Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 73 JANUARY, 1950 NO. 1

SECTION MEETINGS

CLEVELAND Western Reserve University	November 11 and 14, 1949
DISTRICT OF COLUMBIA George Washington University	December 1, 1949
MINNESOTA University of Minnesota	December 22, 1949
NEW YORK New York Academy of Medicine	December 14, 1949

Nutrition of Animal Cells in Tissue Culture. I. Initial Studies on a Synthetic Medium.*† (17557)

JOSEPH F. MORGAN, HELEN J. MORTON, AND RAYMOND C. PARKER.
From the Connaught Medical Research Laboratories, University of Toronto.

Over the past 30 years, the nutritive media that have been employed for the propagation of animal cells in tissue culture have been derived, directly or indirectly, from the organism and have consisted of blood plasma, blood serum, body exudates and extracts of various tissues and organs. But the complexity and variability of these natural substrates has made it almost impossible to employ them in experiments designed to determine the substances that are actually utilized by the cells for survival and multiplication. From time to time, attempts have been made to devise nutritive media comprised of chemically-known ingredients. Thus, Vogelaar and Erlichman(1), Baker(2), and Baker and Ebeling(3), have reported feeding solutions for fibroblasts, epithelial cells and blood macrophages. These media consisted in part of chemically-defined substances, but the addition of serum or other natural substrates was necessary for cell multiplication and survival. Fischer and his associates(4) employed dialyzed plasma and dialyzed chick embryo extract as a base and devised supplementary solutions consisting of compounds of known

* This investigation was supported, in part, by grants from the National Cancer Institute of Canada, and the Ontario Cancer Treatment and Research Foundation.

† Grateful acknowledgment is made to Mrs. M. G. Pearce and Mrs. C. J. Porter for technical assistance.

1. Vogelaar, J. P. M., and Erlichman, E., *Am. J. Cancer*, 1933, v18, 28.

2. Baker, L. E., *Science*, 1936, v83, 605.

3. Baker, L. E., and Ebeling, A. H., *J. Exp. Med.*, 1939, v69, 365.

4. Fischer, A., Astrup, T., Ehrensvard, G., and Oehlenschlager, V., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 40.

biological importance to replace the accessory growth factors removed during dialysis. White(5) reported a culture fluid consisting of constituents of known composition that supported the life of relatively large masses of chick embryo tissue for several weeks. It was found by Jacoby and Darke(6), however, that it was necessary to add serum to White's mixture in order to support the life and activity of pure cultures of blood macrophages.

It is the purpose of the present communication to report the first of a series of studies made in this laboratory over the past 2 years in an attempt to devise a synthetic medium of chemically-known composition that would promote continuous cell multiplication of small amounts of tissue in the absence of blood serum and embryo extract. Although a completely adequate synthetic mixture has not yet been achieved, the results thus far obtained are sufficiently encouraging to justify the present report.

Materials and Methods. All cultures were prepared from the leg muscle of 11-day-old chick embryos. After the tissue had been chopped with cataract knives into extremely fine fragments, and the fragments had been washed with Earle's modification of Tyrode's solution(7), very small amounts of the resulting suspension were transferred with a pipette to the inner surface of standard pyrex test tubes (18 × 150 mm). The culture fluid (2 ml) was then placed in the bottom of the tubes, care being taken to avoid wetting the tissue, the pH was adjusted to 7.2 with a gas mixture consisting of 8% CO_2, 21% O_2 and 71% N_2, and the tubes were tightly stoppered. It was found that a period of 1 hour was required for the tissue to become firmly attached to the glass surface of the tubes before the fluid medium could be washed over it. As soon as the tissues were flooded with the culture fluid, the tubes were inserted in an almost horizontal position in a rotating drum(8) at 38°C.

All cultures received a preliminary feeding mixture containing naturally-occurring ingredients (horse serum and embryo extract) when first made and were left in the rotating drum for an initial period of 3 to 5 days. At the end of this time, they were examined microscopically and any unsuitable cultures were discarded. The remaining cultures were divided into groups of 5 or more, and the preliminary feeding mixture was replaced by various synthetic mixtures. These mixtures were changed 3 times a week. The cultures were examined microscopically at frequent intervals and careful record was made of the development of new tissue, breakdown of the original tissue, and the general appearance of the individual cells. Cultures were maintained in the synthetic mixtures until living cells were no longer apparent. The average survival time of the cultures in each group of a series (Table I) was considered to be a rough indication of the suitability of the experimental media. Throughout the experiments, certain cultures were continued in the preliminary feeding mixture (containing horse serum and embryo extract) as positive controls, and in Earle's solution alone as negative controls.

By careful refinements of technique, it was found possible to decrease progressively the amount of embryo tissue added to the roller tubes. In all of the experiments to be reported, cultures were made with the minimum amount of tissue (Fig. 1) that would yield extensive cell growth.

The non-synthetic, *preliminary feeding mixture* consisted of 4 parts horse serum, 6 parts

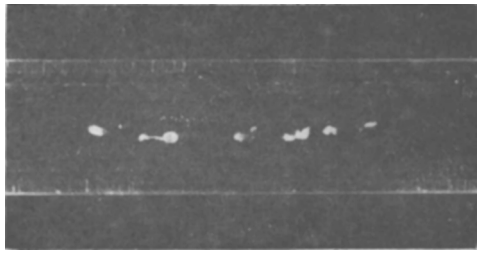

FIG. 1.
Portion of 18 × 150 mm test tube showing amount of tissue employed in preparation of typical culture; photographed after 18 hr incubation. ×1.

5. White, P. R., *Growth*, 1946, v10, 231.
6. Jacoby, F., and Darke, S. J., *Nature*, 1948, v161, 768.
7. Earle, W. R., *J. Nat. Cancer Inst.*, 1943, v4, 165.
8. Gey, G. O., *Am. J. Cancer*, 1933, v17, 752.

Earle's solution, and final concentrations of 1% chick embryo extract(9) (in Earle's solution) and 0.002% phenol red.

The *components of the synthetic media* were of the highest grade obtainable commercially and were employed without further purification. It was found most convenient to prepare a number of basic stock solutions and to combine these in various proportions.

Sol. A: *l*-Arginine monohydrochloride, 14.0 mg; *l*-histidine monohydrochloride, 4.0 mg; *l*-lysine monohydrochloride, 14.0 mg; *dl*-tryptophane, 4.0 mg; *dl*-phenylalanine, 10.0 mg; *dl*-methionine, 6.0 mg; *dl*-serine, 10.0 mg; *dl*-threonine, 12.0 mg; *dl*-leucine, 24.0 mg; *dl*-isoleucine, 8.0 mg; *dl*-valine, 10.0 mg; *dl*-glutamic acid monohydrate, 30.0 mg; *dl*-aspartic acid, 12.0 mg; *dl*-α-alanine, 10.0 mg; *l*-proline, 8.0 mg; *l*-hydroxyproline, 2.0 mg; glycine, 10.0 mg; *l*-glutamine, 20.0 mg; sodium acetate, 16.3 mg. These ingredients were dissolved in 100 ml of double-strength Earle's solution.

Sol. B: *l*-Tyrosine, 200 mg; *l*-cystine, 100 mg. These substances were dissolved with gentle heating in 100 ml of 0.075 N HCl.

Sol. C: Niacin, 25.0 mg; niacin amide, 25.0 mg; pyridoxine hydrochloride, 25.0 mg; pyridoxal hydrochloride, 25.0 mg; thiamin hydrochloride, 10.0 mg; riboflavin, 10.0 mg; calcium pantothenate, 10.0 mg; *i*-inositol, 50.0 mg; *p*-aminobenzoic acid, 50 mg; choline chloride, 500.0 mg; glass-distilled water to 200 ml.

Sol. D: Cysteine hydrochloride, 100.0 mg; glutathione, 50.0 mg; ascorbic acid, 50.0 mg; glass-distilled water to 100 ml. A fresh solution was prepared each month.

Sol. E: *d*-Biotin, 10.0 mg; glass-distilled water to 100 ml.

Sol. F: Folic acid, 10.0 mg; Earle's solution to 100 ml.

Sol. G: Vitamin A, 10.0 mg; ethyl alcohol (95%), 1.0 ml; Tween 80 (5% aqueous solution), 10.0 ml; glass-distilled water to 100 ml. A fresh stock solution was prepared each month.

Sol. H: Calciferol (vit. D_2), 10.0 mg; ethyl alcohol (95%), 1.0 ml; Tween 80 (5% aqueous solution), 10.0 ml; glass-distilled water to 100 ml.

Sol. I: Disodium α-tocopherol phosphate,‡ 10.0 mg; glass-distilled water to 100 ml.

Sol. J: Menadione (vit. K), 10.0 mg; glass-distilled water to 100 ml.

Sol. K: Cholesterol, 10.0 mg; ethyl alcohol (95%), 10.0 ml; Tween 80 (5% aqueous solution), 10.0 ml; glass-distilled water to 100 ml.

Sol. L: Adenine sulfate, 500.0 mg; glass-distilled water to 100 ml.

Sol. M: Guanine hydrochloride, 10.0 mg; xanthine, 10.0 mg; hypoxanthine, 10.0 mg; thymine, 10.0 mg; uracil, 10.0 mg. These substances were dissolved in 100 ml glass-distilled water with the addition of 0.35 ml concentrated NH_4OH.

Sol. N: *d*-Ribose, 100.0 mg; *d*-2-desoxyribose, 100.0 mg; glass-distilled water to 100 ml. A 1.0 ml amount of this solution was incorporated in each liter of Solution A.

Sol. O: Adenylic acid (from muscle), 100.0 mg; glass-distilled water to 100 ml. A 4.0 ml portion of this solution was incorporated in each liter of Solution A.

Sol. P: Adenosinetriphosphate (dibarium salt), 67.6 mg. This substance was dissolved in 50 ml of 0.01N HCl. The barium was precipitated by the addition of a slight excess of saturated Na_2SO_4 solution, removed by centrifuging, and the clear supernatant was diluted to 100 ml with glass-distilled water. A fresh solution was prepared each week.

Sol. Q: $Fe(NO_3)_3 \cdot 9H_2O$, 14.5 mg; glass-distilled water to 100 ml.

Stock solutions A, B, K, L, M, P and Q were employed without further dilution. Solutions C, G, H and J were diluted 1:10 in glass-distilled water before use. Solutions D, E, F and I were diluted 1:100 in glass-distilled water.

All solutions used in the preparation of the synthetic mixtures were sterilized by passage through UF fritted glass filters (Corning). Stock solutions and the combined mixtures were stored in low-actinic glassware. The

9. Morgan, J. F., and Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 665.

‡ Obtained through the courtesy of Dr. J. G. Baxter, Distillation Products, Inc., Rochester, N.Y.

TABLE I.
Average Survival Time of Chick Embryo Roller-tube Cultures in 6 of the 199 Synthetic Mixtures That Were Tested.

Mixture No.	Ingredients	No. of experiments	Total No. of cultures*	Avg survival (days)
Balanced salt mixture	Earle's modification(7) of Tyrode's solution (incorporated in all subsequent mixtures)	6	23	6.6
22	Amino-acid mixture	5	23	14.5
81	Mixture No. 22 + vitamins and Tween 80	5	18	16.8
88	Mixture No. 81 + purines, pyrimidines and cholesterol	5	22	23.4
148B	Mixture No. 88 + ATP, adenylic acid, sodium acetate, glutamine, ribose, and desoxyribose	12	70	28.0
199	Mixture No. 148B + ferric nitrate	11	60	33.0†

* Approximately 3,000 cultures were used to test all of the mixtures studied.
† A few cultures in this mixture have survived beyond 70 days.

majority of these solutions were kept at 4°C, but certain stock solutions, *e.g.* adenine, cholesterol, tyrosine and cystine, which tended to form precipitates in the refrigerator, were stored at room temperature.

In the preparation of the synthetic mixtures, 25 ml of the amino acid basal mixture (Solution A) were taken, and to this were added appropriate volumes of the other stock solutions. Sufficient glass-distilled water was then added to bring the total volume to 50 ml. The isotonicity of the media was confirmed by freezing-point determinations. Each new ingredient, or group of ingredients, added to the basal solution was first studied over a wide range of concentrations to detect possible toxic effects, and was finally incorporated in the mixture at a level that was either beneficial or, at least, non-toxic.

Experimental. Amino acids. Preliminary experiments were carried out in which an acid hydrolyzate of casein, fortified with tryptophane, served as the sole source of nutrient. This solution proved to be completely inadequate, since it supported cell life for only 5 to 7 days. An amino acid mixture was then prepared from pure constituents and was made up in proportions based on analyses of tissue proteins(10). This mixture was tested at several concentrations over the range of 500 to 50 mg/100 ml total amino acids. A level of 100 mg/100 ml was found to be most suitable. At this concentration, cultures remained alive for an average period of 14 days (Table I), while at levels above and below this amount the duration of life was somewhat shorter.

Vitamins. The basal amino acid mixture was next supplemented by the addition of vitamins (Solutions C to J). These were tested over a wide range of concentrations, some singly and some in groups. The addition of vitamins was found to improve the appearance of the individual cells, but was not observed to increase the amount of new tissue.

In these early vitamin experiments, considerable difficulty was encountered with vitamins A and D. The stock solutions of these substances were made up in 95% ethyl alcohol, and the vitamins tended to precipitate out when they were added to the aqueous basal mixture. It was also observed that the presence of more than a trace of these solutions

10. Block, R. J., and Bolling, D., *The Amino Acid Composition of Proteins and Foods*, Springfield, Ill., Thomas, 1945.

markedly decreased the survival period of the cultures. This was attributed to the toxic effect of ethyl alcohol upon the tissue cells.

Tweens. The surface active agents of the Tween series were next investigated as a possible means of supplying the tissues with a water-soluble source of fatty acid. Extensive experiments, to be published separately, have established that concentrations ranging from 0.005 to 0.0005% were suitable for use in the cultures. Since it was considered possible that the cells might require a source of *unsaturated* fatty acid, it was decided to incorporate Tween 80 (containing oleic acid) in all subsequent synthetic mixtures. The surface activity of this compound was also utilized to dissolve fat-soluble compounds. It was found that if vitamin A, vitamin D and cholesterol were first dissolved in a minimal amount of 95% ethyl alcohol, and Tween 80 was added subsequently (Solutions G, H, K), these substances remained in solution and did not precipitate even in high dilution with distilled water.

The addition of vitamins A and D, in Tween 80, to the synthetic mixture was found to prolong the survival time of the cultures and to improve the appearance of the cells. Whether this beneficial effect can be attributed to the Tween 80 or to the elimination of all but minute traces of alcohol from the media has not yet been established.

Nucleic Acid Constituents. Since the synthetic mixture now contained amino acids, vitamins and a source of fatty acid, attention was next directed to the constituents of nucleic acid, with particular emphasis upon the purine and pyrimidine bases. Adenine sulfate was not found to exert any marked effect when tested alone at concentrations from 10 to 0.1 mg/100 ml. A purine and pyrimidine solution, containing guanine hydrochloride, xanthine, hypoxanthine, thymine and uracil, was found to be toxic at 6.0 mg/100 ml, but to be slightly beneficial at 0.6 and 0.06 mg/100 ml. Adenine sulfate at the 1.0 mg/100 ml level, however, resulted in some improvement when added to a mixture in which the purines and pyrimidines had already been incorporated. The pentose sugars, ribose and desoxyribose, did not appear to influence the survival time of the cultures, but were added to the medium in order to complete the list of nucleic acid components.

Accessory Growth Factors. In addition to the several groups of compounds already mentioned, a number of accessory growth factors were also tested for possible stimulating effects upon the cultures; and these were incorporated in the synthetic mixture. Even though some of them showed no apparent beneficial effect when tested singly, there was a progressive improvement that could be attributed to various combinations of these metabolites.

Cholesterol was dissolved in Tween 80, as described previously, and was added to the basal mixture at concentrations from 2.0 to 0.02 mg/100 ml. No effect upon the cultures was noted.

Sodium acetate was tested at concentrations ranging from 5.0 to 0.0005%. Levels of 5.0 and 0.5% were found to be toxic, while lower concentrations had no appreciable effect.

An adenosinetriphosphate (ATP) solution was prepared, as already described, and this compound was tested on the cultures at levels of 100 to 0.01 μg per ml. No effect upon the cultures was noted at any concentration studied.

Adenylic acid (from muscle) was used over the same range of concentrations employed with ATP, but it had no apparent effect upon the cultures. A number of experiments were carried out in which the concentrations of ATP and of adenylic acid were varied in respect to each other. Under these conditions, also, no appreciable effect upon the cultures was observed.

Glutamine was incorporated in the synthetic mixtures at concentrations ranging from 100 to 10 mg/100 ml, and appeared to be slightly beneficial at the highest level tested. Later experiments indicated that this concentration was not effective when added to synthetic mixtures containing ATP and adenylic acid. In the presence of these metabolites, however, considerable improvement was exerted by a lower level of glutamine. This beneficial effect was greater than that obtained previously by the highest level of glutamine in the absence of ATP and adenylic acid. Glutamine, ATP

and adenylic acid were finally added to the synthetic mixture in the proportions that had given this marked improvement.

Trace Metals. Although it is planned to study the possible effects of adding various trace metals to the synthetic mixture, iron is the only one that has so far been tested. Solutions of ferric nitrate and ferrous sulfate were tested over wide ranges of concentration. The results appeared to indicate that low concentrations of both salts were slightly beneficial. Ferric nitrate was finally selected for use in the synthetic mixture (Table I) because it appeared to be the more stable of the two.

Synthetic Mixture No. 199. Since these experiments had established the optimal concentrations of the individual substances tested, it was now possible to combine them as shown in Table II. Embryo tissue cultivated in this mixture was found to put out extensive areas of new growth. This was in striking contrast to the results obtained with simpler mixtures. The majority of the cells that grew out from the original tissue fragments cultivated in Mixture 199 were large, flat and spindle-shaped. They did not develop excessive granulation for about 3 weeks, after which time many of the cultures underwent progressive degeneration.

In repeated experiments, Mixture 199 supported cell life for an average period of 4 to 5 weeks, and certain cultures survived for more than twice that time. Further studies are being undertaken in order to determine, if possible, the additional substances that may be required to make Mixture 199 completely adequate for continuous cell multiplication. The effect of supplementing this medium with small amounts of serum and embryo extract is also under investigation.

Discussion. The studies reported in the present communication, which include the results obtained with approximately 3000 cultures, have shown a progressive improvement in the survival of the tissues that have been cultivated. Although the medium that has been developed is not entirely adequate, it will support cell life for an average period of 4 to 5 weeks and has made it possible to make a number of interesting observations on cell nutrition.

TABLE II.
Synthetic Mixture No. 199.*

	Mg per 1000 ml
l-Arginine	70.0
l-Histidine	20.0
l-Lysine	70.0
l-Tyrosine	40.0
dl-Tryptophane	20.0
dl-Phenylalanine	50.0
l-Cystine	20.0
dl-Methionine	30.0
dl-Serine	50.0
dl-Threonine	60.0
dl-Leucine	120.0
dl-Isoleucine	40.0
dl-Valine	50.0
dl-Glutamic acid	150.0
dl-Aspartic acid	60.0
dl-Alanine	50.0
l-Proline	40.0
l-Hydroxyproline	10.0
Glycine	50.0
Cysteine	0.1
Adenine	10.0
Guanine	0.3
Xanthine	0.3
Hypoxanthine	0.3
Thymine	0.3
Uracil	0.3
Thiamin	0.010
Riboflavin	0.010
Pyridoxine	0.025
Pyridoxal	0.025
Niacin	0.025
Niacinamide	0.025
Pantothenate	0.01
Biotin	0.01
Folic acid	0.01
Choline	0.50
Inositol	0.05
p-Aminobenzoic acid	0.05
Vitamin A	0.10
Calciferol (Vit. D)	0.10
Menadione (Vit. K)	0.01
a-Tocopherol phosphate (Vit. E)	0.01
Ascorbic acid	0.05
Glutathione	0.05
Tween 80 (oleic acid)	20.0
Cholesterol	0.2
Sodium acetate	50.0
l-Glutamine	100.0
Adenosinetriphosphate	10.0
Adenylic acid	0.2
Iron (as ferric nitrate)	0.1
Ribose	0.5
Desoxyribose	0.5

* This mixture also contained a modified Tyrode's solution.

The amino acid requirements of tissue cells have been investigated extensively by Fischer(11), who has shown that cystine is most essential and that arginine, tryptophane, glu-

11. Fischer, A., *Biochem. J.*, 1948, v43, 491.

tamic acid and lysine are also required. On the basis of Fischer's work, the present finding that a mixture of 19 amino acids is greatly superior to an acid hydrolyzate of casein might possibly be attributed to an increased level of cystine, since casein is known to be deficient in this particular amino acid(10). Moreover, Fischer's results indicate that the concept of "essential" and "non-essential" amino acids does not apply to tissue cultures. Hence, the inclusion of all the naturally-occurring amino acids would seem to be a desirable feature of Mixture 199. It should also be pointed out that the medium reported by White(5) contains only the "essential" amino acids and does not include cystine.

The amino acid mixture finally employed in the present experiments contained 11 *dl* (the more easily available) amino acids and 7 of the natural *l* configuration. Comparative tests were carried out with a similar mixture made up entirely of *l* amino acids.§ But since experiments made with the same range of concentrations failed to show any significant differences in the results obtained with the two solutions, it was concluded that the *d* amino acids were not growth inhibiting.

The present studies did not afford any clear-cut evidence that vitamins are required by cells in tissue culture. It seemed advisable, however, to include a complete vitamin supplement in Mixture 199 in the hope that beneficial effects might be evident when the mixture became more complete. It was felt that the preliminary media should include as many known nutritional factors as possible, even though many of them had no apparent effect. If an adequate synthetic medium could be devised in this manner, the unnecessary substances could eventually be eliminated. The results shown in Table I would seem to justify this method of approach.

The addition of nucleic acid constituents, the purines, pyrimidines and pentose sugars, to the basal solution was found to be beneficial. The incorporation of these compounds into Mixture 199 would seem to constitute an improvement over other synthetic mixtures that have been devised. White's medium contained no nucleic acid derivatives; and Fischer's solution included only hypoxanthine and inosinic acid. The important role that has been assigned to the nucleic acids in cellular metabolism(12,13) would suggest that any synthetic medium for animal tissue cells should contain either the nucleic acids themselves or substances from which they may be synthesized by the cells.

It was hoped that the addition of various intermediary metabolites and accessory growth factors to the basal synthetic mixture might increase very greatly the survival time of the cultures through a stimulation of the cellular enzyme systems involved in the synthesis of protoplasm. For this reason, great importance was attached to the incorporation of adenosinetriphosphate, which is known to activate many metabolic processes(14). The fact that this compound, and also adenylic acid, failed to exert significant effects upon the survival time of the cultures was rather disappointing. It is possible, however, that stimulation by ATP may occur only in the presence of phosphorylated intermediates of the glycolytic cycle. Thus, Fischer and his associates(4) found that whereas ATP did not stimulate growth, fructose diphosphate was definitely beneficial. A further study of this interrelationship is being conducted at the present time.

The addition of glutamine to the basal mixture was observed to increase the life span of the cultures, but the improvement observed was not as great as that reported by Fischer and his associates(4). It should be pointed out, however, that the present experiments were made with a basal mixture that contained a high level of glutamic acid, whereas Fischer's results were obtained in the absence of glutamic acid.

When the present studies were undertaken, it was anticipated that certain interrelation-

§ *l*-Threonine and *l*-valine were obtained through the courtesy of Dr. Marvin Darrach, Merck and Co., Ltd., Montreal, Canada.

12. Davidson, J. N., *Symposia Soc. Exp. Biol.*, 1947, v1, 77.

13. Davidson, J. N., *Cold Spring Harbor Symposia Quant. Biol.*, 1947, v12, 50.

14. Potter, V. R., *Advances in Enzymology*, 1944, v4, 201.

ships and possible antagonisms might develop between various groups of growth factors. The only instance of such effects encountered up to the present time is that between glutamine and ATP plus adenylic acid. No explanation of this interesting relationship is available at the present time.

The necessity of cultivating the tissues in naturally-occurring nutritive media for 3 to 5 days prior to the replacement of these natural media by synthetic mixtures is an undesirable feature of the present experiments. During this preliminary period of cultivation, the cells might be able to store up reserve food substances that would serve as a source of nutrient during the subsequent period of cultivation in synthetic media. Still, tissues cultivated in Earle's solution alone degenerated and died within 6 or 7 days (Table I). This would seem to indicate that the effects of the initial period of maintenance in the preliminary feeding mixture are not exerted long after this original fluid is removed. Eventually it is hoped that this early maintenance period in non-synthetic media may be eliminated.

The systematic addition of new ingredients to the synthetic mixture has seldom resulted in a marked improvement that could be attributed to any one substance. But periodic comparisons of the effect of the more recent test solutions with simpler mixtures that had been used earlier (Table I) have shown a progressive increase in the survival time of the cultures and a marked improvement in their appearance. Thus, for example, results obtained with Mixture 199 were vastly superior to those obtained with mixtures containing only amino acids, vitamins and Tween 80.

The results reported in the present communication have established that synthetic Mixture 199 will support the survival of chick embryo tissues for an average period of 4 to 5 weeks. Although this mixture will not support the cells indefinitely, it does appear suitable for use as a basal synthetic medium for further investigations on cell nutrition.

Summary. The nutrition of animal cells in tissue culture has been studied with particular reference to amino acids, vitamins, nucleic acid constituents and various accessory growth factors. The optimal concentrations of these substances for the maintenance of cell life *in vitro* have been established and a completely synthetic feeding solution has been devised. Although this mixture will not support cell life indefinitely, it has been found adequate to maintain chick embryo cells for an average period of 4 to 5 weeks. The mixture is being used as a basal synthetic medium for further studies on the unidentified growth-promoting substances known to occur in natural media.

Received October 4, 1949. P.S.E.B.M., 1950, v73.

III. Influence of Renal Tissue in Inhibition of Protein Catabolism.* (17558)

EDWARD C. PERSIKE (Introduced by Arthur L. Bloomfield)

From the Department of Medicine, Stanford University School of Medicine, San Francisco, Calif.

Data are presented elsewhere(1) which show that the rate of protein catabolism, as measured by the rate of urea formation, is increased markedly in rats after sudden loss of 75% or all of the total renal tissue. A higher rate of protein catabolism was found in rats subjected to complete nephrectomy than in those with 25% of the total renal substance remaining. It was suggested that loss of 75% or all of the total renal tissue might release the mechanism responsible for

* This work was supported by a grant from the Columbia Foundation.

The author gratefully acknowledges technical assistance by Miss H. J. Ureen, Mr. W. Lew, Mr. L. J. Poo, and Mr. W. Wong.

1. Persike, E. C., and Addis, T., *Am. J. Physiol.*, 1949, v158, 149.