

THE UPTAKE OF VALINE AND CYTIDINE BY SEA-URCHIN EMBRYOS AND ITS RELATION TO THE CELL SURFACE

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SUMMARY

The rate of uptake of [¹⁴C]valine and [³H]cytidine into the metabolic pool of early embryos of the sea urchin *Paracentrotus lividus* was measured by giving 5-min pulses of these precursors. The uptake rate rose sharply after fertilization, reached a maximum before first cleavage and then stayed constant until at least the fifth interphase. There were no changes in uptake rate at cell division. A longer-term experiment with valine showed that the uptake rate remained approximately constant until the pluteus stage, and was not altered for the first 10 h by an actinomycin D. Continuous labelling experiments indicated that the uptake rate may be controlled by the level of precursor in the pool. The following experiments indicated that the uptake was by specific transport mechanisms. Both precursors showed an uptake inhibited by competitors, an apparent concentration within the cells, and no back-exchange. In addition, the uptake of valine was inhibited by dinitrophenol and azide, and showed a concentration response with Michaelis-Menten kinetics. Valine uptake was not inhibited by puromycin added at fertilization, though it was somewhat affected if the eggs were pretreated.

It is suggested that the transport mechanisms are mediated by specific carrier molecules at the cell surface. These mechanisms are activated at fertilization through links with metabolism. There is no change in the number of carriers per embryo throughout early development. Since the number of cells and the total cell surface are increasing, this implies that the number of carriers per cell or per unit of surface is decreasing. There is a possible reason for this differentiation of the cell surface.

INTRODUCTION

Sea-urchin embryos will take up amino acids and nucleosides from the surrounding sea water (Hultin, 1953; Giudice, Vittorelli & Monroy, 1962; Nemer, 1962). If labelled precursors are used for a short time, some of the label is incorporated into proteins and nucleic acids, but most of it is present within the embryos in acid-soluble form in the metabolic pool of low molecular weight compounds. In addition, the first few divisions after fertilization are highly synchronous. These embryos, therefore, make an excellent system for investigating how the rate of entry of materials into the cell varies during the cell cycle and between successive cell cycles. There are several pertinent questions that can be asked. One is whether the permeability alters at the time of division when there are considerable mechanical and optical changes at the surface. Another is whether there is a change in the rate of entry after a division,

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since at each division the total cell surface increases by about one-third. We have attempted to answer these questions by measuring the rate of uptake of short and closely spaced pulses of labelled valine and cytidine during the early stages of development. We have also investigated the mechanism of uptake of these precursors.

MATERIALS AND METHODS

The experiments were done at Palermo at room temperature (18–20 °C) with embryos of *Paracentrotus lividus*. The labelled precursors (Radiochemical Centre, Amersham) were DL-valine-1-¹⁴C (specific activity 4.76 mc/millimole) and cytidine-5-³H (specific activity 15 c/millimole). With valine, the standard procedure was to make a suspension of washed fertilized eggs at a concentration of about 2000 eggs/ml. At intervals, 1 ml of the suspension was added to 0.2 ml of [¹⁴C]valine in sea water to give a final valine concentration of 16.7 µg/ml. At the end of the labelling period (usually 5 min) excess non-radioactive valine was added (1 ml of 1 mg/ml) and the resulting suspension filtered at once through an Oxoid membrane filter (2 cm diameter) and washed three times with sea water. For measurements of incorporation as opposed to total uptake, the sea-water washes were preceded by three washes with 5% trichloroacetic acid. The filter was then dried, immersed in toluene-based scintillation fluid and counted in an EKCO scintillation counter. The procedure with cytidine was similar except that the egg jelly was removed before fertilization by treatment with 2×10^{-3} N HCl for 5–10 min in order to get more eggs on the filter. The suspension was made up at about 8000 eggs/ml, the concentration of the [³H]cytidine was 0.1 µg/ml and the concentration of the non-radioactive cytidine was 100 µg/ml. Membrane filters are a quick and convenient way of collecting a limited number of embryos for radioactive counting. The embryos survive washing on a filter and the pool remains intact provided the fertilization membranes are not removed. This method also provides an easy way of determining the concentration of embryos since they can be seen and counted on the dry filters under a dissecting microscope.

RESULTS

Changes in uptake rate during early development

The way in which the rate of uptake changed during early development was followed by taking aliquots of a suspension of fertilized eggs and measuring the amount of labelled precursor which was taken up in a 5-min period. The aliquots were taken before fertilization, at 15 min intervals until 65 min after fertilization, then at 10 min intervals until 165 min after fertilization, and finally at 265 min after fertilization when the embryos had completed five cleavages. The collected results from four experiments with valine (each with eggs from a different female) are shown in Fig. 1. There is a very low rate of uptake in the unfertilized egg. The rate then rises after fertilization and reaches a maximum value at about the time of first cleavage. Thereafter there are no significant changes. There are small fluctuations in the mean but they are less than the standard errors and they show no obvious correlation with the

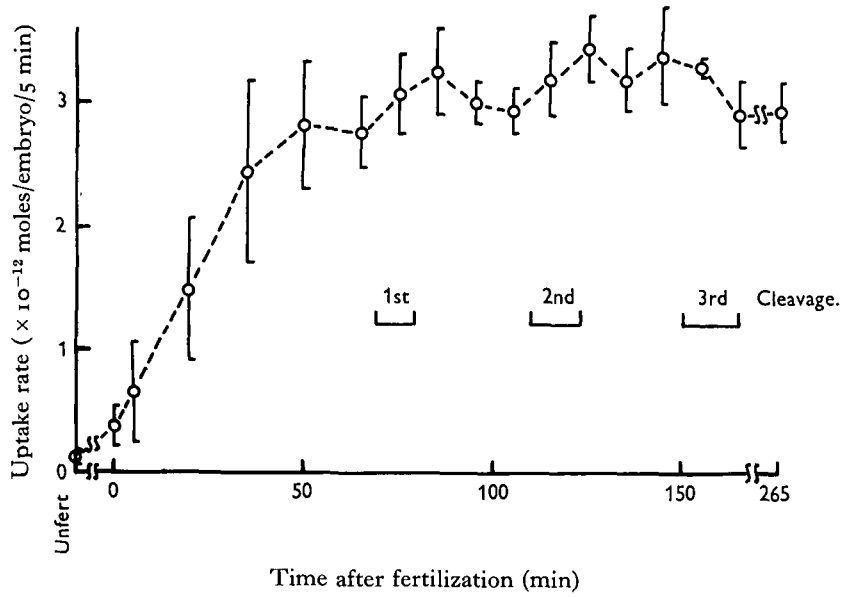


Fig. 1. Rate of total uptake of valine during the first 4½ h after fertilization. Aliquots of embryos taken at times indicated, treated with [¹⁴C]valine (16.7 µg/ml) for 5 min, filtered off, washed and counted. Means and standard errors from four experiments. The four experiments were normalized by equalizing the sum of all values beyond 65 min for each experiment.

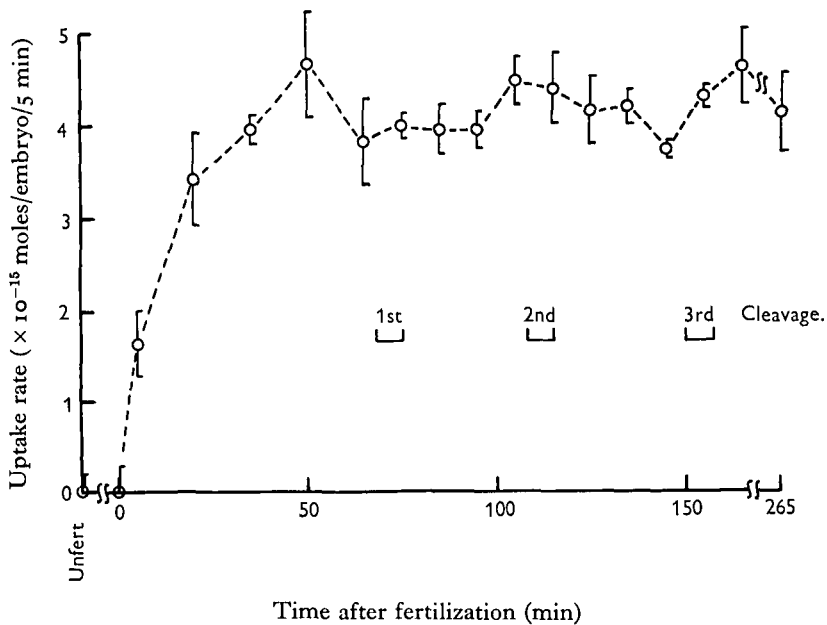


Fig. 2. Rate of total uptake of cytidine during the first 4½ h after fertilization. Aliquots of embryos taken at times indicated, treated with [³H]cytidine (0.1 µg/ml) for 5 min, filtered off, washed and counted. Means and standard errors from five experiments. The five experiments were normalized by equalizing the sum of all values beyond 65 min for each experiment.

cleavages. The results from five experiments with cytidine are shown in Fig. 2. The pattern is very similar to that of valine except that the maximum value is reached a little earlier. In Figs. 1 and 2, the uptake rate has been given in absolute terms. These values are only approximate since the curves have been normalized and there was a variation (range of $\times 1.6$) between the uptake rates of different batches of eggs.

At the end of these 5-min labelling periods, nearly all the labelled material in the cells was in the acid-soluble pool. With eggs at the time of first cleavage, less than 5% of the total radioactivity after five minutes was incorporated in acid-insoluble form.

The relative constancy in the rate of uptake of valine continues for much longer than $4\frac{1}{2}$ h. Fig. 3 shows the results from an experiment in which measurements were made for $1\frac{1}{2}$ days after fertilization. There were no large changes in uptake rate even

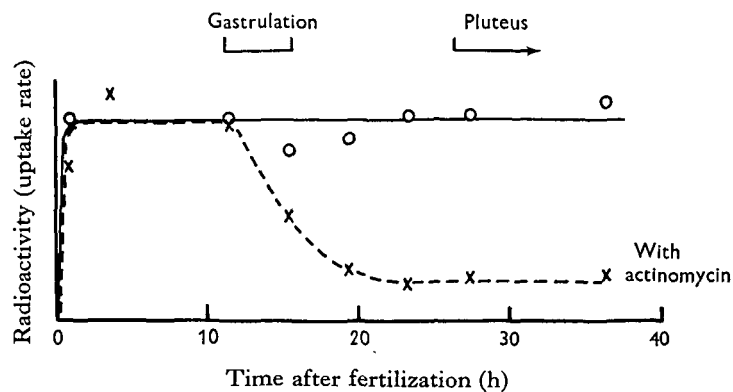


Fig. 3. Rate of total uptake of valine during the first 36 h after fertilization. Same procedure as in Fig. 1. The continuous line is for embryos in sea water. The dashed line is for embryos in actinomycin D ($25 \mu\text{g/ml}$) in sea water from 30 min before fertilization. In both cases, bactericidal agents were added (penicillin, $23 \mu\text{g/ml}$; streptomycin sulphate, $33 \mu\text{g/ml}$; sulphadiazine, saturated).

when the embryos had reached the pluteus stage. Fig. 3 also shows that treatment with actinomycin D did not affect the rate of uptake until the time of gastrulation. The treated eggs then fail to gastrulate (Gross & Cousineau, 1964) and the uptake rate falls. Substantially similar results were obtained on leucine uptake with *Arbacia punctulata* (Mitchison, unpublished).

Absence of exchange between precursors inside and outside embryos

Eggs at an hour after fertilization were labelled for 5 min with valine or cytidine and then divided into two aliquots. The first of them was treated by the standard procedure given in the Methods, while the second was left in the presence of the excess non-radioactive precursor (for 10 min with valine and 60 min with cytidine) before filtering and washing. No significant difference was found between the counts in the two aliquots. This shows that there was no exchange between precursor which had been taken up by the eggs and precursor in the sea water. If there had been exchange, the radioactivity of the second aliquot would have been lower. This result

is important both to validate the use of excess non-radioactive precursor in the standard procedure and also because of its relevance to the next experiment.

Continuous labelling for an hour

Eggs at 45 min after fertilization were left in labelled precursor for an hour with aliquots being taken at intervals through the hour in order to follow the time course of uptake (Fig. 4). The rate of uptake decreased throughout the period with both

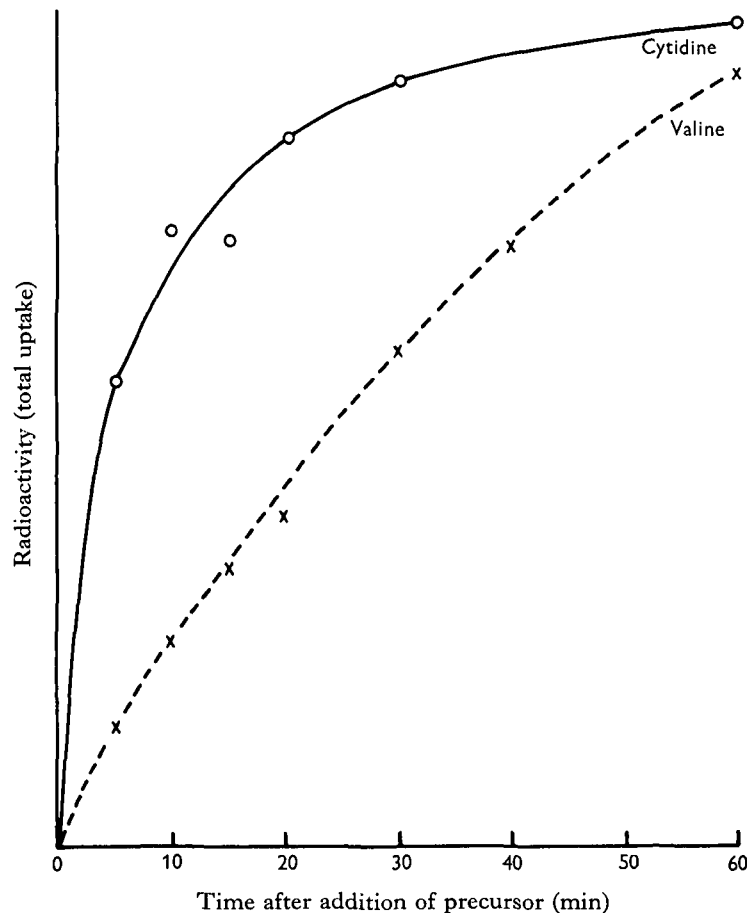


Fig. 4. Continuous labelling with valine and cytidine. Precursors ($[^{14}\text{C}]$ valine, $16.7 \mu\text{g/ml}$; $[^3\text{H}]$ cytidine, $0.1 \mu\text{g/ml}$) added to eggs at 45 min after fertilization. Aliquots taken at times indicated, filtered off, washed and counted. Continuous line for cytidine, dashed line for valine.

precursors, but with cytidine the decrease was much more marked. The final concentration of precursor in the sea water was at least 85% of the initial concentration.

One explanation of uptake curves of this shape is that they are caused by back-exchange across the cell membrane which increases as the pool fills. This, however, is ruled out by the previous experiment. The alternative and most likely explanation

is that the rate of uptake is controlled by the level of precursor in the pool. As the pool fills, it progressively inhibits the uptake mechanism until the rate of uptake equals the rate of incorporation of the precursors into macromolecules. We have used a similar model to explain the uptake of adenine in a fission yeast (Cummins & Mitchison, in preparation).

One reason for this experiment is that it determines the maximum permissible labelling period that can be used as a measure of initial uptake rate. It appears that the cytidine pool is nearly full after 30 min. If, therefore, the embryos had been labelled for 30 min rather than 5 min, the parameter that would have been measured would have been the size of the filled pool rather than the initial uptake rate. As it is, it appears that a 5-min period gives a good measure of the initial rate with valine, and a reasonable one with cytidine.

Concentration of precursors within the embryos

Here, and for most of the remaining experiments, we are concerned with arguments in favour of the uptake of the precursors being mediated by specific transport mechanisms.

One of the characteristics of an active transport mechanism is that it should be capable of moving a substance up a concentration gradient. It can be calculated from the results in Fig. 1 that the apparent concentration of valine in an egg at first cleavage is at least $5.7 \times 10^{-3} \text{M}$ after 5 min, whereas the concentration in the sea water is $1.4 \times 10^{-4} \text{M}$, that is, 40 times less. For cytidine, the apparent concentration in an egg is at least $8 \times 10^{-6} \text{M}$, and in the sea water is $4.1 \times 10^{-7} \text{M}$, that is, 20 times less. With longer labelling periods, the concentration differences would be even greater. Nemer (1962) found similar concentration differences with cytidine and suggested that there might be an active transport mechanism. The qualification which should be made is that the concentration in the egg is only an apparent one based on radioactivity measurements and may not be a real one if the precursor is quickly converted into another compound when it enters the eggs. An analysis of the flow of radioactivity through the components of the pool would be needed to settle this point.

Effect of competitors

Another mark of a specific transport mechanism which distinguishes it from simple diffusion is that the entry of a compound is reduced in the presence of other similar compounds. Tables 1 and 2 show the effects of various competitors on the uptake rates of the precursors. Valine uptake is reduced sharply in the presence of similar amino acids such as leucine and alanine, whereas lysine, a basic amino acid, has much less effect. In the same way, other nucleosides are good competitors for cytidine while the bases are much less efficient.

Concentration response of valine uptake

In a number of specific transport mechanisms, the relation between initial uptake rate and concentration is very similar to that in an enzyme reaction with various substrate concentrations (Michaelis–Menten kinetics). This relationship is linear on

Table 1. Uptake of [¹⁴C]valine in the presence of competitors

Aliquots of embryos 60 min after fertilization labelled for 5 min with [¹⁴C]valine (16.7 μ g/ml) in the presence of competitors at concentrations of 0.1 and 1.0 mg/ml. Results given as amount of radioactivity taken up in presence of competitor as a percentage of that taken up in controls without any competitor.

Competitor	0.1 mg/ml (%)	1.0 mg/ml (%)
L-Leucine	18	13
DL-Isoleucine	25	13
DL-Alanine	27	17
DL-Phenylalanine	30	17
DL-Threonine	48	24
DL-Lysine	87	64

Table 2. Uptake of [³H]cytidine in the presence of competitors

Aliquots of embryos 60 min after fertilization labelled for 5 min with [³H]cytidine (0.1 μ g/ml) in the presence of competitors at concentrations of 0.1 and 1.0 mg/ml. Results given as amount of radioactivity taken up in presence of competitor as a percentage of that taken up in controls without any competitor.

Competitor	0.1 mg/ml (%)	1.0 mg/ml (%)
Uridine	17	16
Thymidine	22	20
Cytosine	59	—
Uracil	91	91

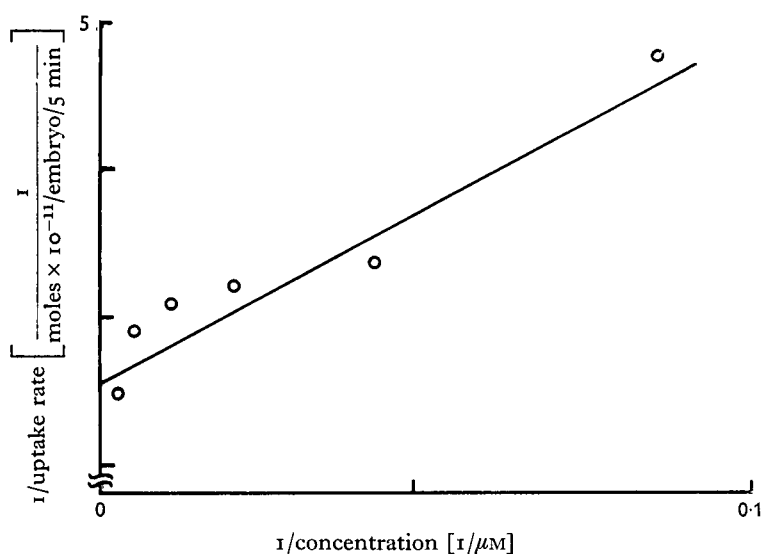


Fig. 5. Rate of uptake of valine at different concentrations. [¹⁴C]valine, at indicated concentrations, added for 5 min to aliquots of eggs at 65 min after fertilization. Aliquots were then filtered, washed and counted. Each point is the mean of two or four measurements.

a reciprocal plot. The concentration response of valine was therefore investigated by measuring the uptake in 5 min with various concentrations of valine. The results are given in Fig. 5 on a reciprocal plot and show an approximately linear relation. From this plot, two constants can be found: $K_m = 9.0 \mu\text{M}$; and V_{max} (max. uptake rate) = 3.9×10^{-12} moles/embryo/5 min.

Effects of inhibitors

An active transport mechanism which is coupled to metabolism should be sensitive to metabolic inhibitors. Table 3 shows the effect of two metabolic inhibitors, 2,4-dinitrophenol and sodium azide, on valine uptake. When added 5 min after fertilization (Table 3A), $2.5 \times 10^{-4}\text{M}$ dinitrophenol cut the uptake rate to a quarter, and $5 \times 10^{-3}\text{M}$ sodium azide completely inhibited it. The inhibitors were somewhat less effective when added an hour after fertilization (Table 3B), but they were also less efficient as inhibitors of cleavage. For example, 10^{-3}M sodium azide blocked development at the streak stage before first cleavage when added 5 min after fertilization,

Table 3. Uptake of [^{14}C]valine in the presence of inhibitors

Aliquots of embryos labelled for 5 min with [^{14}C]valine ($16.7 \mu\text{g/ml}$) at various times after fertilization in the presence of 2,4-dinitrophenol, sodium azide or puromycin. Results given as amount of radioactivity taken up in the presence of inhibitor as a percentage of that taken up in controls without inhibitor.

A. Inhibitors added 5 min after fertilization

Time of labelling period in min after fertilization	Dinitrophenol			Sodium azide		Puromycin	
	10^{-5}M	$5 \times 10^{-5}\text{M}$	$2.5 \times 10^{-4}\text{M}$	10^{-3}M	$5 \times 10^{-3}\text{M}$	10^{-4}M	$4 \times 10^{-3}\text{M}$
20-25	87	65	22	64	0	115	65
40-45	92	51	28	20	0	102	96
60-65	79	43	16	29	0	101	87

B. Inhibitors added 60 min after fertilization

Time of labelling period in min after fertilization	Dinitrophenol		Sodium azide	
	$5 \times 10^{-5}\text{M}$	$2.5 \times 10^{-4}\text{M}$	10^{-3}M	$5 \times 10^{-3}\text{M}$
120-125	45	41	79	39
180-185	37	33	64	24

C. 10^{-4}M Puromycin added 30 min before fertilization

Time of labelling period in min after fertilization

20-25	49
40-45	64
60-65	66

whereas it allowed at least three divisions to go through when added an hour after fertilization.

Table 3 also shows the effect of puromycin, an inhibitor of protein synthesis. This was used in order to test whether the rise in uptake rate after fertilization was dependent on protein synthesis. If the puromycin was added shortly after fertilization, it had no significant effect on the uptake rate (Table 3A). If, however, the eggs were treated with puromycin for 30 min before fertilization, the uptake rate was cut to about two-thirds of the control, although there was still a sharp rise after fertilization (Table 3C). There is therefore some inhibition, though it is less than the inhibition of protein synthesis. Hultin (1961) found that this concentration of puromycin with the same sea-urchin species cuts down the incorporation of valine to 30% of the controls. It is also possible that puromycin may have some effect on metabolism in addition to its direct effect on protein synthesis.

DISCUSSION

Two main types of mechanism have been suggested for the passage of small molecules through the cell membrane (there are recent reviews by Cirillo (1961), and by Wilbrandt (1963)). One is simple diffusion whereby the molecules move through the membrane with the driving 'force' being the concentration difference between the inside and outside of the cell. The other is a more complicated and less clearly defined specific transport mechanism. It is often believed that this mechanism involves a carrier at the cell surface which has a specific affinity for the transported molecule and which moves it across the membrane. Because of this specific affinity and because of the similarity between transport kinetics and enzyme kinetics, the carrier may well be a protein. In many cases, there is a link between transport and metabolism, and the concentration of the transported molecules can be greater within the cell than outside. This kind of specific transport mechanism is present in a wide variety of cells, and we believe that our results indicate that it also occurs in sea-urchin eggs for the uptake of amino acids and nucleosides.

One of the strongest pieces of evidence for a specific transport mechanism comes from the competition experiments. With such a mechanism, one would expect the uptake of a molecule to be inhibited by the presence of similar molecules and to be affected but little by the presence of dissimilar molecules. This happens both with valine and cytidine uptake.

Two other results argue in favour of specific transport and against simple diffusion: the absence of back-exchange and the high apparent concentration within the eggs. As we have pointed out earlier, however, this evidence is not very strong without proof that the precursors are unchanged inside the eggs.

With valine, we have demonstrated two additional characteristics of specific transport. One is the link with metabolism, since uptake is reduced by metabolic inhibitors. The other is that the concentration response follows Michaelis-Menten kinetics.

Assuming then that there are specific transport mechanisms with membrane carriers for the precursors, what deductions can be made about the changes in uptake rate during early development? Before going on to this, we should consider the factors

that can affect uptake rate with this kind of mechanism. First, there is the total number of carrier molecules available at the surface of the cells of the embryo. Secondly, there is the general level of metabolism. Thirdly, there is the amount of precursors present in the pool. The results shown in Fig. 4 suggest that the uptake rate with a full pool is lower than with an empty pool. Fourthly, there is the affinity of the carrier. It is conceivable that this might change (and it would be shown by a change in the K_m) but it is not very likely. We shall, therefore, ignore this possibility in the following argument, and we shall also ignore the possibility of other fundamental changes in the nature of the uptake mechanism.

There are two main phases in the curves of uptake rate during development (Figs. 1-3). The first phase lasts from fertilization until a little before first cleavage, during which time there is a large rise in the uptake rate. It is conceivable that this might be due to a sharp fall in the level of the pool. Kavanau (1954) has shown that there is a fall of 40% in the amount of valine in the pool between unfertilized eggs and the two-cell stage. His absolute values, however, indicate that this fall cannot be responsible for the rise in uptake rate. He found pool valine in an unfertilized egg to be 7.8×10^{-12} mole, and 40% of this is 3.1×10^{-12} mole. This latter figure is the same as that taken up in 5 min by embryos at first cleavage and thereafter (Fig. 1). Fig. 4 shows that the change in the valine uptake rate in 5 min is small, and certainly very much less than the change after fertilization. We have not been able to find equivalent data about the cytidine pool, but it is worth pointing out that Sugino, Sugino, Okazaki & Okazaki (1960) found no significant change in the total acid-soluble deoxyribonucleosides between the unfertilized egg and the 16-cell stage, and a fall thereafter. Another possibility is that this rise is caused by a burst of synthesis of carriers. If, as is likely, these carriers are proteins, the synthesis should be stopped by puromycin. But puromycin, applied at fertilization, does not affect the uptake rate of valine, and even if it is applied before fertilization it inhibits uptake less than it inhibits protein synthesis, and it does not prevent the rise taking place. It seems, therefore, that the most likely explanation of the rise is that it is due to the large increase in the general level of metabolism which follows fertilization (Ballentine, 1940).

The second phase of the uptake-rate curves starts at about the time of first cleavage. From this time on, the rate of uptake stays substantially constant until at least the fifth interphase. The changes thereafter have only been followed with valine (Fig. 3) and not in great detail, but here also there are no large changes in uptake rate for 36 h. In some ways, this is a surprising result. In a growing system, for example a culture of micro-organisms, the uptake rate would increase in proportion to the growth. Although an embryo is not a growing system in the sense that there is no increase of total cytoplasmic volume, it is a dividing one and there is an increase in the total cell surface at each division—the extent of which will be discussed later. In parentheses, the constancy in the initial uptake rate with increasing surface can be used as a further argument against a simple diffusion mechanism.

There are several possible ways of achieving a constant uptake rate with an increasing surface. One would be to have a constant metabolism limiting the uptake rate. This cannot occur with these embryos since measurements of respiration rate show

that metabolism is increasing (Borei, 1948). Another possibility is that there is an increase in the number of carriers which is counterbalanced by an increase in the pool size. It seems unlikely on general grounds that such a balance could be established so exactly, but there is a more cogent argument against this in the case of valine, where Kavanau (1954) has shown that there is very little change in the level of pool valine between the 4-cell stage and the blastula. The results of Sugino *et al.* (1960), mentioned earlier, may also be relevant to cytidine. This leaves as the most likely alternative, that the uptake rate is limited by the number of carriers at the surface and that *this number stays unchanged*. This implies that the number of carriers per unit area of the surface decreases during development.

One problem in the interpretation of these results is the extent by which the cell surface increases during early development. If an egg divided, without change in volume, to give two spherical blastomeres, the surface area would increase by 26%. This happens when the fertilization membrane and the hyaline layer are removed. We attempted to measure the changes in uptake rate under these conditions but the results were inconclusive, probably because of the decreased viability of the isolated blastomeres (compare Harvey, 1956). In normal development, the two blastomeres are constricted after cleavage by the hyaline layer to form two oblate spheroids with a flat area of contact between them. This deformation increases the total cell surface and we have calculated from photographs that the total surface area increase after the first cleavage is 35%. It may be argued that this is an overestimate of the available surface, since the surfaces at the area of contact might not be accessible to the precursors. This is not a very likely possibility since the space between the cell membranes in electron micrographs of the contact area is many times the size of the precursor molecules. But even if this possibility is accepted, there is still an increase in the available surface. If the contact area is subtracted from the figure of 35% given above, it leaves an increase of 11%. Over the five divisions in Figs. 1 and 2 this would give a total increase of 70%. It is also clear that the outer surface of a later morula is considerably greater than that of an egg, and, if the contact areas are taken into account, the factor of increase is larger still. We can conclude then that there is an increase in the cell surface available to precursors during early development, even though the extent of this increase is unknown.

One other objection can be met at this point—that the fertilization membrane might restrict uptake. Giudice *et al.* (1962), however, found that removal of the fertilization membrane did not alter the total uptake of precursors and we found the same in preliminary experiments. Also, there is no change in the valine uptake after the fertilization membrane disappears at hatching (Fig. 3).

Our results are in substantial agreement with those of Giudice *et al.* (1962) on the uptake of methionine and leucine. Although their samples were not so closely spaced in early development, they found a rise in the rate of uptake after fertilization. There was then no change in the uptake rate of methionine between the 4–8-cell stage and the blastula, and only a slight rise and fall with leucine. In later development, they have emphasized changes in the uptake rate of the order of $\pm 15\%$, whereas we have drawn a straight line through the points in Fig. 3. There may well be variations of this

order in the uptake rate, but the important point here is that they are small compared to the increase of total cell surface.

The uptake of phosphate, which has been studied extensively by Whitely and his collaborators, has many points of similarity with our results. The rate of phosphate uptake rises after fertilization and then remains constant from 20 to 60 min after fertilization until at least 300 min (Litchfield & Whitely, 1959). The uptake is markedly inhibited by arsenate, a competitor. It is also inhibited by dinitrophenol although, as in our case, this inhibition is much more effective when the dinitrophenol is added soon after fertilization. In longer-term experiments, however, Bolst & Whitely (1957) found large changes in the uptake rate between early stages and blastulae. These may have been due to changes in the incorporation of phosphate, since the measurements were made after pulses of an hour. It is also possible that a pulse of this length measures the size of the filled pool rather than the uptake rate (compare cytidine in Fig. 4).

We have shown that there are no significant changes in the uptake of precursors at the time of cleavage. It seems that the carrier mechanisms are unaffected by the changes that take place in the optical and mechanical properties of the surface at cleavage (Mitchison & Swann, 1952, 1955), possibly because these changes take place in other regions of the surface such as the cortex. This appears to conflict with the results of workers earlier this century (e.g. Lillie, 1916; Herlant, 1920) who found rhythmic permeability changes at cleavage. It may be that there are such changes in permeability, particularly to substances that enter the cell by simple diffusion rather than with a transport mechanism, but it would be better to confirm this using tracers. Cytolysis and plasmolysis were the main measures of permeability in the early experiments and they are open to several interpretations.

In conclusion, we can present the main points of the model system that we have suggested. Amino acids and nucleosides enter the embryos by specific transport mechanisms mediated by carrier molecules in the cell surface. These carriers are present in the unfertilized egg but the mechanisms are inactive. On fertilization, these mechanisms are activated, presumably because of their link to metabolism, and reach maximum efficiency before the first cleavage. The mechanism is not an adaptive one, since its response to an added precursor is immediate. Nor is it affected by cleavage. There is no change in the total number of carriers per embryo from the unfertilized egg until much later in development. This leads to the important conclusion that the number of carriers per cell or per unit of surface diminishes during development. If the components of the new surface are formed at each division by synthesis, then fresh carriers are not among the molecules synthesized. If, on the other hand, the new surface is formed from a reservoir of membrane material in the cytoplasm, then this reservoir does not contain the carriers. The new membrane is not the same as the original egg membrane because it has fewer carriers, so in this respect there has been a differentiation of the cell membrane. If a reason is to be sought for this, it may lie in the fact that carrier molecules for amino acids and nucleosides may be useful in the developing oocyte which has to take up precursors. But they can be of little use to an early embryo in sea water, and so they are not synthesized afresh until much later.

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NOTE

After this paper was accepted for publication, Piatigorsky, J. & Whiteley, A. H. (1965) published a paper entitled 'A change in permeability and uptake of [¹⁴C]uridine in response to fertilization in *Strongylocentrotus purpuratus* eggs' (*Biochim. biophys. Acta* **108**, 404-418). Their results are in close agreement with those presented here and they also argue in favour of an active transport mechanism for nucleoside uptake.

