THE RACES THAT CONSTITUTE THE GROUP OF COMMON FIBROBLASTS

I. THE EFFECT OF BLOOD PLASMA

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PLATES 26 TO 28

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Since fibroblasts from the heart of the embryo chick were first isolated in pure cultures, it has been known that they multiply in embryonic tissue juice and in fluids containing the larger protein split-products. Adult blood plasma, although the optimal medium for the cultivation of blood and tissue macrophages, was found to be insufficient as a source of nutriment for the heart cells. Later work showed, however, that fibroblasts derived from another part of the organism behaved differently. Thus, morphologically similar cells, isolated from the perichondrium of cartilage, multiplied at a slow but uniform rate when treated with blood plasma and could be kept in good condition over very long periods of time. This led to the discovery that various races of fibroblasts could be isolated simultaneously from the same organism, and that each race, according to its origin, constituted a specific cell type characterized solely by its nutritional properties. Those cell types which exhibited the highest residual growth energy, as determined by their capacity to proliferate in a medium free from nitrogenous food substances, were able not only to subsist on very minute quantities of embryonic tissue juice present in the medium, but also to live on plasma alone. The present communication devotes itself to a consideration of the nutritional properties of fibroblasts with special reference to the effect of blood plasma on their multiplication and behavior in vitro.

4 Parker, R. C., Arch. exp. Zellforsch., 1929, 8, 340.
Materials and Procedures

The material consisted of various pure strains of fibroblasts which were isolated simultaneously from embryo chicks ranging from 13 to 15 days of age. These strains were derived from heart muscle, skeletal muscle, the perichondrium of cartilage, and the periosteum of bone, respectively. Until used for the experiments, which were made in flasks, the strains were carried either by the hanging drop method, or in flasks, on such combinations of plasma and embryonic tissue juice as were favorable for the maximum proliferation of the various cell types. According to the usual procedure, the original explants were very early eliminated and discarded by repeated selection of only the marginal area of outgrowth at the time of transfer, thus insuring against a multiplicity of cell types being carried in the individual strains. Each series of experiments was made from strains of the same age.

The cultures comprising the individual experiments were made and treated as follows: The explants were placed in flasks to which had previously been added 0.3 cc. of chick plasma diluted with twice that amount of Tyrode solution. Coagulation of the plasma clot was allowed to take place spontaneously and without the customary addition of embryonic tissue juice. On the following day, the clots were reinforced by the addition of 0.25 cc. of plasma diluted with an equal amount of Tyrode solution. This was allowed to coagulate of its own accord as before. On the next day, and three times a week thereafter, the cultures were washed for 2 hours with Tyrode solution, after which they were treated with a 50 per cent solution of chick plasma diluted with Tyrode solution. Sufficient heparin had previously been added to the solution in order to prevent its coagulation over a period of 2 hours at 37°C. This mixture was finally aspirated and the cultures were returned to the incubator until the next treatment. Whenever it became necessary to subdivide and transfer the cultures, the subcultures were prepared according to the original procedure, and the same manner of treatment was resumed.

The flasks used were of Pyrex and included two types designed by Carrel. The first, or the D-3 type bearing the oblique neck, has been described many times and is in general use. The second type is more recent (Carrel, A., Compt. rend. Soc. biol., 1930, 105, 826), and has been designated as the microflask. This new flask is 25 or 30 mm. wide and 5 mm. high (inside dimensions). It has a straight neck which is 5 mm. in diameter. At the union of the chamber with the neck, there is a slight depression in the glass which renders possible the maintenance of 1 or 1.5 cc. of medium in the flask without the danger of the fluid running into the neck. The wall is sufficiently thin to permit examination of the tissue cells with a 3 mm. immersion lens. At the termination of an experiment, the tissues may be fixed and stained in situ.

In the concentration used, appropriate experiments have shown that the heparin has no appreciable effect upon the rate of growth of fibroblasts.
Growth curves of the cultures were constructed by the planimetric measurement of outline drawings of the surface area which were made from time to time with the projectoscope. Although the limitations of this method have long since been realized, it remains the only practical one available at the present time.

EXPERIMENTS AND RESULTS

1. Rate and Mode of Growth of Fibroblasts Grown on a Plasma Medium.—In view of the marked differences in food requirements already found to exist between the four types of mesenchyme cells employed in the present experiments, one would expect to find differences of the same order in the ability of the various races to multiply on a plasma diet. In general, this is the case. Of the four types, the heart muscle fibroblasts, which invariably have the lowest residual growth energy and the lowest rate of proliferation on a medium containing embryonic tissue juice, multiply least rapidly in the plasma medium. These differences are as great when the different cell types of the same age are cultivated under identical conditions in separate flasks (Fig. 1) as when they are cultivated side by side in the same medium (Text-fig. 1; Figs. 2 and 3). Occasionally, the heart muscle fibroblasts have been found to succumb soon after having been placed on the plasma diet. When, however, the cells survive a certain period of adjustment, they are usually capable of continual proliferation for very long periods of time. Cultures of heart muscle fibroblasts, together with representative cultures from two other cell strains, namely, fibroblasts from skeletal muscle and the perichondrium of cartilage, have been grown on this medium for as long as 236 days, at which time it was necessary to terminate the experiment (Fig. 6).

The change that occurs in the mode of growth of cell colonies transferred from a medium containing embryonic tissue juice to one in which it is absent is not abrupt. Cells are capable of storing food substances during periods of plenty, which reserves they in turn utilize while becoming adjusted to a poorer medium. But, inasmuch as the medium employed in the present experiments is thoroughly washed with Tyrode solution prior to each treatment with fresh plasma, it is not possible that these reserves could suffice for more than a few days.

Cultures that are transplanted to the plasma medium acquire, almost immediately, features by which they may readily be distinguished from cultures of the same cell type that have access to embryonic tissue juice. In the plasma medium, cell colonies become very dense (Fig. 4). Even at the periphery, the young cells grow in many layers forming a dense entanglement not unlike fresh tissue removed from the organism. This renders it impossible to state definitely that a culture which has ceased to increase in surface area has also ceased to increase in cell population. In fact, there is abundant evidence that all cultures which cease to grow under these conditions are not necessarily dead. Many of the component cells remain, for very long periods of time, in a quiescent state which is perhaps not unlike that of the dormant cells in the adult organism. As an example, reference might be made to a culture belonging to a series which, although regularly washed and treated with fresh plasma, was allowed to remain in the original flasks for 100 days (Text-figs. 2 and 3; Fig. 4). During the last 40 days of this period, the culture referred to showed little increase in surface area. Examination of the peripheral areas revealed the presence of many cells in various stages of disintegration but, together with these, large numbers of cells which, although heavily granulated and relatively inactive, were obviously alive. When, however, the culture was subdivided and transferred to a fresh medium, active cell multiplication was resumed. From the very regular distribution of the new cells which grew out from the various transplants
TEXT-Fig. 2. Culture 392-1. Diagrammatic representation of the increase in area of a culture of heart fibroblasts, in the 12th passage, subjected to the plasma treatment. Tracings made from the projected culture at various time intervals from the moment it was placed in the flask until the 60th day. (Compare Fig. 4.)

TEXT-Fig. 3. Culture 392-1. Curve showing the rate of growth of a culture of heart fibroblasts, in the 12th passage, subjected to the plasma treatment. The units of area were ascertained by planimetric measurement of the tracings represented in Text-fig. 1.
(Fig. 5), it may be concluded that living cells had been evenly scattered throughout the whole mass of the original culture. It is of interest to note that this was a culture of heart muscle fibroblasts which had been subdivided and transferred twelve times before being used for the 100 day experiment.

It is also not uncommon to find marginal areas of local proliferation which very rapidly alter the whole contour of the culture. Such areas may result from local differences in the rate of multiplication while the whole culture is still active, or from cells continuing to multiply at certain points after proliferation has ceased in adjacent areas.

(Text-fig. 4). In other words, under the conditions of these experiments, it is possible to maintain a cell population comprised of individuals of all ages and in all states of functional activity.

It should be mentioned that the character of the cell growth obtained with plasma is very different from that which is obtained with serum, even when the two are prepared from the same sample of blood. The former brings about cell senescence much less rapidly than the latter. This is not surprising when one considers that serum is far more artificial than plasma and, as such, does not exist in the organism. Serum lacks not only fibrinogen, but possibly also substances of importance in cell nutrition which may be removed together with it.
2. Transformations from Fibroblasts to Macrophages.—Whereas certain cell colonies become adjusted to the new environmental conditions without very pronounced changes in the cells themselves, it does not follow that this is always the case. Two sister cultures originating from the same strain and subjected to the same treatment may behave quite differently. To illustrate: After the first series of experiments had been in progress for about 12 days, it was noticed that the

![Text-Fig. 5. Culture 400-4. Diagrammatic representation of the increase in area of a culture of heart fibroblasts in which transformation to macrophages occurred. The circles represent tracings made from the projected culture. The fibroblasts ceased to increase in area after the 11th day. The broken lines represent areas covered by the macrophages up to the 24th day. (Compare Figs. 8 and 13.)](image)

cells of one of the cultures belonging to a strain of fibroblasts derived from skeletal muscle had very suddenly given rise to a broad band of macrophages (Fig. 14). A few days later, a culture of the same age, but belonging to a strain of heart muscle fibroblasts, behaved similarly (Text-fig. 5; Figs. 8 and 13). Less than 2 weeks later, a third culture showed the same phenomenon. This third culture (Figs. 9-11), which had been treated for 28 days in the flask when the transforma-
tion occurred, had been made from a strain of fibroblasts originating from bone periosteum, a strain that had been carried for twelve passages before the experiment was made. It was obvious, therefore, that the phenomenon was not limited to any one cell type.

After these observations had been made, a new experiment was set up in an endeavor to duplicate as closely as possible every step in the treatment of these cultures in the hope that the changes might recur.

Text-Fig. 6. Cultures 401–1 and 401–3. Growth curves of sister cultures of muscle fibroblasts given the same treatment yet placed in separate flasks. Culture 401–1 showed transformation of fibroblasts to macrophages on the 8th day. The broken lines represent the increase in area covered by macrophages. Culture 401–3 did not transform. (Compare Fig. 14.)

This experiment included three cultures of fibroblasts from a heart muscle strain, three from a strain derived from skeletal muscle, and one culture from a strain of fibroblasts isolated from the perichondrium of cartilage. These cultures were made from strains of the same age and had a common past history. After this experiment had been running from 20 to 25 days, the three heart cultures (Figs. 15 and 16) and the three muscle cultures transformed. The culture made from the strain of cartilage fibroblasts, however, did not transform, although
subcultures derived from it were successfully grown on the plasma medium for almost 150 days before the experiment was terminated.

Since but a limited number of the cultures comprising the various experiments showed the phenomenon, it was possible to make a comparative study of the general condition and rate of growth of cultures which had transformed and of those which had not. It was found that those cultures which had not transformed fell into two groups, namely, cultures that responded very favorably to the plasma treatment, as evidenced by the condition of the cells and their rate of growth (Text-figs. 2 and 4; Fig. 12), and cultures in which the cells could not become adjusted to the new medium and were forced to succumb when their residual energy had been utilized. This last condition, which was found but rarely, was restricted to a certain number of cultures which had been derived from the heart strains (Text-fig. 4; Figs. 2 and 3). When transformation did occur, it seemed to take place at some critical period in the life of a culture in which the degeneration process, although evident, was advancing at a relatively gradual rate. It was also found that if two sister cultures belonging to the same experiment had been placed in separate flasks, and one of them transformed whereas the other continued to grow without change, the culture that transformed invariably grew more slowly than the other up to the time when the transformation occurred (Text-fig. 6; Fig. 14).

3. Growth of Carrel's 19 Year Old Strain of Fibroblasts on a Plasma Medium and Transformation to Macrophages.—More recently, a slightly modified form of the plasma treatment was used in connection with cultures from the 19 year old strain originally obtained from embryonic heart muscle. This was done in an endeavor to find a means of retaining the material in the laboratory with less effort than is expended by the very frequent transfer of cultures made necessary by the rapid proliferation of cells treated with embryonic tissue juice. The medium differed from that already described only in that about 1.5 per cent of embryonic tissue juice (frozen) was added to the clot in order to bring about rapid coagulation of the plasma. At regular intervals, the medium was washed with Tyrode solution and treated with heparinized plasma, as described above. At the time of writing, cultures have been successfully carried in this manner for over 100 days, during which time they have been transferred to fresh medium eight
times. Taking into consideration the relatively high concentration of embryonic tissue juice necessary for the continued well-being of all strains of fibroblasts derived from embryonic heart, it is safe to assume that the plasma cultures of the 19 year old strain do not owe their present condition to the minute trace of tissue juice added in the preparation of the solid medium. And should its concentration reach a level sufficiently high to be available for cell growth and multiplication, it would be washed out prior to the first treatment with fresh plasma.

61 days after the first of these series of experiments were started, numerous macrophages appeared in a culture which had been treated for 11 days since the previous passage (Figs. 17 and 18). It was a transformation of exactly the same type as that found in the other strains of fibroblasts. In a second series of experiments, a transformation was observed to occur after 68 days of cultivation in the plasma medium. It was apparent, therefore, that the phenomenon was not restricted to those cell strains that had been more recently isolated from the organism.

The possibility of the macrophages being present in the cultures from the moment of their isolation from the tissues of the organism is entirely ruled out, not only by the very nature of the transformation process itself, as will be disclosed later, but also by the conditions of the experiments. Just as plasma is the optimal medium for macrophages, so is a high concentration of embryonic tissue juice toxic to them. A mixed culture of macrophages and fibroblasts, when cultivated for a time on embryonic tissue juice, will soon be found to consist of fibroblasts alone. The macrophages eventually die. Had the tissue been transferred directly from the organism to the plasma medium, it is quite possible that any macrophages present might have survived. In the present experiments, however, this was not the method of procedure. Before being transferred to the plasma diet, the cultures were maintained for an extended period in embryonic tissue juice. Every cell type has its optimal food requirements, and under the conditions of a given nutritional régime there will be a survival of only that cell type able to subsist indefinitely on that particular medium. The recent transformations which have been observed in cultures of the old strain of heart fibroblasts have occurred after a period of culti-
vation of almost 20 years in high concentrations of embryonic tissue juice. The history of this strain alone affords ample proof of the fact that the macrophages could not have been present from the beginning.

4. Observations on the Transformation Process.—The cells that owe their origin to the transformation process (Figs. 10–11, 14–17, 19, 21–22) have been referred to as macrophages because they were identical with the cells which are commonly referred to as macrophages both in form and behavior.¹ They were independent, very active, highly phagocytic, and showed no tendency to form a tissue. They also developed the undulating membrane. Examination of cultures in which the phenomenon was occurring revealed that the macrophages were being budded off from heavily granulated and distended fibroblasts (Figs. 21 and 22).

In order to study the process in detail, it was necessary to select an active fibroblast belonging to a culture in which the macrophages were appearing, and to observe it at 37°C. in the hope that sooner or later something of interest might occur. Although the experiments that were made with the microcinema⁹ have not yet happened to show the macrophages actually being budded off from the fibroblasts, it was finally demonstrated with the aid of the camera lucida that this took place by one or more unequal divisions of the mother cell. During these divisions there were no indications of the well known phenomena which accompany mitosis and which make it so easy to detect wherever it occurs. For example, the cells did not round up and there was no "bubbling" of the cytoplasm. The divisions were direct, suggesting amitosis. The camera lucida drawings which showed this have been reproduced (Text-fig. 7). Unfortunately, however, the culture that gave the crucial evidence was contained in a flask of the older type, the walls of which were too thick to permit one to follow with utmost precision the nuclear changes that accompanied the final division of the cell into three separate entities. Each of these was nucleated, and one of them became transformed into a typical macrophage. For this reason, it was necessary to omit the nuclei from the drawings made during the most interesting period. 5 hours after the last drawing


⁹ The cinematographic experiments were made with the assistance of Mr. Heinz Rosenberger.
TEXT-FIG. 7 a

TEXT-Figs. 7 a and 7 b. Culture 600-2. Selected camera lucida drawings from a series showing the progressive changes through which a fibroblast passed until its final division into three separate cells, one of which became transformed into a macrophage. Indication is given of the time when each drawing was made.

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TEXT-Fig. 7 b
was made it was found that several other macrophages had been formed. At this time, nothing remained of the original fibroblast but small remnants in the midst of the macrophage group. These were packed with the larger globules and granules that had been contained in the mother cell.

Previous and subsequent study has shown an almost total absence of karyokinetic figures and other evidences of mitotic divisions in cultures subjected to the conditions of these experiments for long periods of time. Cells containing two or more nuclei are numerous.

Text-Fig. 8. Culture 1181. Camera lucida drawings of multinucleate cells and nuclear amitosis from a strain of fibroblasts, in the 19th passage, which had been derived from the perichondrium of cartilage and subjected to the plasma treatment for 178 days. Hematoxylin after Carnoy fixation. X 280.

Nuclear amitosis is exceedingly common (Text-fig. 8; Fig. 20). That these multinucleated cells are very active, and are not degenerating cells, is clearly indicated by studies made with the microcinema. In fact, every observation made has been of such a nature as to suggest that cell multiplication in these cultures, as well as the production of macrophages from fibroblasts, may take place amitotically. It is certain that further work with the aid of the microcinema will ultimately furnish more detailed information as to the nature of this type of cell multiplication and the conditions that predispose it.

The production of macrophages, once begun, usually progressed
with amazing rapidity. As a rule, they wandered out into the medium at a uniform rate from the entire periphery of the culture. It was not uncommon for them eventually to cover an area ten or twelve times as great as that finally covered by the colony of fibroblasts from which they were derived (Text-fig. 5; Fig. 8). It was also characteristic of every transformation observed that, as soon as the macrophages were set free, they invariably migrated to a level which was nearer the surface of the clot than the culture itself. The fibroblasts at any point on the margin, and the macrophages that were located directly above them, could be photographed separately simply by making a slight alteration in the focus of the lens (Figs. 9-11, 17-18).

DISCUSSION

Cells grown in a medium rich in nitrogenous food substances, as for example, in one containing embryonic tissue juice, do not mature for the reason that they are stimulated to incessant division. The daughter cells arising from one division barely recover before they enter the next. The cells remain forever young. It matters little whether they are originally derived from the embryo or the adult. Sooner or later they all acquire embryonic properties. If, however, the rate of proliferation is reduced to a minimum by the substitution of a pure plasma diet for one in which embryonic tissue juice is present, the cells proliferate at a slower rate and can be left undisturbed for a much longer time before the concentration of metabolic products becomes so great as to inhibit further proliferation. When the plasma treatment is combined with the hanging drop system of cultivation, the volume of medium is so small that cultures have to be transferred every few days in order to maintain a sublethal concentration of catabolites. Thus, the cells are not allowed to remain in any one environment long enough to become accustomed to it. And inasmuch as the increase in tissue volume is negligible in so short a time, the original culture is very soon sacrificed through the mere mechanical procedure of carrying it from passage to passage. This undoubtedly accounts for the failure of every attempt to cultivate normal fibroblasts in serum where the flask technique is not employed.

In the case of the present experiments, in which the plasma treatment is combined with the flask techniques, it has been possible to
keep the cells in good condition over very long periods of time by using large quantities of medium and by periodically washing out the accumulated waste products and treating with fresh plasma. Under these conditions, cultures have been retained in a state of continual growth in the same flask for as long as 100 days. It has been found that, within the confines of a single colony of fibroblasts which have been cultivated for an extended period of time in the plasma medium, the cell variations are numerous. Here we may have, at any one moment, cells of all ages and in all conditions of activity. Within a limited area it is not unusual to find young cells which have recently emerged from division lying side by side with old cells that are still active but have not divided for days, with degenerating cells, and the remnants of cells which have actually succumbed. This heterogeneity may in itself account for much that has been observed in the present experiments. Cells that die and disintegrate must certainly give up substances which very profoundly alter the chemical composition of the medium and thus exert an influence upon the living cells. And the living cells, by creating their own immediate environment, in turn contribute to the environment as a whole. Hence, it is only to be expected that no two cultures would behave similarly under the conditions of the present experiment. Each is an individual with its own peculiar properties.

Reference has already been made to the nutritional differences which exist between various strains of fibroblasts isolated from different tissues of the same organism. The present experiments have shown that, by altering the composition of the pericellular medium, mutants may be produced from the cells of any of these strains. Thus, any of these races, when cultivated for a time on the plasma medium, develop nutritional properties similar to those of macrophages. In fact, it has been observed that certain cells may undergo complete structural transformation to the macrophage type. Under the conditions of these experiments, these changes are irreversible. The macrophages, once formed, will live as long as they are given appropriate treatment, and never completely revert to the fibroblast type. That the cells which result from the transformation process should be identical with macrophages is not remarkable when one considers the food requirements of these cells and the nutritional ré-
gime of the present experiments. Carrel and Ebeling\textsuperscript{10} have shown that macrophages are able, not only to utilize plasma and serum substances themselves, but also to elaborate substances which are available for fibroblasts.

Several authors have already reported the occasional occurrence \textit{in vitro} of transformations from fibroblasts to macrophages, although little attempt has been made to define the factors responsible for the changes. In 1926, Carrel and Ebeling\textsuperscript{11} reported that twice in the period of 14 years during which the old strain of heart fibroblasts had been cultivated \textit{in vitro}, they had observed a transformation of scattered peripheral cells into macrophages. Still more recently, a similar phenomenon has been reported by Fischer,\textsuperscript{13} Ephrussi and Hugues,\textsuperscript{13} and W. and M. von Möllendorff.\textsuperscript{14}

The reverse process, that is, the transformation of macrophages to fibroblasts, has also been observed to occur. Thus, Carrel and Ebeling\textsuperscript{15} reported a transformation of large mononuclears into fibroblastic forms in old cultures in which migration and multiplication had practically ceased. Some years later, Fischer\textsuperscript{16} succeeded in obtaining a permanent strain of fibroblasts originally derived from a culture of blood leucocytes. Maximow\textsuperscript{17} and Bloom\textsuperscript{18} agree that monocytes cultivated \textit{in vitro} may hypertrophy and become transformed, first into macrophages and later into fibroblasts. In this connection, it is of interest to note, however, that it is impossible to distinguish between a monocyte removed from the blood and cultivated for several days in plasma and embryonic tissue juice, and a tissue macrophage. These cells are extremely sensitive to changes in the medium. When, for example, macrophages are cultivated in plasma, they are large.

\textsuperscript{12} Fischer, A., \textit{Arch. exp. Zellforsch.}, 1926, 3, 345.
\textsuperscript{13} Ephrussi, B., and Hugues, Y., \textit{Compt. rend. Soc. biol.}, 1930, 105, 697.
\textsuperscript{17} Maximow, A., \textit{Compt. rend. Soc. biol.}, 1917, 80, 225; \textit{Arch. exp. Zellforsch.}, 1928, 5, 169.
\textsuperscript{18} Bloom, W., \textit{Arch. exp. Zellforsch.}, 1928, 5, 269.
and possess a very definite undulating membrane. But when amino peptones are added to the medium, the undulating membrane disappears and the cells become elongated and "snake-like." If a medium is used in which the nitrogenous food materials have been replaced by Tyrode solution, the starved cells become smaller and assume the dimensions and appearance of blood monocytes. These changes, however, are reversible. When brought back to a plasma medium, the cells regain their original features.

Evans and Scott have defended the hypothesis that connective tissue is composed of but two cell types, namely, the phagocytic and the fiber-forming elements (macrophages and fibroblasts). This opinion is based upon the marked difference shown in the response of the two cell types to intravitam acid dyes. The present investigation and consideration of the literature show, however, that the cells comprising either of these races may, under certain conditions, appear either as typical fibroblasts or as typical macrophages. The form which the cell takes is but an expression of its physiological state at any one moment. In other words, the fibroblast and the macrophage represent, in vitro at any rate, extreme functional states of the same cell type. As macrophages, aside from their typical structural and functional characteristics, the cells display staining reactions with vital dyes which are identical with the reactions of the corresponding cell type freshly removed from the organism.

At this point, one may well ask what criteria exist for the definition of cell types and how one cell type may be distinguished from another. The classical histologists are satisfied to define them on the sole basis of their morphological appearance. That such definitions are inadequate, however, and contribute very little to our knowledge of cells is evidenced not only by the general state of confusion to which they have led, but also by the fact that the workers themselves are never in accord as to the relative values of the various systems of classification which have been so devised. Although it would seem to be self-evident that a cell cannot be conceived of apart from the medium in which it exists, this factor is usually ignored. In order, therefore, to

19 Carrel, A., Science, 1931, 73, 297.
gain a precise conception of any cell type, it becomes necessary to
determine and to specify not only its origin, but also the structural and
functional properties which it manifests under given environmental
conditions. In other words, descriptions must be based on the actual
conditions which exist. For this reason, it has been deemed unneces-
sary, in connection with the experiments which have been reported,
to enter into discussion as to whether the cells studied are or are not
identical with the fibroblasts in the tissues of the organism, or what
their status may be in terms of the various systems of nomenclature
which exist at the present time.

Brief reference should finally be made to the nature of the trans-
formation process itself. In this connection, the findings of the von
Möllendorffs are of particular interest. These authors observed
frequent transformations of fibroblasts into macrophages, both in
inflammation induced by the subcutaneous injection of mice and
rabbits with trypan blue, and in cultures of subcutaneous tissue de-
derived from adult rabbits and cultivated over very long periods of time
in a medium consisting of a mixture of rabbit plasma and rabbit spleen
"extract" containing the same dye substance. Furthermore, although
mitoses were occasionally observed in both types of experiments, a
high percentage of the cells gave evidence of amitotic division of the
nuclei. This led them to believe that the macrophages arose chiefly
by amitotic division of the fibroblasts, and that amitosis may be found
to occur wherever the metabolism of the cells is disturbed. It has
been seen that the present experiments have furnished considerable
evidence of this same type of cell multiplication. Whatever its signif-
icance may be, it is certain that it should not be overlooked. The
mere fact that the idea of amitosis may seem to infringe upon the cur-
rent theories of cellular heredity is certainly no sound reason either
for ignoring its existence or for relegating it to the very convenient
category of a degeneration phenomenon.

On the basis of these considerations we may assume, therefore, that
the potencies of living cells are far more varied than is generally be-
lieved and that the properties which they manifest at any particular
moment are functions not only of their inherent capacities, but also
of the composition of the environment in which they live and to which
they contribute.
SUMMARY

1. The ability of fibroblasts to mature and to manifest their various potencies in any particular medium is inversely proportional to the growth energy which they exhibit in that medium. Fibroblasts having access to high concentrations of food substances in their environment do not mature, regardless of their origin or the age of the animal from which they were derived. They behave as embryonic cells.

2. Fibroblasts cultivated in vitro are potentially able to produce cells with the structural and functional properties commonly attributed to macrophages. This is true regardless of their origin or the length of time which has elapsed since their isolation from the origin.

3. The fibroblast and the macrophage are considered to represent extreme functional and structural variations of the same cell type.

4. The structural and functional characteristics displayed by fibroblasts in vitro vary according to their origin and to the changes which take place in the composition of the medium in function of time.

EXPLANATION OF PLATES

PLATE 26

FIG. 1. Cultures 1120–1, 1121–3, and 1122–1. Fibroblasts from heart muscle (H), skeletal muscle (M), and the perichondrium of cartilage (C), respectively. These have been cultivated for 22 days in these flasks in a plasma medium, are in their 8th passage, and were made from strains which have received the same treatment from the moment of their isolation from the same chick embryo. × 1.

FIG. 2. Culture 396–1. Fibroblasts from heart (H), and from the perichondrium of cartilage (C) cultivated side by side in the same medium for 77 days; plasma treatment. These cultures are in their 12th passage and were made from sister strains. The fibroblasts from heart have been dead for more than 50 days; the fibroblasts from cartilage are still active. (Compare Text-fig. 4.) × 108.

FIG. 3. Culture 396–1. The same cultures after having been treated for 98 days. Note the area of dead heart fibroblasts now invaded by the actively growing cells from the adjacent culture. × 108.

FIG. 4. Culture 392–1. Fibroblasts from heart in the 12th passage, photographed after 65 days' cultivation in a plasma medium. This culture was allowed to remain in the flask for 100 days before being transferred. (Compare Text-figs. 1 and 2.) × 8.

FIG. 5. Culture 886–3 (from 392–1). Heart fibroblasts from the margin of a 3 day old culture which had been made from transplants taken from the above culture after 100 days' cultivation in the first flask. When photographed, these
cells were in their 13th passage and had been cultivated for 103 days in the plasma medium. × 115.

Fig. 6. Culture 1177. Peripheral cells from an 8 day old culture of fibroblasts from heart belonging to a strain originally derived from 392-1. When photographed, these cells were in their 18th passage and had been cultivated in the plasma medium for 183 days. × 108.

Fig. 7. Culture 112-2. Serial section of culture of fibroblasts from cartilage, in the 13th passage, cultivated for 44 days in the plasma medium. Stained with hematoxylin after fixation with formol-Zenker without acetic. × 272.

PLATE 27

Fig. 8. Culture 400-4. Transformed culture of fibroblasts from heart, in the 5th passage, cultivated for 15 days in the plasma medium. Note the broad band of macrophages. Fig. 13 shows a photograph of this culture made 3 days earlier. (Compare Text-fig. 5.) × 9.

Fig. 9. Culture 395-1. Degenerated cells from the margin of a transformed culture of fibroblasts from the periosteum of bone. This culture was in its 12th passage and had received the plasma treatment for 29 days. × 111.

Fig. 10. Culture 395-1. Lower magnification of margin of the same culture, showing both macrophages and the shadows of the fibroblasts (out of focus). × 41.

Fig. 11. Culture 395-1. Macrophages from the same culture. × 111.

Fig. 12. Culture 399-1. Active cells from the margin of a culture of fibroblasts from the periosteum of bone, belonging to the same series of experiments. These cells are also in their 12th passage and have received the plasma treatment for 42 days without transformation having occurred. × 111.

Fig. 13. Culture 400-4. Fibroblasts from heart, in the 5th passage, cultivated for 12 days in the plasma medium. 3 days later this culture transformed. (Compare Fig. 8.) × 9.

Fig. 14. Culture 401-1. Macrophages from the margin of a transformed culture of fibroblasts from muscle in the 5th passage. This culture had received the plasma treatment for 12 days before transformation occurred. × 41.

PLATE 28

Fig. 15. Culture 599-1. Transformed culture of fibroblasts from heart, in the 5th passage, after having received the plasma treatment for 21 days. × 21.

Fig. 16. Culture 599-1. Higher magnification of macrophages from the margin of the same culture. × 108.

Fig. 17. Culture 8000 C1. Macrophages from a transformed culture of the 19 year old strain of fibroblasts from heart, after 61 days of cultivation in a plasma medium. × 216.

Fig. 18. Culture 8000 C1. Atypical fibroblasts from the margin of the same culture. For the purpose of reproduction, it was necessary to accentuate the large upper cell. × 216.

Fig. 19. Culture 768 (from 600-2). Macrophages from a culture of fibro-
blasts from muscle which had transformed in the previous (5th) passage. At the time of transformation, the culture belonged to the series of experiments which included Culture 599-1 (Figs. 15 and 16). Both strains were of the same age and had a common past history. × 216.

Fig. 20. Culture 1272-1. Cells from a strain of fibroblasts from muscle, in the 13th passage, which have been cultivated for 26 days on lipoid-free serum protein, and stained supravitaly with neutral red. Note the binucleated cell and the absence of fat globules. × 216.

Fig. 21. Culture 600-2. Two macrophages and an atypical fibroblast from the culture of fibroblasts from muscle referred to above (Fig. 19). Note the distended, irregular, and heavily granulated appearance of the fibroblast-like cell. × 376.

Fig. 22. Culture 600-2. This photograph shows a number of macrophages, one of which is in the process of segregating itself from the remnant of a fibroblast. (Compare Text-fig. 7.) × 376.
(Tysker: Races constituting common fibroblasts, 1)
(Parker: Races constituting common fibroblasts.)