were fed, with the result that on the fourth day pronounced ataxia, loss of sight and hearing, complete anesthesia, and catalepsy were observed, recovery occurring on the next day. After fasting for 24 hours the animal was placed on a diet of fresh lean beef. In five days a recurrence of the above symptoms was noted. The death of the animal occurred on the fifty-ninth day of the experiment, after the dog had undergone a loss of 42% of his weight. Autopsy showed a fistulous opening 2 cm. in length and no collateral circulation. In other cases the symptoms described occurred only after the addition of Liebig's extract to the meat diet.

The administration to normal dogs of sodium carbamate either by mouth or by intravenous injection, gave rise to none of the symptoms observed by Pawlow and associates.

33 (79). **"On chemical fertilization": JACQUES LOEB.** (Presented by **WILLIAM J. GIES.**)

1. In two previous publications the author mentioned the fact that by applying two different methods of treatment to the unfertilized egg of the sea urchin, this egg could be caused to develop in a way which resembled in all its essential features the development of the eggs fertilized with sperm. These two methods consisted, first, in putting the eggs for about two hours in hypertonic sea water (the method used in the early experiments) and, second, in exposing the eggs for from one to two minutes to sea water, to which a certain amount of acetic acid or formic acid had been added. When the old method alone was used the eggs did not form a membrane, nor did the larvas rise to the surface. When the acid treatment alone was used, the eggs formed a membrane and after about six hours divided into from two to six cells, but then died. When the eggs were exposed to the acid for only a short time, *e. g.*, for three-fourths of a minute, not all the eggs formed a membrane when put back into normal sea water; and in this case only those divided into two or four cells and subsequently died within 20 hours, which had formed a membrane, while those eggs which had not been exposed long enough to the acid to form a membrane neither segmented nor died. If both methods of treatment were combined, however, those eggs which had formed a membrane developed at about the same rate as the eggs fertilized

with sperm. A certain percentage of these eggs rose to the surface of the water in the usual way, while the eggs which had not formed a membrane either did not develop at all, or developed in the somewhat abnormal and slow way characteristic of the treatment by hypertonic sea water alone.

The reader will notice that the eggs were submitted first for about two hours to the hypertonic sea water and then exposed to the acid. When the order was reversed, and the eggs were exposed to the acid first and afterward to the hypertonic sea water for about two hours, most of them died without developing. This seemed rather strange, in view of the fact that in the case of sperm fertilization, the membrane formation is the first act in the series of events, while in the above-mentioned experiments it was the last. It occurred to the author that by shortening the time of exposure of the egg to the hypertonic sea water, he might also accomplish the last postulate of a complete imitation of the process of fertilization by physico-chemical means, namely, to get the order of events identical in both cases. This idea proved correct. It was found that when the unfertilized eggs were exposed for about one to two minutes to 50 c.c. of sea water, to which about 3 c.c. $n/10$ acetic acid were added, the majority of the eggs formed the membrane characteristic of the entrance of the spermatozoön. If these eggs were afterward exposed for from 30 to 40 minutes to 100 c.c. of sea water, to which 14 c.c. or 15 c.c. of a $2\frac{1}{2}n$ solution of NaCl were added, those of the eggs which had formed membranes developed into swimming larvas that rose to the surface. The author has raised these larvas and they develop into perfect plutei as fast as the larvas of eggs fertilized with sperm.

It is very remarkable that when the order is reversed and the eggs are put first into the hypertonic sea water for about 40 minutes, and then into the acidulated sea water for about one or two minutes, not a single larva is formed, and the eggs behave on the whole as if they had been exposed to the acid alone. If it is desired to put the eggs into the hypertonic sea water first and then expose them to the acid, it is necessary to expose them to the hypertonic sea water at least an hour and a half in order to obtain larvas. On the other hand, if the eggs are treated with acid first and then exposed to the hypertonic sea water for from an hour and

a half to two hours, most of the eggs die in the early stages of development.

It may also be mentioned in this connection that if eggs are fertilized with sperm first and then exposed to the hypertonic sea water of the above mentioned concentration for about two hours, many more eggs will die without reaching the larval stage than when the order is reversed. It is therefore obvious that the process of membrane formation caused by the spermatozoön modifies the sensitiveness of the egg to the hypertonic sea water in the same sense as the process of membrane formation caused by the acetic acid. If eggs are fertilized with sperm and then exposed to the hypertonic sea water for from about thirty to forty minutes, their development becomes almost identical with that of the unfertilized eggs treated first with acid and then exposed to the hypertonic sea water for the same period of time. The majority of these eggs segment and develop in a normal way.

2. The question arises as to how far the division of labor between the two agencies used in these experiments goes. Does the treatment with acid cause only the formation of the membrane, or does it also set the internal mechanism of nuclear and cell division into motion? And what is the rôle of the treatment with hypertonic sea water? From the author's earlier experiments he had expected that the latter was required to cause the internal changes necessary for karyokinesis. The direct observation, however, of the eggs treated in the above-mentioned way with acetic acid, shows that the acid treatment causes the formation not only of the membrane, but also, in due time, of the karyokinetic spindle; while the eggs exposed for only thirty or forty minutes to the hypertonic sea water do not show any karyokinetic changes nor, in fact, changes of any kind.

It is a striking fact that the spindle formation which can be observed in the living egg of *Strongylocentrotus* seems to be identical in the cases of the fertilized egg and the unfertilized egg treated with acetic acid in the above-mentioned manner. The rôle which the subsequent treatment with hypertonic sea water for from thirty to forty minutes seems to play, is, in the first place, the acceleration of the process of segmentation. When the eggs are treated first with acid and then for about thirty or forty minutes with

hypertonic sea water, they begin to segment at a temperature of about 19° C., in from an hour to an hour and ten minutes after they have been removed from the hypertonic sea water. After this they go on segmenting at the rate and usually in the manner characteristic of the fertilized egg. The eggs treated with the acetic acid alone, after having formed a membrane, do not begin to segment for about five or six hours (if they segment at all) and they do not develop beyond the four or eight-cell stage, dying as a rule within twenty hours. The treatment with hypertonic sea water, therefore, first accelerates the mechanism of cell division originated by the acid treatment, and second, indirectly through or in addition to this acceleration, increases the vitality or prolongs the life of the egg.

It is not yet possible to say how the acid brings about its effects. Several years ago the author ventured the suggestion that the process of membrane formation was due to coagulation. The author's recent experiments, however, contradict such an assertion, inasmuch as the membrane formation never occurs while the eggs are in the acidulated sea water of the above-mentioned concentration, but only after they are taken out and put back into normal sea water. If the process of membrane formation were due to coagulation by acid, it should occur while the eggs are in the acidulated sea water.

The author considers it possible (but far from proved) that the membrane formation by the spermatozoön and possibly the subsequent process of karyokinesis are due to the transitory action of an acid carried by the spermatozoön into the egg or produced transitorily by the spermatozoön in the egg ; and that, in addition, the spermatozoön carries a second agency or substance into the egg, which supplies some of the conditions produced in the above experiments by the brief treatment with hypertonic sea water.

Twelfth meeting.[1]

Laboratory of Clinical Pathology at the Cornell Medical College. May 24, 1905. Vice President Dunham in the chair.

34 (80). **"Contributions to the study of sulfur. 1. The metabolism in brombenzol poisoning": W. MACKIM MARRIOTT** and **C. G. L. WOLF.**

Baumann and his pupils investigated the effect of the adminis-

[1] Reprinted from *Science*, 1905, xxi, p. 986 ; *American Medicine*, 1905, ix, p. 026 ; *Medical News*, 1905, lxxxvii, p. 520.