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EFFECT OF AMINO ACIDS
ON CLEAVAGE
OF SEA-URCHIN EGGS

BY

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AMINO ACIDS

AND

CLEAVAGE

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ABSTRACT

A review of the literature reveals much work relating amino acids to growth but very little concerning their specific effect on cleavage.

In the present study the effect of lysine, arginine, histidine, methionine, glycine, glutamic acid, glutamine, cysteine and the tripeptide glutathione on the cleavage of the eggs of *Paracentrotus lividus* was determined.

The amino acids were added in equivalent weights of 0.273 millimoles/100 cc to the sea-water containing the eggs 10 min. after fertilization. Care was taken to keep the pH of sea-water unchanged(8.1) by properly neutralizing the acidic amino acids with 1N NaOH. Samples were taken from both experimental and control groups at certain intervals after cleavage had started, and fixed in 37% formaldehyde. Counts were made and the percentage of cleaved eggs in both experimental and control groups was then determined.

It was clearly shown that arginine, lysine, histidine, glutamic acid, methionine and glycine accelerated cleavage. On the other hand, cysteine, glutamine and the tripeptide glutathione were inhibitory. All results were statistically significant.

In most cases it was noticed that the curves for the control and experimental groups diverged considerably

at the start and then converged near the end of the cleavage period. This was attributed to a probable action of the amino acids on nuclear events.

The accelerative action was explained by theorizing that some of the amino acids (lysine, arginine and histidine) might have entered into the synthesis of nuclear proteins as they are already suspected to be constituents of chromosin. Three amino acids, i.e. arginine, histidine and glycine, might have played an important role in the nucleic acid synthesis by providing the raw material for the formation of the purine rings. Still others might have acted directly or indirectly as energy sources for cleavage (arginine, glutamic acid, methionine and glycine).

As far as the inhibitory action of cysteine and the tripeptide glutathione is concerned, it was thought that the -SH groups might have reduced certain respiratory enzymes or competed for the oxygen of the medium, thus favouring fermentative metabolism which would result in unfavourable conditions for cleavage. The -SH might have also combined with structural proteins thus causing a colloidal change in the egg cytoplasm. Glutamine retarded cleavage probably because it prevented glutamic acid from acting as an energy source.

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INTRODUCTION

The physiology of cell division has been studied biochemically and biophysically with much enthusiasm about the merits of each procedure by its protagonists. Time will come when the findings of both fields will converge into a unified and clear picture of the mechanism of cell division. At present the two fields are separate and independent.

The literature in both fields is voluminous and no attempt will be made to review it here. Only the trend of thought that led to the present investigation will be outlined.

Biochemical findings

There was a feeling among early workers that the problem of cell division was one of energy supply. Working on eggs of the frog and toad, Stefanelli (1937) measured their oxygen consumption during cleavage. He found that a slight increase in oxidation occurred just before the appearance of the cleavage furrow and then the oxygen consumption fell to its original value. Brachet (1950) who studied the changes in respiration during the mitotic cycle of *Chaetopterus* eggs, believes that oxygen consumption increases slowly during cleavage. On the other hand Whitaker (1933) working on *Chaetopterus* and *Cumingia* eggs proved that oxygen consumption is not directly related to the initiation

of development and sometimes not even concomittant. So interest shifted to another aspect of cell division, that of its inhibition by compounds of metabolic significance.

Some drugs cause cells, which enter or are about to enter division, to be arrested. The use of such mitotic poisons at a certain stage will probably help in understanding the chain of chemical events involved. Heilbrunn (1920) found that before a cell divides its protoplasm gels. Later (1949) he worked with heparin and was able to prevent the so-called mitotic gelation by immersing the eggs in a dilute solution of heparin. Suppression of the mitotic gelation resulted in suppression of cleavage. Dustin (1950) found that in the intestine of the mouse aminopterin and other folic acid antagonists are violent mitotic poisons of the chromosomic type, while Hughes (1950) working on the chick observed that the effect of aminopterin is primarily on the spindle. The effect of the famed mitotic poison colchicine has been studied by Sedar and Wilson (1951). They used the electron microscope to observe the mitotic figures of normal and colchicinized onion root tip cells. The effect of short exposure to colchicine caused a progressive swelling and solubilization of the spindle fiber material. Podophyllin is another of these inhibitory compounds which act specifically on the achromatic figure and cause it to regress. Its effect was tried by Cornman and Cornman (1951) on eggs of the starfish _Asterias forbesii_, the sand dollar _Echinarachnius parma_, the sea-urchins _Arbacia puntulata_,

Tripneustes esculentus and *Lytechinus variegatus*, and the sea slug *Chromodoris.* Exposure to podophyllin resulted in abnormal cleavage of the eggs because the achromatic figure was destroyed.

Inspite of much advance, there is still a great deal to be learned about the chemical events that culminate in cleavage.

Biophysical studies

These studies are concerned with the colloidal changes of protoplasm during cleavage. The investigators in this field are encouraged by the fact that their findings are applicable to almost all dividing cells. They try to attribute the movements of division to protoplasmic gelation.

As mentioned previously, Heilbrunn (1920) noticed that the formation of the mitotic spindle was preceeded by an increase in protoplasmic viscosity which later decreased. Marsland (1950) found that the gelation process is an endothermic reaction. In performing the work of cleavage the cell uses energy which comes from the reserve previously stored during the endothermic formation of the cortical gel structure. Chambers (1951) believes that cell division is caused by the growth of two viscous spheres separated by a liquid zone.

Some investigators prescribe importance to cortical rigidity rather than cytoplasmic gelation.

Schechtman (1937) found that cleavage is initiated by a concentration of the egg cortex at the site of the future furrow. The cortex becomes thicker and buldges towards the interior of the egg. At the same time the surface of the egg is displaced towards the site of thickening. Marsland (1950) devised an apparatus which permits cleaving eggs to be observed at a magnification of 600 diameters while the cells are subjected to pressures up to 16,000 lbs/in^2 and to temperatures ranging between 0°C and 35°C. The gelated cortical protoplasm of the eggs was solated by increasing the pressure and decreasing the temperature. Whenever cortical gelation was brought below a certain value, cleavage failed to occur.

Recently, optical techniques using polarized light found their way in cytological investigation.

Monroy and Montalenti (1946) working with eggs of the sea-urchin Psammechinus miliaris found that there is a disappearance of birefringence at fertilization and a reappearance during the anaphase of the first mitotic division. So they suspected that the cortical layer was undergoing rhythmical variations of its submicroscopic structure. They also proved that this cycle was synchronous with, but independent of, the spindle cycle by inhibiting spindle formation in fertilized eggs with cochicine, without altering the rhythm of birefringence. Swan and Mitchison (1950) observed a wave of reduced birefringence which started from the center of each aster and moved to the cell surface.

Cleavage was observed to start when the wave reached the poles of the egg.

One of the biophysical approaches relates to surface expansion. Dan (1951) noticed that at fertilization of *Arbacia*, some pigment granules got caught in the cortex and thus were used as natural landmarks for following the movements of the cortex. It was apparent that the cortical material accumulated in the region of the furrow after the first cleavage. Later the pigment granules shifted towards the poles where they piled up.

While biochemical and biophysical advances in the study of cleavage were being made, in some laboratories the interplay between nucleus and cytoplasm was gaining ground as the controlling factor of division. Various workers were coming to suspect that the nucleus initiates cytoplasmic division in a manner reminiscent of embryonic induction or hormonal action.

Chambers (1921), and others before him, described how the nuclear membrane breaks down during maturation and the nuclear sap spreads throughout the cytoplasm. This must have an effect on the cytoplasm because, according to Wilson (1903) who worked with the *Nemertine* egg, any non-nucleated fragment taken prior to the dissolution of the vesicle is non-fertilizable contrarily to a fragment taken from a mature egg. Chambers and Fell (1931) observed an immediate increase in the viscosity of the cytoplasm and breakdown of mitochondria on puncturing the nuclei of fibroblasts. In

the same way Peterfi and Naville (1931) found that puncture of the Amoeba nucleus caused cytoplasmic gelation. A more specific study was done by Swan and Mitchison (1950) on birefringence that led them to suspect the existence of an X-substance diffusing out of the chromosomes at anaphase and causing the egg to cleave. Cornman and Cornman (1951) also believed that a furrow organizer was elaborated by the nucleus at the end of prophase and followed the path of the achromatic figure.

Hence, in view of the above theories on the nuclear induction of cleavage, it is important to investigate possible effects of nuclear components on cleavage. In this connection Stedman and Stedman (1943) found that 25% of chromosin, the chief constituent of chromosomes, was composed of basic amino acids, namely lysine 11%, arginine 9.5%, and histidine 5%. Chromosin contained also a large amount of glutamic acid. Other investigators determined not only the amino acid content of the nucleus but that of the egg as a whole. Berg (1950) extracted the free amino acids of Strongylocentrotus purpuratus with 75% ethanol and identified large amounts of glycine and small amounts of alanine, glutamic acid, valine, threonine, lysine, glutamine and leucine. More recently Kavanau (1954) determined the amino acid content of Paracentrotus lividus eggs and found that free amino acids account for 74% of the non-protein portion. Asparagine, glutamine and glutamic acid make up 19%, other

amino acids, principally, cysteine, histidine, phenylalanine and tyrosine, constitute the remaining 7% and are found as peptides.

The study of amino acids has been quite extensive as far as their effect on growth is concerned. Gordon and McLeod (1926) worked on bacterial growth and found that arginine, glutamic acid, l-leucine, d-lysine, tyrosine and valine acted as indifferent amino acids, while taurine, aspartic acid and alanine favored growth and cystine, phenylalanine and tryptophane inhibited it.

Hammett (1940) used Obelia geniculata as experimental material and studied the effect of different amino acids on proliferation and differentiation. He found that d-threonine and S-containing amino acids stimulated proliferation and cell multiplication while l-proline and l-leucine favored differentiation and specialization of the cells. Instead of trying the effect of single amino acids, Wagner (1936) used mixtures of amino acids and observed that they increased the yield of yeast. This effect was ascribed to their particular utilization as C-source and also to the fact that they lowered the surface tension of the fermenting fluid, thus permitting better and increased multiplication of the yeast. Nielsen, Niels and Vagn Hartelius (1939) working on yeast found that asparagine and glutamic acid increased growth. Krukovskaya (1940) on the other hand observed that the growth of Torula utilis was accelerated when asparagine or arginine were added to the mineral medium (3 mg/100 cc.) Fisher and

Astrup (1942) found that tissue cultures of periostal chicken fibroblasts will grow after the addition of nine essential amino acids among which the very important ones were cystine, lysine and glutamic acid. Gudernatsch and Hoffman (1931, 1932) worked with tadpoles and found that arginine, cystine, lysine, phenylalanine permit growth but not differentiation while tyrosine and tryptophane favor differentiation.

Kavanau (1954) studied the metabolism of amino acids during early development of the sea-urchin egg. Cysteine, histidine, phenylalanine, tyrosine, glycine, arginine and glutamic acid were found to decrease during early cleavage. Gaunt (1931) using the eggs of fresh water snails, added cysteine to the water and found that there was no acceleration of cell division as compared with a control group in plain water.

In view of these findings it was deemed very instructive to extend this kind of work to sea-urchin eggs and that is the purpose of this paper i.e. to study the effect of a number of amino acids on the cleavage of these eggs. Amino acids were chosen mainly among those known to be found in the nucleus. Additional ones were also used for comparison.

MATERIALS AND METHODS

As experimental material, the eggs of the sea-urchin *Paracentrotus lividus* were used. A detailed description of the local urchins is given in the Appendix of this paper. This echinoderm is abundant along the Beirut shore and can be easily picked up from rocks not very far from the shore and at a depth of two or three meters below the low-tide mark. The sea-urchins were maintained at room temperature in a sea-water aquarium provided with an air pump.

The eggs and sperm of these animals were obtained in the following manner, a modification of the procedure used by Just (1939):

A circular cut was made around the peristome to expose the coelom. The five gonads occupy most of this cavity and are yellowish orange in males and pinkish orange in females. The ovaries were placed in filtered sea-water while the testes were placed in a dry vessel. The ovaries extruded the eggs which formed a clear layer at the bottom of the vessel. The sperms came out as a thick milky fluid. For each experiment the eggs of one sea-urchin were divided into two groups: a control and an experimental. Both were fertilized in the following way (Just 1939):

One drop of "dry sperm" was diluted in 3 cc. of filtered sea-water and from this milky suspension 3 drops were used to fertilize each group.

The amino acid dissolved in a known quantity of sea-water and properly neutralized was added to the experimental vessel 10 minutes after fertilization to avoid complications with the fertilization process. The volume of each vessel was then made up to 100 cc. with filtered sea-water, thus bringing the concentration of the acid in the experimental solution to the required point.

Fixing and counting

Both groups were examined microscopically from time to time. As soon as some eggs started cleavage in either vessel a sample was taken from each and placed in two stender dishes containing 37% formaldehyde.

Two more samples were taken and fixed in the same manner from each group at two or three minute intervals. In some cases only two samples were fixed for each vessel.

Cleavage usually was completed at 60-75 min. after fertilization at a temperature range of 19°C - 23°C.

Counting of the fixed eggs was done in the following manner:

After shaking the vessel thoroughly, a drop of formaldehyde solution containing eggs from the control vessel was placed on a slide and the number of cleaved and uncleaved eggs counted. An egg was considered as "cleaved" as soon as a furrow appeared. Three or four different fields were examined per drop and a total of thirty counts made per group. The same procedure was used for the experimental eggs. The percentage of divided eggs was

calculated for both the experimental and the control groups, and the standard error of the difference between the two percentages was determined by means of the following formula:

$$\sigma_{D\%} = \sqrt{pq\left(\frac{1}{N_1} + \frac{1}{N_2}\right)}$$

$\sigma_{D\%}$ = Standard error of the difference.

p = total percentage of divided eggs.

q = $1 - p$

N_1 = total number of counted eggs in the control.

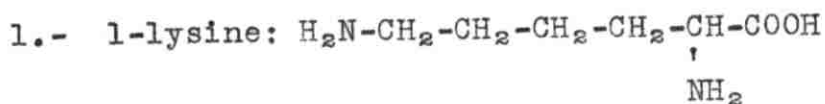
N_2 = total number of counted eggs in the experimental.

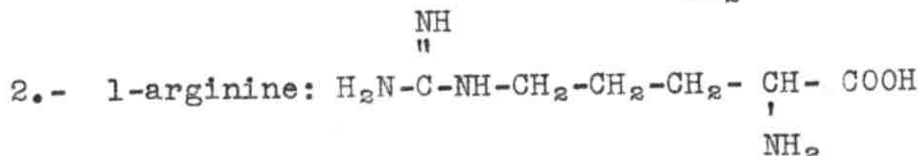
After dividing the actual percentage difference by $\sigma_{D\%}$ we resort to Normal Curve Area Tables to determine the significance of the results. Two experiments were run for every amino acid except in the case of histidine where three experiments were performed.

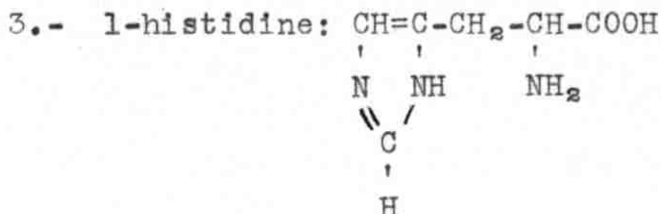
Preparation of the amino acids

The following amino acids were used:

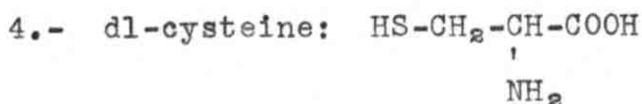
Monobasic diamino acids:

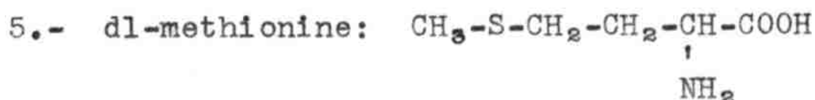

1.- l-lysine: $H_2N\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH$


2.- l-arginine: $H_2N\text{-}C(=NH)\text{-}NH\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH$



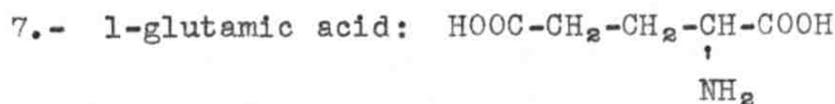
Monobasic monoaminoacids containing sulfur:

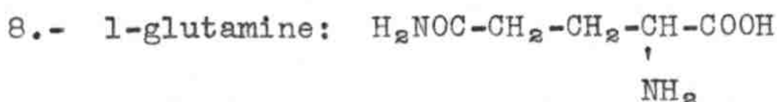




Monobasic monoaminoacids:

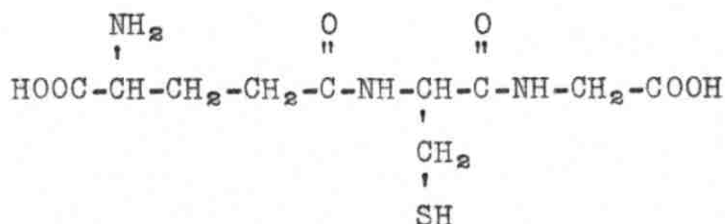
Monoaminodicarboxylic acids:





Glutathione:

To determine the joint effect and possible antagonistic action of amino acid groups, glutathione, a tripeptide was used. It consists of glutamic acid, cysteine and glycine each of which was tested individually.



γ- glutamyl-cysteinyl-glycine.

Of the above mentioned compounds, arginine, histidine,

methionine, glutamic acid and glutathione were products of the Eastman Kodak Co. Rochester, N.Y., while lysine and glutamine were prepared by the Nutritional Biochemical Corporation Cleveland, Ohio, and glycine by Merck Co.

All the amino acids used were soluble in sea-water at room temperature. For every experiment a fresh solution of the amino acid was prepared. The concentration used depended on the optical activity of the acid and was empirically determined by preliminary experiments. In the case of the l-isomers, 0.273 millimoles/100 cc. of sea-water were used but for the dl-forms double that concentration was used, i.e. 0.546 millimoles/100 cc. assuming that only one of the isomers would be taken up by the dividing eggs.

In the case of glutathione, however, two different concentrations were used since there was no indication of its optical activity. The first concentration (84 mg./100 cc.) was equivalent to 0.273 millimoles/100 cc. of the tripeptide, while the second one (168 mg./100 cc.) was equivalent to double that amount.

The pH of sea-water was 8.1. Some amino acids like lysine, arginine, glycine, methionine and glutamine did not affect the pH of sea-water. But glutamic acid and glutathione were acidic in reaction and had to be brought to pH 8.1 with 1N NaOH. Cysteine and histidine were also brought to pH 8.1 because they were received from commercial firms as hydrochlorides. No buffer was used because sea-water

contains carbonates which have a sufficient buffering action.

RESULTS

Of all the amino acids tried, cysteine, glutamine and the tripeptide glutathione proved to retard cleavage while lysine, arginine, methionine, glycine, glutamic acid and histidine accelerated it. In all cases fertilization was successful and resulted in 95% to 100% cleavages at the end of the experiment. Plate I shows two such counts.

l-lysine (.273 millimoles/100 cc.) accelerates cleavage as is clearly shown in Fig. 1. In Experiment I at the instant when 70% of the control eggs were in division, the eggs in lysine showed 81.6% in division, In Experiment II, 67.1% of the eggs in the control group divided as compared with 82.6% of the experimental.

Fig. 2 shows that l-arginine (.273 millimoles/ 100 cc.) caused an acceleration of cleavage in both experiments I and II i.e. 73.3% (Exp. 1) and 17.5% (Exp. II) of eggs dividing in the controls while the experimentals had reached 91.5% (Exp. I) and 33.5% (Exp. II) respectively.

As far as dl-methionine (.546 millimoles/100 cc.) is concerned, Experiments I and II show that it is markedly accelerative (Fig. 3). In Graph 1 it can be noticed that the experimental group takes the upper hand soon after fertilization and continues with a higher percentage of

cleavage. At 67 min. after fertilization it begins to approach the curve of the control, the latter rising sharply until they come quite close to each other (71 min. after fertilization).

That dl-glycine (.546 millimoles/100 cc.) is also an accelerative amino acid is clearly shown in Fig. 4 where only 39.5% of the control eggs have divided as compared to 56.7% of the experimental. Similarly, in Experiment II there are 40.4% cleavages in the control as compared to 55.1% in the experimental.

Figure 5 shows the percentage of cleaved eggs in glutamic acid in two experiments. On comparing the curves for the experimental and control in Graph 2, one can see that there is a very sharp rise at the beginning in the former. At 90 min. after fertilization the curve of the experimental levels down to a plateau while that of the control continues to rise smoothly. It seems that l-glutamic acid (.273 millimoles/100 cc.) stimulates cleavage intensily at the beginning but less so towards the 100% cleavage point.

The three experiments performed with l-histidine (.273 millimoles/100 cc.) proved that it favours cleavage (Fig. 6). Graph 3 shows that the percentages for the control and experimental groups run almost parallel coming very close together at 74 min. after fertilization.

Although l-glutamine is the amide of glutamic acid

and would be expected to have a somewhat similar effect, it was found to retard cleavage (Fig. 7). Graphs 4 and 5 also show this inhibitory effect. They have the same shape as Graph 1 but with the position of experimental and control reversed.

Similarly to l-glutamine, dl-cysteine (.546 millimoles/100 cc.) also retards cleavage (Fig. 8). In Experiment I 54.3% of the eggs in the control group had already divided while the experimental indicated only 26% in division. In Experiment II there is an even more striking difference i.e. 96.1% cleavage in the control and only 29.2% in the experimental. Graphs 6 and 7 show this effect very clearly.

The inhibitory effect of the tripeptide glutathione seems to be directly proportional to its concentration (Figs. 9 and 10). Plotted on the same coordinates (Graph 8) the curve for the control group and the two curves for the experimentals (84 mg./100 cc. and 168 mg./100 cc.) seem to converge towards the same point 61 min. after fertilization.

This convergence is shown in almost all the graphs regardless of the nature of the amino acid used i.e. whether inhibitory or accelerative.

The calculation of the standard error of the difference between percentages is given in Tables 1-9. From there it is seen that the "t" values are very high and

hence the results are statistically very significant.

DISCUSSION AND CONCLUSION

The results obtained in this study are not very different from what was expected.

Lysine, arginine and histidine, being important constituents of the nuclear protein chromosin (Stedman and Stedman 1943), are evidently necessary for the synthesis of new nuclear material. That is probably the reason for their favourable effect on cleavage. By speeding up the nuclear cycle they might have caused an earlier cytoplasmic division. There is, of course, the possibility that they might have acted directly on the cytoplasm causing it to cleave earlier, or that they might have acted favourably on both the nucleus and the cytoplasm.

It is not possible to decide between these possibilities although the shape of the graphs suggests an accelerating action at an early stage when the nuclear changes were taking place. It is possible though that they only indicate a sharp rate of increase at the start which gradually falls off.

According to Ackroyd and Hopkins (1916) histidine and arginine are interconvertible and are considered as purine precursors. As new purine rings have to be manufactured for the synthesis of new nucleic acids in mitosis, it is not surprising to find an acceleration of cleavage in the presence of excess arginine and histidine.

Methionine is a known biological methylating agent and might take part in several methylating processes that would speed up the events of cell division. It might also act indirectly as an energy source by providing the methyl group for the formation of creatine which in turn can be converted to creatine phosphate, a phosphagen, known to be present in sea-urchins (Baldwin 1953).

Glycine is suspected to play an important role in the formation of the purine ring (Baldwin 1953), so it would help in the synthesis of nucleoproteins; it is also concerned with the synthesis of creatine (Baldwin 1953) and so would act indirectly as an energy source.

We have, therefore, three amino acids, arginine, glycine and methionine that contribute to the formation of creatine and that were found to accelerate cleavage.

Glutamic acid acts both as an amino group donor and as a glycogen precursor. By giving up its amino group it becomes a keto acid which ultimately forms glycogen (Baldwin 1953). This is probably used as an energy source during cleavage; the amino group liberated might unite with a keto acid to give rise to a new amino acid which will enter into the synthesis of new proteins.

Glutamine gave unexpected results. As the amide of glutamic acid it has an extra amino group and would be supposed to accelerate cleavage. However, the contrary was observed. A possible reason for this might be that as

glutamine enters the egg, it is deaminated in the presence of deaminase giving rise to glutamic acid molecules and setting free ammonia. This is likely to happen at the pH at which the experiments were run (8.1). Ammonia is a toxic substance which would be harmful to the eggs and consequently to cleavage concentration of glutamine used might also be partly responsible for the retardation of cleavage. As a new supply of glutamine molecules is entering the eggs continuously, ammonia is produced in relatively large amounts. It seems that ammonia is more readily liberated from glutamine (amide) than from glutamic acid.

Cysteine and the tripeptide glutathione can be discussed together because they both contain an -SH group and have the same effect i.e. inhibition. According to Hammett (1940) this particular group accelerates cell division. Sun (1930), on the other hand, found that the development of sea-urchin eggs was retarded by the use of H.HS. As no buffer was used he attributed this effect to the acidity caused by the production of H ions from H.HS.

Gaunt (1931), mentioned in the introduction of this paper, found no acceleration of cell division when cysteine was added to eggs of fresh water snails. It is true that in the case of the present study sea-water was used which contains active iron and heavy metals and has an alkaline reaction (8.1). These factors would tend to favour the oxidation of any sulphydryl group added to the water. It

would be instructive to make tests on cysteine and glutathione after these were added in the reduced form, to find out if the sea-water had altered them. However, as the concentrations used were quite high, some of the molecules must have reached the eggs in an unchaged (reduced) condition. As such they favour the action of succinic dehydrogenase and coenzyme A. (Baldwin 1953); the former is concerned with the glycolytic cycle and the latter with biosynthesis. Both of these processes take place during cleavage, and so -SH groups would be expected to favour cell division. Keeping in mind that cleavage needs large amounts of energy and that the concentration of sulphydryl groups around the eggs is high we can explain the inhibitory effect of cysteine and glutathione in the following way.

The -SH groups, as reducing agents in abundance, tend to reduce the enzymes which would have otherwise acted in the energy-yielding respiratory cycle of the cells. If cysteine and glutathione were oxidized in the sea-water before reaching the eggs, we can theorize that these compounds would have used up a great part of the free oxygen present in the sea-water. The eggs would not therefore be able to perform their respiratory activities properly.

Thus in both cases the dividing eggs would have to resort to their fermentative metabolism. During this process lactic acid would be produced which would make the protoplasm of the cell acidic and favour the presence of

more -SH groups. Under these conditions we would have an excess of such groups inside the eggs. Another alternative would be that the excess of sulphydryl groups might interfere with the colloidal behaviour of the structural proteins by uniting with their -SH groups.

The two concentrations of glutathione used, prove that its inhibition is quantitative i.e., the higher the concentration the more the delay in cleavage.

In concluding it can be said that the three amino acids, supposedly found in the nucleus i.e., arginine, lysine and histidine, are accelerative. Others also favour cleavage probably because they contribute to the formation of the purine rings or because they have a role in energy-yielding systems of the cells.

These findings are in favour of the original hypothesis on the effect of the nucleus in cleavage but they do not prove that nuclear constituents affect it directly. For that it is needed to test the effect of these amino acids on cytoplasmic viscosity or cortical expansion which are important factors in cleavage. Their mode of action might also be clarified if they are administered after nuclear changes have taken place.

For the amino acids that inhibited cleavage, like glutamine, cysteine and the tripeptide glutathione, it can be said that they might have interfered with the energy-yielding constituents of the cell or competed for the

oxygen of the medium.

SUMMARY

The effect of the following amino acids on cleavage was studied: lysine, arginine, histidine, methionine, glycine, glutamic acid, glutamine, cysteine and the tripeptide glutathione.

As experimental material the eggs of the sea-urchin *Paracentrotus lividus* were used.

The amino acids were added to the sea-water containing the eggs in equivalent weights of 0.273 millimoles/100 cc, ten minutes after fertilization and their effect was determined by comparing the percentage of cleaved eggs in the control and experimental groups at different intervals during the experiment.

The results indicated that lysine, arginine, histidine, methionine, glycine and glutamic acid accelerated cleavage while cysteine, glutamine and glutathione retarded it.

It was concluded that the accelerative amino acids might have taken an active part in the synthesis of new proteins and nucleic acids or acted as an indirect energy source for cleavage. On the other hand the inhibiting amino acids were suspected to have hindered some energy-yielding processes of the cell or to have altered some colloidal properties of the structural proteins.

<u>TABLES</u>

1 - 9

Giving the percentage of cleaved eggs in control and experimental groups, the actual difference between them, the standard error of their difference and the "t" values, for every amino acid.

Table 1 - Lysine

	No.of div. eggs	% of div. eggs	No. of counted eggs
Control	314	70%	448
Experimental	454	81.6%	556
Total	768	76.5%	1004
$\sigma_{D\%}$	2.6%		
Diff.between the percentages of the control & experimental	11.6%		
"t"	4.4		

Experiment I

	No.of div. eggs	% of div. eggs	No. of counted eggs
Control	440	67.1%	655
Experimental	402	82.6%	484
Total	842	73.9%	1139
$\sigma_{D\%}$	2.6%		
Diff.between the percentages of the control & experimental	15.5%		
"t"	5.9		

Experiment II

Table 2 - Arginine

	No. of div. eggs	% of div. eggs	No. of counted eggs
Control	404	73.3%	551
Experimental	520	91.5%	568
Total	924	82.5%	1119
$\sigma_{D\%}$	2.2%		
Diff. between the percentages of the control & experimental	18.2%		
"t"	8.2		

Experiment I

	No. of div. eggs	% of div. eggs	No. of counted eggs
Control	88	17.5%	501
Experimental	170	33.5%	509
Total	258	25.5%	1010
$\sigma_{D\%}$	2.7%		
Diff. between the percentages of the control & experimental	16%		
"t"	5.9		

Experiment II

Table 3 - Methionine

	No.of div. eggs	% of div. eggs	No. of counted eggs
Control	275	71.6%	384
Experimental	269	86.6%	301
Total	544	79.4%	685
$\sigma_{D\%}$	3.1%		
Diff.between the percentages of the control & experimental	15%		
"t"	4.8		

Experiment I

	No.of div. eggs	% of div. eggs	No. of counted eggs
Control	184	39.5%	465
Experimental	322	55.5%	580
Total	506	48.4%	1045
$\sigma_{D\%}$	3.1%		
Diff.between the percentages of the control & experimental	16%		
"t"	5.1		

Experiment II

Table 4 - Glycine

	No.of div. eggs	% of div. eggs	No. of counted eggs
Control	184	39.5%	465
Experimental	351	56.7%	619
Total	535	49.2%	1084
$\sigma_{D\%}$	3%		
Diff.between the percentages of the control & experimental	17.2%		
"t"	5.7		

Experiment I

	No.of div. eggs	% of div. eggs	No. of counted eggs
Control	190	40.4%	470
Experimental	251	55.1%	455
Total	441	47.6%	925
$\sigma_{D\%}$	3.2%		
Diff.between the percentages of the control & experimental	14.7%		
"t"	4.5		

Experiment II

Table 5 - Glutamic Acid

	No.of div. eggs	% of div. eggs	No. of counted eggs
Control	65	14.4%	449
Experimental	191	38.2%	499
Total	256	27. %	948
$\sigma_{D\%}$	2.8%		
Diff.between the percentages of the control & experimental	23.8%		
"t"	8.5		

Experiment I

	No.of div. eggs	% of div. eggs	No. of counted eggs
Control	169	55.8%	303
Experimental	287	77.5%	370
Total	456	67.7%	673
$\sigma_{D\%}$	3.6%		
Diff.between the percentages of the control & experimental	21.7%		
"t"	6		

Experiment II

Table 6 - Histidine

	No. of div. eggs	% of div. eggs	No. of counted eggs
Control	233	44.3%	525
Experimental	319	60%	531
Total	552	52.2%	1056
$\sigma_{D\%}$	2.9%		
Diff. between the percentages of the control & experimental	15.7%		
"t"	5.4		

Experiment I

	No. of div. eggs	% of div. eggs	No. of counted eggs
Control	286	54.3%	526
Experimental	393	67.6%	581
Total	679	61.3%	1107
$\sigma_{D\%}$	2.9%		
Diff. between the percentages of the control & experimental	13.3%		
"t"	4.5		

Experiment II

Table 6 - Cont'd

	No.of div. eggs	% of div. eggs	No. of counted eggs
Control	229	53.8%	425
Experimental	252	69.4%	363
Total	481	61%	788
$\sigma_{D\%}$	3.3%		
Diff.between the percentages of the control & experimental	15.6%		
"t"	4.7		

Experiment III

Table 7 - Glutamine

	No.of div. eggs	% of div. eggs	No. of counted eggs
Control	441	85.1%	518
Experimental	330	64.8%	509
Total	771	75%	1027
$\sigma_{D\%}$	2.5%		
Diff.between the percentages of the control & experimental	50%		
"t"	20		

Experiment I

	No.of div. eggs	% of div. eggs	No. of counted eggs
Control	323	96.1%	336
Experimental	206	48.8%	422
Total	529	69.7%	758
$\sigma_{D\%}$	1.3%		
Diff.between the percentages of the control & experimental	47.3%		
"t"	36.3		

Experiment II

Table 8 - Cysteine

	No.of div. eggs	% of div. eggs	No. of counted eggs
Control	286	54.3%	586
Experimental	98	26%	377
Total	384	42.5%	903
$\sigma_{D\%}$	3.3%		
Diff.between the percentages of the control & experimental	28.3%		
"t"	8.5		

Experiment I

	No.of div. eggs	% of div. eggs	No. of counted eggs
Control	323	96.1%	336
Experimental	77	29.2%	263
Total	400	66.7%	599
$\sigma_{D\%}$	3.9%		
Diff.between the percentages of the control & experimental	66.9%		
"t"	17.1		

Experiment II

Table 9 - Glutathione

	No.of div. eggs	% of div. eggs	No. of counted eggs
Control	345	96.1%	359
Experimental	323	91.2%	354
Total	668	93.6%	713
$\sigma_{D\%}$	1.8%		
Diff.between the percentages of the control & experimental	4.9%		
"t"	2.7		

Experiment I: 84 mg/100 cc.

	No.of div. eggs	% of div. eggs	No. of counted eggs
Control	345	96.1%	359
Experimental	164	40.2%	407
Total	509	66.4%	766
$\sigma_{D\%}$	3.3%		
Diff.between the percentages of the control & experimental	55.9%		
"t"	16.9		

Experiment II: 168 mg/100 cc.

GRAPHS

1 - 8

Showing the percentage of cleaved eggs in control and experimental groups at different time intervals.

Black curve = control

Red curve = experimental.

Graph 1 : Methionine (Exp.I)

Graph 2 : Glutamic Acid (Exp.I)

Graph 3 : Histidine (Exp.II)

% of div. eggs.

100
90
80
70
60
50
40
30
20
10

Time after fertil. in min.

70 72 74

Graph 4 : Glutamine (Exp.I)

% of div. eggs.

100
90
80
70
60
50
40
30
20
10

Time after fertil. in min.

60 62 64 66 68

Graph 5 : Glutamine (Exp.II)

% of div. eggs.

100
90
80
70
60
50
40
30
20
10

60 62 64 66 68

Time after fertil. in min.

Graph 6 : Cysteine (Exp.I)

% of div. eggs.

100
90
80
70
60
50
40
30
20
10

70 72 74

Time after fertil. in min.

Graph 7 : Cysteine (Exp.II)

% of div. eggs.

100 90 80 70 60 50 40 30 20 10

Time after fertil. in min.

60 62 64 66 68

Graph 8 : Glutathione.

% of div. eggs.

100 90 80 70 60 50 40 30 20 10

84 mg/100 cc

168 mg/100 cc.

Time after fertil. in min.

56 57 58 59 60 61 62

FIGURES

1 - 9

Showing average cleavage counts for control and experimental groups at a certain time. More than one experiment is illustrated for each amino acid.

Control = black

Experimental = red

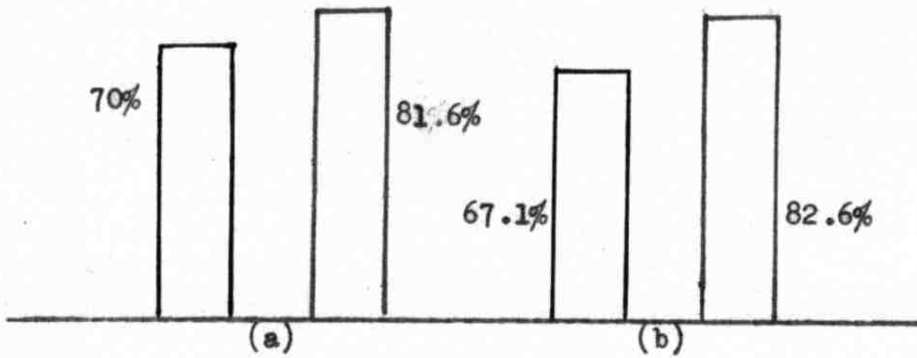


Fig. 1 - **Lysine**

(a) Experiment I, 67 min. after fertilization.
(b) Experiment II, 65 min. after fertilization.

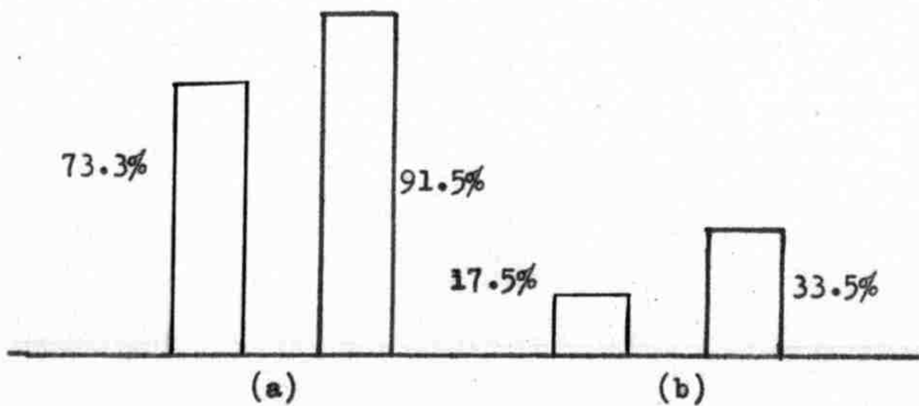


Fig. 2 - **Arginine**.

(a) Experiment I, 75 min. after fertilization.
(b) Experiment II, 73 min. after fertilization.

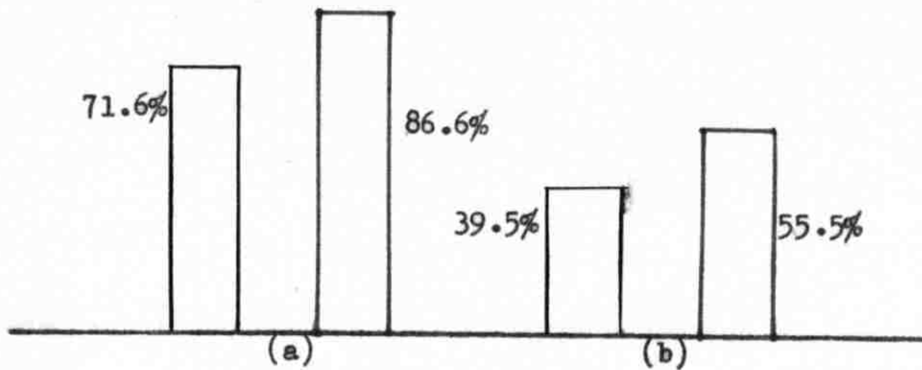


Fig. 3 - **Methionine.**

(a) Experiment I, 69 min. after fertilization.

(b) Experiment II, 65 min. after fertilization.

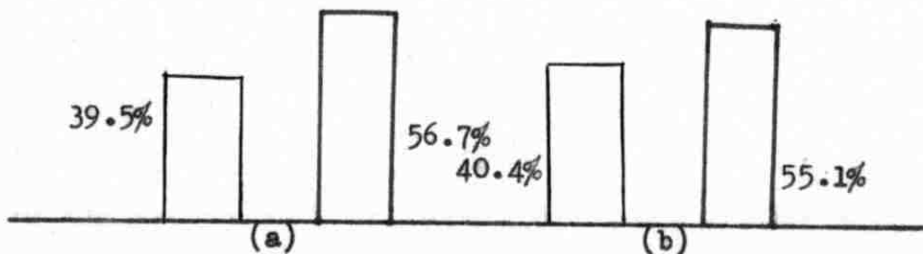


Fig. 4 - **Glycine.**

(a) Experiment I, 65 min. after fertilization.

(b) Experiment II, 62 min. after fertilization.

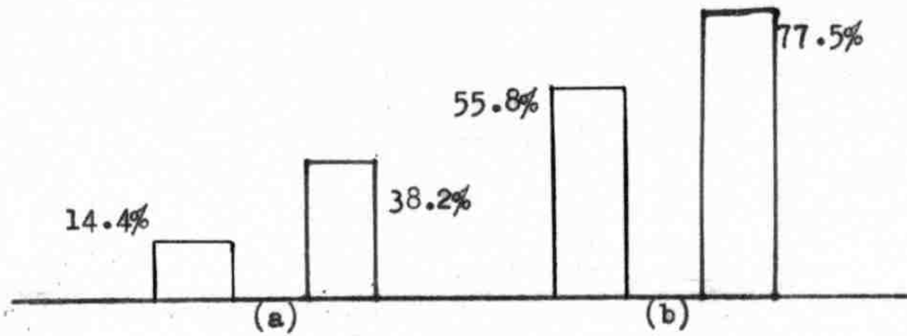


Fig. 5 - **Glutamic Acid.**

(a) Experiment I, 87 min. after fertilization.
(b) Experiment II, 70 min. after fertilization.

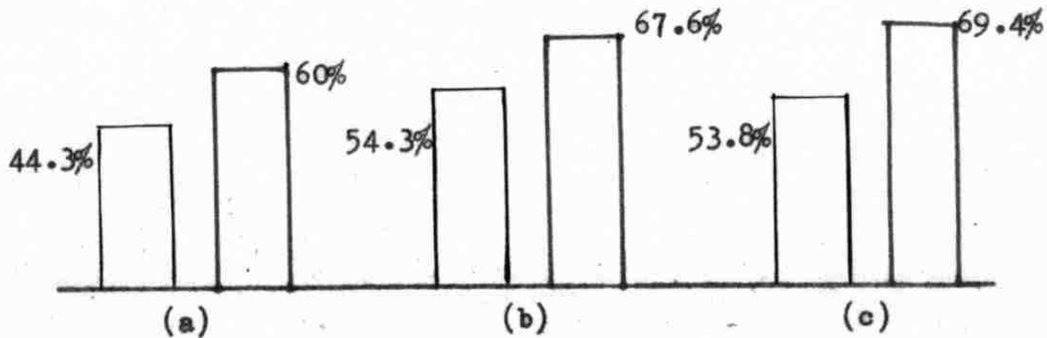


Fig. 6 - **Histidine.**

(a) Experiment I, 70 min. after fertilization.
(b) Experiment II, 70 min. after fertilization.
(c) Experiment III, 67 min. after fertilization.

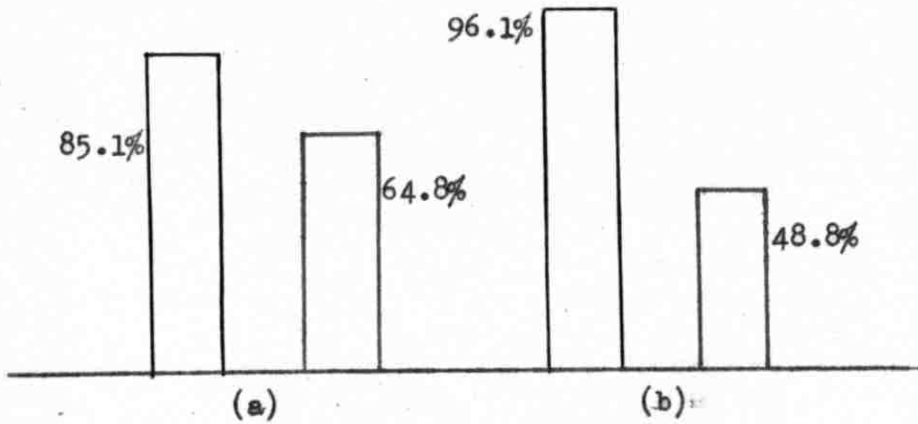


Fig. 7 - **Glutamine.**

(a) Experiment I, 65 min. after fertilization.
(b) Experiment II, 63 min. after fertilization.

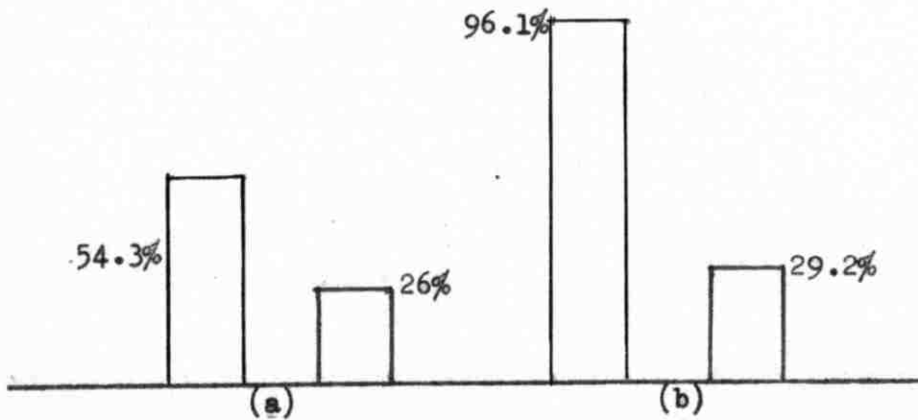


Fig. 8 - **Cysteine.**

(a) Experiment I, 70 min. after fertilization.
(b) Experiment II, 63 min. after fertilization.

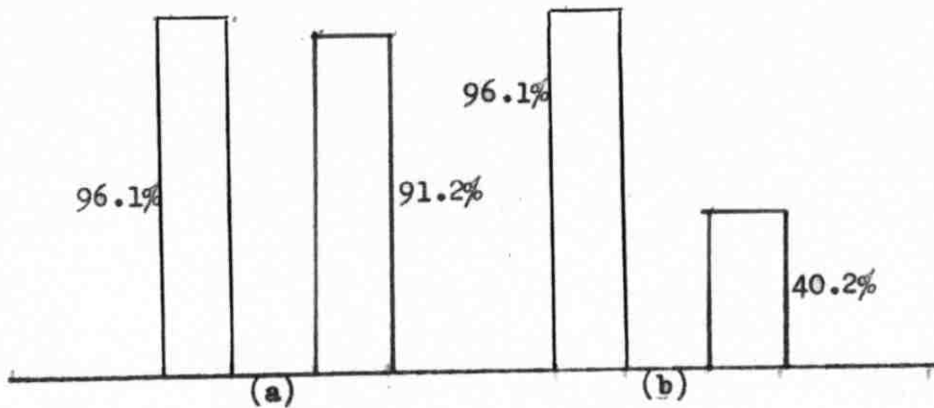


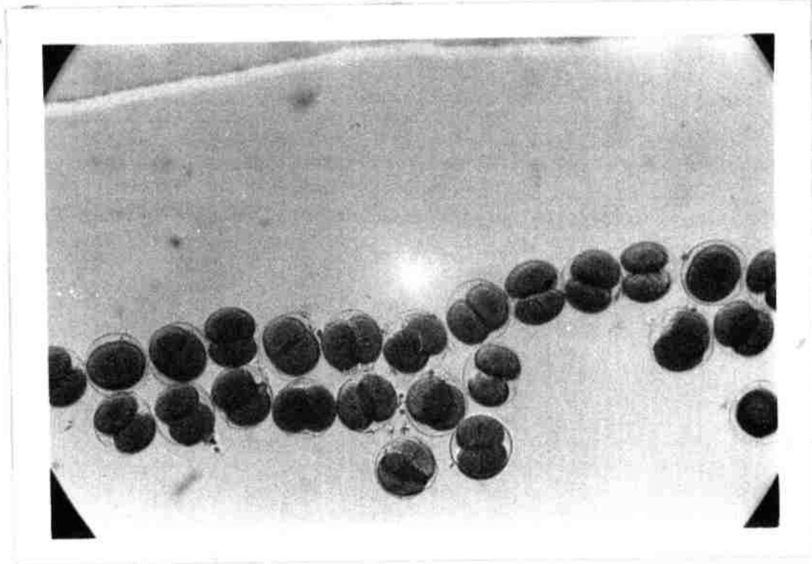
Fig. 9 - **Glutathione.**

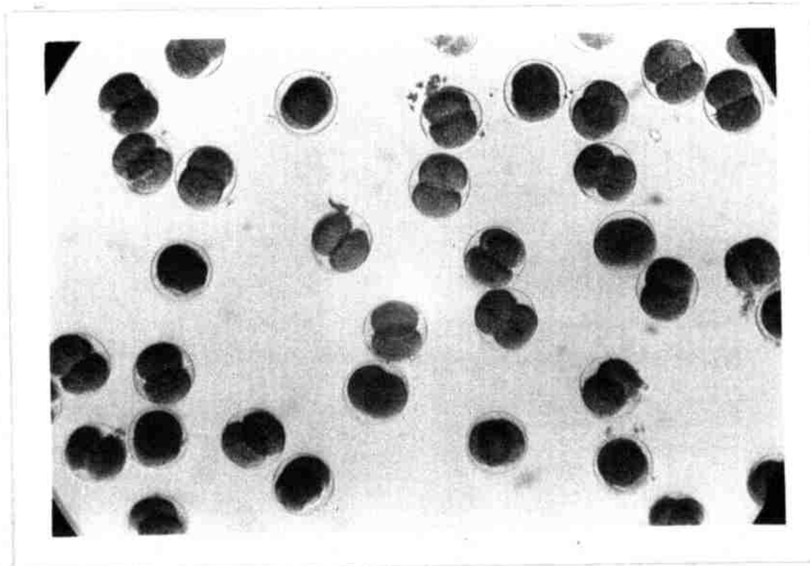
(a) 84 mg./100 cc. , 61 min. after fertilizatinn.

(b) 168 mg./100 cc. , 61 min. after fertilization.

PLATE I

Microphotographs of cleaved *Paracentrotus* eggs in glutathione solution at the end of the experimental period.





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APPENDIX

Although sea-urchins are abundant along the Beirut coast very little work has been done on their taxonomy. Only general studies on animals of Syria, Lebanon and Palestine are found. These include Bodenheimer[1] and Gruvel[2]. The lack of information on the subject, made it necessary to undertake the identification of the urchins of Beirut. A study of a large number collected from the American University Swimming Grounds and from other areas around Beirut showed that we have both *Paracentrotus* and *Arbacia*. *Paracentrotus* is usually found in crevices in the rocks of the littoral zone below the low tide mark, *Arbacia* lives in deeper places.

Characteristics of *Arbacia*.

The body of the animal is flattened dorsoventrally; its test is conical and solid. It has a purplish black dye which comes out on rubbing.

Although the test in both *Arbacia* and *Paracentrotus* has almost the same diameter (4.5 - 5 cm), the former looks

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1. Bodenheimer, F.S., *Animal life in Palestine.* Tel-Aviv, Printing "Sefer". (1935).

2. Gruvel, A., *Les États de Syrie*. Paris, Société d'Éditions Géographiques, Maritimes et Coloniales. (1931)

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larger because its spines are longer i.e. 2 - 2.5 cm (the spines of <u>Paracentrotus</u> being only 1 - 1.5 cm long). <u>Arbacia</u> spines are balck (Plate II) and the tip of the primaries is covered by a hard calcareous cap. Moreover, they converge and cannot aid in locomotion.

The ectoderm is devoid of ciliation and the fecal matter accumulates on the aboral surface. When the animal is kept in a tank this might be the cause of its death, but in its natural habitat the water washes off the dirt and cleanses the animal.

The oral tube-feet have suckers at their tip but the aboral do not.

The peristome contains ten prominent plates perforated by the buccal tube-feet. External gills are present at the edge of the peristome and the gill clefts or the callosities in relation to the gills are deep and expanded.

The periproct is covered by four valve-like plates.

The ambulacral and interambulacral plates of the corona are held together by means of small tooth-like projections which fit into holes at the place of the sutures. They bear tubercles which are not perforated. Moreover, the ambulacral pore-plates near the peristome are united to form the "Arbacioid" pattern. There is one sphaeridium (sense organ) per ambulacral area, found at the edge of the peristome in a small depression of the test.

The auricles form incomplete arches springing from the ambulacral plates around the edge of the peristome. The epiphyses are only projections on the upper side of the alveoli and do not form an arch.

The ovaries and eggs of a mature *Arbacia* contain a red pigment called echinochrome, (Plate II).

The above characteristics and especially the arrangement of the pore-plates are evidence that the "black" urchin found in the Beirut sea-shore belongs to the family Arbaciidae and to the genus *Arbacia*. Determination of the species awaits literature not available in the library of the University at present.

Characteristics of *Paracentrotus lividus*.

Besides the "black" urchin there is also found a "colored" one which was identified as *Paracentrotus lividus* (Plate II). It is the sea-urchin used in the present study and its characteristics are the following:

The test is not as hard as that of *Arbacia* and the epidermis is ciliated (fecal matter does not accumulate on the aboral surface). The color is usually violet and sometimes brown or green.

The peristome contains ten buccal tube-feet while the periproct contains numerous anal plates.

The four kinds of pedicellariae i.e. tridactyl, gemmiform, trifoliate and ophicephalous are present on the corona and all the tube-feet have suckers. External gills

are present but the callosities found under them on the test are shallow. The plates of the corona meet each other in straight simple sutures and do not bear perforated tubercles. They contain 5 - 6 pairs of pores.

The spines are naked, not having a cap at their tip. The auricles form complete arches arising from the ambulacral regions. The epiphyses also meet and form an arch.

The gonads of both males and females are colored orange by carotenoid pigments and are appreciated as food by the people in Beirut.

Upon fertilization *Paracentrotus* eggs get their fertilization membrane higher and faster than *Arbacia* eggs. On centrifugation, 1 - 2 min. in 0.73M sucrose at 400 x g *Arbacia* eggs stratify showing the pigment granules at one pole, a clear zone in the middle and the nucleus with fat and oil droplets on the other pole. For *Paracentrotus* eggs even five minutes of centrifugation at the same speed as above are not sufficient for stratification.

PLATE II

Colored photographs of *Arbacia* (black) and *Paracentrotus lividus* (green).

Fig. 1 The red eggs are seen to be shed by one of the *Arbacia*.

Fig. 2 The specimens are cut open to show the female (red) and the male (white) reproductive organs.

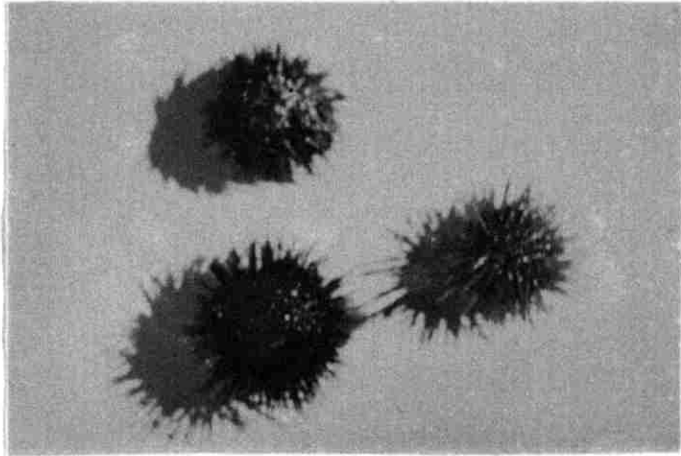

Fig. 1

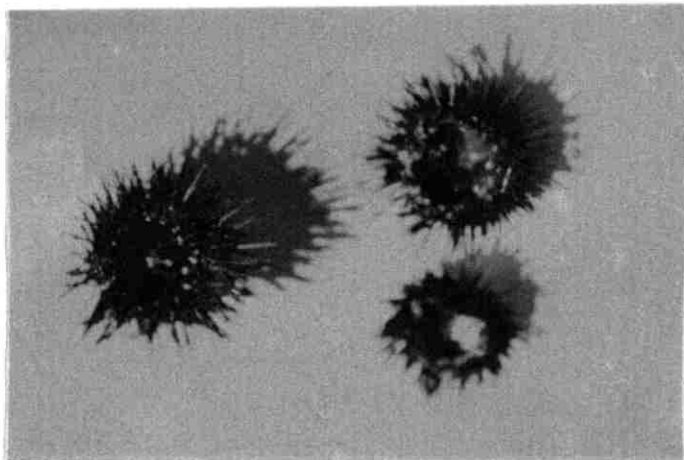

Fig. 2

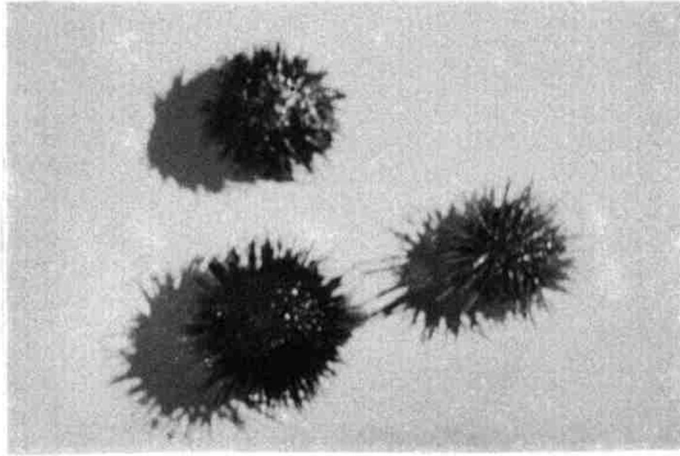

Fig. 1

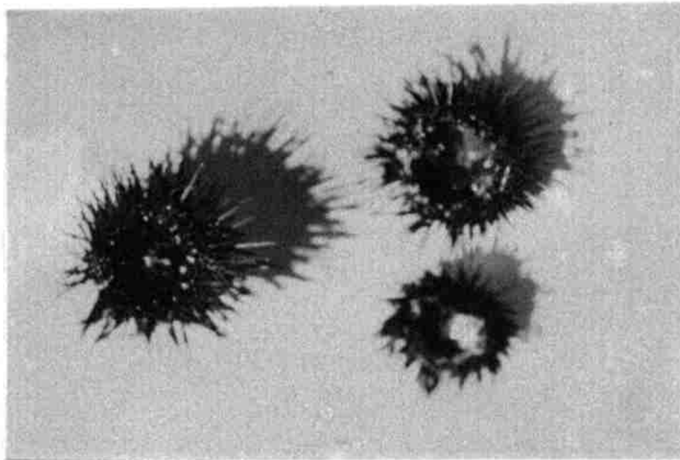

Fig. 2