STUDIES UPON THE EFFECT OF LIGHT ON BLOOD AND TISSUE CELLS.

III. THE ACTION OF LIGHT ON FIBROBLASTS IN VITRO.

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PLATE 17.

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INTRODUCTION.

In the course of a series of observations on the effect produced on leucocytes and erythrocytes by irradiation in vitro (1), it was found that these cells underwent an extreme and rapid degeneration when exposed to light of various regions of the visual spectrum. The data obtained were such as to suggest that substances liberated from the erythrocytes, during their degeneration under the influence of light, played some rôle in the degeneration of the leucocytes when exposed to light. For the further analysis of this problem it seemed essential to obtain the leucocytes free from erythrocytes. Inasmuch as attempts to do this were unsuccessful, it was decided to try the fibroblast, which cell was found to be fairly satisfactory. Further work, detailed in this article, has indicated that the presence of the red blood cells does play a major rôle in degeneration of the fibroblasts under the action of light of the visual spectrum.

Kiaer (3), in 1925, studied the action of light from a Kromayer quartz mercury vapor lamp on pure cultures of fibroblasts. He found that the light from this source exerted a definite impeding action on the growth of the fibroblasts in cul-

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ture, and that an exposure of from 30–60 minutes resulted in the death of the cells.
Light from this source is very rich in ultra-violet wave-lengths and as these are
known to produce striking biological effects, Kiaer's data cannot be considered as
necessarily applying to the longer wave-lengths of light lying within the range of
the visual spectrum, with which the previous articles of this series (1, 2) were
concerned.

**Material and Methods.**

The fibroblasts used in this investigation were obtained from the hearts of
embryo chicks of from 6–12 days incubation. In obtaining the tissue, the chick
was removed from the egg and washed in Tyrode solution (pH 7.4). The heart
was then removed and cut up in the same solution, using a pair of iridectomy
scissors for this work. Fragments of the tissue were then transferred to cover-
glasses by means of a platinum loop, and there covered with a drop of Tyrode
solution (pH 7.4). The cover-glass was then inverted over the well of a hollow
ground slide, and its edges sealed with Salvoline. This whole process was carried
out aseptically in very dim light. The cultures were immediately set in a water-
jacketed incubator in the dark and kept there at 38° until the fibroblasts had
moved out on the cover-slip in a sheet of some size. This required from 10–60
hours. At the end of this time the cultures were divided into two groups; one
group was irradiated, while the other group was kept in the dark incubator as a
control.

The cultures which were to be irradiated were all given a careful examination
at the beginning of irradiation and were discarded at once if any trace of cellular
degeneration was present. Inasmuch as the least examination of the controls
would have vitiated the experiment, these could not be given a preliminary
examination.

The light source and optical system used in this work were the same as those
used in the previous work (1). Spectrograms of this light source, and of this light
source with the special filters described later, have already been shown (Earle (1),
spectra 4, 5, 6 and 7, Fig. 4). In all of these experiments, irradiation and micro-
scopic examination of the cultures were carried out simultaneously.

**Experimental.**

In the preliminary work on this problem, an attempt was made to
irradiate the fibroblasts under conditions as similar as possible to
those under which the white cells degenerated. The factors which
had to be taken into consideration were, (1) that the white cells them-
selves were present (2) that red cells were present in varying num-
bers, (3) that even in cultures of cells in saline, at least traces of
serum were present and (4) that air was present in large volumes
compared with the actual volume of the explant.
In order to secure cultures of fibroblasts in which these conditions prevailed, red blood cells were obtained by puncture, with a capillary pipette, of one of the extra embryonic blood vessels of an embryo chick. These red cells were washed with three changes of Tyrode solution having a pH of 7.4. Cultures were then made using tissue from the heart of the same chick. These cultures were inoculated, as described above, except that a few of the washed red blood cells were also added. Some of these cultures were irradiated while the others were kept in the dark as controls.

An examination of these irradiated cultures showed conclusively that under these conditions the light did cause an extreme degeneration of the fibroblasts. Beginning at about 15–30 minutes after the irradiation was started, the refractive index of the fibroblasts showed a gradual but distinct increase. Normally the fibroblasts in culture have such a low refractive index as to be seen only with difficulty, while the nuclei are exceedingly difficult and often impossible to discern. In these irradiated cultures, however, the refractive index of the fibroblasts increased so greatly that the cells and all of their processes could be outlined with ease. At the same time, the nuclei also became sharply defined.

After the lapse of from 30–140 minutes, vacuoles of low refractive index began to appear throughout the cytoplasm of the cells. These vacuoles did not appear to originate in any single region of the cells, nor did they generally seem to begin as minute sharply defined droplets, increasing in size. Rather, in some region where the cytoplasm had appeared homogeneous, there appeared a small area with a very slightly lower refractive index than the surrounding region, but at first almost indistinguishable from it. The optical distinction between this area and the surrounding region increased rapidly, however, until the droplet appeared as a clear area of low refractive index, a "punched out" looking area in the cell, surrounded on all sides by the more highly refractive cytoplasm. These vacuoles were of all sizes and occurred in different numbers in different cells. When irradiation was continued for 3 or 4 hours, practically every cell in the culture was densely crowded with them. Upon staining the cultures supravitally with neutral red, they took up some of the dye, and were colored orange-red by it. They showed no reaction with Sudan III,
or with osmic acid. From these staining reactions and from their low refractive index, it would seem that their chief content was probably water. About the time the vacuoles began to appear, the cells often showed some tendency to round up. If irradiation was continued until the cells were crowded with vacuoles, this rounding up was almost constant in its appearance, and often occurred to a very marked degree (Fig. 1).

A modified form of this degeneration was frequently seen in these cultures. Instead of the formation of discrete vacuoles in the cytoplasm of the cells, the viscosity of the cytoplasm showed a great decrease, as indicated by the marked increase in the amplitude of brownian movement of intracellular particles. The cells began swelling very rapidly, and, as a result, became almost spherical. None of these cells was ever seen to burst as a result of this swelling.

In the unirradiated fibroblasts in tissue culture, there were generally a few highly refractive droplets staining with Sudan III or with osmic acid, and obviously of a lipoid nature. At the end of several hours of irradiation, these droplets were often perceptibly increased, both in size and in number. The dominant features in this degeneration, however, were the change in the refractive index of the cell and the formation of vacuoles throughout its cytoplasm.

The whole process of degeneration as above described, often took place within 1 hour, and never required longer than 3. This degeneration was remarkably constant in its appearance, although in some cultures it appeared in the modified form previously described. In this modified form the changes seen appeared to be identical with those seen in the polymorphonuclear neutrophils under the action of light.

The protocols from a representative culture of this series, and its controls are here shown as Experiment 1.

**Experiment 1.**

*Culture 801.*—This culture was planted from the heart of an 11 day embryo chick. *16 hours after inoculation:* Irradiation was begun. At this time the fibroblasts had grown out in a sheet. The cells appeared normal; their refractive index was low, and a few fine highly refractive droplets were scattered through them. There was no sign of vacuoles in any of the cells. *40 minutes later:* A marked in-
crease in the refractive index of the cells had occurred; details of cell boundaries and nuclei could be made out. At this time some of the cells showed a single vacuole from one to four times as large as the nucleus. 100 minutes: Many cells showed definite vacuoles, and quite a few were filled with them. In most of the cells the vacuoles were unmistakably of cytoplasmic origin, some of them originating far out in the pseudopodia. 300 minutes: Practically all of the cells were filled with vacuoles. In this respect there had been little change in the last 150 minutes. The cells showed distinct signs of rounding up. The preparation was photographed at this time (Fig. 1).

Cultures 805 and 807.—Control cultures. Kept in the dark incubator. 21 hours after inoculation: The fibroblasts appeared normal. They were of low refractive index, and were very difficult to see. There were only a few fine droplets in the cells, and no vacuoles were present.

The attempt was next made to see if, as in the case of the leucocytes, the degeneration of the fibroblasts was caused by wave-lengths of light

TABLE I.

Experiment 2.

Time Required for the Degeneration of Fibroblasts in Vitro When Irradiated by Light Transmitted by Various Filters.

<table>
<thead>
<tr>
<th>Culture number</th>
<th>Age of chick</th>
<th>Organ from which culture was taken</th>
<th>Age of culture</th>
<th>Time required for degeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>days</td>
<td>hrs.</td>
<td>min.</td>
<td>No filterTransmission 100 per cent</td>
</tr>
<tr>
<td>829</td>
<td>8</td>
<td>Heart</td>
<td>18</td>
<td>120</td>
</tr>
<tr>
<td>831</td>
<td>8</td>
<td>Heart</td>
<td>27</td>
<td>630</td>
</tr>
<tr>
<td>832</td>
<td>8</td>
<td>Heart</td>
<td>39</td>
<td>160</td>
</tr>
<tr>
<td>853</td>
<td>12</td>
<td>Heart</td>
<td>18</td>
<td>About 26</td>
</tr>
<tr>
<td>854</td>
<td>12</td>
<td>Heart</td>
<td>About 26</td>
<td>780</td>
</tr>
<tr>
<td>858</td>
<td>12</td>
<td>Heart</td>
<td>39</td>
<td>570</td>
</tr>
<tr>
<td>859</td>
<td>12</td>
<td>Heart</td>
<td>51</td>
<td>160</td>
</tr>
<tr>
<td>860</td>
<td>12</td>
<td>Heart</td>
<td>60</td>
<td>About 26</td>
</tr>
<tr>
<td>897</td>
<td>9</td>
<td>Heart</td>
<td>35</td>
<td>130</td>
</tr>
<tr>
<td>921</td>
<td>8</td>
<td>Intestine</td>
<td>14</td>
<td>145</td>
</tr>
<tr>
<td>922</td>
<td>8</td>
<td>Intestine</td>
<td>About 16</td>
<td>90</td>
</tr>
<tr>
<td>931</td>
<td>8</td>
<td>Intestine</td>
<td>About 21</td>
<td>160</td>
</tr>
<tr>
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<td>8</td>
<td>Intestine</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>937</td>
<td>8</td>
<td>Intestine</td>
<td>20</td>
<td>70</td>
</tr>
<tr>
<td>1579</td>
<td>8</td>
<td>Heart</td>
<td>About 16</td>
<td>600</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
</tbody>
</table>
lying throughout the range of the visible spectrum. Accordingly, cultures were inoculated and, as in the case of the experiments on the white blood cells (1), were irradiated through Wratten filters Nos. 45, 58 and 29. The time required for the fibroblasts in each culture to undergo degeneration when that culture was irradiated by light transmitted by any one of these three filters is shown in Table I. This table also shows the time required for the degeneration of control cultures irradiated with the same light source but with no Wratten filter inserted in the optical path. Although not presented in this table, other control cultures, inoculated as above but kept in the dark, remained normal.

In preparing this table, the degeneration time for any culture was arbitrarily defined as the time required for the appearance of a marked increase in the refractive index of a majority of the fibroblasts in the microscopic field, accompanied either by the appearance of many vacuoles in the cytoplasm of the cells, or by a marked tendency for the cells to round up and to show a greatly increased amplitude of brownian movement of their intracellular particles. It may be seen from this table that wave-lengths of light transmitted by any one of the three filters used caused the typical degeneration of the fibroblasts.

The next question considered was the possible mechanism of this action of light on the fibroblasts. In this consideration, three possibilities arose. The degeneration of the fibroblasts under irradiation might have been due to, (1) an intrinsic action of light on these cells, or (2) an intrinsic action of light upon the red cells with the formation of toxic substances, which in turn produced a degeneration of the fibroblasts, or (3) to a sensitization of the fibroblasts by some substance liberated from the red cells, with a subsequent action of light on the sensitized fibroblasts.

In order to determine whether the red blood cells did play a rôle in the degeneration of fibroblasts under the influence of light, cultures were prepared as before, except that this time no red cells were added to the culture. These cultures were then placed in two groups, one group being kept in the dark as a control, while the other was irradiated. A further control was kept on these cultures by irradiating similar cultures containing fibroblasts and red cells. All the cultures containing red cells and exposed to the action of light showed a typical
degeneration of the fibroblasts. In one group of eight cultures irradiated, every culture showed a typical extreme degeneration in less than 3 hours. On the other hand, of five cultures, supposedly containing no red blood cells, one showed the typical degeneration only after irradiation for 13 hours. The other four showed only minor degenerative changes at the end of from 13–24½ hours of irradiation. These minor changes consisted of an increase in the refractive index of the cells and the rounding of some of the sharper protoplasmic processes. In these cultures there was no indication whatsoever of any swelling of the cells, of any increase in the brownian movement of intracellular particles, nor of any formation of vacuoles within the cytoplasm. This contrast, between the behavior of the cultures containing red cells and that of the cultures containing no red cells, was augmented by the fact that at the close of the irradiation of the cultures containing no red cells, even some of the controls which had been kept in the dark, were showing traces of degeneration. In every instance, however, this degeneration of the controls was markedly less than that in the cultures kept in the light.

A protocol of a representative culture of this series is presented below as Experiment 3.

Experiment 3.

Culture 823.—Inoculated from the heart of an 11 day embryo chick. 24 hours after inoculation: Irradiation was begun; the cells appeared normal at the time although they contained a good many fine droplets of fatty material. 100 minutes later: There had been no change except a very slight increase in the refractive index of the cells. 270 minutes: There had been no change and the cells appeared normal. 15 hours: The cells showed a somewhat higher refractive index than was normal, and their nuclei were sharply defined. They were not rounding up and showed no vacuoles or swelling. 17 hours: There had been little change. A representative cell was photographed at this time (Fig. 3).

DISCUSSION.

There was a definite similarity between the type of degeneration seen in the fibroblasts and that observed in the erythrocytes and in the leucocytes. The similarity between that shown by the fibroblasts and by the neutrophils was particularly striking. No formation of discrete vacuoles of large size has been observed in the neutrophils,
probably owing to their more fluid cytoplasm and its consequent more general liquefaction. It should be noted, however, that sometimes vacuole formation in the fibroblasts was so slight that no vacuoles could be made out in the unstained cells, the cytoplasm undergoing just such a liquefaction as occurred in the neutrophils. This liquefaction was indicated by a tremendously increased amplitude of brownian movement of particles within the cells. When this occurred the cells rounded up, became almost spherical, and swelled rapidly. Time and again, fibroblasts were seen in this state, and if the complete process of degeneration of the individual cells had not been followed, they would have been indistinguishable from the swollen polymorphonuclear neutrophils seen in the cultures of blood which had degenerated under the influence of light (Earle (1), Fig. 1).

It was found, as in the case of the leucocytes and the erythrocytes, that light of wave-lengths lying within the three zones (a) 430μμ-550μμ; infra-red; (b) 475μμ-630μμ; 690μμ-infra-red; (c) 600μμ-infra-red, was active in causing this degeneration of the fibroblasts. The relative activity of these three zones for the erythrocytes is apparently not the same as for the fibroblasts, for the intensities of light employed. The light transmitted by each of these three filters caused an hemolysis of the red cells within practically the same time, and within the same time that was required by white light of even greater intensity. It was found, however, that when either the blue filter No. 45 (less than 5 per cent total transmission) or the red filter No. 29 (total transmission about 6.6 per cent) was placed in the beam of light irradiating the fibroblasts, the time required for the degeneration of the cells was much greater. A similar, though a less striking phenomenon was also noted in the cultures of leucocytes previously irradiated (1). This difference between the leucocytes and the fibroblasts on the one hand, and the erythrocytes on the other, is most interesting, although at present no explanation for it is offered.

Another interesting point brought out by these data is that when irradiated through the green filter, the fibroblasts consistently seemed to show a more rapid degeneration than when irradiated with white light (100 per cent transmission). This was also found true for the cultures of irradiated leucocytes (1). Before any definite conclusions are drawn on this point, however, these data should be verified and extended by means of a more accurate quantitative technique.
It was found that the fibroblasts, in cultures to which no red cells had been added, had degenerated much less at the close of from 13-24 hours of irradiation than they had at the end of from 2-3 hours irradiation when red cells were present. This observation indicates that the red cells played some major rôle in the degeneration of the fibroblasts. In these cultures, supposedly containing no red cells, the tissue was carefully washed, and red cells were certainly very few in number. However, a very few red cells were seen in the culture which showed the typical degeneration at the end of 12 hours. In the other cultures it is quite possible that either isolated red blood cells or traces of the substances liberated from disintegrated red cells were present. For this reason, it is impossible to say that even the traces of degeneration seen in these cultures were due to an action of light directly on the fibroblasts alone. Certainly any action of light of the visual spectrum on the fibroblasts alone was exceedingly slight, its major influence being conditioned by the presence or absence of red blood cells.

Whether the presence of the red cells is also essential to the degeneration of the leucocytes under the action of light has not as yet been determined. There are, however, several facts which indicate that it plays some rôle. In tissue cultures of leucocytes it was noted that, for any one light source, there was a marked variation in the time required for the degeneration in different cultures of any one series. In these cultures, there was also a marked variation in the number of red blood cells present. Furthermore, in hanging drops of whole blood, the degeneration of the leucocytes rarely occurred before there were marked signs of degeneration of the erythrocytes, and it was never delayed more than a few minutes after the hemolysis of the erythrocytes had begun.

SUMMARY.

1. In the presence of autogenous red blood cells, fibroblasts, grown in vitro from the heart and intestine of embryo chicks of from 6-12 days incubation, underwent a rapid degeneration when exposed to light of the visual spectrum.

2. In this degeneration, the cells showed a marked increase in refractive index, and a massive formation of colorless vacuoles of low refractive index. These vacuoles gave no reaction with osmic acid.
or Sudan III, but took up neutral red. Later in the degeneration the cells showed marked signs of rounding up and of coagulation.

In some cases this degeneration process was modified in such a manner that, instead of the formation of vacuoles, the whole cytoplasm became much less viscid and the cells swelled greatly and became spherical.

3. The similarity of this degeneration to that shown by the erythrocytes, and more particularly, to that shown by the polymorphonuclear neutrophils, under the action of light, is pointed out.

4. Degeneration of the fibroblasts occurred when the cells were irradiated by light of any one of the following wave-length zones: (a) 430\(\mu\mu\)–550\(\mu\mu\); infra-red; (b) 475\(\mu\mu\)–630\(\mu\mu\); 690\(\mu\mu\)–infra-red; (c) 600\(\mu\mu\)–infra-red.

5. This degeneration was much slower for the cells irradiated through the blue or red filters of less than 5 per cent, and less than 6.6 per cent, respective total transmissions. When irradiated through the green filter of 23 per cent total transmission it was much more rapid, and even more rapid than for cultures irradiated with white light of 100 per cent total transmission. These data were obtained on a short series of cultures and must be considered only as suggestive.

6. In the apparent absence of red cells, the fibroblasts underwent only a slight degree of degeneration after irradiation lasting from 13–24\(\frac{1}{2}\) hours. It is possible that even this minor degeneration may depend upon the presence of traces of disintegration products of red cells in the culture. Much more advanced degenerative processes were obtained, when many red cells were present, after only 3 hours irradiation.

BIBLIOGRAPHY.


EXPLANATION OF PLATE 17.

Fig. 1. Culture 801, × 1000 (see text, page 687). Fibroblasts taken from the heart of an 11 day chick embryo, and inoculated with erythrocytes present in the culture. Irradiated for 5 hours. Photographed so that half of the photographic plate was given a longer exposure than the other half. The fibroblasts show a striking increase of refractive index. There was a marked rounding up of the cells and the cytoplasm was filled with vacuoles.

Fig. 2. Culture 820, × 1000. Fibroblasts taken from the heart of an 11 day chick embryo, and inoculated in culture, no erythrocytes being present. Photographed 52 hours after inoculation of the culture and after 13 hours of irradiation. Note that while the refractive index of the cells is somewhat greater than normal, and that their cytoplasm is somewhat more granular than normal, there is no sign of rounding up of the cells, nor are there any vacuoles visible in the cytoplasm.

Fig. 3. Culture 823, × 1000. Fibroblasts taken from the heart of an 11 day chick embryo, and inoculated in culture, no erythrocytes being present. Photographed 40 hours after inoculation and after 17 hours of irradiation. Note that there is some tendency for the cell to round up; its nucleus is clearly outlined, and the refractive index of the cell is slightly increased. There is far less degeneration than was shown in Culture 801, Fig. 1, however.
Earle: Effect of light on blood and tissue cells. III.)