RHYTHMIC SUSCEPTIBILITY CHANGES IN THE CLEAVING *ARBACIA* EGG

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(With Seven Text-figures.)

THE fertilisation reaction initiates a remarkable series of events in the egg. Lyon (1) first showed that *Arbacia* eggs were especially sensitive to heat just before cleavage and most resistant 10–20 minutes after fertilisation. However, the susceptible period for cold seemed to be quite different from that for heat, being almost exactly the same as the resistant period for heat. He (2) further found that eggs were especially susceptible to *M*/100–*M*/200 KCN about 10–15 minutes after fertilisation and that the resistance gradually rose as the time for the first cleavage approached.

Spaulding (3) found that great susceptibility to ether occurred immediately prior to segmentation.

Mathews (4) re-examined Lyon's work on the action of KCN and found that the period of greatest susceptibility coincides with the development of the asters; and the period of greatest immunity coincides with the retrogression of the asters and the development of the nucleus.

In 1909 Lyon (5) demonstrated that following fertilisation there is an increase in the ability of the *Arbacia* egg to catalyse H_2O_2. This he interpreted as an expression of the greater permeability of the eggs to the peroxide after fertilisation. In the next year Harvey (6) showed that neutral red stains fertilised eggs more rapidly than unfertilised, and also that 2–5 minutes after fertilisation there seemed to be an increase in permeability to NaOH. Lyon and Shackell (7) found that artificial activation increased dye intake, and they noted that loss of pigment occurred after fertilisation.

Moore (8) states that resistance to hypertonic sea-water is least immediately before and during cleavage; that maximal resistance is shown 35–45 minutes after fertilisation and just after each division.

Baldwin (9) found that with certain aliphatic alcohols the resistance of the *Arbacia* egg is least after fertilisation, rises to a peak of resistance shortly before cleavage, followed by a rather sharp increase in susceptibility during division.

McClendon (10) demonstrated an increase in conductivity of the eggs after fertilisation, but Gray (11) made more accurate measurements, finding that the

entrance of the sperm into the egg causes an increase in conductivity which attains a maximum within 10 minutes of adding the sperm.

Warburg (12) investigated the respiratory exchange in *Strongylocentrotus* eggs and found that 10 minutes after fertilisation there is a sixfold increase in O_2 consumption over that of the unfertilised egg. Shearer (13) discusses the evidence which seems to point fairly conclusively to the cortical layer of the egg and egg membrane as being the controlling factor in the oxidation processes of the egg. He further suggests that upon fertilisation there is an immediate increase in the glutathione content of the egg. He has shown that within a minute after the addition of sperm to the eggs the O_2 consumption greatly increases and the CO_2 output parallels this increase. The fusion of the male and female pronuclei in the latter phases of fertilisation is correlated with no additional increase in O_2 consumption. Rogers and Cole (14) measured the rate of heat production after fertilisation and found that at the instant of fertilisation the heat produced is ten to twelve times that of the unfertilised egg. It then decreases constantly for 20 minutes when it reaches about 65 per cent. of the value at fertilisation and then remains nearly constant until the first cleavage.

R. S. Lillie, in an important series of papers (15, 16, 17), has shown that following fertilisation the rate of entrance of water into fertilised *Arbacia* eggs in hypotonic sea-water is approximately four times that into unfertilised eggs. The artificial formation of fertilisation membranes (by butyric acid) is followed by a similar marked increase of permeability to water. He further found that the osmotic properties of unfertilised and normally fertilised eggs remain approximately constant during the first eight or more minutes of immersion in dilute sea-water. The essential constancy in the rate of entrance of water (relative to the existing gradient of osmotic pressure) into fertilised and unfertilised eggs, during a period in which the water content of the egg is almost doubled, shows that the difference between the two kinds of eggs is not due to a difference in the condition of the internal protoplasm, but simply to a difference in the resistance of the membrane to the passage of water. Fertilised eggs shrink rapidly, whereas unfertilised shrink slowly in the same hypertonic sea-water or van 't Hoff solution. During the first few minutes after insemination the eggs, when placed in hypertonic sea-water, shrink slowly like the unfertilised eggs. No noticeable increase in permeability was found until 5–6 minutes had elapsed, and the change reached its final equilibrium in about 20 minutes (20–22° C.).

In 1916 Lillie (16) demonstrated that in moderately hypotonic sea-water about the time of formation of the cleavage furrow a marked decline takes place in the resistance of the egg to hypotony, and cytolysis is then rapid and complete.

Just (19) has shown the same type of phenomenon to occur in the *Echinarachinus* egg, and has emphasized the fact that during the period of susceptibility before cleavage the egg in dilute sea-water bursts over the spindle pole indicating a drop in the tensile strength of the hyaline plasma layer. At this time the hyaline plasma layer is moving away from the polar area to the equator. As Just says, the egg is being denuded of hyaline plasma layer in the polar areas. He concluded that

the egg membrane played no rôle in the susceptibility of the egg. Furthermore, in the *Echinarachinus* egg during the process of membrane separation there is a wave of lowered cortical resistance before the period of minimal susceptibility. Just (19) has also studied *Arbacia* eggs, with similar results. He discusses in detail the relation between these susceptibility changes and the mechanism of mitosis and cell division.

We propose to examine the mechanism of this rhythmic change in susceptibility in the *Arbacia* egg.

METHOD.

Eggs shed from the ovaries were placed in sea-water and fertilised. At suitable short intervals of time samples were placed in hypotonic sea-water (75 parts tap-water plus 25 parts sea-water), and the time noted for complete (96–100 per cent.) cytolysis to occur. This cytolysis consists of a discharge of colour from the pigment granules, the change in the colour of the echinochrome from reddish brown to bright pink, swelling and often disruption of the egg, and finally visible change in the character of the cytoplasm.

HYPOTONIC CYTOLYSIS OF NORMAL FERTILISED EGGS.

Fig. 1 is an illustration of many typical experiments made during the summers of 1924–8. The ordinates represent the time in minutes required for complete cytolysis and the abscissae the time in minutes after fertilisation.

The extraordinary ability of eggs to resist cytolysis during the period of 2–6 minutes after insemination is clearly shown. It should be remembered that with less hypotonic solutions the curve is less sharp and the resistance at times carries over to longer periods. In some cases a slight fall in resistance to cytolysis occurs during the first 1 or 2 minutes after fertilisation. A decrease in hypotonic susceptibility can usually also be found after the first cleavage, a fact which is not illustrated in this graph.

The type of cytolysis differs strikingly in eggs treated at different periods after fertilisation has occurred. After eggs have been fertilised 2–6 minutes they become very coarsely granular, vacuolated, frothy in appearance, greatly swollen, pink in colour, and the microdissection needle shows the protoplasm to be coagulated. Sometimes at this period there is a curious tendency for the eggs to become distorted in shape and to resolve themselves into small fragments.

If eggs are placed in dilute sea-water 10 minutes after fertilisation their protoplasm quickly becomes finely granular in texture (later it may or may not become coarsely granular), their colour changes to an intense pink, with much discharge of pigment into the surrounding medium, membranes become irregular, and disruption occurs. Following cytolysis, the contents of the egg usually shrink markedly. The pink colour is probably due to the action of bivalent cations (Ca^{++} and Mg^{++}), as it has been found that eggs may cytolyse or be torn in isotonic NaCl

without change in colour, whereas in solutions containing Ca·· and Mg·· the pink coloration quickly flashes out.

During the phase of resistance to cytolysis the eggs appear not unlike the normal egg except that they are greatly swollen and less coarsely granular. Pigment slowly diffuses out the egg and stains the surrounding jelly pink, giving the egg a coloured halo.

Measurement with a filar micrometer ocular shows that eggs in hypotonic sea-water swell rapidly at first and finally reach a stage in which little or no further

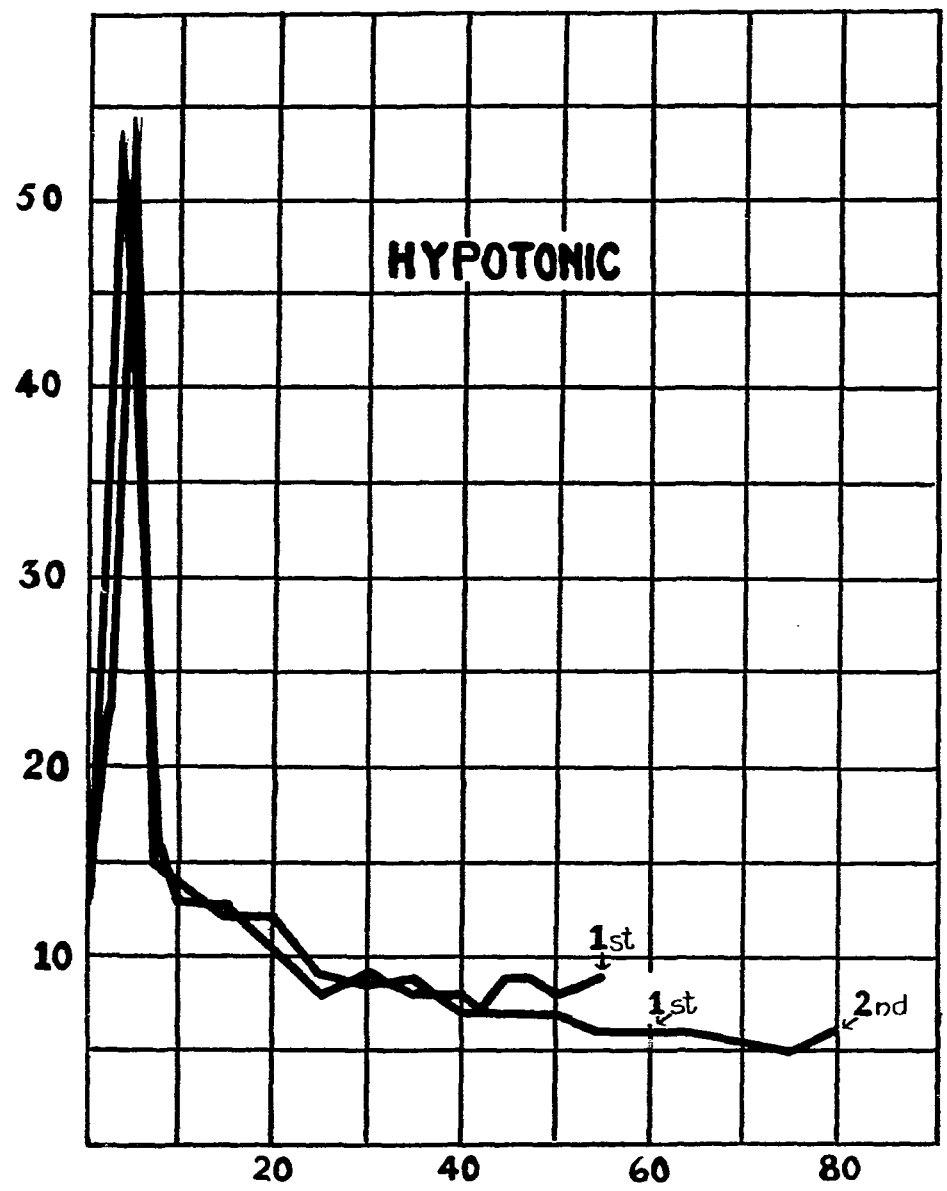

Fig. 1. Cytolysis of *Arbacia* eggs by hypotonic sea-water after fertilisation. (Ordinates represent the time in minutes required for cytolysis and abscissae the time in minutes after fertilisation.)

swelling occurs. Eggs measured during the resistant phase immediately before cytolysis average 10–18 per cent. in diameter larger than eggs examined at a period of increased susceptibility. During the initial resistant phase the eggs seem to keep pace with non-resistant eggs in their rate of swelling, but as the process proceeds the resistant eggs swell less rapidly and tend to reach an equilibrium in size. As the process of swelling proceeds the non-resistant eggs seem to swell less rapidly, shortly bringing the swelling to an end by cytolysing. The rate of swelling at these resistant periods is being further investigated.

ACTION OF ELECTROLYTES.

In order to determine the effect of electrolytes on the normal resistance curve, eggs were gently centrifuged to remove the bathing sea-water, placed for 20 minutes in pure isotonic solutions of the various salts, gently centrifuged, placed in sea-water and fertilised. Samples were removed at suitable time intervals and placed in hypotonic sea-water as before. Figs. 2, 3, 4 and 5 show the results with various

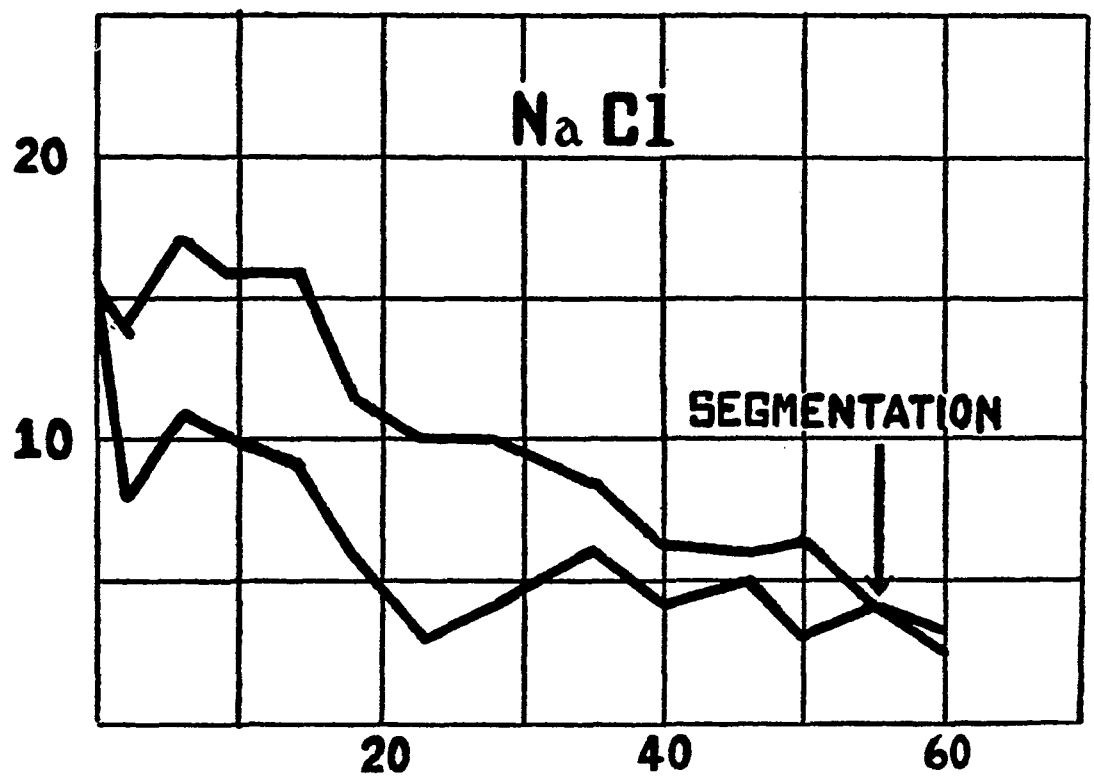

Fig. 2. Cytolysis of *Arbacia* eggs by hypotonic sea-water following isotonic NaCl treatment for 20 minutes, washing in sea-water and fertilisation. 30 per cent. of the eggs divided in 55 minutes and 80 per cent. in 120 minutes.

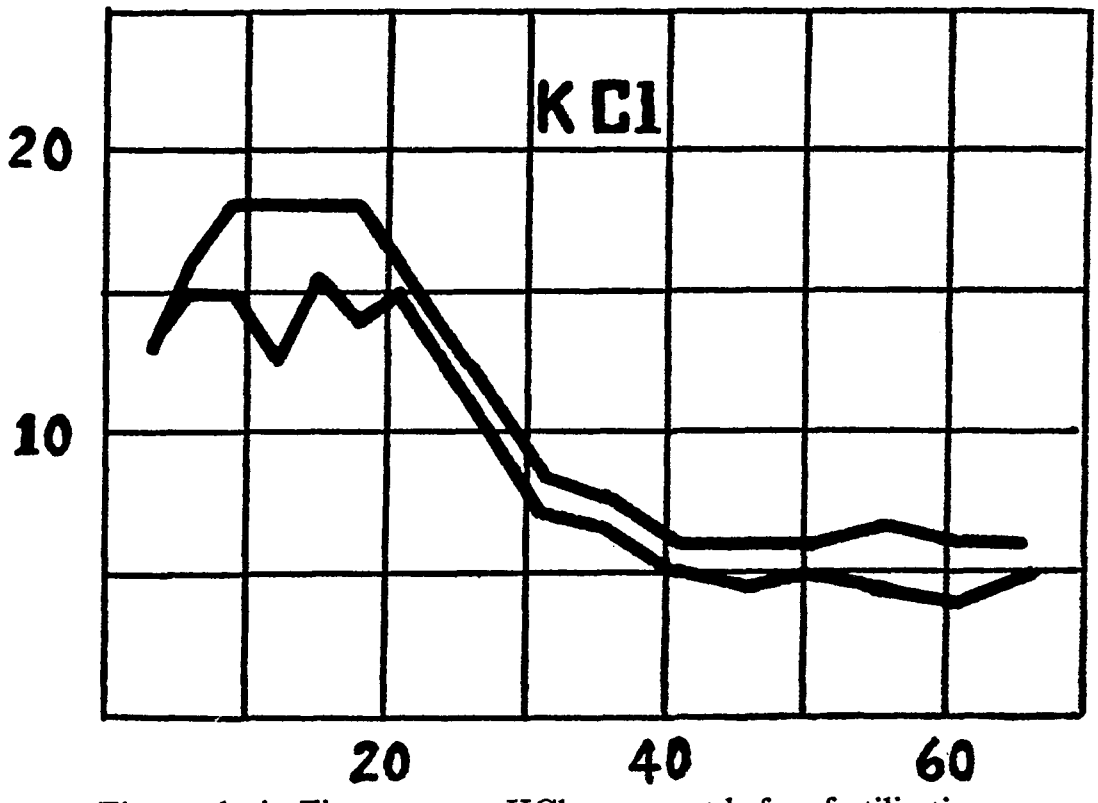

Fig. 3. As in Fig. 2, except KCl treatment before fertilisation.

electrolytes. These experiments were repeated, placing the eggs back into the isotonic electrolyte, after fertilisation in sea-water, with results very similar to those obtained with the procedure detailed above.

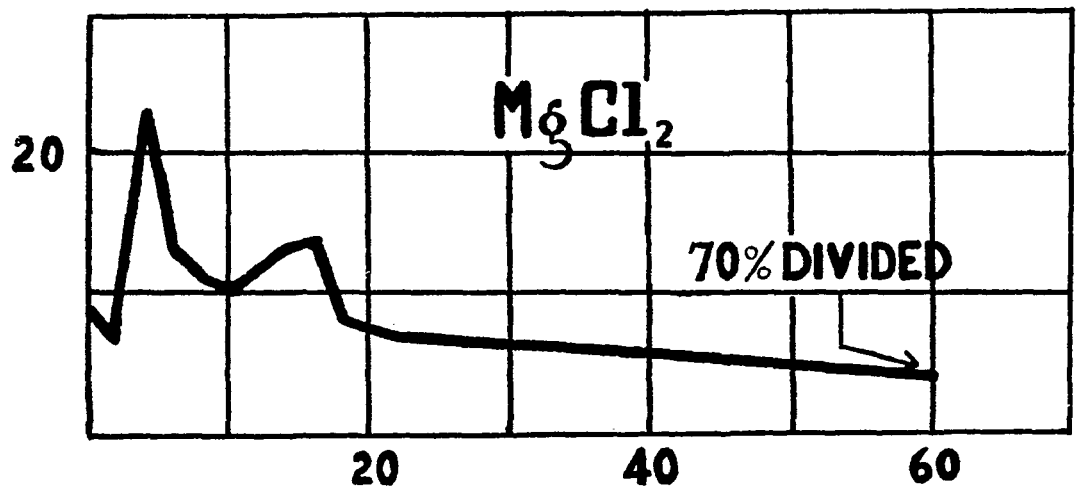

Fig. 4. As in Fig. 2, except $MgCl_2$ treatment before fertilisation.

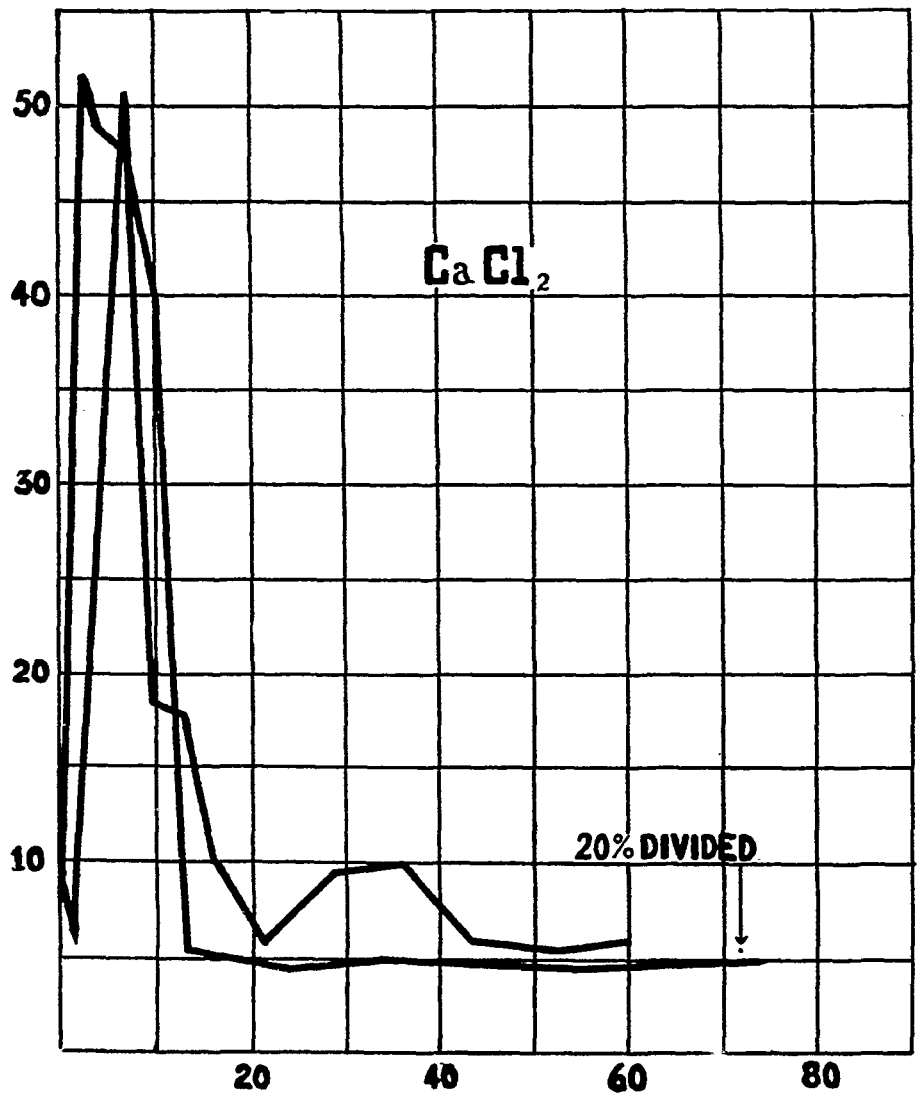

Fig. 5. As in Fig. 2, except $CaCl_2$ treatment before fertilisation.

SAPONIN TREATMENT.

In Fig. 6 are given the results of a typical experiment in which a 0·05 per cent. solution of Quillaja saponin was used as the cytolysing agent instead of the hypotonic mixture. The ordinates represent the time in minutes required for complete

cytolysis and the abscissae the time in minutes after fertilisation when the sample was removed from sea-water for saponin treatment.

Saponin cytolysis is superficially not unlike hypotonic cytolysis in so far as resistance to cellular disruption is concerned.

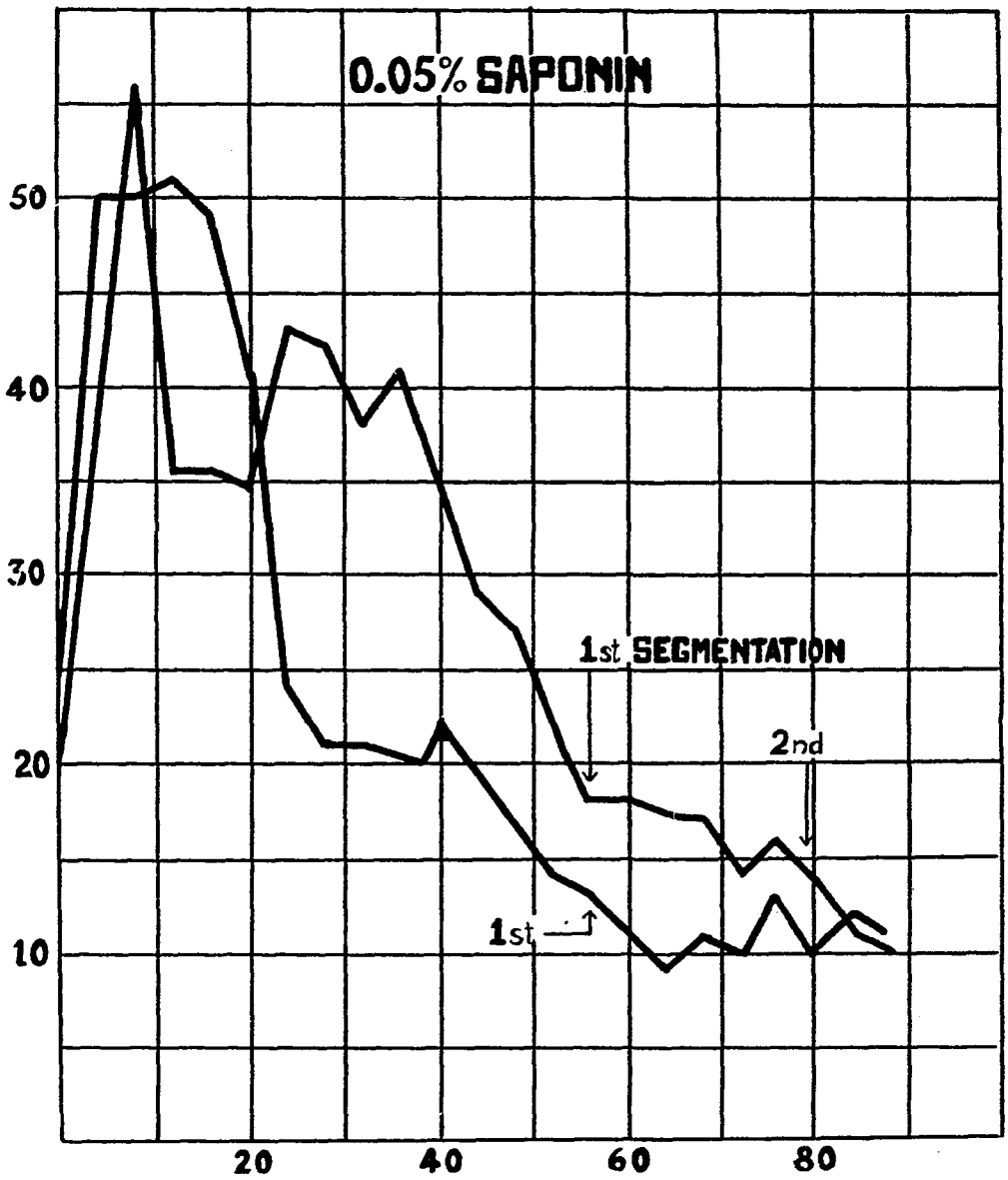

Fig. 6. Cytolysis of *Arbacia* eggs by 0·05 per cent. saponin following fertilisation. (Ordinates represent the time in minutes required for cytolysis and abscissae the time in minutes after fertilisation.)

EFFECT OF AGEING.

It has been found that eggs which have stood for some time in sea-water before fertilisation do not show the resistance curve in nearly so striking a fashion as fresh eggs. Fig. 7 shows such a result, in which the effect of hypotonic solutions on fresh eggs (Curve *A*) is contrasted with that of the same solutions on eggs held for 8 hours prior to fertilisation (Curve *B*).

VISCOSITY CHANGES.

Eggs placed in hypotonic mixtures 2–6 minutes after fertilisation appear to have a less viscous protoplasm than those examined at the end of 10 minutes. This has been tested with the aid of the microdissection needle[1]. The cytoplasm

[1] Through the courtesy of Dr Robert Chambers.

of eggs placed in hypotonic mixtures 4 minutes after fertilisation and examined 30 minutes after this treatment is very much more fluid and less viscous than is that of eggs treated 10 minutes after fertilisation and examined after 30 minutes. On tearing the surface membrane of eggs treated 4 minutes after insemination the contents flow out more readily than do those of a normal egg or of an egg treated 10 minutes after fertilisation. After the fluid contents are disgorged the residual portion of the egg quickly coagulates.

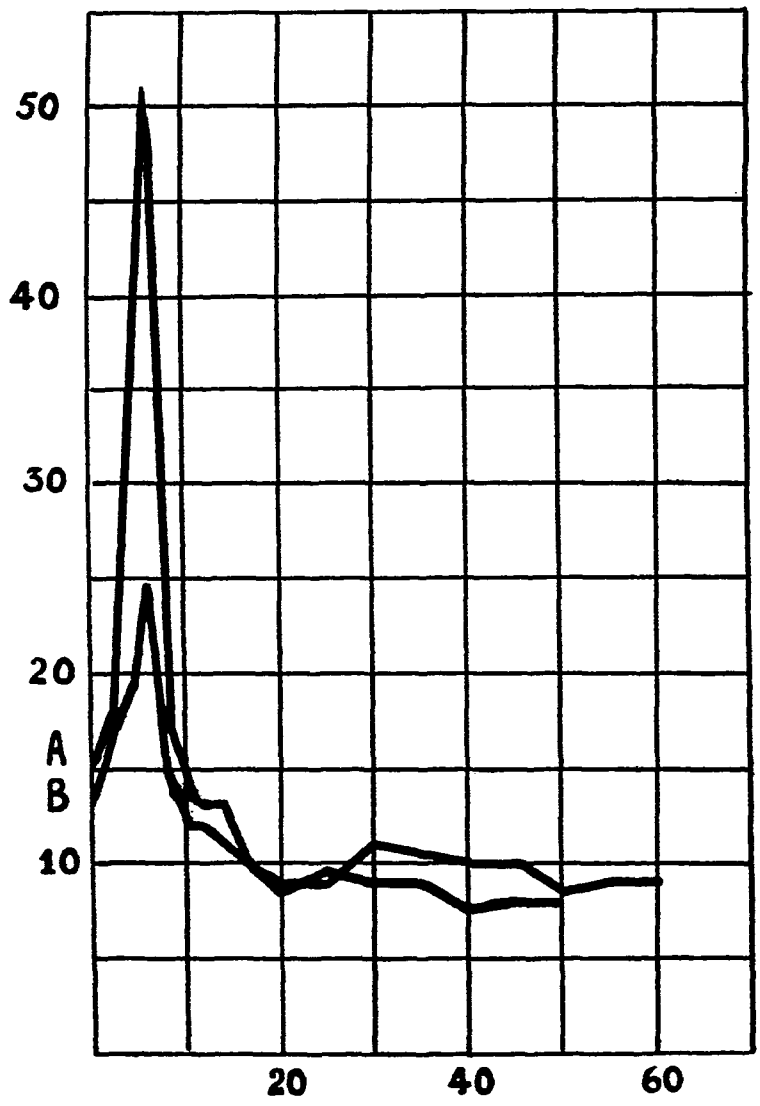

Fig. 7. Effect of ageing eggs before fertilisation. Curve *A* shows the rate of hypotonic sea-water cytolysis after various intervals following fertilisation of eggs removed directly from the ovaries, Curve *B* the cytolysis rate after 8 hours' standing in sea-water. (Ordinates represent the time in minutes required for cytolysis and abscissae the time in minutes after fertilisation.)

DISCUSSION.

The following conclusions seem justified from the data presented above:

1. A marked peak of resistance to cytolysis occurs shortly after fertilisation, and as the time for cleavage approaches the eggs become progressively more susceptible.

2. NaCl and KCl inhibit the formation of the peak of cytolytic resistance, whereas $CaCl_2$, if anything, prolongs the resistant period; the behaviour of $MgCl_2$ is intermediate between NaCl and $CaCl_2$.

3. The visible structure of the eggs placed in hypotonic sea-water during

the period of resistance is quite different from that of eggs placed in this solution after the resistant period has passed.

4. Ageing tends to reduce the period of cytolytic resistance.

5. During the resistant phase the eggs swell to a considerably greater extent before cytolysis occurs than at later periods following insemination.

6. Saponin, though quite different chemically, exhibits a cytolytic action not unlike that of dilute sea-water.

It seems clear from the literature cited that within a few minutes after insemination a number of interesting phenomena occur. This period seems to be the one at which the egg is least susceptible to poisons of various kinds; the period of maximum O_2 consumption and CO_2 and heat production; the period of glutathione production, conductivity increase and change in permeability to water.

There seems to be fairly good evidence (Lillie, *loc. cit.*) for assuming that fertilised eggs are more easily permeable to certain substances than before insemination. But the point that is not clear is just when, after fertilisation, this increase in permeability begins. Lillie (16) believes, though the point has not been exhaustively studied, that the increase in permeability does not occur until after about 6–8 minutes. If this were true it would, of course, place this type of permeability change out of the category of phenomena involved in the rhythmic changes described in this paper. This is a point deserving of further investigation.

Our problem is, then, to explain why "resistant" eggs readily swell but do not cytolyse.

McCutcheon and Lucké (20) have demonstrated quantitatively that NaCl and KCl increase the permeability of the *Arbacia* egg to water while $CaCl_2$ and $MgCl_2$ decrease it. Since we have found that NaCl and KCl abolish the resistant period, whereas $CaCl_2$ and $MgCl_2$ preserve this property, the analogy between the two processes seems clear. It would at least suggest that a period of relative impermeability to water exists ("resistant phase") followed by increased permeability to water ("susceptible phase").

When eggs are exposed to pure isotonic solutions of single electrolytes they cleave less rapidly than do normal eggs. It is possible that NaCl and KCl penetrate the egg, and hence their effects may be carried over after fertilisation, although the eggs are replaced in sea-water before fertilisation. $CaCl_2$ may not penetrate at all and $MgCl_2$ but slightly. Both NaCl and KCl could penetrate into the egg without colour change, as is shown by the fact that cutting the eggs with the microdissection needle in the pure salt produces no colour change. Such a procedure carried out in $CaCl_2$ or $MgCl_2$ immediately causes the granules to swell and the echinochrome to turn bright pink. Chambers (18) has shown that $CaCl_2$ does not have an effect on the viscosity of an egg until the surface is injured, whereas NaCl produces its viscosity change before the egg is manipulated.

The morphological evidence that the resistant phase eggs swell to a greater extent than other eggs, and that more water entered as judged by the greatly decreased viscosity, does not localise the process. Either an increased toughness of

a hypothetical surface layer, or an increased capacity of internal protoplasm to bind water, might account for the observed results.

An interesting speculation, and one which we are now engaged in testing, is the possibility that the water balance of the cell is in part controlled by the $\frac{\text{phosphatide}}{\text{sterol}}$ ratio. Any change in the hydrophilic phosphatide fraction would change the ability of the cell to take up water.

CONCLUSIONS.

1. A marked peak of resistance to cytolysis by hypotonic sea-water occurs very shortly after fertilisation in the sea urchin egg. The same type of "resistance phase" occurs using saponin as the cytolysing agent.

2. NaCl and KCl inhibit the production of the resistant phase, while $CaCl_2$ and $MgCl_2$ tend to prolong this period.

3. There are striking morphological differences in the cytolysis in hypotonic sea-water as it occurs during the resistant or susceptible phase following fertilisation.

4. During the resistant phase the eggs swell to a considerably greater extent before cytolysis occurs than at later periods following insemination.

5. Ageing reduces the length of the period of cytolytic resistance.

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