidneys, adrenals, and liver; and micro-infarcts have been recorded in all organs except the liver. These micro-infarcts are caused by the haemoconcentration and "packing" or "sludging" of the red cells in the capillaries.

Associated Natural Diseases

Natural diseases, other than general senile degenerative changes, were found in a high percentage of the cases of hypothermia examined. The principal diseases identified were:

Cardiovascular

- Hypertension 6
- Advanced coronary occlusion 5
- Advanced aortic stenosis 2

Respiratory

- Cor pulmonale 5
- Suppurative bronchopneumonia 7

(All these cases had recovered or almost recovered from the hypothermic state and had developed the pneumonia as a complication.)

Psychiatric and Mental Disease

- Known mental disease (eg schizophrenia) 4
- Presenile dementia (Alzheimer type) 3
- Senile dementia 2

Cerebral Lesions

- Trauma 1
- Natural cerebral haemorrhage 1
- Parkinson's disease 1

Other Conditions

- Oesophageal ulceration and stenosis 3
- Chronic gastric ulceration 1
- Cirrhosis of liver 2
- Result of crime 2

Uncomplicated senility has not been included. Concomitant heart disease was present in 57 per cent of of the cases, but in only half these cases was the disease considered so advanced that it could cause sudden death at any time without the complication of hypothermia.

The younger group (40—70 years) suffering from known mental disease had all wandered away from their institutions, lived "rough," and were found dead in the open.

Several other contributory factors have been recorded in the literature. These are all diseases where the basal metabolic rate is lowered or causes immobility or wasting. The most important cause, however, is
clearly senility, especially when this is associated with Parkinson's disease.

Drugs

Many drugs in the hypnotic and tranquillizer group have been cited as precipitating agents of the hypothermic state. Routine toxicological analysis is desirable in fatal cases, but, except for acute exposures, the time which normally passes between the commencement of the hypothermic state and death is so long that the chances of recovering most of the drugs are rare. It should be remembered that alcohol is especially dangerous.

Summary

The autopsy findings in 28 cases of fatal hypothermia have been described. Hypothermia, in temperate climates, is one of the complications of longevity. The hypothermic state may be precipitated by a wide range of natural diseases, especially chronic cardiac disease. The classical case of hypothermia may be recognized at post-mortem by the myxoedematous appearance of the patient and the presence of gastric and pancreatic lesions.

References


SLY RM: Perforation of a gastric ulcer in a premature infant exposed to cold. Amer J Dig Dis NS 9: 525, 1964
The Effects of Hypothermia in Pregnant Rats: A Preliminary Report

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Our interest in the effects of hypothermia in pregnancy was aroused by the necessity of using this therapy on our obstetrical service.

A review of the literature revealed that in acute experiments, the fetal temperature fell to the same extent as that of the mother (Assali and Westin, 1962); changes in fetal O₂ saturation, CO₂ content, and pH generally followed similar changes in the maternal host (Vandewater and Paul, 1960). However, we could find only three studies of fetal survival. One, using unanesthetized guinea pigs, showed no change in fetal size or survival (Vandewater and Paul, 1960). Another, using ether anesthesia and hypothermia in mice, revealed some possible adverse effects when used early in pregnancy (Mousse, Boba, and Peck, 1961). The third showed that freezing pregnant hamsters produced death of the fetuses and also gross anomalies related to the day of gestation and duration of the procedure (Smith, 1957). Hehre (1965) reviewed the few reported cases of hypothermia during pregnancy in humans.

It appeared to us that more controlled animal studies were needed to evaluate the effects of hypothermia on fetal and neonatal survival.

Methods

In planning and conducting this study, we realized the importance of a) defining and grading "hypothermia," b) separating teratology from fetology, and c) overcoming the shivering phenomena in the animals subjected to hypothermia.

For the classification of hypothermia, we used the criteria of Swan and Paton (1961):

Moderate hypothermia ............ 37—28 C
Intermediate hypothermia .......... 28—20 C
Deep hypothermia ............... 20— 0 C

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All experiments were carried out between the 17th and 19th days of gestation. At this stage of development in the fetal rat, embryogenesis is complete and teratogenesis, in the strict sense, does not occur (Wilson and Warkany, 1965).

Shivering, known to occur when the rat is subjected to hypothermia, was overcome by the use of different types of anesthesia including pentobarbital, ether, and CO₂ narcosis.

In evaluating the results of the experiments, we took the following factors into consideration: Maternal survival and death, fetal survival and death, litter number, stillbirths, birth weight, sex ratio, and gross malformations.

A total of 60 female rats of the Sherin Wistar Strain, weighing 220-240 grams, were used. For purposes of evaluation, no experiments were conducted until the rats had been maintained through two generations. All females were bred to the same male. The first breeding was used to insure fertility, while the second breeding served as a control or experiment. All were maintained on Purina Lab Chow and water ad libitum in a controlled temperature of 72 F. During the experiments, temperatures were measured with a rectal probe and telethermometer (Yellow Springs Ins. Co.).

All rats were bred for three days and pregnancy was diagnosed by palpation. Those rats which had not delivered by the expected day were sacrificed; pregnancy was confirmed in each rat by the finding of metrial glands. All stillborn fetuses were examined after being fixed in Bouin's solution. The animals were divided into six groups:

Group C-1 Twelve pregnant rats served as the first group of controls.
Group C-2 Twelve pregnant rats were given pentobarbital (30 mg/kg). These served as a second set of controls.
Group E-1 Six pregnant rats were given pentobarbital (30 mg/kg), and cooled by ice water immersion. Their rectal temperatures were maintained at 28-30 C for two hours.
Group E-2 Eight pregnant rats were given pentobarbital (30 mg/kg), and cooled by ice water immersion to 28-30 C for four hours.
Group E-3 Six pregnant rats were given open drop ether anesthesia, and cooled by ice water immersion to 28-30 C for four hours.
Group E-4 Four pregnant rats were given open drop ether anesthesia, and cooled by ice water immersion to 22-24 C for two hours.
Group E-5  Six pregnant rats were given pentobarbital (30 mg/kg), and cooled by ice water immersion to 22-24°C for two hours.

Group E-6  Six pregnant rats were subjected to the closed container technique of cooling (CO₂ narcosis) (Andjus, 1956). The temperature was lowered to 16-18°C by placing them in a hermetically sealed, 1 liter jar at 2°C for two hours.

Results

No gross anomalies or changes in sex ratio were noted in any experimental group. The findings are shown in the Table. There were no adverse effects noted in the experimental groups, E-1, E-3, E-4, or E-6. In group E-2, use of moderate hypothermia for four hours with pentobarbital anesthesia resulted in only one live-born fetus. The rest were either resorbed or stillborn. This deleterious effect was not noted if the same conditions were kept for only two hours (group E-1). Intermediate hypothermia and pentobarbital anesthesia (group E-5) resulted in five of the six animals dying during the experiment, possibly in ventricular fibrillation (Blair, 1964; Covino and Charleson, 1954). Only one animal survived the experiment, and it delivered nine stillborn fetuses.

Comments

The results of this small series of experiments suggest that the use of hypothermia with ether anesthesia (for what may be considered relatively prolonged periods in relation to the gestation period of a rat) causes no harmful effects in the fetus, even if carried to depths of hypothermia rarely used clinically. That the duration of the experiment can be important was noted when cooling and pentobarbital were used for four hours (group E-2). In this instance, there were disastrous effects on fetal survival, whereas when the experiment lasted for two hours no harmful effects

<table>
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<tr>
<th>GROUP</th>
<th>C-1</th>
<th>C-2</th>
<th>E-1</th>
<th>E-2</th>
<th>E-3</th>
<th>E-4</th>
<th>E-5</th>
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<td>6</td>
<td>8(5)*</td>
<td>6</td>
<td>4</td>
<td>6(1)@</td>
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<td>28-30</td>
<td>28-30</td>
<td>28-30</td>
<td>22-24</td>
<td>22-24</td>
<td>16-18</td>
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<td>2</td>
<td>4</td>
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<td>2</td>
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<td>PENTOBARBIT.</td>
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<td>PENTOBARBIT.</td>
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<td>1</td>
<td>57</td>
<td>40</td>
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<td>5</td>
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<td>10</td>
<td>2</td>
<td>1</td>
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<td>6.1</td>
<td>6.1</td>
<td>4.5*</td>
<td>6</td>
<td>5.9</td>
<td>4.3*</td>
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* IN FIVE ANIMALS PREGNANCY WAS CONFIRMED BY METRICAL GLANDS
@ FIVE ANIMALS WERE KILLED BY EXPERIMENT
* SIGNIFICANTLY DIFFERENT FROM CONTROL (p<0.001)
were noted. It is clear that in order to fully evaluate the safety of a given anesthetic agent used to overcome shivering, further studies are required in which the time factor is varied.

The death of five out of six mothers in group E-5 might have been due to ventricular fibrillation. Blair (1964) and Covino and Charleson (1954) reported a higher incidence of ventricular fibrillation at temperatures below 25 C with pentobarbital than with ether anesthesia.

One of the more interesting results was the lack of harmful effects from the combination of deep hypothermia and CO₂ narcosis (group E-6). One might speculate that this would fit in with the finding by Miller and Miller (1966) that hypothermia and hypoxia-hypercapnia protect the fetus and maternal host. These preliminary results need to be confirmed and amplified by further experimentation. Similar studies, performed in the teratogenic phase of the embryo, also seem indicated.

Summary
We have evaluated the effects of varying degrees and durations of hypothermia, combined with anesthesia, on pregnant rats of the Sherin Wistar Strain. Moderate (surgical) and intermediate hypothermia, with ether anesthesia, were safe for both fetus and mother. With pentobarbital anesthesia, however, there were harmful effects, depending on the depth and duration of hypothermia; moderate hypothermia increased fetal mortality, and intermediate hypothermia increased fetal and maternal mortality. The combination of CO₂ narcosis and deep hypothermia induced no ill effects.

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Abstracts of Theses for Graduate Degrees

Medical College of Virginia, June, 1969
Anticholinergic Agents Based on Ariens' Dual Receptor Site Theory

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In the classical receptor theory of competitive antagonism, both the agonist and antagonist are presumed to have an affinity for the same receptor site. However, changes in the structure of cholinergics and competitive anticholinergics do not result in a parallel change in activity. If affinity is the property altered by the structural modification, then classical receptor theory offers no explanation for this. Ariens has suggested that competitive antagonists may be occupying an area that overlaps only a portion of the agonist area and gain affinity through attraction to "additional receptor parts" not utilized by the agonists. After considering these proposed sites at postganglionic parasympathetic synapses and reviewing the chemical structures of potent cholinergic and anticholinergic drugs, a new series of compounds has been designed. These compounds may possess potent anticholinergic properties if Ariens' dual receptor site theory is valid. They contain a cationic head joining two groups—one resembling well-known, potent antagonists and the other resembling classical, potent agonists. The rationale for this structural design is that such compounds may bind to both cholinergic and anticholinergic sites of the receptor simultaneously. Because they should bind to both receptor sites, these compounds may possess a greater affinity than known, potent anticholinergic drugs not containing an agonist moiety and binding to only one receptor site.

The joining of the agonist and antagonist moieties was done by alkylation of basic amines to form the desired quaternary compounds. Seven quaternary compounds were obtained in pure condition for pharmacological testing. They are 2-[(2-acetoxyethyl) methylamino] ethyl benzilate methobromide, 3-[(2-acetoxyethyl) methylamino] propyl benzilate methobromide, 2-[(2-carbamoyloxyethyl) methylamino] ethyl benzilate methochloride, 3-[(2-carbamoyloxyethyl) methylamino] propyl benzilate methochloride, 2-[(2-carbamoyloxyethyl) methylamino] ethyl diphenylacetate methochloride, 2-[(5-methylfururyl) methylamino] ethyl benzilate methiodide, and 3-[(5-methylfururyl) methylamino] propyl benzilate methiodide.

These seven potential anticholinergics were evaluated by means of the pA₂ described originally by Schild. Isolated segments of guinea pig ileum and rat jejenum were used as test systems. Both acetylcholine and carbachol were used as agonists. Four or five dose-response curves were determined on each isolated tissue preparation, and a fresh segment was used for each agonist-antagonist combination. The compounds behaved as true competitive antagonists and were highly specific for the cholinergic receptor.

The pA₂ values were obtained by linear regression analysis. There was no significant difference between the pA₂ values obtained with acetylcholine and carbachol. However, differences between antagonists were significant.

Three basic alterations of the lachesine molecule were made. The ethyl group on the onium nitrogen was replaced by acetoxyethyl, carbamyloxyethyl, and 5-methylfururyl. All of these substitutions decreased the anticholinergic activity compared to lachesine. The anticholinergic activities were in the following order: acetoxyethyl > 5-methylfururyl > carbamyloxyethyl. The distance between the onium nitrogen and the ester group in the benzilate moiety of the molecule was increased from two carbon atoms to three carbon atoms. All of the compounds with a two-carbon chain in this moiety possessed greater anticholinergic activity than their homologues containing a three carbon chain. When the hydroxyl group of the benzilate moiety was eliminated to give the diphenylacetate group, anticholinergic activity was drastically decreased. Thus, all alterations to the lachesine molecule decreased anticholinergic activity. These new compounds would have to possess greater affinity and, therefore, greater anticholinergic activity than lachesine to allow any positive conclusions to be made concerning the general validity of Ariens' dual receptor site theory.

One possible explanation of these results may be that Ariens' dual receptor site theory is not valid for the cholinergic receptor. However, several alternative explanations might be advanced for the lack of higher pA₂ values. (1) The lower affinity might be only apparent due to a lowered ability of the compound to reach the site of action. This seems very improbable with the compounds of this study for the isolated organ systems employed. (2) The receptor sites may not be arranged in a linear fashion. In such a case, the agonist and antagonist moieties of the dual-ended molecule may not bend into a conformation which permits binding with their respective sites simultaneously, thus decreasing their potential affinity. (3) Ariens' dual receptor site theory may not be applicable to the ester-type antagonists chosen for inclusion in the present series of compounds. However, Ariens' theory may be applicable to other compounds such as the well-known, non-ester anticholinergic drugs.

Although no conclusions concerning the general validity of Ariens' dual receptor site theory can be presented, further investigation of carefully chosen molecules may provide enough information to do so in the future.
The Relationship Between Emotionality and Behavioral Performance in a Random Population of Male Charles River Rats

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The capacity of a rat to adapt to the stress of repeated exposure to an open field is the basis for the determination of individual emotionality in a random population of animals. The emotionality of male CD rats was determined by exposing each animal to twelve daily, two minute sessions in a modified Hall open field. During these sessions individual spontaneous activity, rearing, and number of boluses were determined and used as indices of adaptation to this novel stimulus. The relationships between these parameters of emotionality and learning, curiosity and pituitary-adrenal activation were then studied by specific experimentation. The design of these experiments was such that correlations were made possible between open field performance and other behavioral parameters.

Animals exposed to the open field showed adaptation in defecation rate after eight days. Equilibration of activity was apparent following the sixth day. The number of days defecating and mean spontaneous activity during the last four days (9–12) were used for individual emotionality classification. Negative correlations between spontaneous activity and defecation were observed in all experimental groups during this period. Analysis of an intercorrelational matrix of these emotionality parameters further suggested that activity and defecation were two different forms of emotionality. In general, the high emotional rat could be characterized as being a high defecator with low spontaneous activity.

Maze experimentation indicated that all rats tested could learn a double T maze, but running times varied greatly. Correlations between defecation rate (days 9–12) and maze running time alluded to a relationship between emotionality and maze learning. All six of the first six ranked maze learners of Experiment I and five of the first seven in Experiment II were classified as low emotional by defecation scores during days 9–12. It was suggested that in reality, learning was not estimated, but the fear evoked by the maze.

In a second experiment designed to test individual rat curiosity, low defecating rats were also observed to be more active in areas with high illumination. High defecators, on the other hand, were more often found in less illuminated areas, suggesting these latter animals to be more fearful. Correlations between open field performance and curiosity again revealed that a rat which is a high defecator may also be an extremely fearful animal. Food deprivation appeared to reduce these correlations with less of an effect occurring in high emotionality animals. From these data, it was suggested that a second exposure to this test situation may have unmasked a latent curiosity in the emotional rat which surpassed that in the non-emotional animal.

The retention of a one trial avoidance procedure appeared to be greater in the high emotionality rat. This was true 24 and 48 hours after the initial learning session. The fear evoked by a negative reinforcement prevented the high emotional rat from returning to a darkened chamber in which he was previously shocked for one minute. These results supported the hypothesis that high emotionality rats learn passive avoidance procedures more rapidly than non-emotional animals.

High emotional rats exhibited a 50 per cent increase over resting plasma steroid levels in response to an acute psychological stress, while the non-emotional group showed a 27 per cent increase to the same auditory stimulus. Correlations between emotionality factors and plasma levels were not significant. However, positive correlations between defecation and plasma steroid levels further suggested that high emotionality animals were more responsive to this stress. Adrenal steroid levels did not reflect these changes and correlations between emotionality parameters and these endocrine levels were also inconclusive.
Fetal Development and Functional Significance of the Epiphysis Cerebri in Rats and Hamsters: A Light and Electron Microscopic Investigation.

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Reports regarding the significance of the mammalian epiphysis cerebri or pineal organ have for some time presented conflicting or inconclusive data. Out of the confusion arose the concept that this structure in mammals is merely a functionless vestige of a third eye found in some lower vertebrates.

Two significant findings within the last ten years have led to a resurgence of interest in the pineal organ. (1) A previously unknown compound, melatonin (N-acetyl-5-methoxytryptamine) was isolated from bovine pineals and was found to be synthesized only in pineal tissue. (2) Ultrastructural studies revealed photoreceptor cells in the pineals of numerous submammalian vertebrates. It has subsequently been found that the activity of certain pineal substances, including melatonin, is influenced by environmental lighting and that the pineal organs of certain mammals subjected to light deprivation are capable of inducing gonadal atrophy. Information about environmental lighting is transmitted to the pineal in such animals via the lateral eyes and autonomic nerves: the mammalian pineal cells appear to lack the photoreceptive capacity demonstrated by pineal cells of certain submammalian vertebrates. It appears thus that two functional pineal systems have evolved: a photoreceptor system and a secretory or endocrine system. It has been suggested that the cells constituting these two systems may be homologous.

In light of this hypothesis, the present investigation was designed to examine the nature of the mammalian pineal cell which has been variously described as neural, neuroglial, and endocrine. Since light deprivation has been shown to result in pineal-induced gonadal atrophy in rats and hamsters, these rodents were chosen for this study which consisted of two parts. (1) Morphogenesis and cytogenesis of pineal tissue were studied at the light and electron microscopic levels in 16–20 day fetal rats and in 12–16 day fetal hamsters. The pineals in both animal differentiate from ependyma of the dorsal diencephalon and in both, appear to be composed primarily of one type of cell. The resemblance of apical cytoplasmic structures in these cells to certain features of known photoreceptor cells suggests that the former may at least possess the potential to develop into a photoreceptor type of cell. (2) Adult rats and hamsters were subjected to blinding (bilateral orbital enucleation) and to blinding combined with pinealec-
Separation and Partial Characterization of Components Derived from Human Erythrocyte Membranes

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Considerable attention has been focused on the problem of the protein composition of red cell membranes. Evidence has been cited to support the hypothesis that a single protein accounts for the major part of the membrane. To test this hypothesis, a method has been devised of separating the membrane components and analyzing the components for phospholipid and protein composition.

The red cell membrane was isolated by buffer and water washes coupled with homogenization and centrifugation. The alteration in the chemical composition of the membrane during preparation was studied by amino acid, carbohydrate and phospholipid analysis. The final white membrane was similarly characterized and also analysed for hemoglobin and ATPase activity to ensure purity.

The membrane was solubilized in phenol : urea : acetic acid : water (2:1, 2:1:1, W/W/V/V) and subjected to analytical disc gel electrophoresis. After establishing the optimum parameters for separation of the membrane components, the system was scaled up to preparative disc gel electrophoresis. The components were eluted from the preparative acrylamide electrophoresis columns with 50 per cent acetic acid. The effluent was collected in 6.0 ml fractions; a minimum of 400 fractions were collected.

The fractions were analyzed for protein, following hydrolysis in 6N HCl, by the ninhydrin reaction using a Technicon Autoanalyzer. An aliquot of each fraction was also analyzed for phospholipid by phosphorus analysis following perchloric acid digestion. A total of 22 ninhydrin positive peaks and 26 phospholipid components were obtained. Complete amino acid analyses were done on the ten major protein components.

The preparation of the membrane resulted in a loss of protein without concomittant loss of lipid or carbohydrate. This protein must be loosely bound to the membrane since simple water washes removed it. Although this protein may be important to the function of the intact membrane, the classical trilaminar image was preserved as viewed by electron microscopy. The chemical composition of the membrane was: 50 per cent protein, 38 per cent lipid and 12 per cent carbohydrate. Ten per cent of the lipid of the membrane was cholesterol and the remainder was phospholipid. The carbohydrate consisted of 8 per cent neutral sugars, 2 per cent sugar amines and a small amount of sialic acids and fucose. The distribution of amino acids and the chemical composition of the membrane was found to be similar to that reported by other investigators.

The results of the preparative disc electrophoresis show that the membrane was composed of a large number of components. These components were found to be of three types: lipoprotein, protein and lipid. The amino acid analyses of the major components showed that each component was different to the other and to the whole membrane, demonstrating that there were at least 10 different proteins in the human red cell membrane. The per cent of protein of these major components varied from 5 per cent to 17 per cent of the total protein analyzed. Other components contained protein but in quantities too small to obtain accurate analyses. Therefore, 17 per cent was found to be the upper limit for any one component.

It has been postulated that the protein of the membrane consists of a family of proteins. This data leads to a new hypothesis, ie, there are different families of protein in cell membranes. Some of these proteins have an affinity for lipid while others occur as protein alone.
Free Amino Acid Release from Isolated Rat Liver Cells

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The purposes of this thesis were (1) to investigate the amino acid permeability of rat liver cells dispersed with tetraphenylboron (TPB), (2) to investigate the effects of thyroxin and testosterone on the amino acid permeability of TPB dispersed cells, and (3) to present evidence which would serve as proof that the cells are viable.

To determine the amino acid permeability of isolated rat liver cells, measurement of the intracellular and extracellular amino acid concentrations was taken as a function of time. Thyroxin or testosterone were added, in large doses, to demonstrate their effect, if any, on the amino acid permeability of the cells. Respiratory studies were carried out and pictures of the cells were taken to demonstrate two aspects of cell viability.

TPB dispersed rat liver cells were found to maintain a free amino acid ratio across their membrane during incubation in vitro. Addition of testosterone or thyroxin in large doses does not seem to affect this ratio. The cells respire at rates comparable to rates previously seen for liver cells, and the cells are demonstrated to maintain normal gross morphology during the course of the experiments.

Passer Domesticus*

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Study of the house sparrow revealed that there was a shift in predominant lactate dehydrogenase (LDH) isozymes for ventricle, but not for either breast muscle or cerebrum during development, a pattern of differentiation unlike that of chicken heart and breast muscle. Values of pyruvate concentration giving optimal LDH velocity for sparrow ventricle and pectoralis muscle were similar to published values for these two chicken tissues. The absolute pyruvate concentration giving optimal velocity of LDH isozymes in ventricle decreased during development. However, these concentrations of pyruvate failed to differ significantly among five age groups. The same relationship existed for developing cerebrum. There was a gradually increased inhibition of LDH activity produced by high pyruvate concentrations for ventricle, but not for cerebrum. These catalytic properties coincided with the isozymic changes observed in these tissues during morphogenesis. The observed pattern of inhibition resulted in a higher \( \frac{NADH_{t}}{NADH_{a}} \) ratio for adult ventricle than for adult cerebrum or pectoralis muscle. The total LDH activity of cerebrum reached a minimum just before hatching and then it increased as development progressed.

* Presented June, 1968.
The Investigation of the Bradypnea Response in Dogs Following Left Atrial Distention

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The observation of striking decrease in respiratory rate after sudden inflation of a balloon in the left atrium of a dog anesthetized with chloralose warranted further investigation. Respiratory rate, left atrial pressure, and usually tidal volume and femoral and pulmonary arterial pressures were measured in 93 experiments before, during and after left atrial balloon inflation in three groups of dogs: 1) 14 dogs anesthetized with chloralose, 2) 4 dogs anesthetized with sodium pentobarbital, and 3) 10 dogs unanesthetized (mildly sedated in some experiments). Balloon placement was by retrograde catheterization in the chloralose and pentobarbital groups and by surgical implantation in the unanesthetized group. Marked, moderate and slight bradypnea was observed in 7 of 11 chloralosed dogs (3 of 14 disqualified), in 4 of 10 unanesthetized dogs and in none of the pentobarbital group. Alternating bradypnea and tachypnea following inflation, and bradypnea or deflation were two other responses commonly observed in all groups. Tachypnea, seen in only three experiments of the chloralose group, was predominant in the pentobarbital group and occurred in 50 per cent of experiments without anesthesia. The detailed results suggest that two opposing reflex mechanisms are operating during left atrial distention. Tachypnea may result from sensitization of pulmonary stretch receptors secondary to pulmonary vascular engorgement. The hitherto undescribed bradypnea response might be mediated by left atrial receptors, known to exist. Failure to observe bradypnea in chloralosed dogs, in which the left atrial appendage and a single pulmonary vein near its atrial junction were distended by implanted balloons suggests either that these regions of the left atrium are not the sites of the postulated receptors or that an insufficient area was stimulated. The marked bradypnea response in one dog in which the balloon slipped from the atrial appendage on inflation and became lodged in the mitral orifice suggests that receptors mediating the bradypnea response might be situated in tissues surrounding the mitral ring. Assuming this, it may be conjectured that in those animals in which striking bradypnea was repeatedly observed, the inflated balloon was low enough within the atrium to produce adequate distention of the mitral area.

Glucose Dehydrogenase Activity of a Sweet Sensitive Protein From Bovine Tongues

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We have prepared a fraction of the homogenate of bovine tongue which has the ability to combine with sugars. The relative strengths of the complexes formed are in close agreement with previously published data. With a suitable acceptor present, this protein will catalyze the dehydrogenation of D-glucose. The reaction is quite specific for D-glucose, other reducing sugars being oxidized more slowly.

The material was assayed by measuring its rate of dehydrogenase activity with reducing sugars, using NAD as an acceptor. The Michaelis constants were calculated and were compared with the relative sweetness of the sugars tested.

We speculate that what we have isolated from the bovine taste bud is a glucose dehydrogenase, the sugar binding properties which have been exploited evolutionarily to form the receptor molecule of sweet taste perception. It is hoped that further research will be able to link this protein directly to taste.
Hematology Quality Control in a Large Medical Center

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An evaluation of the needs for hematology quality control in a large medical center with multiple laboratories has been conducted. The areas of greatest need have been identified and procedures have been presented for testing the accuracy and precision of the eight laboratories included in the study.

The historical development of quality control with a synopsis of methods currently being used is given. Both general methods as well as those specifically related to hematology are considered.

Laboratory error usually can be traced to one of four sources. Each one of these was studied in preliminary experiments and showed:

1. collection of the specimen—One tenth of the total weekly samples collected contained less than 3 ml of blood. If hematocrit determinations had been ordered on all of these, this constitutes a possible error of 10 per cent. Small blood or fibrin clots were detected in 3 per cent, constituting a second possible source of error in samples.

2. technical ability of the staff—There was a definite difference between the technologist and the technician.

3. the instruments or methods used—Standard deviation target values of 0.3 per cent for hemoglobin and 400 cells per cubic millimeter for white blood cells were established. Determinations from all of the participating laboratories were two or three times values.

4. transcription of results—Errors due to faulty transcription of results were found in less than one per cent of the total week's work. Only three of the eight laboratories were studied.

Expansion of the preliminary investigation into definitive studies focused on the instruments, the methods and the reagents used throughout all of the laboratories. These were without uniformity in individual laboratories. In addition, there was no homogeneous pattern of technical skill represented.

The definitive investigation included performance semi-weekly of hemoglobin and white cell determinations. These were performed in each of the eight laboratories on aliquots of the same samples. The data collected after three months were subjected to statistical analyses which included an analysis of variance (ANOVA) and a Duncan's Range Test. The ANOVA showed a significant difference exists between the results reported from different laboratories and due to a day-by-day laboratory interaction. The Duncan's Range Test grouped the laboratories according to which ones produced similar results. Results of these tests showed that each laboratory maintained a level of performance that resulted in acceptable precision, but poor accuracy. The wide range of results is believed to be due to the variety of instruments and methods used.

Based on these findings, it is possible to conclude that quality control in a large center with a multiplicity of laboratories, methods, and personnel is difficult to achieve. However, quality control with striving to achieve accuracy is the quintessence of a good laboratory.
A Kinetic Study of the Homogeneous Catalytic Hydrogenation of 2-Butyne-1, 4-Diol and cis-2-Butene-1, 4-Diol

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A review of the chemical literature has shown that certain coordination complexes of the transition metals, especially those in the platinum groups, successfully catalyze the addition of molecular hydrogen to unsaturated hydrocarbons and their derivatives in a homogeneous system. The employment of tris (triphenylphosphine)-chlororhodium (I) in homogeneous catalytic hydrogenation studies has been reported by Wilkinson. However, the studies which have been reported were made using hydrogen at pressures of less than one atmosphere.

It was the objective of this study to demonstrate the use of tris (triphenylphosphine) chlororhodium (I) as a catalyst in the Parr hydrogenation system, since this is the system most frequently found in chemical laboratories. Many kinetic investigations carried out with a heterogeneous catalyst in the Parr system have been reported in the literature, and it was of interest to extend these studies, employing a homogeneous catalyst. 2-Butyne-1, 4-diol and cis-2-butene-1, 4-diol were selected as the organic substrates to be hydrogenated.

It was found the hydrogenation of olefins and acetylenes in the Parr system was first-order with respect to hydrogen concentration and zero-order with respect to organic substrate concentration, provided that the initial hydrogen pressure is at least 35 psig, and the minimum amount of substrate used is 0.5 gram. Butyndiol was found to hydrogenate first to the butenediol by taking up one mole of hydrogen. Reaction continued and another two-thirds of a mole of hydrogen was taken up before the reaction ceased. It is thought that complete hydrogenation to butanediol was not observed due to formation of water in the system, resulting from the cyclization of butanediol, the hydrogenation product. The water thus formed moved in to occupy the vacant site in the rhodium complex normally held by a solvent molecule, and a "poisoning effect" resulted. It was found that the hydrogenation of cis-2-butene-1, 4-diol was poisoned much earlier than the 2-butyne-1, 4-diol, due to the fact that butanediol was formed much earlier in this hydrogenation.

The hydrogenations were carried out at four different temperatures, 30°, 35°, 40°, and 45°C. Applying the Arrhenius equation to the rate constants obtained at these temperatures, activation energies for the hydrogenations of 2-butyne-1,4-diol and cis-2-
Structure-Activity Relationships of Tetracyclines: Cell Division, Protein Synthesis and Nucleic Acid Synthesis in *Escherichia coli* W.

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The kinetics of inhibition of *E. coli* in a peptone broth as a function of the concentration of 18 tetracyclines have been determined. These experiments were designed to obtain antibiotic activities suitable for use in structural activity correlations. Viable and total cell count methods were used to measure rates of cell division. Rates of protein and nucleic acid synthesis were determined simultaneously by a membrane filter technique in conjunction with the Folin-Lowry Assay and the orcinol reaction, respectively. The relationship of the experimentally obtained rate constants to the antibiotic concentrations served as an estimate of antibiotic activity under the test conditions. (These activities have been successfully employed by Dr. Peradejordi (another member of the Pharmacy Department) to obtain correlations of electronic properties of the tetracyclines calculated by quantum mechanical methods with their antibiotic activity.) The rate constants obtained in the presence of any given concentration of tetracycline were essentially the same regardless of the methods used to obtain them, ie, rates of protein and nucleic acid synthesis and rates of cell division were equal in inhibited as well as uninhibited cultures. This observation indicates that inhibited cultures are probably growing in balanced growth and invalidates previously proposed mathematical models of tetracycline inhibition. Attempts to formulate new models consistent with all the reported experimental results were not entirely successful. Different times before onset of inhibition of protein synthesis, cell division and nucleic acid synthesis were observed in some kinetic experiments. These differences, if real, can be interpreted as consistent with the theory that the mode of action of these drugs is a primary inhibition of protein synthesis. Changes in broth pH also caused changes in these times of onset of inhibitions under certain conditions. These broth induced changes can be explained as due to the existence of a finite time of tetracycline permeation in *E. coli* W cultures in a high peptone media.

A Time Course Study of *in Vivo* Effects of Hydrazine on Rat Liver Protein and Nucleic Acid Content

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Hydrazine and its derivatives are used as pharmaceuticals, insecticides and rocket propellants. Exposure to hydrazine by many routes of administration results in variable physiological effects, ranging from minor skin irritations to convulsions. The outstanding pathological finding is lipid accumulation in the liver, which is generally accompanied by depletion of liver glycogen and elevation of liver protein.

A time course study was undertaken to determine the effect of a single sub-convulsive dose of neutralized, anhydrous hydrazine administered intraperitoneally to rats. Saline- and hydrazine-injected animals were sacrificed at 4, 12, 24, 36 and 48 hr periods after treatment. The results indicated that *in vivo* alterations in liver protein metabolism were greatest from 24 to 48 hr after treatment. Significant elevations in liver wet weight, total protein and RNA contents, and the uptake of C^14 leucine into liver protein were demonstrated in hydrazine-treated animals compared to control animals, starting at 24 hr. Prior to 24 hr, significant, hydrazine-induced alterations in all these parameters were not observed. Hydrazine did not produce changes in liver total DNA content during the experimental period. When protein, RNA and C^14 leucine uptake were expressed on a DNA basis, the effects of hydrazine on these cellular constituents were described.

Considered in terms of the total liver or the liver cell, the data were consistent with an hydrazine-induced net stimulation of hepatic protein biosynthesis which was maximal at 24 hr. These results parallel ultrastructural changes in the nucleolus and rough endoplasmic reticulum reported by others. The action of hydrazine appeared to involve a "degenerative" phase over the initial 4 hr period followed by a "regenerative" phase from 4 through 24 hr and, finally, the leveling off to a new steady state.
Ionic Contribution to the Genesis of the Frog Skin Potential

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Although the problem has been extensively studied, there is at present no completely satisfactory explanation of the genesis of the potential difference which exists across frog skin.

In the present study the electrochemical basis of the skin potential has been approached from three points of view. First, the recently proposed “diffusion delay theory” to explain the electrical response of the epidermis to Na⁺ has been tested. Second, the potentiometric response of the skin to several anions and the mode of handling of the anions by the skin has been investigated. Third, the potential profile of the frog skin has been determined using a carefully designed microelectrode experiment.

1. To test the “diffusion delay theory” net Na⁺ fluxes were measured isotopically in skins bathed on the epidermal side in solutions of varying Na⁺ concentration and containing as an anion the impermeable SO₄²⁻ ion. The net fluxes found satisfied the equation
   \[ \frac{1}{J_n} = 6.75 + 30(1/\text{[Na}⁺\text{]}) \]
   The net flux to permeability ratio \( J_n/P_n \) necessary to explain the electrical response of the epidermis to Na⁺ was found to be in excellent agreement with data extracted from recently published kinetic studies on Na⁺ transport in frog skin.

   In addition to flux measurements, the theory was tested potentiometrically by determining the potential response of the epidermis to varying Na⁺ concentrations in NaHCO₃ solutions. The “diffusion delay theory” was found to adequately explain the results obtained.

2. The potentiometric response of the frog skin to anions was determined by monitoring the skin potential during changes in the solutions bathing either the epidermal or corium side of the skin. The anions utilized Cl⁻, HCO₃⁻, SO₄²⁻ and NO₃⁻. The order of permeability of the skin to these anions was Cl⁻ \( \gg \) HCO₃⁻ \( \gg \) SO₄²⁻ and NO₃⁻. In addition it was found that when solutions containing Cl⁻ were substituted on the epidermal side of the skin for solutions containing any of the other anions there was an occasional reversal of the sign of the potential difference across the skin. No effect on the skin potential was found with replacement on the corium side of the skin.

   To determine the mode of handling of Cl⁻ and HCO₃⁻ by the skin, net fluxes of the anions were measured isotopically and chemically in open-circuited and short-circuited skins, respectively. It was found that under conditions of low Cl⁻ (12 mEq/1) at the corium side of the skin there was a net inward active transport of Cl⁻. The HCO₃⁻ ion was handled passively by the skin.

3. The potential profile of skins was determined by monitoring the potential difference between a glass microelectrode which was driven through the skin and a macroelectrode in the bathing solution at the epidermal or corium side of the skin. The skin was bathed in Cl⁻-Ringer’s solution on both sides. Two discrete potential steps were found as the skin was penetrated, increasingly positive in the direction epidermis \( \rightarrow \) corium. The steps were separated by a distance of 10 µ and were considered to occur at the outer and inner borders of the stratum germinativum.

   The potential profile of skins bathed in low Cl⁻ solutions (12 mEq/1) at the corium side were also determined. Two discrete potential steps were found, increasingly negative in the direction epidermis \( \rightarrow \) corium. These steps were separated by a distance of about 10 µ and were considered to occur at the outer and inner borders of the stratum germinativum.
Inheritance and Characterization of a Type A Blood Subgroup

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Blood group A subtypes were examined in 198 Caucasians and 78 Negroes. The frequencies of A₁ (A intermediate) and A₂ subtypes were much higher for Negroes than for Caucasians.

The A antigen strength was quantitatively measured by an immunohemolytic test and by lectins Dolichos biflorous and Phaseolus limensis. The H antigen was quantitated by the lectin Ulex europaeus. The Caucasian distribution of antigenic strength was discontinuous with clear separation between A₁ and A₂ subtypes. Negroes exhibited a continuous distribution in antigenic strength, the mean of A₁ samples falling between the means for A₁ and A₂. However, individual A₁ samples overlapped considerably with A₁ and A₂ samples.

The A₁ samples showed a distinct agglutination pattern with lectins; high titers were found with both Ulex and Dolichos. Family studies indicated A₁ type to be inherited by one or more alleles. A₁, therefore, appears to be a distinct inheritable subtype with unique lectin agglutinations and antigenic strength.

A Scheme for the Rapid Identification of the Enterobacteriaceae

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There is great need for an inexpensive and rapid technique for the identification of microorganisms. The present system employed in most laboratories involves the use of standard size tube cultures requiring from 18 to 24 or more hours of incubation. Usually, after primary isolation, a step by step procedure in the selection of biochemical tests is used until the organism is identified.

Many attempts to circumvent this time barrier have been tried. Three types of medium systems have been reported: the Multitest, the Microtest, and the Minitest. The multitest system consists of media in which two or more reactions can be observed simultaneously. The microtest system decreases the volume of media and utilizes a heavy inoculum. The minitest system utilizes the reagents and sometimes media contained in tablets or impregnated paper.

Two different kinds of microtest have been developed; both are rapid when compared with standard methods, and in almost all cases, the results correlate with them. The two kinds use different principles; in the first, a heavy inoculum is grown in a small volume of medium, previously warmed to 37 C. This type of test can show both preformed and induced enzymes. In the second kind, living suspensions in water, saline or buffer are added to the test substrate. Multiplication does not occur, and the test reveals only those enzymes that are preformed. For certain tests, it is necessary to grow the organism on special medium (to induce enzyme formation) before making the suspension. The present study describes the application of the first type of microtest system for the rapid identification of the Enterobacteriaceae. A comparative study was made on each organism using both the micro and standard methods.

Most of the organisms in this study were fresh human isolates grown from urine, sputum, wounds and feces. A few were obtained from the Virginia State Health Department Laboratories. A total of 455 organisms from different genera were studied. The criteria used to identify the organism were the same as those described by Edwards and Ewing.

The media in this study were dehydrated Difco products made according to the directions given on the bottle. The media selected were those presently used in the blood culture section of the microbiology laboratory at the Medical College of Virginia. The inoculum used was a heavy suspension of the organism in saline and the techniques involved were essentially
those already employed in a routine microbiology laboratory.

There was good correlation in the results obtained by both methods. To test the efficacy of the microtest system under diagnostic laboratory conditions, a blind study was conducted using routine blood cultures from the diagnostic laboratory. These results also correlated well.

The microtest system, as used in this study, has been most useful in the identification of enteric gram-negative organisms as found in a diagnostic clinical laboratory. This system can be converted to routine laboratory use with little difficulty. It is a time-saving system which decreases the operational expenses. Its greatest feature will be the speed and accuracy with which the laboratory will be able to assist the physician in the patient's care.

### Transient Electro-optic Kerr Effect in Spheroidal-like Particles

**ALLEN KENT WRIGHT, Ph.D.**

*Department of Biophysics*

The transient electro-optic Kerr effect has been calculated for ellipsoids of revolution. The derived expression consists of a double exponential function of time, in which the exponential parameters are linearly related to the corresponding rotational diffusion constants. This is to be compared with the single exponential equation derived by Benoit for an ellipsoid of revolution.

The types of molecular information available from the experimental techniques of the transient electro-optic Kerr effect, depolarization of fluorescence, and dielectric dispersion are discussed and compared and it is shown that, heretofore, the Kerr effect has provided only half of the information available from the other two techniques. The equation derived in this thesis predicts that for the case of an ellipsoid of revolution the Kerr effect should provide the same amount of information as the other two effects. In fact, when the limiting behavior of this equation is examined for the case of spherical as well as for long rod shaped particles the number of time exponentials is reduced from two to one, in agreement with the equation of Benoit. It would appear, therefore, that Benoit's equation is applicable only to a sphere and not to an ellipsoid of revolution as originally intended.

Experimental results on several molecular species can be accounted for in terms of 2 relaxation times as predicted by the double exponential for solute particles which are ellipsoids of revolution. This increase in information with respect to ellipsoids of revolution permits the particle dimensions to be obtained, by a computer analysis, without the requirement of additional information concerning the size of the particle.
Studies of Potentially Useful Agents in Urolithiasis

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Department of Chemistry and Pharmaceutical Chemistry

In a review of the literature on urolithiasis the following successful chemical treatments of this disease found: treatment of uric acid and cystine stones with allopurinol and d-penicillamine respectively, treatment of less than six months old calcium oxalate stones by irrigation of the kidney with ion chelating solutions of EDTA or of less effective citrate, and finally treatment of non-infected cases with methylene blue.

The use of an agent which has the ability to chelate calcium more specifically than magnesium would be desirable. Such an agent, ethyleneglycol bis (betaamino-ethyl ether)- N,N,N',N'-tetraacetic acid, EGTA, was evaluated as to its acute and subacute toxicity, and for its absorption and excretion properties. The toxicology studies required a feasible method for determining calcium, magnesium, and chelating agents in biological fluids. Such a procedure was developed and calcium was determined using a solution of Na,EGTA as the titrant for calcium at an initial pH of 8.0, followed by titration of magnesium at the same pH with a solution of Na,EDTA. Chelating agents were determined using Na,EGTA in a back titration after addition of an excess of cadmium nitrate to the sample containing chelates at an initial pH of 4.0-4.5. A sharp break in the pH plot indicated the equivalence point in these methods.

In toxicological evaluation the oral LD₅₀ for Na,H,EGTA in male Holtzman rats was found to be 9.43 mmole/kg. compared with 6.25 mmoles/kg. reported for Na,H,EDTA. In subacute studies EGTA was compared directly with EDTA by feeding up to 10 per cent by weight of the disodium salt of each agent in the regular chow to rats for 90 days. Both EDTA and EGTA were absorbed to less than 5 per cent of the subacute dose and recovery from the urine and feces ruled out significant metabolism. The proportion of calcium not bound to chelate decreased as the amount of dietary chelate increased. EGTA was better tolerated in the diet and was less irritating than EDTA. Because EGTA appeared to be much less toxic and more palatable than EDTA, further study of the use of this agent and its derivatives as potentially effective agents in urolithiasis or in elimination of heavy metals should be made.

Clinical study of organic dyes has been limited to methylene blue. This agent caused both reduction of stone size and prevention of recurrent stone formation in patients producing the common calcium oxalate and phosphate stones.
Contributors

Edward R. Berry (The Effect of Ruby Laser, Xenon-Light Coagulator, and Diathermy on Vitreous Protein) is a research associate in biophysics at the Medical College of Virginia. After graduation from the University of Virginia, he was a research associate in biochemistry studying plasma proteins, lipids, and blood storage. Since his arrival at MCV his research interests have been burn toxins, and protein changes produced by absorption of energy from various sources.

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Arthur Keith Mant (*Accidental Hypothermia in Medico-Legal Practice*), a native of England, teaches forensic medicine at a number of hospitals, including St. Mary’s, Guy’s, King’s College, and at London University and the Police College. After graduating from Denstone College, Staffordshire, and St. Mary’s Hospital Medical School, he took advanced training in pathology. His published work has dealt with the investigation of obscure deaths, poisoning, and war crimes. Dr. Mant was the 1963 A.D. Williams Distinguished Scholar for the department of legal medicine at the Medical College of Virginia.

Thomas W. Nooney (*Solar Retinopathy Following the Eclipse of March 7, 1970*) is an NIH special fellow in the department of ophthalmology and a Ph.D. candidate in the department of physiology at the Medical College of Virginia. His other degrees are B.A., B.S., M.S., and O.D. After a long career in the Naval service as an aviator, optometrist, and radiobiologist Dr. Nooney came to MCV where he was appointed assistant professor in the department of radiology. His research and teaching interests are in visual physiology and psychophysics. Presently on faculty leave he will resume his duties as associate professor of ophthalmology in June, 1970.
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Joseph R. Svoboda *(Solar Retinopathy Following the Eclipse of March 7, 1970)* is a clinical instructor in the department of ophthalmology at the Medical College of Virginia. Born in Great Falls, Montana, he attended undergraduate school at Montana State College, and obtained his medical degree at George Washington University School of Medicine, Washington, D. C. Dr. Svoboda interned at D. C. General Hospital, and did his residency in ophthalmology at the Medical College of Virginia, after which he served as chief of ophthalmology at the U. S. Naval Hospital in Key West, Florida.

Elam C. Toone, Jr. *(Rheumatoid Arthritis and Malignancy)* is professor of medicine and chairman of the division of connective tissue disease at the Medical College of Virginia. Dr. Toone received his college education at Hampden-Sydney and his medical training at the Medical College of Virginia. He has been vice-president of the American Rheumatism Association, and was recently the recipient of the Distinguished Service Award of the Arthritis Foundation.
Marion Waller (Rheumatoid Arthritis and Malignancy) is associate professor of medicine and serologist for the division of connective tissue disease at the Medical College of Virginia. She received her A.B. degree at Hunter College in New York City, and earned her Ph.D. in immunology from the Graduate School of the Medical College of Virginia. Dr. Waller also spent a year at the Lister Institute in London, England, under an Arthritis and Rheumatism Fellowship.
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<td>hyoscyamine sulfate</td>
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Warnings: Caution patients about possible combined effects with alcohol and other CNS depressants. As with all CNS-acting drugs, caution patients against hazardous occupations requiring complete mental alertness (e.g., operating machinery, driving). Though physical and psychological dependence have rarely been reported on recommended doses, use caution in administering to addiction-prone individuals or those who might increase dosage; withdrawal symptoms (including convulsions), following discontinuation of the drug and similar to those seen with barbiturates, have been reported. Use of any drug in pregnancy, lactation, or in women of childbearing age requires that its potential benefits be weighed against its possible hazards.

Precautions: In the elderly and debilitated, and in children over six, limit to smallest effective dosage (initially 10 mg or less per day) to preclude ataxia or oversedation, increasing gradually as needed and tolerated. Not recommended in children under six. Though generally not recommended, if combination therapy with other psychotropics seems indicated, carefully consider individual pharmacologic effects, particularly in use of potentiating drugs such as MAO inhibitors and phenothiazines. Observe usual precautions in presence of impaired renal or hepatic function. Paradoxical reactions (e.g., excitement, stimulation and acute rage) have been reported in psychiatric patients and hyperactive aggressive children. Employ usual precautions in treatment of anxiety states with evidence of impending depression; suicidal tendencies may be present and protective measures necessary. Variable effects on blood coagulation have been reported very rarely in patients receiving the drug and oral anticoagulants; causal relationship has not been established clinically.

Adverse Reactions: Drowsiness, ataxia and confusion may occur, especially in the elderly and debilitated. These are reversible in most instances by proper dosage adjustment, but are also occasionally observed at the lower dosage ranges. A few instances syncope has been reported. Also encountered are isolated instances of skin eruptions, edema, minor menstrual irregularities, nausea and constipation, extrapyramidal symptoms, increased and decreased libido—all infrequent and generally controlled with dosage reduction; changes in EEG patterns (low-voltage fast activity) may appear during and after treatment; blood dyscrasias (including agranulocytosis), jaundice and hepatic dysfunction have been reported occasionally, making periodic blood counts and liver function tests advisable during prolonged therapy.

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