

Scarcely less welcome are the glimpses of Pringle's highly individual personality and the sidelights on his methods of work which the diaries afford. One can only regret that his early journeys to the Pacific states

in 1880-1884 and the brief visits to Cuba in his last active years could not have been included.

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## SPECIAL ARTICLES

### SURVIVAL OF ASCARIS EGGS AFTER CENTRIFUGING<sup>1</sup>

FERTILIZED eggs of *Ascaris suum* in the uncleaved stage, in the 2-cell stage and in the 4-cell stage of development were exposed to a centrifugal force of approximately 400,000 times the force of gravity for one hour in the ultracentrifuge recently developed by J. W. Beams. The eggs were then removed from the rotor, placed in depression slides and their stratification and stage of development noted and charted by aid of the microscope. They were found stratified into 3 distinct layers: (1) a layer of yolk at the centrifugal pole; (2) a middle clear and apparently homogenous protoplasmic layer; and (3) a layer of fat at the centripetal pole. In some cases the fatty layer was observed to be separated from the rest of the egg.

Twelve hours after the eggs had been centrifuged it was observed that they had lost their stratified condition and some of them had undergone mitosis. After 48 hours at least 90 per cent. of them had divided once. They were observed at intervals under the microscope until they had developed beyond the 8-cell stage, which extended over a period of approximately 3 or 4 days.

In a second set of experiments eggs in the same stages of development as those used in the first were exposed to a centrifugal force of approximately 150,000 times the force of gravity for 4½ days. They were studied in the same manner as those mentioned above. Here also, at least 90 per cent. of the eggs were observed to be alive and to develop at about the same rate as the controls. In still other cases, eggs have undergone cleavage while rotating at 100,000 times gravity.

These results seem to bear directly upon the questions recently discussed by Taylor<sup>2</sup> concerning living and non-living colloidal systems. He states:

If, therefore, a centrifugal force applied to the ground substance (protoplasm) were sufficiently great, it, too, would suffer a stratification of its colloidal components no less definite than that of its grosser, visible inclusions as effected by ordinary centrifuging. Indeed, in recent times many non-living colloidal systems have been successfully stratified by means of the ultracentrifuge as perfected and employed by Svedberg (1928) and others. But to what extent the living ground substance would endure the rigors of such enormous forces (10,000-100,000

times gravity) and remain living, is, of course, exceedingly problematical. If the basis of protoplasmic organization is molecular—a postulate which now applies to colloidal systems generally—we may reasonably suppose that the living substance physically owes its being to the condition and maintenance of its unique structure. This qualification of the spatial relation of its component parts, once violated by mechanical or other forces sufficient to disrupt that spatial relationship, is thereby relinquished and the living substance disintegrates and dies.

It is of interest to note here that according to Bodine and Boell<sup>3</sup> practically no change in the oxygen consumption of blocked grasshopper eggs occurs after centrifuging at 400,000 times gravity, although such eggs do not recover. We have shown that the protoplasm of *Ascaris* eggs in the early stages still remains living after being exposed to forces equal to the maximum employed by Svedberg to separate from solution many artificial colloids and native colloids, such as proteins. However, we have been unable to determine whether or not a stratification of the protoplasmic components under such strong centrifugal force has taken place. If such does take place, it is of particular interest, for then the normal spatial relationship of the separate elements can not be of vital importance for the maintenance of life. However, if, as we are inclined to believe, little or no separation or stratification of the components has taken place in this material, they must be held together in a firmer way than those in the colloidal systems examined by Svedberg. In other words, the conditions present in this living colloidal system (protoplasm) seem to be different from those in non-living ones.

We are of the opinion that the killing of cells by the present methods of centrifugation is usually due to mechanical distortion or disruption (prevented in *Ascaris* eggs by the presence of a very resistant shell) rather than to a disturbance of the spatial relationship of their molecular parts.

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### THE SEMIQUINONE OF THE FLAVINE DYES, INCLUDING VITAMIN B<sub>2</sub>

SINCE it has been shown that many derivatives of phenazine, such as pyocyanine,<sup>1</sup>  $\alpha$ -oxyphenazine<sup>1</sup> and

<sup>3</sup> J. H. Bodine and E. J. Boell, *Jour. Cell. and Comp. Physiol.*, 7: 455, 1936.

<sup>1</sup> L. Michaelis, *Chem. Reviews*, 16: 243, 1935.

<sup>1</sup> Aided by grant from the Rockefeller Foundation for Research in cellular biology.

<sup>2</sup> C. V. Taylor, *Physiol. Zool.*, 4: 423, 1931.