extract in patients with pernicious anemia. Mermod and Dock point out that this observation, together with the known effectiveness of Congo Red in neutralizing certain toxic substances, calls for a further exploration of the old theory that pernicious anemia is due to the presence of some toxic agent. During the past two years we have accumulated a certain amount of evidence for the presence of a toxic factor in pernicious anemia.

Sterile morning urine specimens obtained from eight untreated pernicious anemia patients (two males and six females) were injected intramuscularly into pigeons. Eight pigeons were used for each urine. Four of the birds received 0.1, 0.5, 1.0 and 1.5 cc of urine per 100 gm of pigeon respectively for five successive days. The other four pigeons were given corresponding amounts of urine previously heated to 100° C. for two hours. Daily reticulocyte counts were made for two weeks prior and for six weeks subsequent to the first injection. Each of the eight unheated urines produced a decrease in the reticulocytes of the pigeon below the significant level of 5 per cent. (Repeated daily counts on more than one hundred fifty control pigeons by a method already reported[4,5] have never shown a minimum reticulocyte percentage of less than 5.) The significant minimal counts varied from 0 to 4 per cent. with an average of 2 per cent. The peaks of the decreases were obtained on the 5th to the 8th days. The reticulocyte decreasing substance is thermolabile, inasmuch as none of the pigeons injected with the heat-treated urines showed a reduction in reticulocytes. Likewise, pigeons receiving corresponding doses of six normal human urines, unheated and heated, failed to show a reticulocyte decrease. The four urines containing the greater concentrations of the reticulocyte decreasing principle were definitely toxic, since 15 of the 21 birds injected with these urines died in from one to eight days following the first injection. Indeed one urine was so toxic that smaller doses (.025 and .05 cc) were necessary in order to demonstrate the reticulocyte decreasing effect. On the other hand, of the 32 pigeons injected with the heat-treated pernicious anemia urines which were without reticulocyte decreasing effect, all but one survived. Likewise the 48 pigeons receiving the normal unheated and heated human urines survived. The toxicity, therefore, is associated with the reticulocyte decreasing factor and is very probably due to it.

Following the primary decrease in reticulocytes, most of the surviving pigeons showed a subsequent reticulocytosis. This reticulocyte stimulating effect was partially retained by the heat-treated pernicious anemia urines. A detailed consideration of the significance of this reticulocyte stimulating substance previously reported to be present in normal human urine[4,5] is outside the scope of this progress note. However, the possibility of the identity or similarity of this second urinary substance and the anti-pernicious anemia principle is suggested by the reports of Decastello[6] and one of us.[7] Obviously more work is necessary, although these findings also throw doubt on the current deficiency theory as a complete explanation of the pathogenesis of pernicious anemia.

Thus far, we have examined the urines of two treated pernicious anemia patients and have found the toxic, reticulocyte decreasing substance absent. Interestingly enough, the concentration of the reticulocyte stimulating principle in these two urines was definitely decreased.

It is apparent, therefore, that urine from untreated patients with pernicious anemia contains both a thermolabile, comparatively toxic, reticulocyte decreasing factor and a partially thermostable, relatively nontoxic, reticulocyte stimulating principle for the pigeon. Normal human urine contains the latter but not the former, or at least not in the quantities of urine used. What relation the urinary reticulocyte decreasing principle bears to the toxic substances reported by Macht,[8] Mermod and Dock,[9] and Kingisepp[10] as present in the plasma of untreated pernicious anemia patients is largely speculative at present. If our impression that the principle acts through depressing erythrogenesis in the bone-marrow should prove to be correct, the production of experimental pernicious anemia in mammals by the separation and administration of sufficient quantities of the reticulocyte decreasing factor is not beyond the realm of possibility.

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SOME OBSERVATIONS ON ULTRA-VIOLET IRRADIATED AMEBAS

PRELIMINARY to a study of the manner in which various saline solutions modify the action of ultra-violet light on protoplasm a number of exploratory experiments were done upon *Amoeba proteus*. In the course of these experiments certain results of more general interest were obtained.

(1) Changes in the prominent food vacuoles of approximately 15 amebas were observed during and

[4] G. E. Wakerlin, H. D. Bruner and J. M. Kinsman, *Proc. Am. Physiol. Soc.*, p. 136, 1935.
[5] G. E. Wakerlin and H. D. Bruner, *Arch. Int. Med.*, in press.
[6] A. Decastello, *Med. Klin.*, 31: 377, 1935.
[7] G. E. Wakerlin, *Proc. Soc. Exp. Biol. and Med.*, 32: 1607, 1935.
[8] D. I. Macht, *Jour. American Medical Association*, 89: 753, 1927.
[9] C. Mermod and W. Dock, *Proc. Soc. Exp. Biol. and Med.*, 32: 373, 1934.
[10] G. Kingisepp, *Klin. Wochnschr.*, 13: 1820, 1934.

following irradiation. In most cases a "Cold Quartz" lamp was used with which 10 irradiations each of approximately 10 seconds duration were given at 10-minute intervals. Most of the light of this lamp is of wave-lengths ranging from 2540Å to about 1800Å. A few were observed under irradiation from a Kromayer quartz mercury arc, which gives relatively much more energy at longer wave-lengths, including an appreciable amount of heat and visible light. These radiations were of such intensity that morphological changes, as described by Black,[1] were noticeable after about 30 seconds exposure. Digestion seemed to stop with the first appearance of these morphological changes and did not resume during the remaining life of the now moribund amebas. Ultimate death, with cytolysis, was often delayed for from 12 to 24 hours after irradiation. Upon disintegration of the amebas the food particles could be seen apparently in the same condition as when they were examined before the commencement of irradiation. This suggests that irradiation inactivated the digestive enzymes or stopped their secretion or elaboration. It might also be supposed that proenzymes or the coenzymes necessary for their activation were affected. It is well known that ultra-violet light inactivates many enzymes and similar substances *in vitro*. Induced impermeability of the walls of the food vacuole must also be considered in seeking an explanation of the stoppage of digestion.

(2) Five amebas were exposed to an oblique beam of ultra-violet light from a Kromayer lamp. They all oriented and moved away from the source. The angle of incidence of the ultra-violet light was made as nearly parallel as possible to the surface upon which the amebas were moving. Although the radiation included a good deal of visible light, this was not great relative to the bright diffuse daylight which was not oriented. The response, therefore, appears to have been evoked by ultra-violet light. It is hardly possible on the basis of the available evidence to say whether it may be a direct effect upon the contractile mechanism; such as increased viscosity or gelatin of the sol in the more intensely illuminated side before the plasmagel is broken down.

There are several reports of modifications of the degree of motility or the direction of movement of amebas as a result of irradiation by visible light, but ultra-violet light appears not to have been studied with regard to tropism.

Harrington and Leaming[2] found that intense white or violet light thrown on an active *Amoeba proteus* produced instantaneous stoppage of protoplasmic streaming. This observation was confirmed by Mast[3] and Folger.[4] Mast found that a sudden increase in light intensity caused all streaming to stop and that the effect was repeated as one passed from one wave-length to a shorter one, while no effect was obtained when the wave-length was changed in the reverse direction. Visible light was used throughout.

Davenport,[5] using white light, found negative phototropism in *Amoeba proteus*. Mast[3] confirmed this and observed that the process of orientation is associated with inhibition of pseudopod formation on the more highly illuminated side. Mast[6] says that *A. proteus* orients negatively in directive illumination in strong light but probably positively in very weak light.

(3) The surface membranes of amebas are often regarded as extensible,[7] but there appear to have been no quantitative measurements of this extensibility or elasticity. We found that after amebas had been made to round up by the action of ultra-violet light the membrane appeared relatively inextensible. Many camera lucida drawings of outlines of the amebas were made, and they indicate that the increase in diameter can not have been greater than of the order of 10μ for an ameba 150μ in diameter. This represents an increase in surface of less than 14 per cent.

It is quite possible that ultra-violet light leads to increased internal pressure, and this in turn to increase in volume and attendant rounding up of the amebas. At first the volume may increase simply by approach to a spherical form without greatly stretching the membrane, but further swelling thereafter must stretch it, and unless it is extensible further irradiation must ultimately rupture it with little or no expansion. The ultimate cytolysis of irradiated cells often has the appearance of rupture of the membrane as a result of internal pressure.

It seems necessary to conclude that the surface membranes of irradiated amebas are relatively inextensible. Whether this conclusion may be generalized to include unirradiated amebas is doubtful. The extensibility of the cell membrane of an irradiated ameba may be very different from that before irradiation, probably much less.

SUMMARY

(1) Observations upon irradiated amebas indicate that digestion of food is stopped by ultra-violet light

[1] W. A. Black, "Experimental Modifications of the Effect of Ultra-violet Light on Protoplasm.—I. *Amoeba proteus.*" In process of publication.

[2] N. R. Harrington and E. Leaming, *Amer. Jour. Physiol.*, 3: 9–18, 1900.

[3] S. O. Mast, *Jour. Exp. Zool.*, 9: 265–277, 1910.

[4] H. T. Folger, *Anat. Rec.*, 29: 96–97, 1924.

[5] C. B. Davenport, "Experimental Morphology," 280 pp., London and New York, 1897.

[6] S. O. Mast, *Zeitschr. vergl. Physiol.*, 15: 139–147, 1931.

[7] J. G. Edwards, *Brit. Jour. Exp. Biol.*, 1: 571–596, 1924.

and that cessation occurs with the appearance of morphological symptoms of impairment.

(2) *Amoeba proteus* shows negative phototropism toward ultra-violet light.

(3) The cell membrane of irradiated amebas is relatively inextensible.

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A CONTRIBUTION TO THE PHARMACOLOGY OF PHYSOSTIGMINE[1]

WHILE investigating the peripheral action of barbiturates, we observed that in all experimental animals where the cardiac vagus response to weak faradic stimulation had been abolished by barbiturates doses of physostigmine salicylate ranging from 0.2 to 0.35 mgm per kgm showed no detectable spontaneous effect on the heart rate. However, if two to three minutes were allowed to elapse after intravenous administration of this drug and then the peripheral vagus was stimulated with the same weak, or even weaker, faradic current as used above profound cardiac inhibition was produced. Occasionally the slowing of the heart which was produced by stimulation of the peripheral vagus persisted for several minutes after stimulation was discontinued. A similar cardiac slowing was noted following injection of acetyl choline, but it was not as marked and consistent.

This physostigmine sensitization of the vagus to stimulation lasted for about 30 minutes and was promptly antagonized by intravenous injections of further doses of barbiturates.

On the assumption that barbiturates produced their vagus-impairing effects by ganglionic depression, we employed in another series of experiments nicotine salicylate in doses varying from 2 to 4 mgm per kgm to produce ganglionic paralysis in dogs and rabbits. After sufficient nicotine salicylate had been administered intravenously to render the peripheral vagus non-responsive to even strong faradic stimulation, physostigmine (0.2 to 0.3 mgm per kgm) was injected intravenously. In every case within three minutes following the injection of physostigmine, faradic stimulation of the peripheral end of the vagus nerve produced marked cardiac inhibition. In other words, physostigmine antagonized the synaptic paralysis produced by nicotine.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A PROPOSED METHOD FOR THE DIRECT MEASUREMENT OF CORRELATION

ATTENTION is called to the possibility of applying certain relations given by Yule to the development of a device for the physical determination of the correlation coefficient. The relations are:

$$\Sigma_1^2 + \Sigma_2^2 = \sigma_1^2 + \sigma_2^2$$

$$r^2 = 1 - \frac{\Sigma_1^2\ \Sigma_2^2}{\sigma_1^2\ \sigma_2^2}$$

where Σ_1 and Σ_2 are the maximum and minimum standard deviations of the scatter about the intersection of mean$_1$ and mean$_2$, and σ_1 and σ_2 have the usual connotations.[1]

Given the correlation surface actually constructed, the measurement of all these standard deviations in terms of moments should be feasible. The equivalence of σ^2 to the moment of inertia of the distribution about its mean is well known. It is possible that a physical method has not yet been applied to correlation because of the difficulty of obtaining cross-products. If Yule's Σ_1^2 and Σ_2^2 can be physically measured, this difficulty is eliminated by the proposed method.

A moment of inertia is measurable as $\frac{T\,t^2}{2\,\theta}$, where T is torque applied (constant), and t is time to reach θ, which is resultant angular displacement. What would seem necessary then would be a device utilizing unit rotational force acting through unit time, whence a moment would be found in terms of distance rotated. A spark marker could be used to measure this distance. Having found the means of the constructed correlation surface by balancing, σ_1^2 would be obtained by rotation of the surface about the line through mean$_1$ parallel to the axis of the other variable, and correspondingly for σ_2^2. The axes of rotation for finding Σ_1^2 and Σ_2^2 would be passed diagonally through the intersection of mean$_1$ and mean$_2$ (center of gravity). The angles of these axes would be varied until the minimum or maximum rotation had been noted. Inspection of the surface would reveal the angles for these axes closely for correlations higher than .30.

For plotting the scatters there could be provided light, rigid trays having twenty cells each way and a small post in each cell. With thin but heavy metal coins having holes in the centers, the plotter could construct the surface as rapidly as one plots the usual scatter on paper. The coins should fit snugly and be thin enough to give the surface a relatively small vertical dimension. The physical measurements of the

[1] From the Department of Pharmacology and Materia Medica, Georgetown University, School of Medicine, Washington, D. C.

[1] "Introduction to the Theory of Statistics," p. 322, 1919.