

Thirty fourth meeting.

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President Lee in the chair.*

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**The comparative toxicity of sodium chloride and of
staining solutions upon the embryo of *Fundulus*.**

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of Pennsylvania ; and from the Marine Biological
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What substances enter cells and upon what conditions the entrance of various substances into cells and the permeability of organized animal membranes generally depends, is as yet only very imperfectly understood. The following experiments may not be without interest in this connection.

In studying the toxicity of stains upon star-fish eggs, we find thionin, Bismarck brown, methylene blue and neutral red all to be very poisonous, if the solutions are exposed to light. Among these, neutral red is perhaps less poisonous than the other stains. Solutions of eosin are very much less toxic than the other substances. Thionin, Bismarck brown, methylene blue and neutral red easily penetrate into ova and stain them in a characteristic way. Eosin does not stain living cells, but only enters in combination with the dead protoplasm.

Very different is the degree of toxicity of these stains towards the eggs of *Fundulus*. Here, Bismarck brown, thionin, methylene blue and eosin are devoid or almost devoid of toxic action, whilst neutral red alone possesses any marked degree of toxicity, if the solution acts in the light. And the latter stain is likewise the only one able to enter the healthy ova of *Fundulus* and to stain certain parts of the embryo. We are therefore justified in the conclusion that in the case of stains the toxicity of these substances towards

ova is dependent upon and is an indicator of their combination with the protoplasm of certain cells of the embryo.

Now, it is not without interest to state that the toxicity of neutral red varies according to the stage of development at which the eggs are exposed to the influence of the staining solutions. Ova immersed in a solution of neutral red and exposed to the light, inside of sixteen hours after fertilization are most severely affected; ova exposed approximately twenty to thirty hours after fertilization are somewhat more resistant, and ova which are exposed to the light as late as two to four days after fertilization are affected only to a very slight degree.

Correspondingly, we find that the older the embryo becomes, the less is it liable to be stained with neutral red and in embryos five days old we usually find almost the whole embryo unstained with the exception of the newly developed liver which appears in an orange-yellow color.

We may therefore conclude that the embryos of *Fundulus* and their cells become less and less permeable for neutral red as the development advances and that its toxicity decreases correspondingly.

A curve of toxicity almost parallel to that of neutral red we find in the case of isotonic sodium chloride solutions. During the first sixteen hours isotonic solutions of sodium chloride are extremely toxic to the embryo of *Fundulus*; from twenty to thirty hours there is noticeable a certain decrease in toxicity; while embryos two to four days old develop in $5/8$ N. sodium chloride solutions almost as well as in sea water. We see, therefore, that the similarity of the curves is very great and inasmuch as in the case of the neutral red the variations in toxicity seem to depend upon variations in the staining ability of this substance and therefore probably upon the permeability of certain membranes or of the protoplasm of certain cells to the stain, we may assume as the most plausible explanation that in the case of sodium chloride the variations in toxicity also depend upon the permeability of certain organized structures to the latter substance, and that therefore the conditions of permeability in the embryo of *Fundulus* depend upon the same conditions in the case of the lipid soluble neutral red and in the case of lipid insoluble inorganic salts, a conclusion which is at

variance with the views of Overton and Hoeber, but agrees with the observations made by Jacques Loeb, Robertson and by the botanist, Ruhland. We are well aware of the number of variable factors which are to be taken into account in the interpretation of these phenomena which may perhaps later necessitate a somewhat more complicated explanation; but we believe that comparative studies in the toxicity of stains and of various other substances will prove to be of value in the elucidation of the problems of cell permeability and of the cause of toxicity.

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The influence of calcium chloride and of adrenalin upon the secretion of urine and upon absorption from the peritoneal cavity.

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I. Intravenous injection of calcium chloride diminishes the secretion of urine. Porges and Pribram ascribed this effect to the lowering of blood pressure which follows the intravenous injection of this substance. Our experiments, we believe, show such an interpretation to be erroneous for the following reason:

If we add adrenalin to sodium chloride solutions the blood pressure rises during the intravenous injection of this fluid and we also find a noticeable increase in diuresis under the influence of adrenalin. If we now add calcium chloride to the adrenalin-sodium chloride mixture the blood pressure remains likewise very high during the intravenous injection and the ultimate fall due to the influence of calcium chloride is delayed for a considerable time, but notwithstanding the high blood pressure produced by adrenalin which is in itself a substance favorable to diuresis, the addition of calcium chloride again causes a marked decrease in diuresis. The effect of calcium chloride in diminishing the secretion of urine can therefore not be ascribed to its action on the blood pressure, but to some other condition, most probably to its direct influence upon the epithelial cells of the kidney, an interpretation originally given by John B. MacCallum.