THE METABOLISM OF TISSUE CULTURES*

I. Preliminary Studies on Chick Embryo

By HILDEGARD WILSON, ELIZABETH B. JACKSON, and AUSTIN M. BRUES

(From the Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University)

(Received for publication, January 23, 1942)

The study of tissue metabolism by classical methods has certain serious limitations which are obviated by the use of tissue cultures. In investigating the mechanism of growth it is particularly desirable to use intact cells, whose growth may be controlled during the period of observation. The present study deals with metabolic processes in cultures of embryonic tissue grown under normal conditions.

All experiments were conducted on chick embryo tissue, usually 9 to 13 days old. Muscle, heart, and liver tissue were explanted together and grown by the roller bottle technique described by Shaw, Kingsland, and Brues (1). Unless otherwise stated, cultures were grown in an artificial medium similar to that described by Baker (2), but without added serum.[1] In any one experiment all culture bottles contained the same amount of medium per unit weight of wet tissue; a typical culture thus had 160 mg. tissue and 4 cc. of medium. Changes in composition of the medium resulting from metabolism of the explanted tissue were then followed by analytical methods.

Glucose Utilization

Previous observations have shown that tissues can use an amount of glucose greater than their own dry weight in 48 hours (Krontowski (3)), and that the

* This is reprint No. 550 of the Cancer Commission of Harvard University.

[1] 100 cc. of the modified Baker's solution contained:

	mg.
NaCl	720
KCl	18
$CaCl_2 \cdot 2H_2O$	23.8
$MgCl_2 \cdot 6H_2O$	9
$NaH_2PO_4 \cdot H_2O$	5.2
$NaHCO_3$	100
Witte's peptone	675
Glucose	100
Cysteine HCl	9
Glutathione	1
Ascorbic acid	0.25
Thiamin HCl	0.5

amount used decreases with the age of the tissue (Lebensohn (4)). The effect of glucose concentrations higher than those found in physiological media has received considerable attention. Demuth (5) stated that growth in cultures increased with increasing glucose concentration. Ebeling (6) observed increased growth in cultures of fibroblasts whose glucose was raised from 200 to 1150 mg. per cent; at higher levels glucose appeared to inhibit growth and to become toxic. Willmer (7), using saline mixture media, found the total amount of cell migration to be directly proportional to glucose concentration up to 1000 mg. per cent, although the tissues were more active in concentrations of 200 mg. per cent as long as enough glucose was present.

TABLE I

Glucose Utilization in Relation to Initial Concentration

Concentration expressed as mg. per cent in media.
Rate = mg. glucose per 100 mg. tissue per day.

Initial concentration	622		407		198		86	
Rate on 1st day		2.6		2.7		2.0		1.4
Concentration at 1 day	476		260		110		29	
Rate on 2nd day		1.8		1.1		1.3		0.5
Concentration at 2 days	372		197		52		7	
Rate on 3rd and 4th days		0.3		0.5		0.5		0.2
Concentration at 4 days	337		147		6		0	
Rate on 5th and 6th days		0.1		0.6		0		0
Concentration on 6th day	332		82		5		0	

We have investigated quantitatively the following phases of glucose utilization:—

Rate.—With media containing 100 mg. per cent glucose we have done 10 experiments comprising 40 observations on 24 cultures. Glucose was used initially at rates from 0.5 to 2.0 mg. per 100 mg. wet tissue per day, the rate falling sharply as glucose disappeared.

With a range of higher initial concentrations 7 experiments were done, comprising 64 observations on 29 cultures. Initial rates of glucose utilization were higher, more or less in proportion to concentration, up to 300–400 mg. per cent. Above this level, utilization was at first rapid, but was either greatly slowed or entirely stopped after a few days, while much glucose yet remained. Figures for one such experiment are shown in Table I. The results are in close agreement with those of Gemmill, Gey, and Austrian (8) on Walker rat sarcoma 319.

Total Glucose Used.—From a range of higher initial glucose concentrations, total utilization before onset of degeneration similarly appeared to show an optimum at 300–400 mg. per cent, as shown in Table II.

If exhaustion of glucose is prevented by frequent replacement of the regular medium, it continues to be used for a long time and in great amount, especially with daily changes. 2 cultures were followed with daily replacement of medium, 1 for 14 days. This 1 used increasing amounts of glucose each day up to 11 days, totalling 22 mg. of glucose consumed per 100 mg. wet tissue, or an average of 1.6 mg. per day. When cultures were changed every 2 or 3 days so that the media were more nearly depleted, total utilization was less, as shown in Fig. 1.

TABLE II

Total Glucose Used from Various Initial Concentrations

Initial glucose concentration	Glucose used per 100 mg. tissue		
	Experiment 6	Experiment 9	Experiment 7
mg. per cent	*mg.*	*mg.*	*mg.*
500–600	4.6	4.0	5.3
350–400	5.1	8.2	5.9
175–200	4.2	4.0	4.4
Duration of experiment, days.........	4	5	6

Figures represent the total glucose which 100 mg. of tissue had used at the end of each experiment.

Exhaustion of glucose may also be prevented by daily additions of concentrated solution without other change in medium, thus maintaining the total concentration near 100 mg. per cent. 9 such cultures had used more glucose at 4 and 6 days than those whose medium was replaced.

Effect of Colchicine and Phlorhidzin.—Isolated observations indicate little or no effect of these substances. 1 series of cultures with phlorhidzin and 2 with colchicine were followed.

Colchicine, in concentrations of $1:10^6$ and $1:10^7$ did not appreciably alter glucose utilization, although all cells entering mitosis were stopped permanently in metaphase.

Phlorhidzin, in concentrations of 33, 100, and 300 mg. per cent appeared not to prevent the utilization of most of the glucose present, or to be itself destroyed.[2] Phlorhidzin increased the number of mitoses seen in stained cul-

[2] At each concentration of phlorhidzin the level of reducing substances reached a base line corresponding to the amount of phlorhidzin used, and glucosazones could not be obtained in these depleted solutions.

tures. The appearance of the mitoses suggests that it acts through prolonging the mitotic process rather than by stimulating growth. Mitotic counts are seen in Table III.

Absence of Glucose.—In explanted tissue not supplied with glucose, degenerative processes are greatly increased. Nevertheless some tissue remains alive for 2 or 3 days. 12 such cultures were followed. At 2 days, they showed a

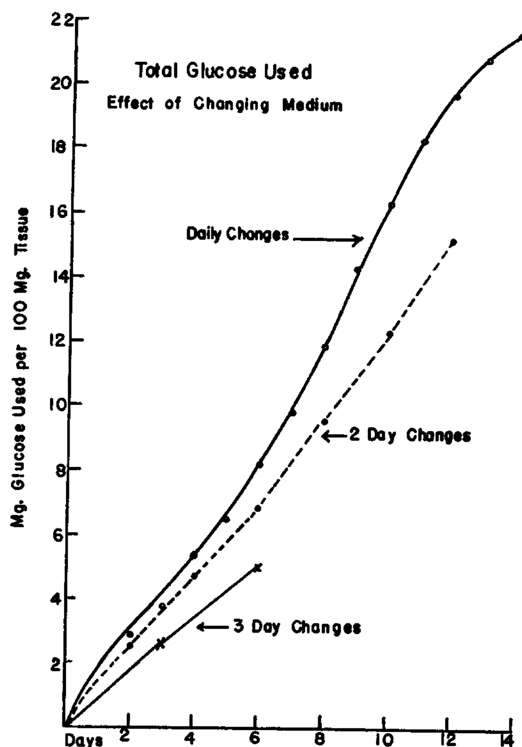

FIG. 1. Cumulative curves showing total glucose used by 1 culture changed daily, and 2 other cultures changed at longer intervals.

slight but definite migration of cells, without mitoses. Most of the tissue, however, degenerated before it began to migrate, and at 4 or 5 days degeneration was complete. Hence metabolic findings, to be discussed later, are of value only if interpreted with caution.

Nitrogen Metabolism

Amino Nitrogen Production.—True growth, involving formation of new tissue, must be accompanied by incorporation of protein derived from nitrogenous substances in the medium. Carrel and Baker (9) have shown that

proliferation of fibroblasts is rapid when higher cleavage products of protein are supplied, as by Witte's peptone. If cells do not take up exclusively whole peptone molecules but first hydrolyze some of them to smaller units by means of proteolytic enzymes, an increase of the amino nitrogen of the medium during growth should be seen.

We have followed amino nitrogen production in Baker's solution. 31 observations were made on 26 cultures. The original amino nitrogen concentra-

TABLE III

Mitotic Counts on Various Types of Culture

Medium	Experiment No.	Per cent mitoses				
		Days				
		1	2	3	4	5
Baker's 100 mg. per cent glucose	10	1.3	1.7	0.9		0
Baker's 500 mg. per cent glucose	10	1.6	1.6	1.4		0
Baker's No glucose	18	0			0	0
Baker's No glucose* Added lactate	15		2.0		0	0
Baker's No glucose* Added pyruvate	22	0.7	0.15			
Baker's Added phloridzin 1.0 mg. 0.3 mg.	20		4.3 8.2			
Amino acid medium	14	1.8	2.1	1.4		

* These cultures showed widespread degeneration. Mitoses were counted in the relatively small areas of healthy cells.

tion was about 14 mg. per cent which is one-seventh of the total nitrogen. This value rose after contact with tissue grown under our standard conditions. Hydrolysis was rapid at first, but decreased markedly in rate after a day or two when the same medium was left on the culture. A maximum rise of 24 to 31 mg. per cent in 6 days was observed under these conditions. Daily change of medium for 3 days appeared to increase the daily production of amino nitrogen.

Total Nitrogen.—Utilization of total nitrogen from the medium during growth is difficult to demonstrate in these experiments due to the high protein content of the small amount of plasma used, and to partial autolysis of explants. De-

terminations on cultures grown in nitrogen-free Tyrode solution showed that up to 15 mg. per cent protein nitrogen may thus be added. Total nitrogen values in Baker's solution usually remained quite constant or showed a rise. It therefore seemed advisable to look for utilization of amino nitrogen from a medium whose nitrogen was mainly in this form.

An amino acid solution was prepared by hydrolyzing Witte's peptone with sulfuric acid. This was then used to replace the peptone in a culture medium containing all other ingredients plus 10 mg. per cent tryptophane. The final amino nitrogen content was 65 mg. per cent or about 400 mg. per cent amino acids, which is roughly 4 millimols per 100 cc.

With this medium we obtained excellent growth over 6–8 day periods, with mitotic rates comparable to or exceeding those in the peptone medium (see Table III). Certain differences in behavior from that in Baker's solution were, however, observed. In cultures of embryo muscle a number of giant multinuclear cells were seen using this medium, but never in regular Baker's solution. These cells bear some resemblance to those seen in regenerating adult muscle. The two types of growth are contrasted in Fig. 2. Our amino acid solution was identical with Baker's solution in every possible respect except that the peptone had been hydrolyzed. These effects, therefore, are probably due to specific actions of amino acids, singly or as a group.

We ran 5 experiments using this medium, comprising 60 observations on 29 cultures. In 2 experiments amino nitrogen remained constant for 5–7 days. In 1 case this was associated with tissue degeneration but in the other experiment the tissue appeared in good condition. In the remaining experiments amino nitrogen fell, the tissues grew well, and used glucose at about the same rate as in Baker's solution. Although the variation in results has not been satisfactorily explained, it may be concluded that nitrogen can be progressively used from an amino acid medium. An initial rate of about 0.1 mg. amino nitrogen used per 100 mg. tissue per day dropped to about one-third that value after 2 days. This lessened uptake bears some relation to exhaustion of glucose, for when the glucose level was maintained near 100 mg. per cent, amino nitrogen utilization continued at a somewhat higher rate. Typical results are graphically shown in Fig. 3.

In cultures depleted of glucose the amount of urea plus ammonia nitrogen found accounted for a large proportion of the amino nitrogen lost, whereas a negligible amount of urea plus ammonia appeared when glucose was added. This would seem to indicate that amino acids are deaminated and burned in the absence of glucose, while in the presence of glucose they are stored. Total nitrogen values were somewhat lower in the culture media to which glucose had been added, indicating nitrogen utilization.

Lactic Acid Production

Previous workers have found extensive lactic acid production by chick fibroblasts. It has been noted that a decreased growth rate resulted in an increased

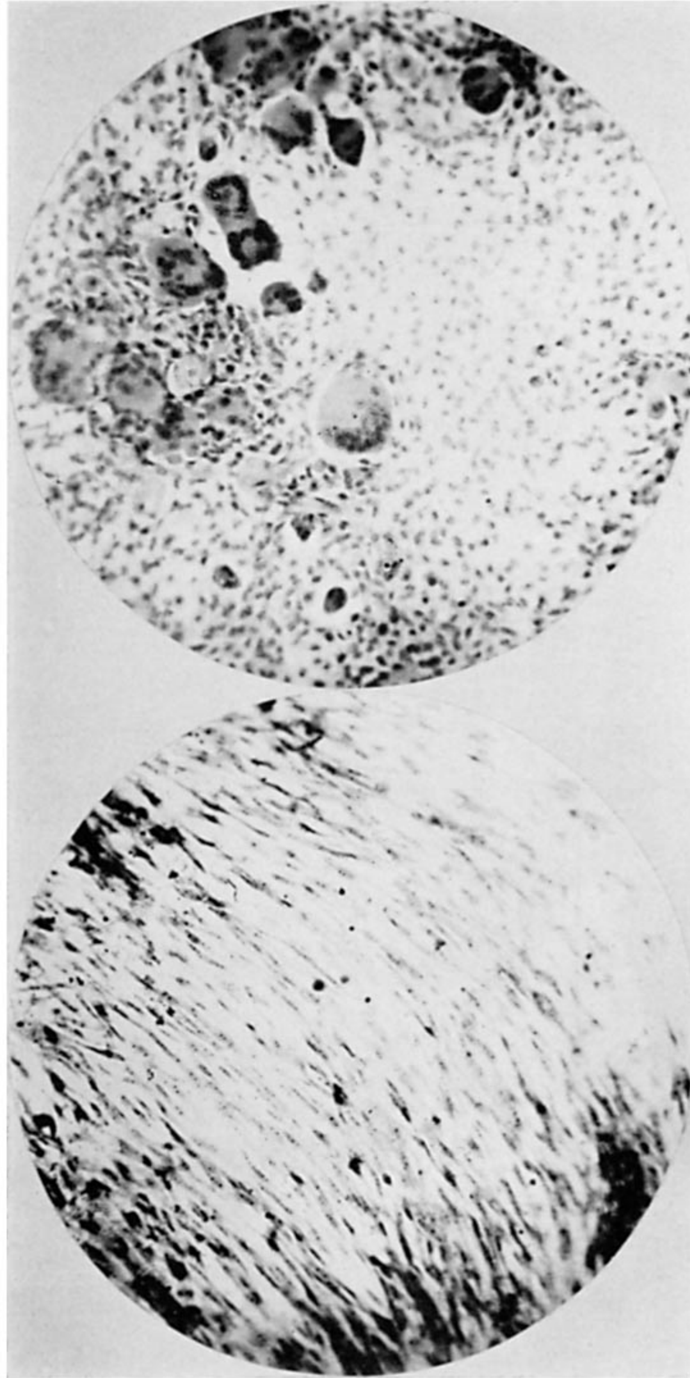

FIG. 2*a* FIG. 2*b*

FIG. 2. Photomicrographs of embryo muscle cultures. (*a*) Grown in peptone medium (Baker's). (*b*) Grown in medium similar to Baker's except that the peptone had been hydrolyzed to amino acids. Stained with hematoxylin. × 80.

ratio of lactic acid to size of cultures (Demuth and Meier (10); Meier (11)). About 60 per cent of the glucose used in 48 hours has been accounted for as lactic acid, and glycolysis has been considered characteristic of the chemodynamics of tissue cultures (Krontowski (3)). Pomerat and Willmer (12) offer much indirect evidence that non-phosphorylating glycolysis is essentially linked with growth in cultures of embryo tissues. However, measurements on cultures transferred to Warburg vessels (Lipmann (13, 14)) appear to show that glycolysis is not the characteristic energy source of growth.

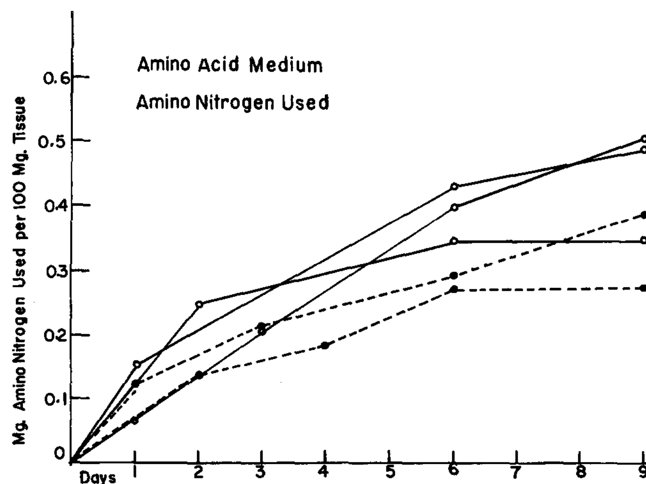

FIG. 3. Amino nitrogen utilization from the amino acid medium. Each curve represents a single culture sampled for analysis at the times shown by points. Resulting changes in volume have been taken into account. Solid lines represent cultures to which glucose was added at 2, 4, and 6 days. Dotted lines represent cultures to which no glucose was added.

Our studies suggest a relation between glycolysis and glucose utilization. They may be divided into six groups.

A. When 100 mg. per cent initial glucose is used up by the tissue: 8 experiments were done, with 32 determinations on 23 cultures. Lactic acid invariably appeared while glucose was falling rapidly, rising during the first 48 hours to values between 25 and 45 mg. per cent. After glucose had disappeared, decreased amounts of lactic acid were found in 3 of these experiments.

B. When initial glucose is 500–600 mg. per cent and is not all used up by the tissue: 5 experiments were done, comprising 17 determinations on 17 cultures. Lactic acid appeared more rapidly than at lower glucose concentration and continued rising to 100 mg. per cent or over in 4 to 6 days.

C. With daily change of medium: in 1 culture given fresh medium and

analyzed daily, from 25 to 42 mg. per cent lactic acid was formed each day for 13 days. Production reached a peak at 10 and 11 days, after which smaller amounts were formed. For the first 11 days 60 to 70 per cent of the removed

TABLE IV

Lactic Acid Production under Various Conditions

Group	Conditions	Experiment No.	Constituent	Amount per 100 mg. tissue									
				At start	Days								
					1	2	3	4	5	6	12	13	14
				mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
A.	100 mg. per cent glucose	10	Glucose used	0	0.7	1.0	2.4		2.4				
			Lactate	0	0.6	0.7	0.4		0.2				
	100 mg. per cent glucose	31	Glucose used	0		2.1		2.3		2.4			
			Lactate	0		0.9		0.6		0.7			
B.	600 mg. per cent glucose	34	Glucose used	0	5.0					8.4	10.8		
			Lactate	0	1.3					2.1	2.7		
C.	Daily change of medium	33	Glucose used	0	1.7	1.2	1.1	1.7	1.2	1.6	1.3	1.1	0.7
			Lactate	0	1.2	1.3		1.1	0.9	1.1	1.2	1.1	0.7
D.	No glucose	18	Lactate	0		0.3		0.4	0.6				
E.	Constant glucose	19	Lactate	0		0.1		0.7		2.0			
		29	Lactate	0			2.0			3.3			
F.	Added lactate	15	Glucose used	0		1.5				3.6			
			Lactate	0.7		1.3				0.7			
	Added lactate; no glucose	15	Lactate	0.6		0.8		0.6		0.6			

All cultures were grown in Baker's solution containing 100 mg. per cent glucose, with modifications as shown.

Figures represent total glucose used and total lactate appearing in an unchanged medium except in group C (daily change), where the figures represent daily glucose consumption and lactate formation.

glucose usually appeared as lactic acid. On the 14th day and last day, the proportion had risen to 96 per cent; at this time the cells were healthy but there was extensive liquefaction of the plasma.

D. In the absence of glucose: 1 experiment was done, with 3 determinations on 3 cultures. Lactic acid was formed, but reached only 15 mg. per cent. It is likely that this rise is associated with tissue degeneration.

E. At "constant" glucose level: 2 experiments were done, with 5 deter-

minations on 6 cultures. When glucose was maintained near 100 mg. per cent by daily additions, lactic acid rose progressively.

F. Added *dl*-lactate: 17 mg. per cent was originally included in the culture medium. Determinations on 5 cultures showed that the lactic acid content first rose and then fell to the original level, but not below. This was true both with and without glucose present.

Typical experiments illustrating these findings are shown in Table IV. Results are given in milligrams per 100 mg. tissue in order to make the findings of different experiments comparable.

Pyruvate Utilization

2 experiments comprising 10 cultures and 10 determinations were conducted with 50 mg. per cent pyruvic acid (neutralized) added to the medium, one in the presence of the usual amount of glucose, the other without glucose. The fall in bisulfite-binding values of both media showed rapid utilization of added pyruvate, with values close to zero after 28 hours. Incubation of the pyruvate medium without tissue caused little fall in bisulfite bound, and so it appears that with and without glucose the tissue rapidly disposes of pyruvate. In the absence of glucose, pyruvate allowed slight but definite growth to occur, as with lactate. This growth was much less than that seen with a corresponding amount of glucose, which is confirmed by the mitotic counts shown in Table III.

DISCUSSION

To understand these results we must consider carefully the test object. The life of a tissue culture includes four periods whose chief attendant features are: (1) injury of removal, (2) tissue growth, (3) regressive change, retardation of cell division, and visible alterations such as vacuolation, and (4) degeneration and autolysis.

In the period of injury we have largely intact cells plus those which have been cut or injured so that they die. The injured cells may reach 50 per cent of the amount explanted (15). Cell contents, autolytic products, and intracellular enzymes are thus added to the medium. This probably continues for a few hours under normal conditions, after which the metabolism of the culture is that of intact growing cells.

Thus we are dealing with the activity of a complex physiological system similar to that in the whole animal. The substrate is outside an intact cell surface, and localization of enzyme systems within the cell is preserved. In tissue extract experiments catalyzed reactions are occurring in a single phase system without cells, while in tissue slice experiments observations are made during the period of injury and under conditions precluding recovery and growth. Hence study of tissue cultures during the period of growth gives a more faithful picture of metabolic reactions in intact cells.

It is to be expected that an unchanged medium loses efficiency by loss of essential constituents and by accumulation of end products. The increased life of cultures bolstered by additions of glucose to an otherwise unchanged medium indicates that glucose loss may be a limiting factor in this regard. Daily changes of the whole medium increase the life span of cultures still further.

The amount of glucose used appears from our observations to have no direct relation to growth. Cultures treated with colchicine in concentrations which block all mitoses continue to use glucose at the usual rate. Moreover cultures grown in 500 mg. per cent glucose use more than those grown simultaneously in 100 mg. per cent, but examination of the fixed tissues at various periods shows about the same area of migration and closely similar mitotic counts in both groups. This observation appears to be in contrast to those of some previous workers whom we have quoted; the difficulties involved in estimating true growth in cultures must be emphasized. Probably the energy requirements for life and growth are easily supplied, and higher amounts of glucose do not increase growth rate because it is already limited by factors such as permeability, rate of intracellular synthetic reactions, or availability of essential foodstuffs. The enzyme systems for disposal of glucose appear to be stimulated to greater activity, possibly by a simple mass law effect. Conversion to glycogen, either in muscle or liver, has not been demonstrated by staining methods in our cultures. Presumably more energy is evolved and somehow absorbed when the rate of glucose removal is increased.

Glycolysis appears to be associated with the period of growth, for lactic acid production was related in rate and amount to glucose utilization. Lactate is also produced during the period of regressive change. While the amount evolved is less than in a healthy culture, it tends to represent a greater proportion of the total metabolism. The small amount of lactate formed in cultures not supplied with glucose increased with time and with the onset of degeneration.

Our experiments afford some evidence that tissues can utilize lactate after their glucose is used up. At this point lactate values frequently showed a tendency to fall from levels reached earlier in the life of the culture. When *dl*-lactate was added to cultures without glucose no loss was observed, but microscopic observations showed more migration and less vacuolation with than without lactate. Moreover many mitoses were seen after 2 days when control cultures showed no dividing cells (see Table III) indicating that in cultures deprived of glucose lactate favors cell division although it is little used. Degeneration was delayed for about 2 days by added lactate. Pomerat and Willmer (12) report similar findings in cultures of osteoblasts without glucose to which lactate was added.

Lactate found in cultures without glucose may originate from glucose formed from certain amino acids. In support of this hypothesis is the fact that in an

amino acid medium without glucose tissues produced over ten times as much urea plus ammonia nitrogen as those kept supplied with glucose. Deamination of certain amino acids prior to their conversion to glucose would explain this difference.

Although utilization of nitrogen must obviously occur during growth, the quantitative demonstration of this process when amino nitrogen is practically the only available form of nitrogen is of special interest. There has been considerable question as to whether cells in culture could make use of nitrogen supplied as amino acids, although Vogelaar and Erlichman (16, 17) believe this may be possible under optimal conditions.

The definite morphological stigmata of cells grown in our amino acid medium indicate that the medium affects certain processes differently from a peptone medium. Vogelaar and Erlichman believe that amino acids may be preferentially adsorbed on the cell surface to such an extent as to interfere with normal processes. In this case morphological changes may result from specific effects of the amino acids upon the cell surface. It must be considered, however, that these variations may be explained by an altered metabolism within the cell: in the case of a peptone medium nitrogen may be taken up in the form of peptides and utilized as such, while amino acids taken up directly might follow a different course. Although our experiments have not yielded proof of the utilization of nitrogen in peptide form, morphological observation suggest this possibility.

EXPERIMENTAL

Planting Technique.—Tissue to be explanted was removed from the embryo under the usual sterile conditions and placed in small, weighed, sterile Petri dishes. It was then cut up and weighed to the nearest 0.1 mg. as rapidly as possible. Tyrode solution was then added and the tissue transferred to the inside surface of a roller bottle previously coated with a thin film of plasma, which formed a holding clot when in contact with the tissue. Care was taken to introduce all the weighed tissue into the bottle, and to remove the entire excess of plasma and Tyrode solution used in the transfer before adding the culture medium. Thus a known wet weight of tissue was explanted.

General Procedure.—Bottles were placed in the roller apparatus in an incubator at 37°C., removed serially at intervals up to 7 or more days, and the entire fluid decanted for analysis. Thus a progressive picture of events was obtained. For some determinations it was possible to sample one bottle at intervals without greatly changing the volume. Results obtained in this way showed great reproducibility, especially in the case of glucose utilization.

All cultures were examined microscopically every day. This necessitated no interference with their environment except when media were sampled or changed. Areas of fibroblastic and epithelial migration were qualitatively noted, as well as the extent of changes such as rounding of cells, vacuolation, liquefaction, or focal or generalized degeneration. The migration rate of cells was estimated when desired by measure-

ments on camera lucida drawings of explants. At the end of each experiment the cultures were fixed, the coverslip was removed by immersion in acetone, and the tissue was stained with Harris' hematoxylin and mounted. Microscopic examination was then made and in certain cases mitoses were counted in groups of 1000 peripheral cells. Typical mitotic counts are given in Table III.

Ordinarily, tissue cultures under standard conditions grew normally for at least 3 days with mitoses decreasing on the 3rd day. After 5 days mitoses were usually absent in unchanged media, and rounding or vacuolation had begun, but little frank degeneration occurred until later. During the period of most active growth the daily radial increase was 0.5 to 0.8 times the radius of the explant, and about 1.5 per cent of the cells were in mitosis.

Analytical Methods.—All determinations of changes in composition of media must of course be controlled by parallel determinations on original media. This is especially true since observed values for some metabolites in Baker's solution differ from those in solution in water. A substantial non-glucose-reducing value is found which is not diminished by tissues in the absence of glucose, and for which corrections must be applied. Interfering substances also give values for lactate, and affect lactate determinations so that special modifications are necessary. The metabolites listed below were determined by the following methods.

Glucose.—Folin's micro method using potassium ferricyanide reduction, as adapted for the Evelyn photoelectric colorimeter.

Total Nitrogen.—Kjeldahl digestion and distillation followed by titration with 0.02 N alkali.

Amino Nitrogen.—The Van Slyke gasometric procedure, using the manometric gas apparatus.

Ammonia Plus Urea Nitrogen.—The manometric sodium hypobromite method of Van Slyke (18).

Lactic Acid.—The gasometric procedure of Avery and Hastings (19), using the Van Slyke manometric apparatus, was first employed. For reasons of greater specificity and convenience our later determinations have been done by Edwards' modification of the aeration method of Friedemann, Cotonio, and Shaffer (20, 21). We further, altered the procedure partly according to the suggestions of Friedemann and Kendall (22) for peptone media, using 0.06 M phosphoric acid and 0.025 N potassium permanganate. Each of these methods was carefully developed as to time of reaction, etc., to give nearly quantitative recovery of lithium lactate added to Baker's solution, and substantial agreement was obtained when both methods were applied to the same solution. Even so, our results are indicative of changes in lactate rather than true values, and are probably accurate to not more than $\pm$ 10 per cent.

Pyruvate.—The bisulfite-binding method of Clift and Cook (23).

Preparation of Amino Acid Solution.—Witte's peptone was hydrolyzed with 20 per cent sulfuric acid until amino nitrogen had risen to an almost constant level of 78 per cent of the total nitrogen. Barium hydroxide was added to pH 7, and the precipitate of barium sulfate filtered off and washed. The filtrate was again carefully adjusted with sulfuric acid and barium hydroxide to the lowest obtainable content of both barium and sulfate ions. The final filtrate was concentrated as far as possible without causing precipitation of insoluble amino acids.

SUMMARY

1. The metabolism of chick embryo tissues has been followed by analysis of the culture media after various periods of incubation in roller bottles.

2. The initial rate of glucose utilization is increased by increasing glucose in the medium from 100 to 500 mg. per cent. Total glucose used can be increased in the same way or by daily addition of small amounts. Glucose is used in greatest amount when the medium containing 100 mg. per cent is replaced daily.

3. Although glucose consumption appears necessary for survival of cultures it may be used at a rate far in excess of that required for life and maximal growth. Complete blocking of mitosis by colchicine does not alter the rate of glucose utilization.

4. Proteolytic activity of the cultures is shown by an increase in the amino nitrogen of the peptone medium after incubation with tissue.

5. Utilization of nitrogen from an amino acid medium is shown by a decrease in the amino nitrogen of this medium. Cells obtaining their nitrogen from amino acids proliferate as rapidly as those grown in a medium identical except for the substitution of peptone, but the cell type is markedly different, in that embryo muscle forms cells resembling regenerating adult muscle.

6. Lactic acid was formed in both the presence and absence of glucose. Its formation increased with increased glucose utilization. There is some evidence that lactate may be utilized, and that it favors growth in the absence of glucose.

7. Added pyruvate was rapidly metabolized by the tissues. It, too, favors growth slightly in the absence of glucose.

BIBLIOGRAPHY

1. Shaw, D. T., Kingsland, L. C., and Brues, A. M., *Science*, 1940, **91**, 148.
2. Baker, L. E., *Science*, 1936, **83**, 605.
3. Krontowski, A. A., *Arch. exp. Zellforsch.*, 1931, **11**, 93.
4. Lebensohn, E. G., *Arch. exp. Zellforsch.*, 1934, **16**, 264.
5. Demuth, F., *Arch. exp. Zellforsch.*, 1931, **11**, 98.
6. Ebeling, A. H., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 886.
7. Willmer, E. N., *J. Exp. Biol.*, 1927, **4**, 280.
8. Gemmill, C. L., Gey, G. O., and Austrian, R., *Bull. Johns Hopkins Hosp.*, 1940, **66**, 167.
9. Carrel, A., and Baker, L. E., *J. Exp. Med.*, 1926, **44**, 503.
10. Demuth, F., and Meier, R., *Biochem. Z.*, Berlin, 1929, **212**, 399.
11. Meier, R., *Biochem. Z.*, Berlin, 1931, **231**, 253.
12. Pomerat, C. M., and Willmer, E. N., *J. Exp. Biol.*, 1939, **16**, 232.
13. Lipmann, F., *Biochem. Z.*, Berlin, 1932, **244**, 177.
14. Lipmann, F., *Biochem. Z.*, Berlin, 1933, **261**, 157.
15. Brues, A. M., Cohn, W. E., and Wilson, H., unpublished data.

16. Vogelaar, J. P. M., and Erlichman, E., *Am. J. Cancer*, 1936, **28,** 301.
17. Vogelaar, J. P. M., and Erlichman, E., *Am. J. Cancer*, 1938, **33,** 246.
18. Peters, J. P., and Van Slyke, D. D., Quantitative clinical chemistry, Baltimore, The Williams & Wilkins Co., 1932, **2,** 379.
19. Avery, B. F., and Hastings, A. B., *J. Biol. Chem.*, 1931, **94,** 273.
20. Friedemann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, **73,** 335.
21. Edwards, H. T., *J. Biol. Chem.*, 1938, **125,** 571.
22. Friedemann, T. E., and Kendall, A. I., *J. Biol. Chem.*, 1929, **82,** 23.
23. Clift, F. P., and Cook, R. P., *Biochem. J.*, London, 1932, **26,** 1788.