THE ACTIVATION OF STARFISH EGGS BY ACIDS.

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INTRODUCTION.

In the activation of starfish eggs (*Asterias forbesii*) by temporary exposure to solutions of fatty acid the essential factors are concentration of acid, duration of exposure, and temperature. In order to effect complete activation at any temperature (within the physiological range) the eggs must be exposed to a given concentration of acid for a time which is definite within somewhat narrow limits.[1] For example, exposure to 0.002 M butyric acid (dissolved in balanced non-buffered salt solution) for 12 to 14 minutes at 20°C. typically causes all eggs to develop into active free-swimming blastulæ. If the eggs are exposed for a few minutes longer or shorter than this critical time, activation is partial or defective (membrane formation, cleavage slower or less regular than after the optimum exposure), and the eggs cease development and die at an early stage. In general, the rate of activation[2] by fatty acid, within a range of 0.001 M to 0.005 M, is closely proportional to the concentration of acid. The relation between duration of exposure to a given solution and degree of activation may be represented graphically by plotting against the durations the percentages of eggs reaching a free-swimming stage. Any such curve has a time range which varies with temperature in the manner shown (Fig. 1). The position of the optimum is more sharply defined at the high temperatures, and the rate of activation shows a high temperature coefficient which increases rapidly as the temperature approaches 28°C.[3] At this temperature heat-activation begins to

[1] Lillie, R. S., *Biol. Bull.*, 1915, xxviii, 260; *J. Biol. Chem.*, 1916, xxiv, 233.

[2] This rate is defined as the reciprocal of the optimum time of exposure.

[3] Lillie, R. S., *Biol. Bull.*, 1917, xxxii, 131.

appear; it is noteworthy that the rate of heat-activation, within the effective range of temperatures, 28–38°C., is almost doubled by each rise of 1°,[1] a result indicating its dependence on some physical or structural change in the protoplasmic colloids. Complete heat-activation at a given temperature also requires a definite time of exposure, and the relations between duration of exposure and degree of activation are closely similar to those found in activation by fatty acid. Heat-activation and acid-activation can in fact be substituted

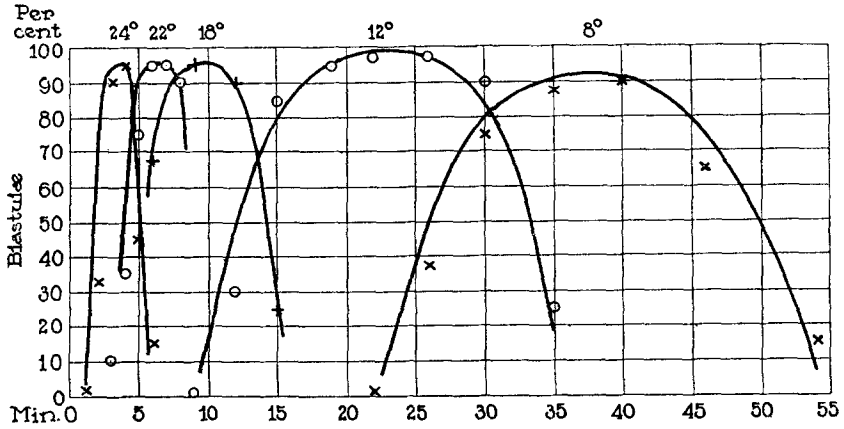

FIG. 1. Relation between durations of exposure to butyric acid solution (0.006 M dissolved in sea water) and percentages of eggs forming blastulæ at different temperatures (Table I, *Biol. Bull.*, 1917, xxxii, 138). Ordinates: percentages of blastulæ; abscissæ; exposures.

for one another, a brief exposure to either heat or butyric acid being used, for example, to complete a previous partial activation by either treatment.[4] It seems probable, from the resemblance between the curves correlating degree of activation and duration of exposure in the two cases, that high temperature acts indirectly by freeing acid in certain regions of the egg; *i.e.*, that heat-activation is in reality acid-activation. The latter would thus appear to be the fundamental phenomenon in activation and its conditions demand special study.

[4] *Cf. Biol. Bull.*, 1915, xxviii, 284.

In the *Asterias* egg acids have a special relation to activation. Bases (ammonia) are entirely ineffective.[6] It is significant that only the more rapidly penetrating and surface-active acids show the above described definite relation between time of exposure and degree of activation; also, it is only with such acids that complete activation is obtained in a large proportion of eggs. These acids include the fatty acids and other penetrating acids such as benzoic and carbonic acids. The difference between the activating effectiveness of these acids and that of the mineral acids and hydroxy-acids is striking. Hydrochloric acid is only slightly effective; in numerous experiments with this acid, using concentrations between 0.00075 N and 0.01 N (dissolved in balanced isotonic salt solution), I found the greatest effect with 0.01 N HCl acting for 20 minutes (at 20°C.); with this treatment about 25 to 30 per cent of the eggs formed blastulæ, mostly feeble and irregular. This is an exceptionally high proportion of blastulæ for activation with HCl;[7] with concentrations between 0.001 N and 0.005 N and exposures ranging from 1 to 20 minutes, it is unusual for more than 1 per cent of eggs to reach this stage. Oxy-substituted fatty acids (lactic acid) are more effective than mineral acids, but much less so than fatty acids.[8] Loeb's experiments with the membrane-forming action of acids on the eggs of *Strongylocentrotus* gave a similar result; fatty acids and benzoic acid proved much more effective than mineral acids and oxy-acids.[9] It seems clear that the readiness with which the acid penetrates the egg protoplasm is a chief factor in its physiological action. Acids belonging to the first named group may be called activating acids; rapid penetration of protoplasm and low ionization are apparently their chief characteristics in relation to the activating

[5] Heat and penetrating acids are by far the most effective parthenogenetic agents. Other agents, radiation, electric currents, neutral salt solutions, hypertonic sea water have only partial action.

[6] *Cf. J. Biol. Chem.*, 1916, xxiv, 237.

[7] Possibly higher concentrations than 0.01 N may be more effective, but I have not experimented sufficiently with such solutions. The action of 0.005 N fatty acids is too rapid (at 20°) for satisfactory measurement (see below).

[8] See below, page 350.

[9] Loeb, J., Artificial parthenogenesis and fertilization, Chicago, 1913, Chapter 14, 133.

effect. It is significant that surface activity is also favorable; this fact indicates a localization of the critical reaction with reference to structural surfaces within the egg protoplasm.

A difference between the action of the activating acids on sea urchin and on starfish eggs is that in the latter case complete activation is produced by a single properly timed exposure. No supplementary treatment with hypertonic sea water is required. The problem of the nature of the activating process thus appears simpler in this egg than in the sea urchin egg. The indications are that a single localized change in the egg system, involving the participation of a definite quantity of acid, is the primary condition of the critical physiological change in activation. This change brings the egg into a state in which it proceeds automatically with its development to an advanced stage.

Method.

The experiments were performed at the Marine Biological Laboratory during the summers of 1924 and 1925. The chief acids used were the early members of the fatty acids series, including formic, acetic, propionic, butyric, isobutyric, valeric, isovaleric, caproic, isocaproic, and caprylic acids.[10] Mono-, di-, and trichloracetic acids were also used to test the effect of ionization-increasing substitutions. Other acids used were benzoic, carbonic, lactic, and hydrochloric.

The concentrations of fatty acids most suitable for activation (at 20°) lie between 0.001 M and 0.004 M. Below 0.001 M the action is too slow (except with formic) and above 0.004 M too rapid for satisfactory measurement. In all experiments the acids were dissolved in isotonic balanced non-buffered salt solution;[11] this was done to avoid the disturbing effects of the carbonates and other weak acid salts of sea water. In most cases three concentrations, 0.001 M, 0.002 M, and 0.003 M

[10] The higher fatty acids from isobutyric on were obtained from the Eastman Company of Rochester, New York. Stock solutions were standardized by titration with NaOH, using brom-thymol blue as indicator.

[11] Either van't Hoff's solution (100 volumes 0.5 M NaCl + 7.8 volumes 0.5 M $MgSO_4$ + 3.8 volumes 0.5 M $MgCl_2$ + 2 volumes 0.5 M KCl + 2 volumes 0.5 M $CaCl_2$) or a mixture of 100 volumes 0.52 M NaCl + 5 volumes 0.5 M $CaCl_2$. No difference of action was found between equally concentrated solutions of an acid in these two solvents.

were used with the same lot of eggs, and the experiments with these three solutions were carried out simultaneously. The eggs, previously thoroughly washed in sea water, were exposed during the prematuration period (between 15 and 50 minutes after removal from the animal). Equal quantities (usually the mixed eggs from several animals) were placed in each of three small beakers; after the eggs had settled the surplus sea water was removed with a pipette, and to the remaining dense suspension (about 2 cc.) 100 cc. of the solution of acid (at 20°) was added. The three solutions were added to the three beakers successively at intervals of 15 seconds, and the transfers of eggs from each solution to sea water (after the lapse of the desired time of exposure) were made at the same intervals; this was done to ensure precise equality of exposure in the corresponding experiments of each series. The intervals between the successive transfers from each acid solution to sea water were varied in different experiments; when the rate of activation is gradual intervals of 2 minutes are usually sufficient to determine the optimum exposure with sufficient definiteness; but in many experiments with more active acids or with higher concentrations intervals of 1 minute or less were used. The eggs were examined next day and the proportion developing to a blastula stage with each exposure was determined.

General Results with Fatty Acids.

All the above named fatty acids except caprylic give the same kind of result. The earliest indication of activation is the separation of the fertilization membrane; this effect is produced by very brief exposures; 1 minute in 0.001 M acid is sufficient. The membranes are typical, but the eggs break down shortly afterwards, either without cleaving or after a few slow and irregular cleavages. As the exposures are progressively lengthened the cleavages become more frequent and regular until the optimum is reached at which most eggs develop to blastulæ or later stages. Still further increase of exposure is unfavorable; the cleavages become again irregular and slow; fewer and fewer eggs form blastulæ, until eventually even membranes fail to separate.

The proportion of eggs developing to a blastula stage is a good index of the favorability of the conditions. When 90 per cent or more so

develop activation may be regarded as complete; to produce this effect a definite time of exposure is required which at a given temperature and concentration is characteristic of the acid.

TABLE I.

Acid.	A. Range of exposures giving blastulæ. B. Optimum exposures. Time in min. Temperature 20°.		
	0.001 M	0.002 M	0.003 M
	min.	*min.*	*min.*
Formic, *A*	3–10	1–5	1–3
B	7– 8	3–4	*Ca.* 2
Acetic, *A*	18–26+	8–18+	6–12
B	*Ca.* 26	12–14	*Ca.* 8
Propionic, *A*	16–28+	6–16	2–8
B	26–28	10–12	*Ca.* 6
Butyric, *A*	16–28+	8–16	4–10
B	22–24	*Ca.* 10	*Ca.* 6
Isobutyric, *A*	14–28+	6–16	4–10
B	22–26	12	*Ca.* 6
Valeric, *A*	16–28	6–12	4–6
B	20–22	8	*Ca.* 6
Isovaleric, *A*	14–26	6–12	2–8
B	20	8	4–6 (4 min. better).
Caproic, *A*	10–20+	2–8	<2–4
B	16–18	4–6	*Ca.* 2
Isocaproic, *A*	12–20	4–6	2
B	14	*Ca.* 6	*Ca.* 2

With formic acid the transfers from the acid solution to sea water were made at intervals of 1 minute; with the other acids at intervals of 2 minutes.

The + sign means that at the longest exposure of the series a considerable number of eggs still formed blastulæ.

Table I gives a list of typical single experiments with the fatty acids, selected from a large number. The agreement between corresponding experiments with the same acid is good, but the time relations show

some variability. In particular the optimum times of exposure tend to become shorter toward the middle or end of the summer[12] and the variability is then greater. The experiments cited were all performed during June, at which time the eggs are most uniform in quality and behavior. The table gives the range of exposures resulting in blastulæ, together with the optimum exposure in each case; with the lower concentrations of acid two or even three successive exposures (differing by 2 minutes) may give equally good results; with 0.003 M acid the slope of the curve is steeper, and the optimum is more sharply defined.

The rate of activation is seen to be greatest with formic acid, the strongest of the group; acetic, propionic, and butyric acids are closely similar, with a rate of action between one-third and one-fourth that of formic acid; from butyric to caproic the rates increase somewhat rapidly. This is well shown in another experiment (June 23, 1925) in which the effects of 0.002 M butyric, valeric, and caproic acids were compared, using the same lot of eggs with transfers to sea water at intervals of 1 minute; the optima (80 to 90 per cent blastulæ) were: butyric, 10 to 12 minutes; valeric, 8 to 9 minutes; caproic, 6 to 7 minutes. There is thus a distinct increase in the rate of action from butyric to valeric acid and a more decided increase from valeric to caproic. The almost equal activity of acetic, propionic, and butyric acids, as contrasted with the definite increase shown on passing from butyric to valeric, indicates that the progressive increase in the capillary constants of the acids first assumes importance at this stage; *i.e.*, that adsorption as a factor in the action of the acid first becomes relatively important with valeric acid. Since the dissociation constants of these acids (with the exception of formic) are almost equal, this seems the most probable explanation of the increase of activity. In the qualitative nature of their effects the acids are practically indistinguishable. Under favorable conditions all have produced 75 per cent or more (frequently 90 per cent) of active blastulæ. No significant difference was found between the normal acids and the iso-acids.

Caprylic acid, on the other hand, proved definitely toxic; in twelve experiments (12 separate solutions from 0.0005 M to 0.003 M) the high-

[12] *Cf. J. Biol. Chem.*, 1916, xxiv, 244.

est proportion of blastulæ was *ca.* 5 per cent, obtained with 0.0005 M acting for 14 to 18 minutes. This acid is much more rapid in its action than the earlier members of the fatty series and has a strong cytolytic effect.

The three chlorine-substituted acetic acids are also strongly cytolytic and ineffective as parthenogenetic agents. CCl_3COOH formed fertilization membranes and a few blastulæ (< 1 per cent) in concentrations so low as 0.00025 M, acting for 1 minute; its disintegrative action is very rapid. $CHCl_2COOH$ and $CH_2ClCOOH$ acted similarly, but less rapidly; 0.0005 M $CHCl_2COOH$ formed membranes in most eggs with exposures of 2 minutes, while 0.0005 M $CH_2ClCOOH$ required 4 minutes to produce the same effect. The rate of activation, as measured by membrane formation, thus increases with the number of Cl-atoms; but the cytolytic action is so strong that only an occasional egg (< 1 per cent) reached the blastula stage in any experiment (concentrations 0.00025 M to 0.001 M; exposures 1 to 12 minutes).

In the case of any single acid the rate of action increases with increase of concentration in approximately linear proportion; this rule holds especially for the earlier members, up to butyric, and indicates that the rate of action for a given molar concentration—or what may be called the molecular rate of action—is an approximate constant for each acid. There is evident, however, especially with valeric and caproic acids, a falling off in the molecular rate of action at low concentrations; the same decline of activity at high dilutions is seen also in benzoic and carbonic acids and is apparently general. Presumably what is effective is the excess of acid above a certain low concentration which is fixed by the normal variation of intracellular acidity in the unfertilized egg.

In an earlier paper I suggested with reference to this tendency to a linear relationship between concentration of acid and rate of activation that the velocity of the activation reaction might be determined by the velocity of interaction between the penetrating acid and some colloidal or structural component of the egg system.[13] On this hypothe-

[13] Lillie, R. S., *Biol. Bull.*, 1917, xxxii, 131. The rate of penetration, as such, is not a factor, since penetration is rapid compared with the rate at which the acid reacts within the egg. This is shown by the fact that activation is initiated by an exposure of less than 1 minute to 0.001 M acid, while the whole process

sis the proportionality would represent a simple case of chemical mass action. It is assumed that the chemical and physical properties of the protoplasmic component are thus altered in a definite manner and that the characteristic metabolic and formative sequences of activation are a secondary result of this alteration. That the initiatory process involves a structural alteration in the egg is indicated by many facts of parthenogenesis and normal fertilization.[14] It is conceivable, however, that the acid may act in some other manner; *e.g.*, indirectly by facilitating some other combination having a critical relation to the sequence of events determining activation. Some reaction having a rate proportional to the H ion concentration at some definite region within the egg system is indicated.

It seems likely that the ability to activate the starfish egg completely, at a rate determined by the concentration and special characteristics of the acid, is a property of all weak acids, not too toxic, which can penetrate the surface layer freely. Of other acids exhibiting this action, I have so far experimented with only benzoic and carbonic acids. Both show the same behavior as the fatty acids. A typical series with benzoic acid is the following (Table II).

TABLE II.

June 23, 1924. Benzoic acid. Temperature 20°.

Concentration.	Results.
M	
0.00025	Most eggs form membranes with an exposure of 2 min.; 1 min. is insufficient. No blastulæ are formed up to 15 min. (at 15 min. an occasional blastula — <1%).
0.0005	Optimum at 9 to 10 min. (80–90% blastulæ).
0.00075	Optimum at 5 to 6 min. (60–70% blastulæ).
0.001	Optimum at *ca.* 3 min. (75–85% blastulæ).

lasts 20 minutes or longer in this solution; also by its being promptly arrested at any stage by returning the egg to sea water. The acid thus diffuses rapidly in either direction.

[14] For example, the phenomena of heat-activation already cited. Purely physical agents such as mechanical action, radiation, and the electric current cause partial activation, and the surface layer of the egg changes in permeability and tensile strength during the process.

Benzoic acid is thus rapid and complete in its parthenogenetic effect, its molecular rate of action being approximately from four to five times that of caproic acid. This difference between the two acids corresponds approximately to the difference between the dissociation constants (caproic: $k = 1.45 \times 10^{-5}$; benzoic: 6.6×10^{-5}).[15]

Carbonic Acid.[16]

In the first series of experiments a saturated solution of CO_2 was made by bubbling the gas from a generator for some hours through the isotonic balanced salt solution (van't Hoff's solution), and the various concentrations were obtained by diluting this saturated stock solution with the salt solution. The solutions were kept in corked 100 cc. flasks, and were added to the eggs in flasks of the same size; these were kept corked during the intervals between the transfers to sea water. In other respects the procedure was the same as in the experiments already described. The molecular concentration of the solutions was estimated from the known solubility of CO_2 in salt solutions at the room temperature (20–22°).[17] In a second series, to be described later, CO_2 was generated in known concentration by adding definite quantities of 0.5 M HCl to isotonic salt solution containing 0.05 M $NaHCO_3$ (see p. 360).

Solutions of the gas in van't Hoff's solution exhibit an action which is physiologically identical with that of the fatty acids, and the same proportionality between concentration and rate of action is seen. The following series will illustrate (Table III).

[15] See below, page 364.

[16] The effectiveness of carbon dioxide as an activating agent with starfish eggs has been well known since Delage's experiments in 1902 (Delage, Y., *Arch. zool. exp. et gén.*, 1902, x, 3. série, 213).

[17] The solubility of carbon dioxide in a 0.5 M solution of an alkali salt (which may be regarded as equivalent to sea water in its solvent properties) is about 93 to 95 per cent of its solubility in distilled water (*cf.* Landolt, H., Börnstein, R., Tabellen, Berlin, 5th edition, 1923, 768). At 22° and 760 mm. Hg such a solution dissolves *ca.* 0.8 volumes of the gas. The molecular concentration of the saturated solution under these conditions is thus ~~0.8/22.4 × 295/273 = 0.034 M.~~

$0.8 \div (22.4 \times 295/273) = 0.034$

TABLE III.

Carbon dioxide dissolved in van't Hoff's solution.

Concentration.	Optimum exposures.
Series 1 (July 17, 1924).	
Saturated (0.034 M).....	10 min. (80–85% blastulæ).
0.75 saturated (0.025 M).....	14–16 min. (70–80% blastulæ).
0.5 " (0.017 M).....	20–22 min. (60–70% blastulæ).
Series 2 (July 25, 1924).	
0.6 saturated (0.02 M).....	12–14 min. (80–90% blastulæ).
0.5 " (0.017 M).....	16–18 min. (75–85% blastulæ).
0.4 " (0.014 M).....	18–20 min. (80–90% blastulæ).
Series 3 (July 28, 1924).	
0.4 saturated (0.014 M).....	*Ca.* 19 min. (80–90% blastulæ).
0.3 " (0.01 M).....	25–28 min. (*ca.* 50% blastulæ).
0.2 " (0.007 M).....	31–36 min. (55–65% blastulæ).
Series 4 (Aug. 1, 1924).	
0.15 saturated (0.005 M).....	*Ca.* 45–55 min. (<50%).
0.1 " (0.0034 M).....	No blastulæ up to 60 min. exposure; 35–45 min. required to form membranes in most eggs.

The proportionality between concentration and rate of activation is approximately linear up to 0.2 saturation, but with 0.15 saturation the molecular rate of activation shows a falling off; with 0.1 saturation activation is incomplete even after an exposure of an hour or more.

Hydrochloric and Lactic Acids.

Hydrochloric acid, as already described, is only slightly effective as an activating agent. Since this ineffectiveness is apparently connected with its inability to penetrate the surface layer of the egg, the attempt was made to modify the rate of action by using as solvents a series of isotonic salt solutions having varying influence on permeability. Three series of experiments were carried out, using the concentrations 0.001 M, 0.002 N, and 0.003 N. In each series the acid was dissolved in the four solutions:

A. Pure 0.5 M NaCl.

B. 95 volumes 0.5 M NaCl *plus* 5 volumes 0.5 M $CaCl_2$.

C. 0.35 M $CaCl_2$.
D. van't Hoff's solution (corresponding to buffer-free sea water).

The eggs were exposed to the solutions of each series at 20° for periods of 1 to 15 minutes. The results were essentially negative; *i.e.*, in *pure* NaCl solution (permeability-increasing) the acid showed the same action as in the pure $CaCl_2$ solution (regarded as permeability-decreasing) and in the two balanced solutions. A small minority of eggs formed blastulæ in each series; the proportion was greatest with 0.003 N HCl acting for 15 minutes. The regular and progressive increase in degree of activation with time of exposure up to a definite optimum (the characteristic effect with penetrating acids) is absent in solutions of hydrochloric acid; for example, with 0.001 N and 0.002 N HCl a small and variable proportion of blastulæ (from 1 to 5 per cent) was formed in all exposures from 2 to 15 minutes; the longer exposures were most favorable on the whole, but no regular progress was found. The proportionality between rate of action and concentration is also ill defined in solutions of HCl; stronger solutions act more rapidly, but in such a way as to suggest a penetration by alteration of structure rather than by simple diffusion. The conditions indicate that the penetration to the site of the activating reaction is gradual and not readily modified by changes in the external salt content.

Lactic acid is more rapid and complete in its action than hydrochloric acid, but less so than the fatty acids. It resembles HCl in not being readily affected by changing the salt-content of the external medium. Five concentrations were used: 0.001, 0.002, 0.003, 0.004, and 0.005 M. In 0.001 M lactic acid (in van't Hoff's solution) the minimal exposure for membrane formation was 3 to 4 minutes (as compared with 1 minute or less in 0.001 M fatty acid). With 0.003 M solutions (five experiments) a moderate proportion of eggs (10 to 30 per cent) formed blastulæ with exposures of 12 to 15 minutes. The most complete results were obtained with 0.005 M solutions: of fifteen series with exposures of 1 to 15 minutes, ten showed optima between 10 and 15 minutes; at these optima about 50 per cent of the eggs formed blastulæ; the remaining five series also showed well defined optima between 8 and 15 minutes, but the yield of blastulæ was lower (15 to 35 per cent). Lactic acid is thus less efficient as an activating agent

than the fatty acids and benzoic and carbonic acids; the optima are also less sharply defined. Little difference was found between the effects in the different salt solutions. In pure isotonic NaCl solution, however, membranes were formed with somewhat briefer exposures than in the balanced solutions (*B* and *D* above), while in 0.35 M $CaCl_2$ membrane formation was appreciably retarded. This result was obtained more particularly in experiments in which the eggs were first placed for 10 minutes in the neutral salt solution, from which they were transferred to the same solution containing the acid (0.005 M at 20°).

Lactic acid is thus intermediate in its action between the strong acids and the penetrating or activating acids. Its molecular rate of action is one-third to one-fourth that of propionic acid, and this in spite of its much higher ionization.[18] The retarding influence of the OH group is thus apparent. The presence of this group has been shown by the studies on permeability to interfere with penetration.

Fatty Acid in Presence of Its Salt.

Although the foregoing experiments with different acids show that the physiological effectiveness of an acid is determined by quite other conditions than the H ion concentration of the external solution, it remains possible that ionization at the site of the activation reaction in the egg-interior may be an essential factor. That this is the case is suggested by the difference between acids of equal penetrating power but unequal ionization, such as formic and acetic, or caproic and benzoic. There is also the possibility that penetration of the anions from without rather than of the H ions may be the initiatory event in activation. Or the effect may depend on penetration of the undissociated molecules of the acid; the effectiveness of weak acids is favorable to this interpretation. The experimental results of changing the relative proportions of ions and undissociated molecules in the external medium may be expected to give an answer to such questions, and I have accordingly investigated the effects of the addition of Na-acetate to solutions containing acetic acid in activating concentrations. Changes in the relative proportions of the three components of the

[18] The dissociation constant is 0.00014, and in 0.005 M solution, in the absence of lactate, the dissociation is about 17 per cent.

acetic acid solution (acetate ions, H ions, and acetic acid molecules) in the external solution need not involve corresponding changes in the egg-interior, for the chances are that not all components will penetrate equally. The penetration of ions of one sign is also restricted by the condition of electroneutrality which must be fulfilled. We may assume, however, that the relative concentrations in the egg-interior will be determined by the same conditions of equilibrium as in the external solution, since the solvent concerned is water in both cases.[19]

Acetic acid was used in four concentrations, 0.001, 0.002, 0.003, and 0.004 M, dissolved in the balanced NaCl-$CaCl_2$ solution. Na-acetate was added in concentrations of a similar order, as shown in Table IV. The dissociation of the acetic acid added is represented by the ratio of the H ion concentration to the total concentration of the acid. The H ion concentrations were calculated from the equilibrium equation, $C_{H+} = \frac{kC_{acid}}{\gamma C_{salt}}$, k being the dissociation constant and γ the degree of dissociation of the acid. As a sufficiently close approximation the concentration of acetate ions in the solutions containing $NaCOOCH_3$ was regarded as equal to that of the $NaCOOCH_3$ multiplied by the degree of dissociation, which was taken as 0.64.[20] The equation as used has the form $C_{H+} = \frac{C_{acid} \times 1.8 \times 10^{-5}}{C_{acetate} \times 0.64}$. In the solutions containing acetic acid without added acetate the H ion concentration is given by the formula $C_{H+} = \sqrt{C_{CH_3COOH} \times 1.8 \times 10^{-5}}$, since anions and cations are present in equal concentration.[21] Table IV gives the calculated H ion concentrations and percentages of undissociated acetic acid in the several mixtures used.

[19] Organic solvents (lipoids) are present in protoplasm, but it seems unlikely that reactions within these solvents are of physiological importance. The indications are that the reactions occur within the aqueous phase, although catalyzed by conditions at the boundary surfaces of protoplasmic structures.

[20] This is the dissociation in 0.5 M concentration (*cf.* Seyler, C. A., and Lloyd, P. V., *J. Chem. Soc.*, 1917, cxi, 138) and is assumed to be the dissociation of the Na-acetate in the solution containing 0.5 M NaCl (*cf.* Washburn, E. W., Principles of physical chemistry, New York, 2nd edition, 1921, 345). The dissociation of Na-bicarbonate is very nearly the same as that of Na-acetate.

[21] *Cf.* Michaelis, L., Die Wasserstoffionenkonzentration, Berlin, 2nd edition, 1922, pt. 1, 34.

Comparison was made between the rates of activation observed in the solutions containing acetic acid alone and in the solutions containing in addition $NaCOOCH_3$. It will be noted that the series of solutions of a constant acid content and variable salt content present a wide range of variation in the concentration of H ions and of acetate ions; but, as will be seen from Table IV, the variation in the concentration of free acid is comparatively small. For example, in the

TABLE IV.

Acetic acid added.	Na-acetate added.	$C_{H^+} \times 10^{-5}$	Acid dissociated.	Acid undissociated.
M	M		*per cent*	*per cent*
0.001	0	13.4	13.4	86.6
"	0.001	2.8	2.8	97.2
"	0.002	1.4	1.4	98.6
"	0.004	0.7	0.7	99.3
"	0.008	0.35	0.35	99.65
0.002	0	19.0	9.5	90.5
"	0.002	2.8	1.4	98.6
"	0.004	1.4	0.7	99.3
"	0.008	0.7	0.35	99.65
0.003	0	23.0	7.7	92.3
"	0.004	2.1	0.7	99.3
"	0.008	1.1	0.35	99.65
"	0.016	0.5	0.18	99.82
0.004	0	27.0	6.5	93.5
"	0.004	2.8	0.7	99.3
"	0.008	1.4	0.35	99.65
"	0.016	0.7	0.18	99.82

solutions containing 0.002 M acetic acid the addition of the acetate has the effect of increasing the concentration of undissociated acid by 9 to 10 per cent, and the effect is almost the same in the several solutions of the series.

Tables V and VI give the results of two typical series of experiments with 0.002 M acetic acid, alone and in the presence of Na-acetate. The acid produces the same effect in the presence of acetate as in its absence, but the time required for a definite degree of activation is

very distinctly shortened. It will be noted that the increase in the rate of activation is not large, the exposure required for complete ac-

TABLE V.

July 1, 1925.

Composition of solutions (20°).	Durations of exposures (min.) and percentages of blastulæ.						
	8	9	10	11	12	13	14
A. 0.002 M acetic acid (alone)	3–4	30–40	65–75	50–60	*Ca*. 50	*Ca*. 50	25–30
B. 0.002 M acid *plus* 0.002 M Na-acetate	55–60	60–65	45–50	20–25	1–2	<1	0
C. 0.002 M acid *plus* 0.004 M Na-acetate	60–70	60–70	*Ca*. 50	*Ca*. 10	1–2	<1	0
D. 0.002 M acid *plus* 0.008 M Na-acetate	*Ca*. 50	*Ca*. 50	*Ca*. 50	1–2	<1	<1	0

TABLE VI.

July 7, 1925.

Composition of solutions (20°).	Durations of exposures (min.) and percentages of blastulæ.							
	4	5	6	7	8	9	10	11
A. 0.002 M acetic acid (alone)	<1	<1	*Ca*. 1	*Ca*. 5	*Ca*. 50	>50	60–70	*Ca*. 50
B. 0.002 M acid *plus* 0.008 M Na-acetate	*Ca*. 1	<5	*Ca*. 50	50–60	50–60	20–25	<1	0
C. 0.002 M acid *plus* 0.016 M Na-acetate	*Ca*. 1	5–10	30–40	*Ca*. 60	*Ca*. 50	20–30	*Ca*. 5	0
D. 0.002 M acid *plus* 0.032 M Na-acetate	<1	<5	25–35	55–60	*Ca*. 50	20–25	*Ca*. 1	0

tivation being shortened by 1 to 2 minutes, indicating an acceleration of the order of 10 per cent; further that the variation in the concentration of the salt has little influence upon the degree of acceleration

observed. This is the result to be expected if the effect depends upon the increase in the concentration of undissociated acetic acid.

A similar acceleration was observed in six other similar series with mixtures of acetic acid (0.002 to 0.004 M) and Na-acetate (0.002 to 0.032 M). The total number of separate experiments showing a definite result of this kind was twenty-two; no exceptions were observed when the concentration of acetic acid was 0.002 M and higher. The degree of acceleration varied somewhat in different experiments, but typically lay between 10 and 20 per cent. In some experiments it was larger than could be accounted for by the estimated increase in the concentration of acetic acid molecules.[22] In the experiments with 0.003 M and 0.004 M acid the acceleration, while distinct, was less than in those with 0.002 M acid, a result also consistent with the present hypothesis.

The fact that the increase in the rate of activation is moderate, and that it is almost the same with the several concentrations of acetate, indicates that the increase in the undissociated acetic acid is the essential factor in the effect. The conclusion therefore seems justified that the rapid penetration of the acetic acid molecules to the site of the activating reaction is the primary condition of activation. The results obtained by the addition of HCl to bicarbonate-containing solutions (to be described below) also indicate an effect which is independent of the penetration of ions from without. Once inside the egg, the penetrating molecules dissociate, yielding H ions and the anions of the acid.

We need not conclude that the acetate ions and hydrogen ions are

[22] Some other factors may enter, but their nature is not clear. It is possible that the dissociation constant of acetic acid in the 0.5 M NaCl solution is greater than in pure water. The degree of the salt effect (equivalent to increase in dissociation of the acid) is uncertain. According to Michaelis, pK_1, the negative exponent of the altered dissociation constant (activity coefficient), is 4.48 for acetic acid in 0.5 M NaCl solution (Michaelis, L., and Krüger, R., *Biochem. Z.*, 1921, cxix, 307), corresponding to a dissociation constant of 3.2×10^{-5}. If this value is used, instead of 1.8×10^{-5}, the agreement with the experiments described above is better. The relations between activity and dissociation are not clear in the case of the weak acids (whose dissociation is still usually regarded as partial), and provisionally we may regard activity and dissociation as equivalent terms.

entirely unable to penetrate the egg from without. Smith and Clowes[23] have recently found that the inhibiting influence of fatty acids and of carbonic acid on cell division in echinoderm eggs is decreased in the presence of the salts of these acids, to a degree which is explained satisfactorily on the hypothesis that the anions also penetrate. My own observations on activation with weaker solutions of acetic acid (0.001 M) are consistent with this view; they indicate, however, that the anions penetrate much more slowly than the undissociated molecules. If the penetration were equally rapid it would be difficult to understand (in view of Smith's experiments) why the presence of acetate in the external medium should not *retard* rather than hasten the activation reaction. If, however, the acetate ions penetrate slowly, one might expect that the retarding effect would not appear unless the rate of activation were also slow; there would then be time for the anions to reach the site of the activation reaction before the latter was completed. Such a retarding influence is in fact seen in 0.001 M acetic acid, as will shortly be described.

We should also expect that in stronger solutions of acid the hastening influence of acetate would be replaced by a retarding influence if the concentration of the salt were sufficiently increased. Systematic experiments to determine what occurs under these conditions have not yet been performed, but in several of last summer's experiments with 0.002 M acetic acid it was noted incidentally that the accelerating action was distinctly less marked with the stronger solutions of the salt (0.016 and 0.032 M) than with the weaker. This result, otherwise difficult to explain, becomes intelligible on the assumption of a penetration of anions which is the more rapid the higher the external concentration of salt. In such a case the anions are assumed to act (as in Smith's experiments) by decreasing the dissociation and hence the effectiveness of the acid at the site of the activating reaction.

It should be noted that Na-acetate alone, added to the neutral balanced salt solution used as solvent, is entirely without activating effect. This shows that the acetate anions by themselves are indiffer-

[23] Smith, H. W., and Clowes, G. H. A., *Am. J. Physiol.*, 1924, lxviii, 183. Smith, H. W., *Am. J. Physiol.*, 1925, lxxii, 347.

ent in their action. We infer that they influence the action of acetic acid by depressing dissociation, either outside or inside the egg. In the former case they accelerate, in the latter they retard the rate of activation.

Experiments in which HCl was added to isotonic NaCl-$CaCl_2$ solution containing Na-acetate gave results similar to those just described. In such mixtures acetic acid is formed by the union of hydrogen and acetate ions, and the proportions of undissociated acid and ions may be calculated from the equilibrium equation. The presence of acetic acid in the solution is shown by the physiological response of the eggs.

TABLE VII.

July 13, 1925.

Composition of solutions (20°).	Durations of exposures (min.) and percentages of blastulæ.									
	1	2	3	4	5	6	7	8	9	10
A. 0.001 M HCl *plus* 0.004 M Na-acetate...	0	0	0	0	0	<1	*Ca.* 1	10–20	35–40	65–70
B. 0.002 N HCl *plus* 0.004 M Na-acetate...	0	0	10–20	40–50	*Ca.* 10	<1	0	0	0	0
C. 0.003 N HCl *plus* 0.004 M Na-acetate...	0	10–15	20–25	0	0	0	0	0	0	0
D. 0.004 N HCl *plus* 0.004 M Na-acetate...	0	40–50	0	0	0	0	0	0	0	0

HCl added alone to the NaCl-$CaCl_2$ solution has only slight activating effect, as already shown; the further addition of an equivalent quantity of Na-acetate corresponds essentially to a substitution of acetic for hydrochloric acid, and imparts typical activating properties to the solution. Still further addition of acetate, by depressing dissociation, increases the concentration of undissociated acetic acid and increases correspondingly the rate of activation.

Table VII gives the record of a series of experiments in which HCl in the four concentrations, 0.001, 0.002, 0.003, and 0.004 M was added to the NaCl-$CaCl_2$ solution containing 0.004 M Na-acetate.

The increase in the rate of activation with increasing concentration

of acid is well shown in this series. The action of the weakest solution (optimum at about 10 minutes) is relatively slower than that of the other three, which show a rate of action closely proportional to the concentration of acid. A similar result was obtained in several other series of the same kind.

Table VIII illustrates the effect of adding varying quantities of Na-acetate to the balanced solution containing 0.002 N HCl. When HCl and Na-acetate are present in equal concentrations the rate of activation is similar to that observed in 0.002 M acetic acid; when further Na-acetate is added the rate is decidedly increased.

TABLE VIII.

July 15, 1925.

Composition of solutions (20°).	Durations of exposures (min.) and percentages of blastulæ.					
	3	4	5	6	7	8
A. 0.002 N HCl *plus* 0.002 M Na-acetate	<1	<1	*Ca.* 1	10–20	40–50	65–75
B. 0.002 N HCl *plus* 0.004 M Na-acetate	*Ca.* 5	*Ca.* 50	50–60	*Ca.* 10	*Ca.* 1	0
C. 0.002 N HCl *plus* 0.008 M Na-acetate	*Ca.* 10	>50	10–15	<1	0	0
D. 0.002 N HCl *plus* 0.016 M Na-acetate	5–10	*Ca.* 50	15–20	<1	0	0

Retarding Action of Acetate in Weak Solutions of Acetic Acid.

It is remarkable that while in all of the numerous experiments[24] in which Na-acetate was added to NaCl-$CaCl_2$ solution containing 0.002 M to 0.004 M acetic acid the rate of activation was distinctly increased, in experiments with weaker solutions (0.001 M $HCOOCH_3$, or 0.001 N HCl *plus* Na-acetate) the reverse result was obtained; *i.e.*, the addition of Na-acetate retarded rather than hastened the activation process. This effect is usually not distinct until the ratio of salt

[24] In all 28 in number, including those in which HCl was added to the NaCl-$CaCl_2$ solution containing Na-acetate.

to acid is 8 to 1 or higher, but is quite definite and unmistakable. When the proportion of acetate is less, the effect is slight or of the already described accelerating kind. Table IX gives the record of a typical series of experiments.

The significant feature is that this retarding effect is seen only when the rate of activation is relatively slow. The results described in the preceding section indicate that activation follows upon the penetration

TABLE IX.

July 20, 1925.

Composition of solutions (20°).	Durations of exposures (min.) and percentages of blastulæ.											
	8	9	10	11	12	13	14	15	16	17	18	19
A. 0.001 M acetic acid (alone)....	*Ca*. 1	20–30	40–50	40–50	50–60	35–40	35–40	25–30	25–30	15–20	10–15	*Ca*.10
B. 0.001 M acid *plus* 0.004 M Na-acetate....	1–2	10–20	25–30	40–45	60–65	60–65	60–70	65–75	60–70	*Ca*.50	*Ca*.50	10–20
C. 0.001 M acid *plus* 0.008 M Na-acetate....	*Ca*. 1	*Ca*. 1	20–30	30–40	50–60	50–60	*Ca*.50	*Ca*.50	*Ca*.50	60–70	50–60	25–35
D. 0.001 M acid *plus* 0.016 M Na-acetate....	*Ca*. 1	1–2	5–10	10–15	20–30	30–35	40–50	*Ca*.50	50–60	60–70	60–65	*Ca*.60

of the undissociated molecules of acid to some definite region in the egg protoplasm. At this region the critical reaction of activation occurs, at a rate proportional to the concentration of acid. The relation already pointed out between the strength of the activating acids and their rate of action indicates further that ionization of the acid at the site of activation is an essential factor in its physiological action. It is reasonable therefore to assume that the retardation depends on a depression of ionization by acetate anions which diffuse

relatively gradually into the egg. A noticeable feature of the series *B*, *C*, and especially *D* (Table IX) is that the optimum is less sharply defined than in the control series without acetate (series *A*); *i.e.*, there is a prolongation of the time during which the eggs exposed to the acid remain capable of development. This is a further indication of retardation, such as might be expected to result from a gradual increase in the concentration of anions at the site of the activating reaction. In series *D*, after 19 minutes in the solution, the majority of eggs still form blastulæ when returned to sea water, and little change in the proportion of developing eggs is apparent during the last 5 minutes of exposure.

Smith has shown that the cleavage-inhibiting action of the fatty acids is lessened by the addition of their salts to the external medium;[23] his quantitative data indicate further that the anions of the added salt are concerned in the intracellular equilibrium, and that equal retarding effects correspond to equal H ion concentrations within the protoplasm. The activation reaction in the starfish egg is evidently a process of quite special type, requiring for its production much higher external concentrations of acid than those which just inhibit cell division. It seems probable, however, that in certain general respects the conditions determining the velocity of activation are similar, and that in this case also the rate of the critical activating reaction is determined by the H ion concentration at its site in the cortical layer of protoplasm[25] (see below, page 363). Retardation would thus be expected to result from penetration of anions.

Experiments on the Addition of HCl to NaCl Solutions Containing $NaHCO_3$.

Carbonic acid is generated in known concentration in the interior of a solution containing bicarbonate when definite quantities of HCl or other strong acid are added to the solution. When the bicarbonate is present in excess and the salts of other weak acids are absent the effect is essentially equivalent to a substitution of carbonic acid for the

[25] Chambers has shown that the membrane-forming reaction, which is an integral part of the primary activation process, is confined to the cortical region of the egg protoplasm (*cf.* Chambers, R., *J. Gen. Physiol.*, 1921–22, iv, 41; *Biol. Bull.*, 1921, xli, 318).

HCl added. The H ion concentration of such a mixture may then be calculated from the equilibrium equation:

$$H^+ = \frac{H_2CO_3}{\gamma NaHCO_3} \times 3.2 \times 10^{-7}$$

the concentration of the remaining unneutralized bicarbonate and its degree of dissociation, γ, being known. The carbonic acid is present almost entirely in the undissociated form, and, since the equilibrium ratio between the anhydride CO_2 and the acid H_2CO_3 $\left(\frac{CO_2}{H_2CO_3}\right)$ is apparently of the order 50 or 100 to 1, probably chiefly as the dissolved gas CO_2.[26]

TABLE X.

Volumes 0.5 M HCl added in 100 cc. of mixture.	Total concentration of $RHCO_3$ in mixture.	Concentration of H_2CO_3 in mixture.	Concentration $NaHCO_3$ remaining unneutralized.	pH (calculated).
cc.	M	M		
1	0.0495	0.005	0.0445	7.25
2	0.049	0.01	0.039	6.9
3	0.0485	0.015	0.0335	6.65
4	0.048	0.02	0.028	6.45
5	0.0475	0.025	0.0225	6.25
6	0.047	0.03	0.017	6.05
7	0.0465	0.035	0.0115	5.8
8	0.046	0.04	0.006	5.5

In the present series HCl was added to isotonic NaCl solution containing $NaHCO_3$. A mixture of 90 volumes 0.5 M NaCl *plus* 10 volumes 0.5 M $NaHCO_3$ was used.[27] Volumes of 0.5 M HCl ranging from 1 to 8 cc. were added to this solution (in 100 cc. flasks) so as to

[26] The symbol H_2CO_3 in the present paper is used to indicate total dissolved CO_2, in whatever form (*i.e.* H_2CO_3 *plus* CO_2). The work of Thiel and Strohecker (Thiel, A., and Strohecker, R., *Ber. chem. Ges.*, 1914, xlvii, 945) and of Pusch (Pusch, L., *Z. Elektrochem.*, 1916, xxii, 206) indicates that the equilibrium ratio CO_2/H_2CO_3 is of the above order.

[27] $CaCl_2$ was omitted, to avoid any disturbing effects resulting from the precipitation of carbonate. The NaCl-$NaHCO_3$ solution has a slight activating effect (like that of pure NaCl and other Na-salts). This, however, is negligible in comparison with that of the H_2CO_3 formed by adding HCl.

make the resulting total volume 100 cc. in each case. The flasks, almost completely filled with the solution, were kept tightly corked to avoid loss of CO_2. The solutions were added to the eggs (1 to 2 cc. of a dense suspension in sea water) in 100 cc. flasks; these were kept corked in the intervals between the transfers to sea water.

Table X gives the composition of the mixtures used and the estimated concentrations of H_2CO_3; also the approximate pH of each solution as calculated from the equilibrium equation. The degree of dissociation of the $NaHCO_3$ is regarded as 0.64,[28] and the first dissociation constant of H_2CO_3 as 3.2×10^{-7}.[29]

The following results were obtained in typical experiments with

TABLE XI.

Concentration of H_2CO_3.	Optimum exposures and percentage of eggs forming blastulæ.
M	
1 and 2.	Few or no membranes formed with exposures up to 10 min. (the longest exposure in this series).
0.005 and 0.01	
3. 0.015	All eggs form membranes with 1 min. exposure; no blastulæ with exposures up to 10 min.
4. 0.02	A few blastulæ (3 to 4%) with exposures of 10 min. (optimum not reached).
5. 0.025	Optimum at 15 min. (35–45%).
6. 0.03	Optimum at 11–13 min. (35–50%).
7. 0.035	Optimum at 12–13 min. (50–60%); a second less favorable series showed an optimum at 9–10 min. (20–25%).
8. 0.04	Optimum at 5–6 min. (30–50%).

good controls (Table XI). Only slight activation was obtained with concentrations of H_2CO_3 below 0.02 M; in these experiments, however,

[28] The value given by Seyler and Lloyd[20] for 0.5 M $NaHCO_3$. The dissociation of the $NaHCO_3$ is to be regarded as the same in all of the solutions, *viz.* that of the 0.5 M solution, this being (approximately) the total concentration of Na-salt in the solution (*cf.* Washburn[20]).

[29] The value for 20° (to the first decimal) corresponding to Kendall's recent determination (Kendall, J., *J. Am. Chem. Soc.*, 1917, xxxviii, 1480). The dissociation of H_2CO_3, as of other weak acids, is presumably increased under the influence of the salts present (the effect seen in the salt error of indicators),[34] but this value is used since it (or 3.25×10^{-7}) was found by Smith and Clowes[23] to agree satisfactorily with their observations on CO_2 tensions in sea water to which $NaHCO_3$ and HCl were added.

the exposures were insufficiently prolonged; with higher concentrations typical activation, showing well defined optima at certain durations of exposure, was obtained, as in the experiments with dissolved CO_2 gas described above.

It will be seen on comparing these results with those of Table III that the activating effects of solutions of equivalent CO_2 content in the two series correspond closely. The action of the HCl-$NaHCO_3$ solutions appears, however, to be somewhat slower than that of the solutions of the gas. This retardation may be regarded as indicating a penetration of HCO_3 ions, but further experiment is required before the degree of this effect can be estimated.[30]

Jacobs[31] has shown the remarkable readiness with which CO_2 penetrates the protoplasmic system; and the results given in Tables III and XI show that its physiological effects are identical with those of other penetrating acids. This is somewhat surprising in view of its constant presence in living protoplasm. The CO_2 tension of the parthenogenetically effective solutions is, however, much higher than that normally found in protoplasm. What is remarkable is that the maintenance of this heightened CO_2 tension for a certain definite time brings the egg into the same automatically developing state as does the entrance of a spermatozoon. The results with valeric and caproic acids indicate that the critical activating reaction is localized and is a surface reaction, the surfaces concerned being apparently situated in the cortical or ectoplasmic layer of the egg. Apparently, therefore, a sufficient increase of CO_2 tension in this region, lasting for a sufficient time, produces an effect equivalent to activation.

The molecular effectiveness of carbonic acid is low, apparently from one-tenth to one-twentieth of that of the fatty acids;[32] this is to be expected in view of its weakness as an acid, and suggests the question whether the parthenogenetic action of weak acids in general is a pure H ion effect. If this is the case, we should expect that the H ion con-

[30] Other factors, such as oxygen tension, which is evidently higher in the HCl-$NaHCO_3$ mixtures, may play a part; but this remains to be determined.

[31] Jacobs, M. H., *Am. J. Physiol.*, 1920, li, 321; liii, 457.

[32] It will be noted that if the ratio CO_2/H_2CO_3 is of the order of 100 to 1, and if carbonic acid (the true ionizing compound H_2CO_3) alone is effective, its effectiveness is greater than that of formic acid.

centrations of equally effective solutions of the penetrating acids would be identical—assuming that the concentrations of acid at the intracellular site of the activating reaction are the same as in the external solution. This may not always be the case, especially when the acids are strongly adsorbed at the protoplasmic surfaces where the reaction occurs. Nevertheless many of the facts described above point to this conclusion, *e.g.* the difference between formic and acetic acid, the approximate equality in the action of acetic, propionic, and butyric acids, the comparative effectiveness of caproic and benzoic acids, and the high concentrations of the effective solutions of CO_2. It is therefore of interest to estimate the H ion concentrations of solutions having the same activating effect.

TABLE XII.

Concentrations of Penetrating Acids Which Produce Complete Activation in 10 Minutes at 20°.

Acid.	Concentration (A).	Dissociation constant ($k \times 10^6$).	Calculated C_H[33] ($C_H \times 10^4$).
	M		
Formic	0.0008	22	4.2
Acetic	0.0025	1.8	2.1
Propionic	0.0024	1.4	1.8
Butyric	0.0022	1.4	1.75
Valeric	0.0018	1.4	1.6
Caproic	0.0014	1.45	1.4
Benzoic	0.0005	6.0	1.7
Carbonic	0.035	0.032	1.1 (corrected 1.6).[34]

In Table XII the concentrations of the different penetrating acids required to effect complete activation in 10 minutes (approximately)

[33] By the formula $H^+ = \sqrt{A \times k}$, A being the concentration of the acid and k its dissociation constant.[21]

[34] Warburg (Warburg, E. J., *Biochem. J.*, 1922, xvi, 153) finds the first dissociation constant (k_1) for H_2CO_3 in the presence of 0.29 M NaCl to be 6.3×10^{-7} ($pk_1 = 6.2$); *cf.* page 253 of his paper. If, as an approximation, we regard k_1 in sea water or in the egg as having the value 7×10^{-7} at 20°, C_H becomes *ca.* 1.6×10^{-4} (*i.e.* $\sqrt{0.035 \times 7 \times 10^{-7}}$). (Provisionally we are not distinguishing between the dissociation and the activity of the weak acids.[22])

at 20° are given in the second column. These concentrations are derived by interpolation from the results of typical experiments such as those of Table I. The dissociation constants are given in Column 3. Column 4 gives the calculated H ion concentrations of these solutions. We assume provisionally that the acid is present at the site of activation in the same concentration as in the external solution and that its salts are absent; also that the dissociation constants are of the values usually accepted (*i.e.* neglecting the salt effect).

Solutions of these acids in the concentrations given in Table XII cause activation in approximately the same time, under similar conditions of temperature and physiological state of the eggs. The estimated H ion concentrations are evidently of the same order, especially in the case of benzoic acid and the fatty acids. The series of fatty acids shows a progressive decrease in the external C_H as the molecular weight rises; this may be taken as indicating that the concentrations at the intracellular site of the activation reaction are in reality higher (because of adsorption), especially with valeric and caproic acids, than in the outside solution. Formic acid is less effective and carbonic acid more effective than the external C_H would lead us to expect. The reasons for these discrepancies are not entirely evident at present,[34] but may be indicated by further experimental study of the conditions. Other acids should also be used. So far as they go the data indicate that the C_H adjoining certain structural surfaces in the cortical region of the egg is a main factor determining the inception and rate of progress of the activation reaction.

The observed proportionality between the rate of activation and the concentration of acid, within the effective range, would then indicate a rate of reaction dependent on the C_H existing at the protoplasmic site of the reaction. What is further implied by this relation is difficult to say. Specific unions of complex bodies are apparently concerned in activation and fertilization; there are also structural changes, the whole process forming a definite and complex sequence of interdependent events. One can imagine some reaction holding a critical position in the sequence to be held in check at a certain C_H (*e.g.* because of dependent polarization effects) and released when the C_H is changed. Another possible factor is H ion catalysis; or the critical reaction may occur only within the pH range of

some enzyme; or there may be a relation to the isoelectric points of certain structural proteins: the C_H value found for most acids in Table XII, 1.6 to 1.8 $\times$ 10^{-4} (pH = 3.7 to 3.8) is consistent with this interpretation. Little is gained for the present by pursuing such suggestions in detail, since each step in the investigation must be controlled by experiment.

SUMMARY.

1. Exposure of unfertilized starfish eggs to dilute solutions of weak acids (fatty acids, benzoic and carbonic acids) in isotonic balanced salt solution causes complete activation with the proper durations of exposure. For each acid the rate of activation (reciprocal of optimum duration) varies with concentration and temperature; at a given temperature and within a considerable range of concentrations (*e.g.* 0.00075 to 0.004 M for butyric acid[1]), this rate is approximately proportional to concentration. We may thus speak of a molecular rate of action characteristic of each acid.

2. In general the molecular rate of action increases with the dissociation constant and surface activity of the acids. In the fatty acid series (up to caproic), formic acid has the most rapid effect, acting about four times as rapidly as acetic; for the other acids the order is: acetic = propionic $\gtreqless$ butyric < valeric < caproic. Carbonic acid acts qualitatively like the fatty acids, but its molecular rate of action is only about one-fourteenth that of acetic acid.

3. Hydrochloric and lactic acids are relatively ineffective as activating agents, apparently because of difficulty of penetration. Lactic acid is decidedly the more effective. The action of both acids is only slightly modified by dissolving in pure (isotonic NaCl and $CaCl_2$) instead of in balanced salt solution.

4. The rate of action of acetic acid, in concentrations of 0.002 M to 0.004 M is increased (by 10 to 20 per cent) by adding Na-acetate (0.002 to 0.016) to the solution. The degree of acceleration is closely proportional to the estimated increase in undissociated acetic acid molecules. Activation thus appears to be an effect of the undissociated acid molecules in the external solution and not of the ions. Acetate anions and H ions acting by themselves, in concentrations much higher than those of the solutions used, have no activating effect.

The indications are that the undissociated molecules penetrate rapidly, the ions slowly. Having penetrated, the molecules dissociate inside the egg, yielding the ions of the acid.

5. When the rate of activation is slow, as in 0.001 M acetic acid, the addition of Na-acetate (0.008 M to 0.016 M) has a retarding effect, referable apparently to the gradual penetration of acetate ions to the site of the activation reaction with consequent depression of dissociation.

6. An estimate of the C_H of those solutions (of the different activating acids) which activate the egg at the same rate indicates that their H ion concentrations are approximately equal. On the assumptions that only the undissociated molecules penetrate readily, and that the conditions of dissociation are similar inside and outside the egg, this result indicates (especially when the differences in adsorption of the acids are considered) that the rate of activation is determined by the C_H at the site of the activation reaction within the egg.