The importance of developing artificial media that can be used in the place of serum for maintaining the life of tissues and organs outside the body hardly needs to be emphasized. Many of the studies for which the organ culture technique was developed, as well as others that can be carried on by the simpler methods of tissue culture, depend for their success on the creation of suitable artificial media. These media are needed to reduce the cost of experimentation, to make possible extensive cultivation of human organs and those of small animals from which serum in sufficient quantity cannot be obtained, and for all studies in which the production of serum and other protein substances is to be investigated. For the latter purpose, media that are serumless and free from protein will be required. But for other work media that contain serum as one constituent may be used.

Several artificial media designed to promote rapid growth of cells in tissue culture have already been described. But these are not...
suitable for studies in which the functioning of organs and tissues is to be investigated. For such studies media that will maintain cells without promoting growth are required. The purpose of this report is to describe media devised for this purpose, and the results obtained when they were used to sustain the life of a pure strain of fibroblasts in vitro. Experiments in which they were used for organ cultivation will be described in another communication. 4

Composition of the Media

In the course of this work many different media with varying combinations of constituents have been used. For the sake of brevity only four will be described. Their composition will be given first, and then a description of the way in which they can be prepared.

Medium I.—
Whole blood digest, as described further on, in amount to give 30 to 60 mg. per cent nitrogen
Serum 2 or 3 per cent
Phenol red 5 mg.
Tyrode's solution

Medium II.—
Whole blood digest to give either 30 or 60 mg. per cent nitrogen per 100 cc.
Cysteine hydrochloride 9.0 mg.
Insulin 0.1 unit
Thyroxine 0.001 mg.
Hemin 0.004 mg.
Vitamin A (containing some D) 100.0 units dissolved in serum
Vitamin B1 0.1 gamma
Vitamin B2 3.4 gammas
Ascorbic acid 0.3 mg.
Glutathione 1.2 mg.
Glucose 200 to 300 mg.
Phenol red 5.0 mg.
Potassium iodide 0.13 mg.
Salts as in Tyrode's solution

4 Some experiments in which organs have been cultivated in these media have already been described by Carrel, A., and Lindbergh, C. A., The culture of organs, New York, Paul Hoeber, Inc., 1938.
5 The vitamin A was prepared from haliver oil and contained 1 unit vitamin D for each 5 units of vitamin A.
Many of the constituents used in this medium were selected because they had previously been found either by Vogelaar and Erlichman or by Baker to prolong the life of cells in artificial, growth-promoting media. The concentrations of the individual constituents have been adjusted to those that seemed best suited to maintenance.

To bring the vitamin A into a form in which it could be taken up by the cells, it was dissolved at high concentration in serum. Then a small amount of this serum, about 0.07 per cent, was used in the medium.

Medium III.---This contained all the constituents listed under medium II and in addition:

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophane</td>
<td>5 to 10 mg.</td>
</tr>
<tr>
<td>Witte's peptone to give</td>
<td>6.0 mg. nitrogen</td>
</tr>
<tr>
<td>Sodium glycerophosphate</td>
<td>57.5 mg.</td>
</tr>
<tr>
<td>Urea</td>
<td>2.4 mg.</td>
</tr>
<tr>
<td>Glycerine</td>
<td>0.2 cc.</td>
</tr>
<tr>
<td>Thymus nucleic acid(^7)</td>
<td>20.0 mg.</td>
</tr>
<tr>
<td>Antuitrin</td>
<td>0.2 cc.</td>
</tr>
<tr>
<td>Adrenalin chloride(^8) (1:1000)</td>
<td>0.1 cc.</td>
</tr>
<tr>
<td>Eschatin (suprarenal(^8) cortex hormone)</td>
<td>0.1 cc.</td>
</tr>
<tr>
<td>Pitressin(^8) (pituitary hormone)</td>
<td>0.1 cc.</td>
</tr>
</tbody>
</table>

Medium IV.---This contained all the constituents used in medium III with the exception of the vitamin A. It was, therefore, a serumless medium.

Preparation of the Blood Digest.—Approximately 700 cc. of cow blood is obtained

\(^6\) Though many experiments have been made in the course of this work, with the individual constituents, to ascertain the nature of the substances needed and the concentrations at which each should be used, it was not always possible to arrive at final conclusions. Thus, nucleic acid was incorporated in medium III on some evidence obtained with it in simpler media but, after the completion of the experiments described here, some additional experiments of rather short duration were made in which medium III was used with and without the nucleic acid. These experiments indicated that nucleic acid at the concentration used made the cells more granular than they were when it was omitted. Probably, therefore, with further experimentation, it will be possible to devise media that are simpler and still more satisfactory than those developed thus far.

\(^7\) The authors are indebted to Dr. P. A. Levene of The Rockefeller Institute, who was kind enough to prepare and furnish this substance.

\(^8\) Excellent results have been obtained with the whole thyroid gland in a medium containing only 1/5 of this quantity of hormone.
from the slaughter house. Coagulation is prevented during delivery by having the blood collected in a bottle containing 50 mg. of heparin\(^9\) dissolved in 20 cc. of Ringer's solution. 450 cc. of this blood is shaken with 225 cc.\(^9\) of chloroform and placed in an incubator at 37°C. overnight. The next day the solidified mass is broken up, either by macerating it in a mortar, or passing it through a meat chopper. It is then diluted with distilled water to a volume of 6 liters. \(\text{N/1}\) sodium hydroxide solution is added in quantity sufficient to bring the pH of the mixture as measured by the glass electrode to 10.2.\(^{11}\) Then 10 gm. of Armour's pancreatin is added and the mixture is incubated for 24 hours, being shaken at half hour intervals during the first few hours. The next day, alkali is again added to bring the pH to 8.3. Then 4 gm. more pancreatin is added and digestion is continued without further adjustment of pH for 2 days. After this, the mixture is filtered through glass wool, and then through filter paper. The volume of the filtrate is measured, and trichloracetic acid is added in quantity sufficient to make its concentration 2.5 per cent. After standing at room temperature for 16 hours, the clear, supernatant fluid is siphoned off, and the remaining cloudy fluid centrifuged. The siphoned fluid and that obtained on centrifuging are combined and boiled in an open basin to approximately half their original volume. This destroys the enzyme, decomposes the trichloracetic acid, and removes the chloroform. The cryoscopic point is determined and the fluid made isotonic by addition of salt or water as required. Total and amino nitrogen determinations are made and the fluid is sterilized by autoclaving. The ratio of amino to total nitrogen of digests so prepared varies from 0.42 to 0.45. Samples of the digest saturated with ammonium sulfate show only the faintest trace of precipitate.

Preparation of Medium I.—To prepare 250 cc. of medium, calculate the amount of blood digest required to furnish 75 or 150 mg. nitrogen (i.e., 12.5 or 25 cc. of a digest containing 600 mg. per cent nitrogen). Add to this 5 or 7.5 cc. homologous serum and 1.25 cc. of a 1 per cent solution of the sodium salt of phenol red. Then dilute with Tyrode's solution to 250 cc.

\(^9\) Obtained from the Connaught Laboratories, Toronto.

\(^{10}\) 225 cc. of chloroform were used in making the digest for this work. A subsequent experiment has shown that by reducing this quantity to 50 cc., a digest of the same characteristics is obtained. Thus, when a given lot of blood was divided into two parts, one of which was treated with 225 cc. of chloroform, and the other with 50 cc., the following analytical results were obtained: Total nitrogen in the first digest, 439 mg. per cent; and in the second, 451 mg. per cent. Ratio of amino to total nitrogen, 0.445 in the first, and 0.477 in the second. As yet no comparative tests of these digests for their ability to support cell life have been made.

\(^{11}\) In case a glass electrode is not available, it is advisable to add the pancreatin before adjusting the pH, and then to add \(\text{N/1}\) NaOH solution until the mixture is just alkaline to phenolphthalein paper.
Preparation of Media II, III, and IV.—Stock solutions containing the most stable and inexpensive constituents at concentrations four times as great as those desired in the final medium are prepared first. These stock solutions are sterilized by passing them through a Berkefeld filter. They may be preserved at ice box temperature for 4 or 5 weeks. Solutions of the less stable and more expensive constituents are prepared in small quantity at frequent intervals. These are combined from time to time with small amounts of the stock solution to make the complete media. For organ cultivation, it is advisable to prepare a liter or more of the stock solution at a time. For culture work, much smaller quantities are advised. The freezing point of the medium should be determined before it is used and should lie between -0.62 and -0.66°C. The pH should be adjusted between 7.4 and 7.6. All media when completed should be sterilized by Berkefeld filtration.

To Prepare 1 Liter of Stock Solution for Medium II.—Calculate the volume of blood digest required to give 1200 or 2400 mg. nitrogen as desired (i.e., 400 cc. of digest containing 600 mg. per cent nitrogen if a final medium containing 60 mg. per cent nitrogen is to be used). Measure out this volume and add:

- Solution of sodium salt of phenol red, 1 per cent: 20.0 cc.
- Insulin, Squibb's (10 units per cc.): 0.4 cc.
- Thyroxine-hemin solution containing 1 mg. thyroxine and 4 mg. hemin in 100 cc.: 4.0 cc.
- Potassium iodide, 2.8 per cent, diluted with water 1:100: 18.6 cc.
- Double strength Tyrode's solution: 22.2 cc.
- Cysteine hydrochloride, solid: 360.0 mg.
- Isotonic sodium bicarbonate solution, 1.4 per cent: 6.8 cc.

Then dilute to 1 liter with Tyrode's solution modified to contain 300 mg. per cent glucose.

To Prepare 250 cc. of Medium II.—Take 62.5 cc. of the stock solution just described and add:

- Ascorbic acid-glutathione solution (see below): 1.5 cc.
- Vitamin B 1 (0.1 mg. per cent solution betaxin): 0.25 cc.
- Vitamin B 2 (1.0 mg. per cent solution riboflavin): 0.85 cc.
- Vitamin A serum, containing 1400 international units per cc.: 0.18 cc.

(or that amount calculated to furnish 250 international units)

Then dilute to 250 cc. with Tyrode's solution containing 300 mg. per cent glucose.

To Prepare 1 Liter of Stock Solution for Medium III.—Calculate the volume of blood digest needed to supply 2400 mg. nitrogen (or 1200 mg. nitrogen, if a final medium containing 30 mg. per cent nitrogen is desired). Add to this:

- Tryptophane: 200 or 400 mg.
- Solution of sodium salt of phenol red, 1 per cent: 20.0 cc.
- Insulin, Squibb's (10 units per cc.): 0.4 cc.
- Thyroxine-hemin solution containing 1 mg. thyroxine and 4 mg. hemin in 100 cc.: 4.0 cc.
- Cysteine hydrochloride, solid: 360.0 mg.
ARTIFICIAL AND SERUMLESS MAINTENANCE MEDIA

Potassium iodide, 2.8 per cent solution diluted 1:100 with water 18.6 cc.
Urea 96.0 mg.
Vitamin B₁ (0.1 mg. per cent solution betaxin) 4.0 cc.
Vitamin B₂ (1.0 mg. per cent solution riboflavin) 13.6 cc.
Witte's peptone, 7.5 per cent solution in water 24.8 cc.
Sodium glycerophosphate 2.3 gm.
Glycerine, Kahlbaum's sp. gr. 1.23 8.0 cc.
Glucose 3.0 gm.
Sodium bicarbonate 270.0 mg.
Water, triple distilled 243.0 cc.

Then dilute to 1 liter with a Tyrode's solution from which the glucose has been omitted, and which contains sodium chloride at a concentration of 7.78 gm. per liter.

To Prepare 250 Cc. of Medium III.—Take 62.5 cc. of the stock solution just described and add:

Ascorbic acid-glutathione solution 1.5 cc.
Vitamin A serum containing 1400 international units per cc. 0.18 cc.
(or that amount calculated to furnish 250 international units)
Antuitrin, Parke-Davis 0.5 cc.
Adrenalin chloride,¹ 1:1000 solution 0.25 cc.
Pitressin, pituitary hormone, Parke-Davis 0.25 cc.
Eschatin, suprarenal cortex hormone, Parke-Davis 0.25 cc.
Thymus nucleic acid dissolved in Ringer's solution with the aid of a few drops of N/1 NaOH 50.0 mg.
Water¹² 7.0 cc.

Then dilute to 250 cc. with Tyrode's solution containing 300 mg. per cent glucose.¹⁰

Phenol Red 1 Per Cent Solution of the Sodium Salt.—Weigh 1.000 gm. of phenol red. Grind this in a mortar with 28.2 cc. of exactly N/10 NaOH solution until it is all dissolved. Dilute to exactly 100 cc. with water, using a part of the water to transfer the dye to a graduated flask.

Thyroxine-Hemin Solution (Prepared as Described by Vogelaar and Erlichman).³—To 5 mg. thyroxine, add 6 cc. absolute alcohol, 2 cc. of 1 per cent NaOH, and 2 cc. water. Boil down to 3 cc. Add water to 10 cc. Then add 20 mg. hemin. Dilute 1:50 with water to obtain a solution having 1 mg. thyroxine and 4 mg. hemin in 100 cc.

Double Strength Tyrode's Solution.—A solution containing all the constituents of Tyrode's solution at twice the usual concentration. Sterilize by filtering.

Tyrode's Solution Modified to Contain 300 Mg. Per Cent Glucose.—Follow

¹² The stock solution for medium III is hypertonic. Therefore water is added in making the medium. The stock solution cannot be made isotonic unless the volume of digest required does not exceed 554 cc.
directions for making ordinary Tyrode's solution, reducing the sodium chloride to 7.78 gm. per liter and increasing the glucose to 3 gm. Sterilize by filtering. It is sometimes convenient to make this solution without adding the glucose since such a solution can be preserved for a few days in the ice box without filtering. Then the glucose may be added as each stock solution or medium is made.

**Ascorbic Acid-Glutathione Solution.**—Dissolve 40 mg. glutathione (Hoffman-La Roche) and 10 mg. crystalline vitamin C (natural, Abbott Laboratories) in 20 cc. Ringer's solution. Sterilize by passing through a 1 inch Berkefeld filter. The presence of glutathione is necessary to stabilize the vitamin C and protect it from oxidation. The solution should be made fresh every 10 days or 2 weeks and preserved in the ice box.

**Witte's Peptone Solution, 7.5 Per Cent.**—15 gm. of Witte's peptone is added to 200 cc. triple distilled water in a pressure bottle. Then this is autoclaved for 15 minutes at 30 pounds pressure or 15 pounds gauge pressure.

**Vitamin A Serum.**—To prepare vitamin A serum, a potent concentrate of vitamin A must be obtained. This may be prepared as described by Baker$^{13}$ or it may be obtained commercially.$^{14}$ As soon as this concentrate is received, it should be divided into small lots of approximately 0.75 cc. each, and sealed in small tubes under an atmosphere of CO$_2$.

Before the vitamin is incorporated in the medium, it must be dissolved in serum. To do this, place approximately 0.5 cc. of the concentrate in a small Erlenmeyer flask and add 30 cc. of serum. Shake the flask violently for 20 or 30 minutes in a shaking machine so as to obtain a finely divided suspension of the vitamin concentrate. Then allow the mixture to stand overnight at room temperature. The next day, filter the serum through a Seitz clarifying filter, and then sterilize it by passing it through a 1 inch Berkefeld filter. Sera containing as much as 1800 to 2600 international units of vitamin A per cc. may be obtained in this way. The amount dissolved varies with the individual sera and the species of animal from which it is taken.

To protect the vitamin A serum from oxidation it is divided into portions 1 to 2 cc. in volume, distributed in small glass tubes, and sealed under CO$_2$. If all the oxygen is removed, the vitamin serum will retain its original potency for 3 or 4 months. When the serum is kept in stoppered tubes without removing the oxygen, approximately half the vitamin is lost in a week. Moreover, the resulting oxidation product is somewhat toxic.

The concentration of vitamin A in the serum may be determined by a method worked out in this laboratory by La Rosa. The procedure depends on the development of the typical blue color of the Price-Carr reaction. This is changed to a purplish red color on heating. The latter color is compared with a series of


$^{14}$ That used throughout this work was prepared and donated for this purpose by the Abbott Laboratories.
standards made up of sodium alizarin sulfonate. Details of the method are described by Parker.\textsuperscript{15}

**General Procedure Used in Testing the Media**

The procedure used in testing these media for their ability to maintain fibroblasts was as follows: Cultures from a 26 year old strain of chicken heart fibroblasts were embedded in Carrel D-3 flasks in coagula containing 0.25 cc. chicken plasma and 0.75 cc. of the medium being used. To remove the serum from this coagulum, the cultures were washed on the following day and every 2 days thereafter for 2 hours at 37°C. with 2 cc. of the medium. Then this wash fluid was withdrawn and 0.5 cc. of new medium was supplied. The serum originally present in the coagulum disappeared under this treatment within 12 to 14 days.\textsuperscript{16} The washing was continued, nevertheless, throughout the entire period of cultivation. Before the flasks were sealed, the pH of the medium was brought to 7.4 by using a gas mixture containing 3 per cent CO\textsubscript{2}, 21 per cent O\textsubscript{2}, and 76 per cent N\textsubscript{2}. To ascertain the effect of the various media, the cells were examined microscopically at frequent intervals. Then, at the end of the cultivation period, which extended from 43 to 56 days, the vitality of the cells and their ability to proliferate were tested by transplanting them into a growth-promoting medium (plasma and embryo juice). A sister colony was cultivated in each case in a control medium the nature of which is indicated under each experiment.

**RESULTS**

*Medium I.*—When tested in the manner just described, medium I was found to be an excellent maintenance medium. Fibroblasts culti-
vated in it remained alive and in good condition for 43 days. During the first few days, i.e., while considerable serum was still present in the coagulum, the cells proliferated at a very slow rate. After the concentration of the serum was reduced to that of the nutrient fluid, they were maintained with little or no proliferation. Then, when a little plasma was added on the 43rd day, to reinforce the coagula, the cells began to proliferate again. Control colonies that were cultivated in blood digest and Tyrode's solution died soon after all the serum had been removed from the coagulum. The colonies kept in digest supplemented with serum at 1 per cent concentration lived longer than those kept in digest alone, but not as long as those that received serum at 2 or 3 per cent concentration with the digest. Photographs illustrating the condition of the cells that were cultivated in blood digest alone, in blood digest supplemented with 1 per cent serum, and in blood digest supplemented with 2 and 3 per cent serum are shown in the figure below.

\[ \text{Text-FIG. 1. Experiment 10816-C. Comparison of the rate of growth and the duration of life of fibroblasts cultivated in blood digest, supplemented with 1, 2, and 3 per cent serum, with that of sister colonies cultivated in blood digest without serum. Nitrogen concentration of the blood digest, 60 mg. per cent. The increase in growth on the 43rd day is due to patching the coagula with a small amount of plasma. Radius in mm. × 16.} \]

\[ \text{In another experiment in which blood digest and Tyrode's solution were the only substances supplied in the nutrient fluid but in which the coagulum was reinforced once in 2 or 3 weeks by adding 2 drops of plasma, the tissue remained alive and in good condition for 70 days and proliferated again on being transferred to a growth-promoting medium.} \]
cent serum (medium I) are shown in Figs. 1 a to 1 d. Growth curves showing the duration of life of these cultures and that of the control colonies are shown in Text-fig. 1.

Medium II.—Chicken heart fibroblasts cultivated in medium II lived for 50 days. The control colonies, cultivated in blood digest diluted with Tyrode's solution to the same nitrogen concentration as that used in the medium, died during the 3rd week of cultivation. The cells in the experimental medium remained in good condition for 6 weeks. During the 7th week of cultivation, the cells at the periphery of the colony became somewhat scattered and began to look starved. As it seemed probable that longer cultivation in this medium would not be feasible, the colonies were transferred on the 50th day to a new coagulum and given growth-promoting nutrients. Active proliferation ensued. It would seem, therefore, that this medium can maintain the cells for a considerable time, but not indefinitely. Photographs illustrating the condition of the cells in medium II when made with digest at a nitrogen concentration of 30 mg. per cent, and also when containing digest at a nitrogen concentration of 60 mg. per cent, are shown in Figs. 2 a and 2 b. Growth curves showing the duration of life of colonies cultivated in these media and that of sister cultures kept in digest and Tyrode's solution are shown in Text-fig. 2. The second curve in each case is that of the experimental colony after it was transplanted and given a growth-promoting medium.

Medium III.—This was devised in an attempt to improve medium II. Four of the ten new constituents that were added, antuitrin, tryptophane, Witte's peptone, and sodium glycerophosphate, were found, when added separately to medium II, to improve the nutritive and maintenance value of that medium. Beneficial action was also observed with the other six substances when they were used together. But the differences observed when each was tested separately were too small to constitute definite proof that they were all essential. To illustrate the effect of the complete medium, an experiment is cited in which a comparison was made of the maintenance of power of medium III and that of medium II already improved by the addition of antuitrin and tryptophane. Sister colonies of fibroblasts were cultivated in these two media for 49 days. Almost from the begin-
Text-Fig. 2. Experiment 10835-C. Comparison of the rate of growth and duration of life of fibroblasts, cultivated in artificial medium II, with that of sister colonies cultivated in blood digest and Tyrode's solution, showing growth of the former after transplantation on the 50th day into a growth-promoting medium. Nitrogen concentration of the blood digest, 30 and 60 mg. per cent.

Text-Fig. 3. Experiment 10915-C. Comparison of the rate of growth and the duration of life of fibroblasts cultivated in artificial medium II to which antuitrin and tryptophane have been added, with that of sister colonies cultivated in artificial medium III showing growth in all cases in medium III and in some cases in medium II after transplantation on the 56th day into a growth-promoting medium. The increase in the rate of growth on the 49th day was due to patching the coagulum with a small amount of plasma. Nitrogen concentrations, 30 and 60 mg. per cent.

Text-Fig. 4. Experiment 11004-C. Curve showing the rate of growth and duration of life of a colony of fibroblasts cultivated for 56 days in an artificial and serumless medium (medium IV), showing growth after transplantation on the 62nd day to a growth-promoting medium. Nitrogen concentration, 60 mg. per cent.
ning of the experiment, the colonies in the more complete medium presented a better appearance. The tissue was thicker and the cells larger and clearer than were those cultivated in the simpler medium. In the experiments in which the digest was used at a nitrogen concentration of 60 mg. per cent, an exceedingly slow growth was observed. After 7 weeks of cultivation, the cells in the simpler medium began to look starved, as they had in the experiments with medium II cited above, while those in the more complete medium still seemed to be well nourished. On the 49th day, a small amount of plasma was added to reinforce the coagula. The colonies that had been given the more complete medium responded to this treatment by increasing in size. Those given the simpler medium did not respond. On the 56th day, the colonies were transferred to new coagula and given growth-promoting nutrients. All of those that had been cultivated in medium III grew actively, while only 25 per cent of those that had received the simpler medium were able to proliferate. Growth curves showing these results are reproduced in Text-fig. 3. Photographs illustrating the condition of the cells in medium III are shown in Fig. 3.

Medium IV.—This medium is serumless. It differs from medium III only by the omission of vitamin A and that small amount of serum that was required to dissolve it. Vitamin A had been incorporated in media II and III because it is a normal constituent of serum and because it had also been found to be an essential constituent of artificial, growth-promoting media. But no evidence indicating that it is essential to maintenance has been obtained. To ascertain, therefore, if it, and the serum that had been used to dissolve it, might be eliminated from the maintenance medium, an experiment was made in which sister colonies of fibroblasts were cultivated in medium III made up with and without vitamin A. The colonies that were kept in the serumless medium lived for 56 days. And the cells within those colonies remained a little cleared and appeared to be in better condition than those that had the vitamin and serum at their disposal. On the 56th day of cultivation, a little plasma was added to reinforce the coagula. Then on the 62nd day the cells were transplanted and given a growth-promoting medium. Those that had been maintained in the serumless medium as well as
those that were given the medium containing the vitamin responded by proliferating actively. A curve showing the duration of life and the slow growth of one of the colonies in the serumless medium is reproduced in Text-fig. 4. The second and third curves show the growth of this colony on two successive transfers. A photograph illustrating the condition of the cells cultivated in the serumless medium is given in Fig. 4. As will be seen, the cells in this medium have become quite large and show less polarity than those in the other media. However, when they were transplanted and given growth-promoting nutrients, they reverted immediately to their original form.

SUMMARY

Several media designed for maintaining the life of cells and organs outside the body have been described. Cultures from a pure strain of fibroblasts have been maintained in these media in vital condition and with little or no proliferation for periods varying from 43 to 56 days. One of these media is very simple, inexpensive, and easy to prepare; and one is serumless.

12 to 14 days should be deducted to calculate the time the tissues lived in the absence of serum.
EXPLANATION OF PLATE 19

Fig. 1 a. Control culture. Fibroblasts cultivated 32 days in blood digest and Tyrode's solution, showing degenerated cells. × 206.

1 b. Fibroblasts cultivated 32 days in blood digest, Tyrode's solution, and 1 per cent serum, showing cells in the process of degeneration. × 206.

1 c. Fibroblasts cultivated 38 days in blood digest, Tyrode's solution, and 2 per cent serum (medium I). × 206.

1 d. Fibroblasts cultivated 38 days in blood digest, Tyrode's solution, and 3 per cent serum (medium I). × 206.

Nitrogen concentration of the blood digest, 60 mg. per cent in each case.

Fig. 2 a. Fibroblasts cultivated 37 days in medium II containing the blood digest at a nitrogen concentration of 30 mg. per cent. × 206.

2 b. Fibroblasts cultivated 32 days in medium II containing the digest at a nitrogen concentration of 60 mg. per cent. × 206.

Fig. 3. Fibroblasts cultivated 32 days in medium III containing blood digest at a nitrogen concentration of 60 mg. per cent. × 206.

Fig. 4. Fibroblasts cultivated 56 days in a serumless medium (medium IV), containing the blood digest at a nitrogen concentration of 60 mg. per cent. × 206.
(Baker and Elbling: Artificial and serumless maintenance media)