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[1]

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[2] 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[3] 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_2

SINCE it has been shown that many derivatives of phenazine, such as pyocyanine,[1] α-oxyphenazine[1] and

[1] Aided by grant from the Rockefeller Foundation for Research in cellular biology.

[2] C. V. Taylor, *Physiol. Zool.*, 4: 423, 1931.

[3] J. H. Bodine and E. J. Boell, *Jour. Cell. and Comp. Physiol.*, 7: 455, 1936.

[1] L. Michaelis, *Chem. Reviews*, 16: 243, 1935.

chlororaphine,[2] form a semiquinone radical as an intermediate state of reduction, it was suggestive to search for such a reaction in the closely related group of dyes consisting of the derivatives of isoalloxazine, including what is called lactoflavin, the dye-stuff component of Warburg's yellow respiration enzyme,[3] and identified with vitamin B_2 by Kuhn.[4] In fact, Kuhn and Wagner-Jauregg[5] showed that in a very acid solution an intermediate red form of the dye can be observed. At pH >1 no trace of this red form could be found.

Now, in the case of pyocyanine it has been shown[6] by a potentiometric method that even around neutrality a definite although small amount of the semiquinone can exist in equilibrium with the oxidized and the reduced form. Accordingly, Kuhn and Moruzzi,[7] Stern[8] and later Stare[9] have attempted to show that the same holds for the flavines. They applied the potentiometric method, basing calculations on what has been called the index potential.[10] Stern especially made much use of this method. In a paper to be published soon it will be shown that qualitatively the results of these authors are acceptable, those obtained by Stern even more so than those obtained by Stare, but quantitatively they have to be subjected to considerable improvement before they finally lead to a clear picture of the situation. However, even this evidence is based on very delicate observations of the index potentials in that range of its value where an error of a few tenths of a millivolt makes a big difference in the equilibrium percentage of semiquinone. Under these circumstances it is desirable to have supplementary even though only qualitative evidence for the existence of the semiquinone in neutral solutions. The following experiment gives satisfactory evidence.

A solution of any representative of the flavines (including vitamin B_2), saturated at 70°–80° C., in a buffer anywhere between pH = 4 and 10, and always kept about this temperature to avoid precipitation, is mixed with a suitable amount of solid sodium hydrosulfite. The color changes from intensely yellow through a dirty olive green to pale yellow, and on reoxidation by air the whole color change is reversed.

The color of the semiquinone in approximately neutral solution is, therefore, green. In very acid solution it is red. This is due to a different state of ionization, there being a dissociation constant of the semiquinone, pK = 1.0, approximately. In the very low concentration obtainable at room temperature in weakly acid or neutral solution it is more difficult to observe this phenomenon, although it can be observed on looking through the whole length of a test-tube.

The existence of this intermediate form in neutral solution, which previously rested on rather tenuous evidence, has now been made much more certain by this color reaction. It is easy to imagine that this property, in general very rare in dye-stuffs and just encountered in a group of dyes, one of which has such an important physiological property, if it contains a side chain of a definite steric structure (d-Ribose), should be of physiological significance. It is suggestive to think that in some cases the active form of certain enzymes might be the semiquinoid form. Here, a definite level of *oxidation-reduction,* observed in only a few dyes, is the active form, in analogy to the fact that in some other enzymes one definite state of *ionization,* as determined by the pH, is the kinetically active form.

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ELECTRICAL BRAIN WAVES AND TEMPERATURE

IN a previous note in this journal,[1] I reported that values of the critical thermal increment of approximately 8, 11 and 16 thousand calories had been found for the frequencies of the alpha rhythm (most commonly called the "Berger rhythm" by Adrian and others) in a group of 6 patients whose temperatures were elevated by diathermy. Recently Jasper[2] has criticized the identification of these values, since he finds fluctuations of several cycles per second in a run of an hour or two without, he says, any temperature change.

Fig. 1 is a plot according to the Arrhenius equation of frequencies (F) of the alpha cycles as a function of absolute temperatures (T). If the equation fits, one should get a straight line of negative slope by plotting log F against $1/T$. μ in calories is determined directly from the slope of the line. All the data involved in my first report are embodied in this figure as well as data from 4 additional subjects—10 in all. The lower curve of the figure ($\mu = 8000$ calories) corresponds to 7 daily experiments on 3 normals, 8 daily experiments on 2 mild general paretics and 5

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