tomized rats and their pair-fed controls is within the range of error inherent in the system of grading. Thyroidectomized rats showed less fatty metamorphosis; the difference is not great but may be significant. The livers of rats fed *ad libitum* showed extremely little damage except the one plus fatty metamorphosis. The only known differences between these rats and the pair-fed controls of this experiment were the greater amount of food consumed and a faster growth rate.

The few sections made through the "thyroid area" of each operated rat failed to reveal any thyroid tissue. The thyroid gland of all thiouracil fed rats showed marked epithelial hypertrophy with severe depletion of colloid. The thyroid glands of all other rats were histologically normal.

Discussion. The inhibition of thyroid activity suggested by Forbes *et al.*[2] as a possible explanation for the liver protective effect of sulfanilamide is not supported by our findings. It should be pointed out, however, that in their experiment the CCl_4 was administered by inhalation.

György *et al.*[3] and Handler *et al.*[5] found that a decreased level of thyroid activity protected the livers of rats against parenchymal necrosis in dietary cirrhosis studies. In view of the findings in the present study it would seem that the mechanism of protection against carbon tetrachloride necrosis[2] and the necrosis occurring in dietary cirrhosis is different. In this connection Handler *et al.*[5] found that sulfasuxidine lessened the tendency of the livers of choline-deficient rats to become necrotic and scarred even though the thyroid glands of these animals were essentially normal.

Summary. The lowering of thyroid activity by (1) thyroidectomy and (2) the feeding of thiouracil in a stock diet, failed to prevent or suppress hydropic degeneration or necrosis of the rat liver induced by subcutaneous administration of carbon tetrachloride.

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Effect of Temperature on Transmission of Light by Cell Free Body Fluid in *Phascolosoma gouldii.*

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The biological fluids of higher animals have been studied intensively and extensively from the point of view of chemical, physical, and physiological characteristics. The physical nature of the serum of mammals, for example, has been investigated with great thoroughness by Du Noüy who showed that at 56°C "profound modifications in the structure of the proteins and of the lipoprotidic complex" become evident.[1]

On the other hand, very little is known about the physical behavior of invertebrate body fluids. In order to ascertain the relationship of invertebrate fluids to those of higher animals, a series of experiments was made to test the effect of heating on the transmission of light by the cell free body fluid in *Phascolosoma gouldii.*

Material and methods. In general the procedure was similar to that followed by Du-Noüy in studies on horse serum. Body fluid was carefully removed from 10 phascolosomas, using a 5 cc hypodermic syringe fitted with an 18 gauge needle. Approximately 1 to 3 cc of pink fluid were obtained from each worm. The pooled fluid was then centrifuged for about 10 minutes in an ordinary clinical cen-

[1] Du Noüy, L., *Studies in biophysics. The critical temperature of serum (56°).* New York, 180 pp. 1945.

trifuge. The cell free supernatant fluid was drawn off with a pipette and introduced into a soft glass test-tube about 6" by 5/8" in size. The open end of the tube was then quickly drawn out and sealed in a gas flame. Another tube, carefully matched with the first, was similarly prepared but was filled with distilled water. It was used as a control. Altogether 12 tubes, representing over 100 worms, were prepared.

Some of the tubes were heated for 5 minutes in a carefully controlled water bath at temperatures from 24° to 90°C. Other tubes were heated for 20 minutes at like temperatures. An electric photometer, with a 610 mμ filter, was adjusted to register 100% light transmission on the galvanometer scale, when the control tube was in place. (The light source, filter, absorption tube, and photocell were linearly arranged.) This value was recorded as incident light (I_o). Before each test the needle setting was adjusted with the control tube in place. The control tube was replaced by the tube containing the heated serum (which was cooled to room temperature) and the value on the galvanometer scale was recorded as transmitted light (I). The temperature of heating for each tube was increased after each photometric test until coagulation of the cell free fluid took place.

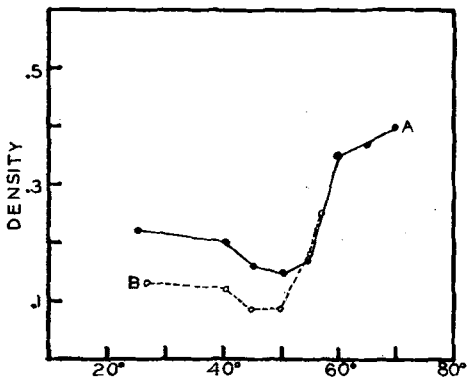

FIG. 1.

Graph obtained by plotting the optical density ($d = \log I_o/I$) against the temperature, in degrees Centigrade, at which the body fluid was heated. Curve A, fluid heated for 5 minutes; curve B, for 20 minutes.

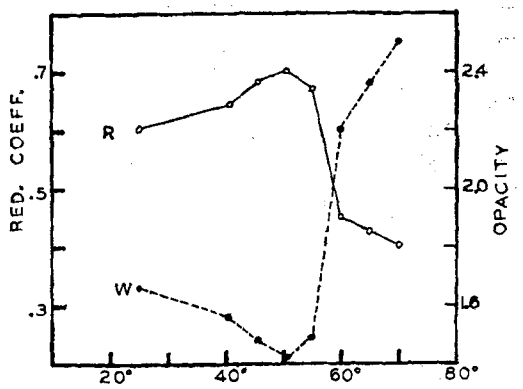

FIG. 2.

Graph showing the curves obtained by plotting the reduction coefficient ($r = I/I_o$) and the opacity ($W = 1/r = I_o/I$) against the heating temperature in degrees Centigrade. Curve R, reduction coefficient; curve W, opacity.

Results. The results are summarized in Fig. 1 and 2. Fig. 1 shows the results of plotting optical density ($d = \log I_o/I$) against temperature of heating. Curve A represents fluid heated for 5 minutes; curve B for 20 minutes. The results show that at about 40°C there is a decrease in optical density which suddenly increases rapidly beginning at about 50°C. (There is an evident increase in light transmission at about 40°C followed by a decrease beginning at 50° to 55°C).

Fig. 2 shows the results of plotting reduction coefficient ($r = I/I_o$) and opacity ($W = 1/r$) against temperature of heating. The curves are for fluid heated for 5 minutes. It is evident that at 40°C there begins an increase in reduction coefficient and a decrease in opacity. At about 50°C the reduction coefficient begins to fall, at first slowly, then rapidly and the opacity increases similarly. Curves for fluid heated for 20 minutes have the same configuration but are higher or lower on the graph.

The temperature for coagulation of the fluid varied greatly with different samples. This agrees with the results of Andrews who says that the fluid may coagulate at any temperature from 63° to 80°C.[2]

Discussion. The pooling of a series of experimental individuals to obtain directly a median value has been used for various investigations with favorable results.[3] In the

[2] Andrews, E. A., *Johns Hopkins Univ. Circ.*, 1890, **9**, 65.

[3] Greiff, D., and Pinkerton, H., *J. Exp. Med.*, 1945, **82**, 193.

present work it was essential in order to have large enough samples for measurement. The median values are quite consistent.

If it is assumed that the body fluid contains protein particles dispersed in a fluid medium, then if the particles become smaller the light transmitted shifts toward the red end of the spectrum.[4,5]

It is well known that electric photometers are ideal for measuring the light transmitted in a parallel direction through a turbid medium.[6] In the present work a red filter was used in such an instrument. Any shift, therefore, in light transmission toward the red end would be reflected in a decrease in density and opacity values of the fluid. Such a decrease is evident after heating the body fluid to 40° or 50°C. This increase in transmitted light may be interpreted as indicating a decrease in the size of the particles in solution. Continued heating above 50° C results in a pronounced increase in optical density indicating a similar increase in the particle size until finally coagulation takes place.

No measurements were made of light scattered in relation to light absorbed by the body fluid. Consequently, it is not certain whether scattering or absorption or a combination of both is responsible for the decrease in transmitted light. Measurements of scattered light are now in progress. However, in other investigations there has been found a consistent parallelism in serological tests between results obtained from measurements of scattered light and those of transmitted light.[7]

The present results are especially interesting if compared with those obtained by Du Noüy using horse serum. His figures indicate no change in light transmitted until the serum is heated to about 56°C at which point there is a pronounced decrease in transmitted light. The consistent preliminary increase in light transmitted after heating to 40° to 50°C, which is found in the body fluid of *Phascolosoma*, does not obtain in horse serum.

Apparently, heating causes the particles in the body fluid in *Phascolosoma* first to decrease in size, perhaps by giving up water, and then to increase in size either by hydration or by aggregation.

The results indicate what might be called 2 critical temperatures for the cell free fluid in *Phascolosoma*: a) 40°C above which heating brings about a decrease in particle size; b) 50 C above which particle size is increased possibly by "intramolecular hydration".[1]

The present method gives a new approach to the study of the phylogenetic relationships of animal body fluids. It is anticipated that a series of investigations covering representative invertebrates and vertebrates will be made with the view to ascertain the evolutionary development of the complex sera of higher animals.

Summary. Cell free body fluid from *Phascolosoma gouldii* was heated in sealed tubes at temperatures from 24° to 90°C. Measurements of light transmitted by the heated fluid indicate that there are 2 critical temperatures for the fluid: a) 40°C above which heating brings about a decrease in particle size; b) 50°C above which particle size is increased.

[4] Burns, D., *An introduction to biophysics*, Macmillan, New York. 580 pp. 1929.

[5] Ostwald, W., and Fischer, M. H., *An introduction to theoretical and applied colloid chemistry*, New York, 266 pp. 1922.

[6] Drabkin, D. L., Photometry and spectrophotometry, in *Medical Physics*, Chicago, 1744 pp., 1944.

[7] Baier, J. G., *Physiol. Zool.*, 1947, **20**, 172.