

contained appreciable traces of phosphates. I have obtained a much purer preparation by a process consisting, essentially, in precipitation from the serum by acetone, extraction of the precipitate with hot N/10 HCl, exactly neutralizing the extract with Ba(OH)₂, re-dissolving the precipitate in N/10 H₂SO₄ and re-precipitating it with acetone. The yield from a liter of ox-serum lies between 10 and 40 milligrams.

The substance which is thus obtained is soluble in dilute acids, alkalis and salt-solutions. It is much more rapidly dissolved when these solvents are hot. Its solutions are not coagulated by boiling. It is thrown out of solution by chlorides of the alkaline earths. It yields the Millon, Acree-Rosenheim and xanthoproteic tests for protein. One part of the substance rubbed up in 512,000 parts of sea-water caused membrane-formation in 80 per cent. of *Strongylocentrotus purpuratus* eggs which had previously been sensitized by 4 minute's immersion in 3/8 m SrCl₂.

The active substance, therefore, is either a protein or a peptone, or else, by the above methods of preparation, is precipitated together with a protein or peptone.

I find that Witte's "peptone" contains the membrane-forming substance, since one part of Witte's "peptone" dissolved in 16,000 parts of sea-water caused membrane-formation in 32 per cent. of sensitized *purpuratus* eggs. Hence the membrane-forming agent is digested either with difficulty or not at all by pepsin.

I find that the addition of 0.08 per cent. of lecithin or cholesterolin to rabbit serum does not affect, either qualitatively or quantitatively, the membrane-forming and cytolyzing action of the serum. It would appear very unlikely, therefore, that the active substance is a lipid.

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On the influence of lecithin upon the development of sea-urchin embryos. (Preliminary communication.)

By **T. BRAILSFORD ROBERTSON.**

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When the eggs of *Strongylocentrotus purpuratus* are fertilized by sperm in a mixture of 50 c.c. of sea-water and 5 c.c. of a 1.7

per cent. suspension of lecithin in $m/2$ NaCl, the inner and outer fertilization-membranes are slowly dissolved by the lecithin, with the result that in the course of some six hours the cleavage-cells which have formed fall apart and ultimately disintegrate.

The eggs of *Strongylocentrotus purpuratus* female were divided into two portions. Both portions were placed in sea-water and fertilized with sperm. After 24 hours both lots of eggs had developed into free-swimming blastulæ. One portion was now transferred to a mixture of 50 c.c. of sea-water and 5 c.c. of a 1.7 per cent. suspension of lecithin in $m/2$ NaCl for a period of 24 hours and then returned to normal sea-water. The other portion was left in normal sea-water. The following table shows the relative development of the two portions:

Time After Fertilization.	Portion 1 (Controls).	Portion 2.
1 day.....	Blastulæ.	Blastulæ. (These were now transferred to the lecithin mixture for 24 hours.)
2 days.....	Gastrulæ.	Blastulæ. (These were now transferred to normal sea-water.)
3 days.....	Gastrulæ.	Blastulæ.
4 days.....	Gastrulæ and early plutei.	Blastulæ.
5 days.....	Fully developed plutei.	Blastulæ and 25 per cent. gastrulæ.
6 days.....	Advanced plutei.	Early gastrulæ with narrow, unbranched intestine and large, clear body-cavity.
7 days.....	Advanced plutei.	Unchanged.
8 days.....	Advanced plutei.	The gastrulæ are now retrograding; the intestine has almost disappeared.
9 days.....	Unchanged.	Unchanged.

Bacterial invasion terminated the experiment after the 9th day. It is evident that the immersion of blastulæ for 24 hours in a 0.15 per cent solution of lecithin enormously retards their development.

If *purpuratus* eggs are fertilized by sperm in more dilute solutions of lecithin in sea-water (0.003 per cent. to 0.015 per cent.) the fertilization-membranes are not dissolved sufficiently rapidly to affect the development. In these solutions development is not appreciably retarded until the blastula stage is reached. Thereafter development is very markedly retarded, and the retardation is greater the greater the concentration of the lecithin. The

embryos are not injured by the lecithin, however, as they will ultimately develop to normal plutei if left in these solutions for a sufficient time.

If cholesterol, suspended in a mixture of m/100 sodium oleate and m/2 NaCl be mixed with the lecithin in equal proportions the retarding action of the lecithin upon the development of *purpuratus* eggs is almost completely neutralized. The slight retardation which is observed in these mixtures may be due to the sodium oleate which is employed to keep the cholesterol in suspension, since sodium oleate is very toxic for sea-urchin eggs and embryos.

Cholesterol itself, when added to sea-water, has no influence upon the rate of development of the eggs. The emulsions of cholesterol are, however, coagulated by the salts in sea-water and the cholesterol is completely thrown out of suspension in the form of coarse flocculi.

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On acid agglutination as a method of differentiation of bacteria.

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Michaelis and several other workers following him have claimed for the phenomenon of acid agglutination a specificity comparable with that of specific serum agglutination. The reaction is specific, they say, in that optimum agglutination is produced in suspensions of bacteria of a single species by a definite concentration of hydrogen ions, irrespective of the acid used, and in that this concentration is, in general, different for different species. Their method has been to prepare solutions of definite hydrogen ion concentrations by using mixtures of a weak acid with its sodium salt, the concentrations being calculated from the formula

$$C_H = k \cdot \frac{C_{\text{acid}}}{C_{\text{salt}}},$$

where k represents the dissociation constant of the acid used. The differences between the constants obtained in this way for