STUDIES ON THE METABOLISM OF CELLS IN VITRO.

I. The Toxicity of α-Amino-Acids for Embryonic Chicken Cells.

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Plate 13.

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A synthetic medium suitable for the growth of tissue cells outside of the animal organism has not been discovered up to the present time. Since the preparation of such a medium would lead directly to a better understanding of cellular metabolism, this problem has stood forth as one of the most important of those presented by the tissue culture method.

From earlier observations evidence had already been obtained which showed that the ingredients essential for the building of new cells and the liberation of energy in the cultures comes directly from the tissue fragments. The growth observed is a manifestation of a simple transfer of materials from the more central portions of the fragment to the cells at the periphery, or, in other words, the preying of one cell in a more suitable environment upon its neighboring cells in an unsuitable one. This is true in cultures where simple isotonic salt solutions have been used as the medium. That it is also true in the case of the plasma culture can be readily shown by repeatedly changing the medium or transplanting the cells to drops of fresh medium. All activity ceases after a few transplants, or when the cells within the fragment have become exhausted.

One of us,[1] in a recent study, has shown that tissue cells are not highly organized elements. Their various manifestations of life are the result of reactions between themselves and a specifically

[1] Read before Section VIII, Subsection E, Second Pan American Scientific Congress and the American Cancer Society, Washington, January 6, 1916.

organized environment. The form of the manifested change is determined by the peculiar organization and composition of the environment. Growth associated with mitotic cell division is seen only in cells which have become passively placed at the interspace between certain insoluble substances and the medium. These substances are liberated from the cells when they are removed from their normal habitat to an oxygen-containing plasma or salt solution. The substances are almost transparent, their refraction is not very different from that of the original medium, and they accumulate at the surface of the medium to form a membrane. They are liberated in large amounts from a tissue fragment rich in cells.

A cell brought in contact with the surface of this transparent substance adheres to it and flattens over its surface. Such a cell grows and divides by mitosis when oxygen is present and the waste products remain below a certain concentration.

A single isolated cell when placed in a drop of fresh plasma does not grow and divide. It may show movement. Connective tissue cells often assume spindle and irregular stellate shapes. These simple activities lead, however, apparently to the exhaustion of these cells. They come to rest after a short period and show evidences of deterioration through vacuolization and failure to stain sharply.

All cells that grow in these cultures grow at the expense of their neighbors. In the culture the cells at the border of the fragment grow; they have come in contact with a layer of the transparent substances mentioned above, which was liberated by the large number of cells deteriorating in the center of the fragment. These cells within the fragment about which the border cells are growing actively soon become reduced to mere shadows. This deterioration goes on to such an extent that they no longer stain.

With the increase in the concentration of the waste products the activity of the cells in the outer medium not only becomes less and less, but there is also a decrease in the liberation of substances from the fragment. Eventually the whole system comes to a state of complete inactivity or equilibrium. Such a system remains without further change often for a long period of time, sometimes as long as 6 months, at incubator temperature and in the presence of an ample supply of oxygen. If the cells and the fragment at any time during

this period are removed to fresh medium, normal activity is again observed, provided the fragments and the cells had not been transplanted frequently at a previous time, or become exhausted of this substance, or of substances which lead to its formation.

If cells are placed in a medium where the mechanical conditions are such that these substances cannot diffuse out from the fragment over a surface, or the cells are prevented from coming in contact with this surface, growth followed by mitotic division does not take place, but other forms of change result. By merely changing the mechanical organization of the culture or changing the differential surface tension of the cell it has been found possible to change its form, structure, and the manifested activity associated with it. Heart muscle cells when placed in a medium where they may be passively carried against the flat surface of these substances lose their myofibrillar structure and grow and divide by mitosis. They resemble in every way growing sarcoma cells. The same cells may again be caused to grow to form a syncytium, their nuclei dividing by a mitosis. Again they may be caused to round off and resemble large mononuclears, or to differentiate into myofibrils and *vice versa*. The contracting heart muscle cell is a cell which has become stretched through a serum cavity between the surface formed by the substances diffusing from the fragment and an elastic band of fibrin. One end is adherent to the surface and the other to the end of the band of fibrin. The cell is elongated and spindle in shape. Its elongated shape is maintained by the stress of the fibrin. The surface of the cell, which is unattached to the fibrin or the surface of the substances coming from the fragment, is sharply defined. It has a high surface tension. The points of active change in such a cell are evidently the points of contact.

The cells of higher animals are, therefore, strikingly different from many unicellular organisms, such as the paramecium, etc., which contain within themselves all the necessary organization for their growth. Growth and the various manifestations of life of the animal cell are differential surface tension phenomena which are under the control of the environment. Their manifestations of life are manifestations of "tissue rather than cellular reaction."[1]

The growth of the cells in the culture depends upon the presence

of certain specific substances which are liberated by their neighbors and a specifically organized environment. The growth itself is evidently a highly specific reaction. It is a reaction which one might readily assume would continue as long as these specific substances are liberated from the fragment, the proper mechanical organization is maintained about the cells, ample supply of oxygen is present, and the waste products of the reaction are removed.

The questions that confronted us primarily in a further study of these cultures are: What is the nature of these substances? How are they formed in the body under normal conditions? Can any part of the body live an independent existence when isolated from the other parts? Is the organization of the culture such that it will be able to form these substances from simple food bases? The unicellular organism is capable of breaking down complex substances and building its various component parts from the products liberated. Are these systems which have been isolated, tissue cultures, also capable of an independent existence? Is the failure to demonstrate any actual increase of protoplasm in the plasma culture indicative that these organizations are not able to live an independent existence, to build these substances from the food bases, or is it indicative merely of the lack of the proper food bases in the plasma which has been used as a medium?

The fact that plasma contains carbohydrates, proteins, and fats made it seem plausible to assume that these systems are unable to utilize them directly as food substances. That in the ordinary plasma cultures these food bases are insufficient for the building of new cells can be shown definitely not only by the failure of the cells to continue to grow after several transplants, but further by actual measurements. The sum of the masses of new cells formed in each transplant is always less than the original mass. One could readily consider in a well regulated series that it represents the original mass minus the energy of transfer.

EXPERIMENTAL.

In the present series of experiments we tried to determine whether the addition of any substance to the plasma would prolong the growth of the cells. The addition of certain carbohydrates and fats did not

affect the growth to any degree. During the course of further experiments we had the opportunity to try certain hydrolytic products of the protein of egg yolk. Since the results of these experiments have a certain interest in themselves, we decided to report them separately at this time.

Method.

The tissues used for these experiments were heart muscle and pieces of the body wall of chick embryos and fetal chickens. The control medium for the experiments consisted of one part of fresh plasma containing a moderate amount of fat and one part of a 0.9 per cent sodium chloride solution. In the experiments the same proportions were used: one part of the isotonic solution of the substance to be tested was added to one part of the same plasma.

Fragments of heart muscle 1 mm. in diameter are placed in a layer of medium spread over the surface of a cover-glass. This layer has a thickness of 0.5 mm. A hollow ground slide is inverted over the drop, and the edge of the cover sealed to the slide with vaseline and paraffin. The cultures are placed in an incubator kept at a constant temperature of 39.4°C. They are examined under the microscope, which is kept in a chamber heated to the same temperature as that of the incubator.

After 3 days' incubation the growth of each is recorded and the fragment with the cells about it from each of the cultures is carefully transferred to a new drop of the medium, in which it has been previously grown. At the end of another 3 days the growth is again recorded and the fragments and cells again transplanted, and so on until no more activity is seen.

The growth in these cultures takes place as a rule along a single plane. A rough estimate of activity can be made by measuring the area covered by new cells about a fragment. There are always, however, a number of cultures in which the cells grow in several planes. On this account we have decided that a more accurate estimate can be given by the use of a relative term +, rather than by the direct numerical measurements. In the tables we have used this method of recording activity.

Plasma has been used in these experiments not only because it was of interest to test this medium more carefully, but also because

when it is used it is possible to obtain a constant and readily predicted cellular activity in the control cultures. This has not been found to be true for any of the simple liquid media. The proper mechanical conditions in the latter cultures are difficult of manipulation.

In the first series of experiments we prepared peptones from the yolk of eggs; that is, they were prepared from the specific food proteins of the embryonic chicken cells. These peptones were obtained by the use of the general method described by Abderhalden[2] and slightly modified by us as follows: 240 gm. of coagulated egg yolk are dried and pulverized, and treated with 1,000 gm. of 70 per cent (by weight) of pure sulphuric acid. The coagulated yolk does not dissolve as readily as one might wish, so the mixture is shaken vigorously for an hour. A thick scum forms on top, the rest of the material going into solution. This scum is removed. It proves to be fat. The brownish purple solution is next allowed to stand at room temperature for 3 days. It is frequently shaken during this time and then transferred into a 20 liter mixing jar, the latter being packed in a freezing mixture where it is cooled thoroughly. 10 liters of water are then added very slowly, while stirring, keeping the temperature below 20°C. After this, enough barium hydroxide in crystalline form is added to give the solution a neutral reaction towards litmus. This process requires great care. It is best to calculate the exact quantity of barium hydroxide needed, to add nearly this quantity, and then proceed gradually. The solution must be stirred constantly in order to keep the temperature below 20°C. at all times. The voluminous precipitate of barium sulphate is best separated from the digested solution by means of a centrifuge. This solution is next treated alternately with 0.1 N barium hydroxide and 0.1 N sulphuric acid until the addition of an excess of either to a filtered or centrifuged sample does not give a further precipitate of barium sulphate. Of course, an addition of barium hydroxide to a peptone solution will sometimes give a precipitate of barium peptonates, but these are soluble in dilute nitric acid, while barium hydroxide is not. Thus an absolutely neutral solution is prepared. This solution of over 10 liters in quantity is next concentrated under

[2] Abderhalden, E., Defensive Ferments of the Animal Organism, New York, 1914, 204.

reduced pressure until about 100 cc. of a syrupy, light yellow liquid results. This is treated with about 2 liters of hot methyl alcohol and filtered into 10 liters of chilled ethyl alcohol. A white precipitate results, and is quickly filtered off by the aid of a suction pump and dried in a desiccator. The color of the resultant powder is light yellow.

These peptones gave all the true peptone reactions. They were absolutely soluble in a saturated solution of ammonium sulphate and remained unchanged as regards color on boiling with a solution of sodium hydroxide to which a drop of lead acetate solution had been added. They were dialyzable through a dialysis tube, impervious to albumin, and the dialysate gave a typical blue ninhydrin reaction. Finally, they gave a reddish biuret reaction, and their microscopic appearance was similar to that of commercial silk peptone.

A large amount of this peptone, namely, 2.5 per cent, can be dissolved in 0.85 per cent sodium chloride solution, without making the solution hypertonic, and thereby killing the cells. This solution is mixed half and half with plasma. The growth, as is shown in Table I, Cultures 1, 2, 3, 4, and 5, continued regularly up to the sixth transplant. After this the various fragments became exhausted. The control cultures, 6, 7, 8, 9, and 10, behaved in exactly the same manner. From this we conclude that peptones do not in any way enter into this growth reaction. They did not prolong it, and no change of any kind was noted by their addition.

We next turned our attention to the study of α-amino-acids. The complete hydrolysis of egg yolk promised a yield of α-amino-acids in the same proportions as they actually occur in the protein molecule, the only α-amino-acid which does not give a yield on acid hydrolysis being tryptophane. This seemed to be the easiest and most direct way of procedure. Consequently a digestion mixture was prepared by dissolving dried egg yolk from which the fats had been extracted with ether by means of a Soxhlet apparatus, in a 70 per cent (by weight) solution of sulphuric acid. After the protein had gone into solution, enough water was added to make this a 25 per cent solution of sulphuric acid. The entire mixture was then heated in a flask with a reflux condenser until a biuret reaction was no longer obtainable. This acid solution was next neutralized with

TABLE I.

Tissue: Fragments of the heart muscle of a 12 day old chicken embryo.
Medium: Isotonic solutions of peptone, 2.5 per cent, in sodium chloride solution, 0.85 per cent, 1 part.
Plasma, 1 part.
Control Medium: Sodium chloride solution, 0.9 per cent, 1 part.
Plasma, 1 part.

Solution used.	Culture No.	Growth of transplants.						
		1st.	2nd.	3rd.	4th.	5th.	6th.	7th.
Peptone.	1	++++	++++	++	+	0	0	0
	2	+++	++++	+++	++	Few cells.	0	0
	3	+++	+++++	+++	+++	+	Few cells.	0
	4	++++	++++	++++	+++	++	Few cells.	0
	5	++++	+++++++	++++++	++++++	0	0	0
Control.	6	+++	+++++	—*	—	—	—	—
	7	+++++	++++++	++++	++	0	0	0
	8	+++	++++	++	++++++	++	Few cells.	0
	9	+++++	+++++++	++++++	++++	0	0	0
	10	+++	++++	++	++	Few cells.	0	0

* In the tables the dashes indicate that the culture was lost through breaking of the glass slide, infection, or other causes.

barium hydroxide in exactly the same manner as stated above for the preparation of peptones. After this the solution was concentrated under reduced pressure at 40° C. until evaporation to dryness gave a 3 per cent residue, which we believe consists for the most part of α-amino-acids. Plasma was diluted with this mixture. To our surprise all the cells were dead in 48 hours, while the control cultures in plasma alone grew and kept on growing as usual when they were transplanted. We did not consider this peculiar result due to the possible toxicity of the α-amino-acids as such, but thought that traces of sulphuric acid or barium hydroxide caused the death of the cells. In order to make sure that this was the case, a part of the digestion mixture was further concentrated to one-fifth of its

volume, divided in two portions, and each portion tested with an excess of 0.1 N sulphuric acid and 0.1 N barium hydroxide respectively. No precipitation occurred.

Discarding the idea that a trace of sulphuric acid or barium hydroxide could be the cause for failure, we considered hypertonicity as a possibility. A simple way of testing whether a solution is isotonic or not towards chicken cells is by the use of chicken erythrocytes. The red blood corpuscles are nucleated and consequently have a bulge in the middle of the disc. This bulge is especially conspicuous when the corpuscle is seen on end. If a solution is hypotonic, the surrounding disc will swell and this bulge will become less marked. If, on the other hand, the solution is hypertonic, one sees characteristic shrivelling around the edge of the disc. It is, of course, understood that extremely hypotonic or hypertonic solutions will cause a dissolution of the corpuscles, a dissemination of the hemoglobin, and give a characteristic laked appearance.

Making up various dilutions of the digestion mixture, we transferred 1 cc. of the dilutions to suitable test-tubes and added 0.25 cc. of a 5 per cent suspension of washed chicken erythrocytes in 0.9 per cent sodium chloride solution. We then incubated it for 4 hours and compared it with the form of our control diluted with 0.9 per cent sodium chloride solution and treated in exactly the same manner. Using the dilution which showed neither shrivelling nor bulging, we prepared an isotonic digestion mixture.

Renewed experiments with the digestion mixture gave practically the same results as before. A repetition of the experiment with a new digestion again resulted in the death of the cultures. A portion of the last digestion mixture was treated with hydrochloric acid gas to the point of saturation and allowed to stand in the ice box for several days. We obtained a voluminous precipitate of glutamic acid hydrochloride, showing its typical crystalline form. This gave the final proof that α-amino-acids were present. Even now we did not attribute our results to a toxic influence of the acids in themselves but rather to the influence of the brown coloring matter in the solution, said to consist of caramelized carbohydrates, humin substances, etc. We therefore tried pure α-amino-acids.

We used ten of the acids in our first experiment; namely, glycocoll,

alanine, cystine, valine, leucine, phenylalanine, tyrosine, tryptophane, oxyproline, and asparagine.[3] Some of the substances were synthetic (Merck and Kahlbaum preparations), while others were obtained by the hydrolysis of proteins.[4]

To be sure of the substances which we used, the total nitrogen of each of the α-amino-acids was determined, their melting points were ascertained, and their crystalline forms observed. All these tests proved that we were dealing with chemical substances of the right molecular constitution containing no demonstrable impurities.

Hot saturated solutions of these acids were prepared. The solutions were cooled, and each solution was tested with chicken erythrocytes for isotonicity by the methods described above. The more insoluble substances, such as cystine, were primarily dissolved in isotonic salt solution, as we could hope to obtain isotonicity in no other way. All these solutions were diluted half and half with plasma and used as culture media.

Tables II, III, and IV give a survey of the experiments. In every case the addition of a solution of α-amino-acids to the plasma results in the ultimate death of the growing cells, while the controls keep on with their regular development. The discrepancy between the time required to kill the cultures described in Tables II and III, on the one hand, and in Table IV, on the other, the cultures of the latter series dying more quickly, is explained by the age of the embryo used. The cells of the younger embryo grow much faster and are less resistant to toxic influences than are those of an older embryo, as is known from numerous reported experiments.

[3] We used asparagine in spite of the fact that it has not been found as a constituent of hydrolyzed animal proteins. The effect as shown in the tables is exactly the same as in the case of the other α-amino-acids. Fig. 2 is a fair representative of the appearance of any one of the cultures in which α-amino-acids had been added to the medium. This culture was selected for photographing because it was prepared practically at the same time as the controls. The tissue that was used was most similar and had suffered practically the same exposure as that used for the controls.

[4] We wish to thank Dr. P. A. Levene, of The Rockefeller Institute for Medical Research, Dr. D. W. Wilson, of the Physiological Chemical Laboratory of Johns Hopkins University, and Dr. R. F. Ruttan, of McGill University, for supplying us with some of the acids more difficult to manufacture.

TABLE II.

Tissue: Fragments of the heart muscle of a 12 day old chicken embryo.
Medium: Isotonic solution of the α-amino-acid, 1 part.
Plasma, 1 part.
Control Medium: Sodium chloride solution, 0.9 per cent, 1 part.
Plasma, 1 part.

Solutions used.	Culture No.	Growth of transplants.	
		1st.	2nd.
Glycocoll, 35 per cent solution,* 1 part, and water, 19 parts.	1	+++	Few cells.
	2	+++	" "
	3	+++	" "
	4	+++	0
	5	+++	—
Alanine, 11 per cent solution, 1 part, and water, 13 parts.	6	++	+
	7	+++	Few cells.
	8	+++	+
	9	+++	0
	10	+++	Few cells.
Valine, 8 per cent, 1 part, and water, 4 parts.	11	+++	Few cells.
	12	++	0
	13	+++	Few cells.
	14	+++	0
	15	++	—
Leucine, 1.66 per cent, 1 part, and water, 2 parts.	16	++++++	0
	17	++++	0
	18	+++	0
	19	++++	0
	20	+++++	0
Cystine, 0.1 per cent solution, not diluted.	21	++	0
	22	+++	Few cells.
	23	++	+
	24	+++	+
	25	+++	0
Control.	26	+++	—
	27	++++++	++++
	28	++++++	—
	29	+++++	+++++
	30	++++	++++

* All percentages given are approximate.

TABLE III.

Tissue: Fragments of the heart muscle from an 11 day old chicken embryo.
Medium: Isotonic solution of the α-amino-acid, 1 part.
Plasma, 1 part.
Control: Sodium chloride solution, 0.9 per cent, 1 part.
Plasma, 1 part.

Solutions used.	Culture No.	Growth of transplants.			
		1st.	2nd.	3rd.	4th.
Valine, saturated solution, cold, 1 part, and water, 3 parts.	1	+	Few cells.	0	
	2	+	0	0	
	3	+	0	0	
	4	Few cells.	0	0	
Glycocoll (Merck), saturated solution, cold, 1 part, and water, 24 parts.	5	++	++	0	
	6	+++	+++	0	
	7	++	+++	0	
	8	+	+++*	0	
Asparagine, saturated solution, cold, 1 part, and water, 3 parts.	9	++	+*	0	
	10	+	+	0	
	11	+	+	0	
	12	+++	++	0	
Cystine, saturated solution, cold, 3 parts, and water, 2 parts.	13	++++++	+++	Few cells.	
	14	++++	++++	" "	+ Cells rounded off.*
	15	++++	++++	—	
	16	++++	—	—	
Controls.	33	+++++	++++++	+	+
	34	++	+++++	+	+
	35	++	++++++	+	+
	36	++++++	+++++++++	+	+

* All the pieces are contracting actively.

In Table V, we tried more dilute solutions, and, as this table indicates, the cells tend to resist this medium for a longer time. However, none of these cultures grew as long as the controls, which shows that even in very low dilutions the α-amino-acids are toxic. It is of interest in this series to note evidences of stimulation. This stimulation as the table indicates is not a stimulation of growth, but a stimulation of the contraction of the heart muscle fragments.

TABLE IV.

Tissue: Fragments of the body wall of a 5 day old chicken embryo.
Medium: Isotonic solution of the α-amino-acid, 1 part.
Plasma, 1 part.
Control Medium: Sodium chloride solution, 0.9 per cent, 1 part.
Plasma, 1 part.

Solutions used.	Culture No.	Growth after 48 hrs.	Growth after 72 hrs.
Glycocoll, 35 per cent solution, 1 part, and water, 19 parts.	1	+	The cells have all rounded off and show distortion.
	2	Few cells.	
	3	" "	
Alanine, 11 per cent solution, 1 part, and water, 13 parts.	4	+	The cells have all rounded off and show distortion.
	5	Few cells.	
	6	" "	
Cystine, 0.1 per cent solution, not diluted.	7	+	The cells have all rounded off and show distortion.
	8	+	
	9	+	
Valine, 8 per cent, 1 part, and water, 4 parts.	10	+	+++ The cells have all
	11	++	+++ rounded off.
	12	++	+++
Leucine, 1.66 per cent, 1 part, and water, 2 parts.	13	++	++ The cells have all
	14	++	++ rounded off.
	15	+++	+++
Phenylalanine, 2 per cent, slightly hypotonic, not diluted.	16	++	The cells have all rounded off and show distortion.
	17	—	
	18	+	
Tyrosine, 0.285 per cent in 0.85 per cent sodium chloride, 2 parts, and water, 1 part.	19	+	+ The cells have all
	20	++	+++ rounded off and
	21	++	++ show distortion.
Tryptophane, 1.66 per cent in 0.85 per cent sodium chloride, 1 part, and wafer, 2 parts.	22	++	The cells have all rounded off and show distortion.
	23	+++	
	24	++	
Oxyproline, 10 per cent, 1 part, and water, 2 parts.	25	+	The cells have all rounded off and show distortion.
	26	++	
	27	++	
Asparagine, 15 per cent, 1 part, and water, 1 part.	28	Few cells.	The cells have all rounded off and show distortion.
	29	" "	
	30	" "	
Control.	31	+++	++++++
	32	+++	++++
	33	+++	+++++

All the fragments contracted actively with the exception of the controls. This contraction continued to the complete exhaustion of the fragments.

TABLE V.

Tissue: Fragments of the heart muscle from an 11 day old chicken embryo.
Medium: Isotonic solution of the α-amino-acid, 1 part.
Sodium chloride solution, 0.9 per cent, 1 part.
Plasma, 2 parts.
Control: Sodium chloride solution, 0.9 per cent, 1 part.
Plasma, 1 part.

Solutions used.	Culture No.	Growth of transplants.			
		1st.	2nd.	3rd.	4th.
Valine, saturated solution, cold, 1 part, and water, 3 parts.	17	++++	—	—	—
	18	++++	+ Few cells.	0	0
	19	++	—	—	—
	20	+++	++++	+++*	+++* The cells have all rounded off.
Glycocoll (Merck), saturated solution, cold, 1 part, and water, 24 parts.	21	++++	+++++*	+++*	++++* The cells have all rounded off.
	22	+++++	+++++	+++*	0
	23	++++	++++	—	—
	24	—	—	—	—
Asparagine, saturated solution, cold, 1 part, and water, 3 parts.	25	++++	+++++*	—	—
	26	++++	+++++*	++++*	++++*
	27	—	—	—	—
	28	—	—	—	—
Cystine, saturated solution, cold, 3 parts, and water, 2 parts.	29	+++*	++++++*	+*	0
	30	+++++*	++++++	+++*	0
	31	++++*	++++++*	++++*	0
	32	++++++*	++++++*	+*	0
Controls.	33	+++++	++++++	+++	+++++
	34	++	++++++	++++	+++++
	35	++	++++++	++++	++++
	36	++++++	++++++++	++++++	+++

* All the pieces are contracting actively.

DISCUSSION AND SUMMARY.

Summing up these results, we found that all the ten α-amino-acids used inhibited the growth of the cells and finally killed the cultures. This inhibition is preceded by a short period of activity. The typical effect on the cells is shown in Figs. 1 and 2. The first (Fig. 1) is a control culture showing the usual growth of cells and their typical spindle shape form. The second (Fig. 2) is a culture in plasma plus asparagine,[3] showing the cells rounded off and beginning to undergo dissolution.

We do not wish to draw too extensive conclusions from these experiments, but we believe that the toxicity of α-amino-acids towards growing cells has been shown beyond a reasonable doubt; while we have found that compounds of higher molecular weight, namely, the peptones of egg yolk and proteins, are non-toxic. This toxicity depends upon the concentration and the time that the cells are exposed to their action. As these factors are reduced, the toxicity is decreased. In this respect, these substances are similar to all cell poisons.

Applying these results to the work done on the intravenous injection of digestion mixtures, we believe that we have found a reason for the death of the experimental animals when the hydrolyzed proteins were injected too rapidly. Buglia[5] found that large amounts of α-amino-acids could be injected into the circulation without causing deep-seated changes in the renal and intestinal functions, provided they were injected slowly enough; in fact, that enough of these mixtures could be injected in this way to cover the nitrogen consumption of the body. This injection, however, was always accompanied by an α-amino excretion through the urine and an increase of the peristalsis of the intestine, with resultant liquid stools. As is well known, a sudden great concentration of these substances in the blood of an animal causes death. These results agree with our findings.

Folin and Denis[6] demonstrated the fact that α-amino-acids probably pass into the circulation through the intestines. Van Slyke

[5] Buglia, G., *Z. Biol.*, 1912, xviii, 162.

[6] Folin, O., and Denis, W., *J. Biol. Chem.*, 1912, xii, 141.

and Meyer,[7] by means of Van Slyke's[8] nitrogen method, have practically proven this, and Abel, Rowntree, and Turner,[9] and Abderhalden[10] have lately succeeded in obtaining α-amino-acids in crystalline form from the blood. Van Slyke and Meyer[11] have shown that the tissues take up α-amino-acids to a certain point, but that after this the limit of saturation is reached. This is not so in the liver, which continually desaturates itself by metabolizing the α-amino-acids that it has absorbed, and consequently maintains indefinitely its power of removing them from the circulation, as long as they enter it no faster than the liver can metabolize them. Marshall and Rowntree[12] have shown that there is an increase of the α-amino-acid concentration in the blood after injuries to the liver, which have caused deep-seated anatomical changes. Our experiments prove that tissue cells in general are unable to live in the presence of any great concentration of these acids.

At the present time we do not feel able to give an explanation of the significance of this evident toxicity. However, the fact in itself seems to indicate that we should expect stimulation from a certain increase of the α-amino-acid concentration in the body, or the concentration of any one of the acids, while a greater increase would lead to marked disturbances of the metabolism.

EXPLANATION OF PLATE 13.

FIG. 1. The living cells which had grown from a fragment of the body wall of a 5 day old chick embryo into a medium consisting of 1 part of plasma and 1 part of isotonic salt solution, 0.9 per cent.

FIG. 2. The degenerated cells which had grown from a fragment of the body wall of the embryo used in the culture in Fig. 1 into a medium consisting of 1 part of plasma and 1 part of an isotonic solution of asparagine.

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[7] Van Slyke, D. D., and Meyer, G. M., *J. Biol. Chem.*, 1912, xii, 399.

[8] Van Slyke, D. D., *J. Biol. Chem.*, 1911, ix, 185; 1912, xii, 275.

[9] Abel, J. J., Rowntree, L. G., and Turner, B. B., *J. Pharm. and Exp. Therap.*, 1913–14, v, 275, 611.

[10] Abderhalden, *Z. physiol. Chem.*, 1913, lxxxviii, 478.

[11] Van Slyke and Meyer, *J. Biol. Chem.*, 1913–14, xvi, 213.

[12] Marshall, E. K., and Rowntree, L. G., *J. Exp. Med.*, 1915, xxii, 333.

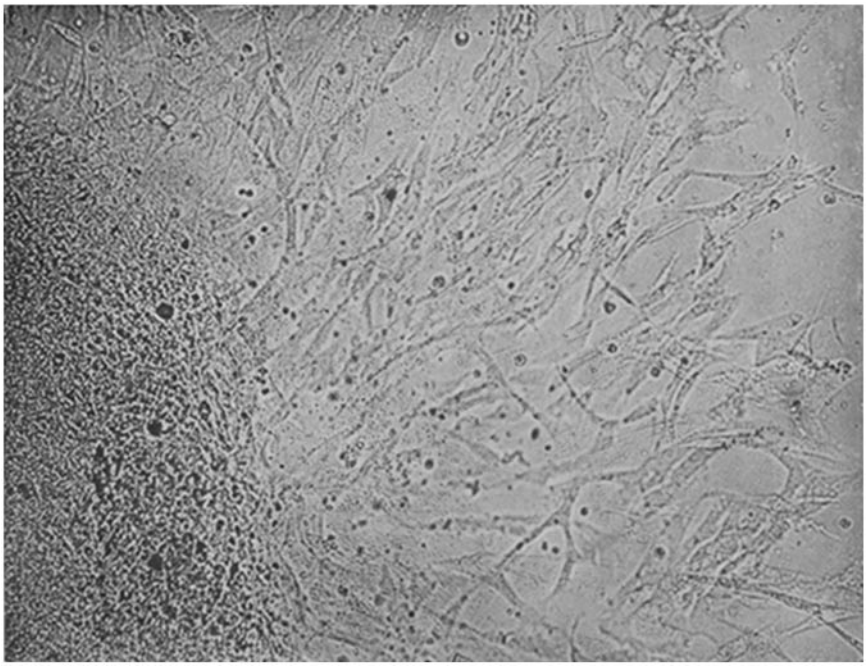

FIG. 1.

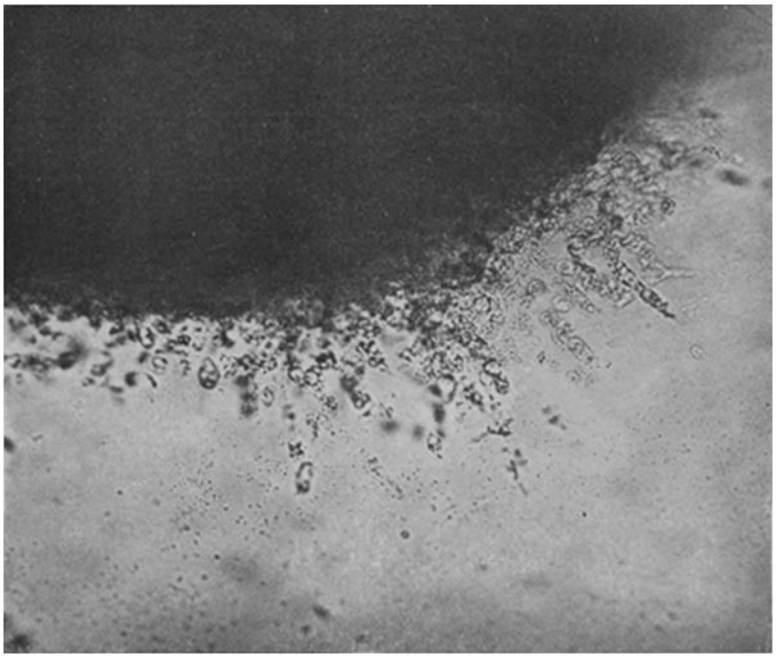

FIG. 2.

(Burrows and Neymann: Metabolism of Cells *in Vitro*. I.)