THE ACTION OF LIGHT ON THE LEUCOCYTE COUNT.

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It has been generally supposed that light produces some effect on the blood, but the experiments which have been done to test this point are many of them unsatisfactory, and the results reported are inconclusive.

In speaking of light one includes, not only the visible spectrum, from 400 to 760 $\mu\mu$, but also the ultra-violet region from 100 to 400 $\mu\mu$, and the infra-red of wave-length longer than the visible. The ultra-violet should be divided into two parts; the near ultra-violet, i.e., the wave-lengths just below the visible, from 300 to 400 $\mu\mu$, and the far ultra- violet, from 100 to 300 $\mu\mu$. The physiological effects produced by these two regions are strikingly different. In previous work practically no attempt has been made to study different wave-lengths of light separately. Experiments have usually been made with sunlight which extends only to 291 $\mu\mu$ in the ultra-violet, and includes, therefore, near ultra-violet and visible light and a large amount of infra-red. Owing to the absorption of water vapour in the atmosphere, very little infra-red of wave-length longer than 400 $\mu\mu$ is transmitted.

The effect produced by light on the erythrocyte count has been found in general to be negative. Long-continued absence of light certainly produces no marked change in the number of red cells. Blessing (1), who acted as physician to Nansen during his expedition in the Fram, published a report in 1898, showing that members of the party exhibited no evidence of anemia during the trip. Similar results were reported for animals by Grober and Sempell (2) in 1919. They examined horses that had worked for years in coal mines, and found no anemia in any case where a satisfactory nutritive condition existed. Borissow (3) kept rabbits and dogs in the light and the dark and reported negative results on the erythrocyte count.

A number of investigators, however, have performed similar experiments with positive results. Graffenberger (4) in 1893 reported

that rabbits showed an increase in the percentage of hemoglobin in the light and a decrease in the dark. This result was confirmed by Marti (5) in 1897. He found that rats kept in the dark showed a decrease in the red count and in the percentage of hemoglobin, while those exposed to sunlight, in the day, and an arc light at night, had an increased count. Orum (6) reports similar results, although he introduces a new factor in that he lays emphasis upon changes in blood volume caused by light. Exposure to darkness or to red light causes an initial decrease in the red count and the percentage of hemoglobin, but after a month or two the count and percentage of hemoglobin are increased, owing to a marked concentration of the blood. In white light, and especially in blue light, there is, on the contrary, a condition of plethora, together with an increase in the total hemoglobin. It would seem to be highly desirable to have these experiments repeated with more modern methods of determining the blood volume. If the effects on volume of long continued exposures to red and violet lights are as striking as described by Orum, they would indicate an actual decrease in red corpuscles in the dark (or red), and an increase in the white (or violet), compensated more or less in each case by a diminished or an increased volume of the blood.

All the positive results reported agree and give considerable evidence for believing that there is a decrease in the red count and in the percentage of hemoglobin in the dark, and an increase in the light. These changes, however, are not permanent and the blood count later returns to normal.

The leucocytes show a greater and more definite response to short exposures to light than the erythrocytes. Polito (7) exposed rabbits to direct sunlight for periods of time ranging from fifteen minutes to an hour. The length of exposure made practically no difference, and he reports an increase in the leucocyte count immediately after exposure, amounting to from 2,000 to 5,000 in the total count, with most of the increase in the mononuclear cells. After an hour the blood returned to normal, and unfortunately was not studied further. These animals were exposed to direct sunlight at a temperature of 38° to 42° C., so that the light effect was seriously complicated by a possible heat effect. Murphy (8) has shown that five minutes' exposure of an animal to dry heat, at 55° to 65° C., will produce a rise in lymphocytes of 100 to 200 per cent. over the normal count.

Aschenheim (9) reported that an exposure of infants to direct sunlight, for an hour, usually brought about an increase in the num-

ber of leucocytes, the neutrophiles being relatively diminished and the lymphocytes relatively increased.

In 1919 a paper was published by Taylor (10) on the effect of exposure to the sun on the circulating lymphocytes in man. Thirty-eight people were examined before and after a summer at Woods Hole, Massachusetts. In twenty-five of the thirty-eight there was an appreciable increase, both percentage and absolute, in the number of circulating lymphocytes. The differential counts showed an average increase from 27.4 to 35.6 per cent. Eight subjects showed a decrease and five no appreciable change. Of these six failed to tan and three were very dark to begin with. It seems hardly justifiable to attribute this increase solely to ultra-violet light when other agencies were active, such as change in climate and mode of life

It is, however, interesting in this connection to find that the blood counts made by Chamberlain, Vedder, Phalen, Wickline and others, on Americans in the Philippines and on native Philippinos, show an increase in the percentage of lymphocytes and a decrease in polymorphonuclears, as a result of residence in the tropics. Their results have been summarized briefly in the following table, from Chamberlain and Vedder (11) (see Table I).

TABLE I.-

Observer.	Race.	Number observed.	Polymorphonuclears.	Small lymphocytes.	Large lymphocytes.	Total count.
Wickline (12).....	Americans, 18 months' service	104	54.9	33.4	6.1	6,831
Guerrera (13) and Sevilla.....	Philippinos	129	51.6	34.5	4.1	—
Phalen (14).....	Americans, more than one year's service	115	58.7	32.6	4.6	—
Chamberlain (15).	Igorots	40	46.9	37.2	5.7	—
Chamberlain (11) and Vedder.....	Americans, 14 months' service	72	56.8	31.7	6.9	7,304
Chamberlain (11) and Vedder.....	Philippinos	50	52.2	29.9	6.6	9,248
Simon (16).....	Normal count, temperate climates	—	60 to 70	20 to 30	1 to 6	5,000 to 10,000

Wickline (12) has shown that these low polymorphonuclear and high lymphocyte counts, for Americans living in the Philippines, developed gradually. He made counts on 104 soldiers, on arrival and 3, 12, and 19 months later. After 3 months the mononuclear cells

averaged 31.4 per cent., after 12 months 34.4 per cent., and after 19 months 39.5 per cent. The small mononuclears, which undoubtedly correspond to the small lymphocytes, give a percentage of 21.8, 26.6, and 33.4 at the three examinations. During this change the total count stayed nearly constant, being 6,943 at the preliminary examination, and 6,699, 6,624, and 6,831 after 3, 12, and 19 months, respectively. How much of this effect is due to the action of tropical light is impossible to say. There has been a very prevalent idea that the solar spectrum extends further into the ultra-violet in the tropics. This is not true. The solar spectrum has been photographed at Manila, by means of a Rowland grating, by Freer (17) and was found to extend to 291 $\mu\mu$. Miethe and Lehman (18) made measurements at Assuan, Berlin, Zermatt, Görnergrat and Monte Rosa and found the limit of the spectrum always the same, 291.55 $\mu\mu$ to 291.21 $\mu\mu$. Neither latitude nor altitude thus makes any appreciable difference in the extent of the solar spectrum in the ultra-violet. However, light in the tropics has greater intensity than in temperate zones, so that in this way there may be a greater exposure in the tropics to ultra-violet light, as well as to visible light and heat. Most of the effects produced on soldiers in the tropics, such as increased pulse rate, respiration, and body temperature, can also be produced by moist heat. (Phalen 14.) Whether the blood changes are due to this cause, or whether they result from an increased exposure to ultra-violet light, is at present impossible to say.

In general, however, one can conclude from former work that exposure to sunlight results in an increase in lymphocytes. The experiments have all been complicated by the action of heat and are suggestive rather than conclusive.

In the experiments reported here an attempt was made to study separately the effects of different regions of visible and ultra-violet light, using short exposures.

Method.

Rabbits were exposed to the light of an iron arc for one hour, at a distance of ten inches. At this distance there was no heat effect, the temperature remaining below 25° C. The rabbits were enclosed in a box, with only the head exposed, and the eyes were covered during exposure, so that only the ears were radiated. The hair was clipped from the ears before radiation, so that the skin was well exposed. Blood counts, both total and differential, were made before

and after exposure, and the animals were then returned to a basement room, very dimly lighted by diffuse light, in which all animals had been kept some weeks or months before being used. The blood was counted daily until a normal condition seemed to be reestablished. This usually occurred in about three weeks.

The iron arc gives a brilliant light which, when examined spectroscopically, is so rich in lines that it almost amounts to a continuous light source. It reaches to about 238 $\mu\mu$, and is strong throughout the ultra-violet. No quantitative work has been done on the relative

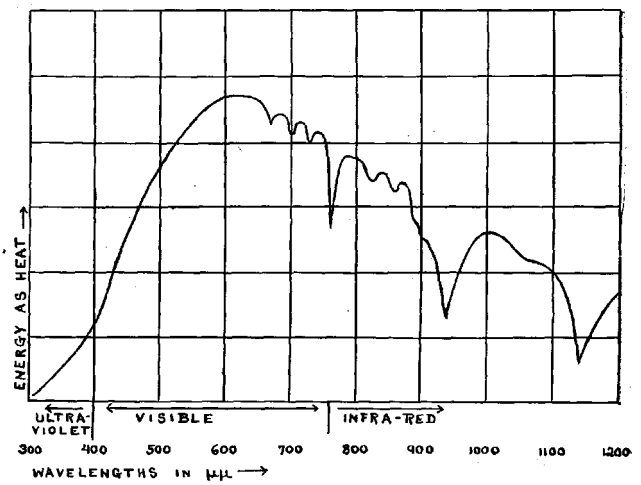

FIG. 1. Distribution of energy in the solar spectrum (Langley). Ordinates —energy as heat, units arbitrary. Abscissae—wave-lengths in $\mu\mu$.

intensity of the iron arc throughout the spectrum. Pflüger (19) investigated the energy distribution in the spark spectrum of iron, with a bolometer, and found the greatest energy in the ultra-violet. The distribution in the arc is very different from that in the spark, but it can be assumed that the ultra-violet region is at least as intense as the visible, and probably of greater intensity. In Fig. 1 the distribution of energy in the solar spectrum is given, showing that in the sunlight there is a great exposure to visible light and a very weak exposure to ultra-violet, while the reverse is true of the iron arc.

In order to investigate separate regions of light a number of different filters were used. The absorption of these is well known, but the particular filters were tested by photographing the transmitted light with a meter focus concave grating spectrograph. The transmission of these filters is given in Table II.

TABLE II.

Source.	Transmission ($\mu\mu$).
Iron arc unscreened	238 →
Iron arc through glass	320 →
Iron arc through ultra-violet glass*	330 → 390
Iron arc through picric acid	450 → 750
Iron arc through red glass	650 →

It is impossible to isolate the ultra-violet below 300 $\mu\mu$ by means of a filter, as this region is strongly absorbed by all substances except those, such as quartz or fluorite, which also transmit the visible. By comparing the effect of the radiation with and without the plain glass screen it is possible to estimate which part of the effect is due to the far ultra-violet below 300 $\mu\mu$.

The absorption of blood is compared with the transmission of the filters in Fig. 2. It might be expected that the effect of light on the blood would be due to the absorption of light energy by some constituent of the blood, with resultant physical and chemical action. It can be seen that the region from 230 to 300 $\mu\mu$ is absorbed by the hemoglobin and also by the blood serum. The hemoglobin also absorbs the region from 300 to 450 $\mu\mu$, and has two absorption bands in the yellow, between 530 and 590 $\mu\mu$.

It is impossible to say anything very definite in regard to the absorption of the skin, although the experiments reviewed by Jesionek (20) show roughly that light of wave-length shorter than 300 $\mu\mu$ is absorbed by the epidermis, and that bloodless tissue will transmit some rays throughout the visible.

Technique of blood counting.

A word should be said about the technique of blood counting used in this work. Previous work, by the author, on rabbits had proved unsatisfactory, owing to large and inexplicable variations in the white count, although the red counts had been reasonably constant.

* This ultra-violet glass was obtained from Dr. R. W. Wood. It absorbs the visible and transmits the near ultra-violet, just below the visible.

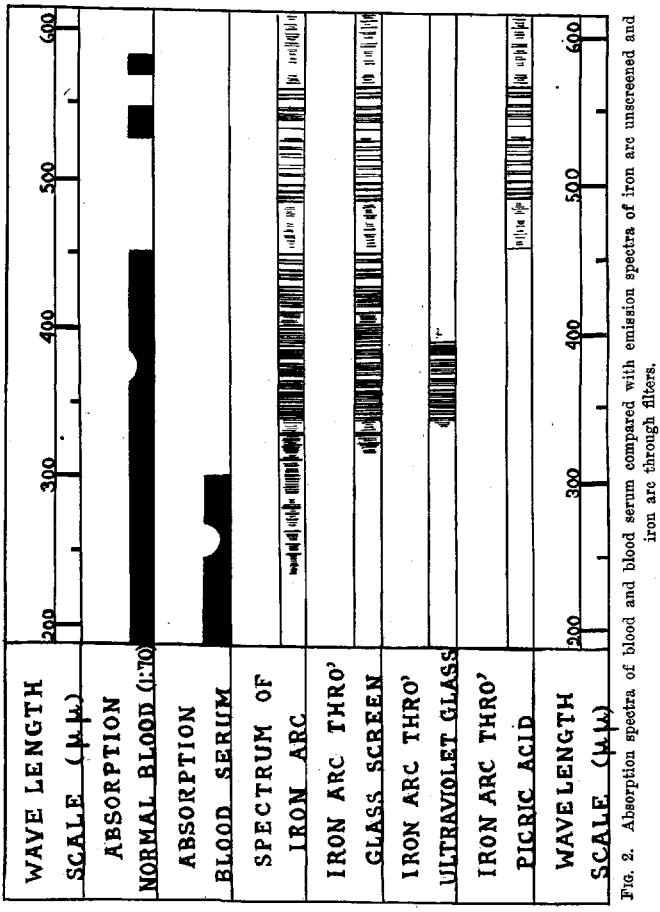

FIG. 2. Absorption spectra of blood and blood serum compared with emission spectra of iron arc unscreened and iron arc through filters.

Believing this to be due to different conditions of circulation in the ear when the counts were made, the leucocyte counts on the normal, untreated ear, and the ear when well flushed with hot water (45° C.) were compared. The results obtained are shown in Table III.

TABLE III.

Leucocyte Counts.

	Untreated.	Flushed once.	Flushed twice.
Rabbit I.....	16,640	11,700	—
Rabbit II.....	16,930	12,510	11,900

Apparently, on flushing the ear and getting a rapid circulation through it, the white count drops to a steady level, which is presumably that of the circulating blood. By following this technique very consistent counts were obtained. Some of the rabbits used gave very constant counts from day to day, when not subject to radiation. Others showed variations, and these responded to radiation with larger changes than the rabbits with constant counts, although the changes were of the same type.

In making the differential count the smears were stained with Jenner's stain and the following classification was followed; large mononuclears (cells in which the ratio of nucleus to cystoplasm is relatively small); lymphocytes (all cells, both large and small, in which this ratio is relatively large); polymorphonuclears (these are called pseudo-eosinophiles by Klieneberger and Carl (21), and correspond to the neutrophiles in man); eosinophiles, and mast cells. A normal differential count, as given by the non-radiated rabbits, is roughly as follows:

	Large mononuclears.	Lymphocytes.	Polymorphonuclears.	Eosinophiles.	Mast.
Per cent.....	1	64	30	1	4

Results.

In the first experiments the total red and total white counts were followed for each animal, but no differentials were made. The erythrocyte count gave negative results. The leucocyte count, however, showed marked and consistent variations after exposure to light.

The red count was therefore abandoned, and total and differential white counts were made throughout the rest of the work.

RESULTS OF EXPOSURE TO THE UNSCREENED IRON ARC (FIGS. 3, 4 and 5).

Immediately after the exposure there is a sharp drop in the total count (Fig. 3). This is contrary to Polito's results (7), but the conditions of the experiment were also different from his. The differ-

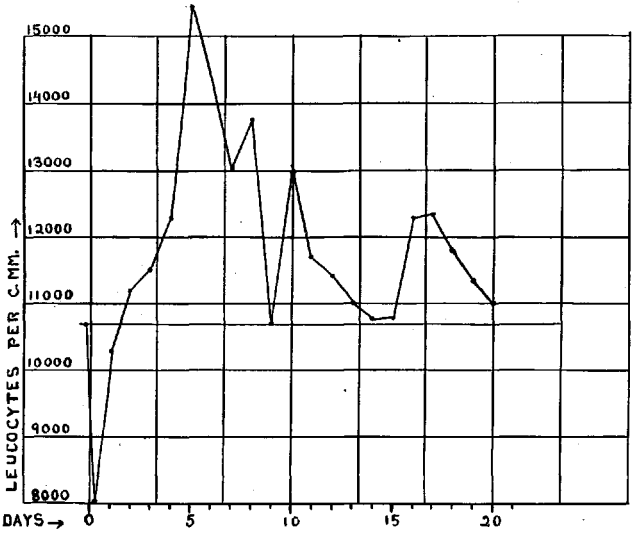

FIG. 3. Total leucocyte count, after exposure to unscreened iron arc (average of five rabbits). Ordinates—leucocytes per c.mm. of blood. Abscissae—time, in days, after exposure.

ential (Fig. 4) shows this to be due entirely to a drop in the number of lymphocytes, so that it cannot be attributed to an increase in blood volume. By the next day the total count (Fig. 3) was approximately normal, and then rose steadily reaching a maximum on the fifth day. In the first experiments there was a sharp rise above normal on the second day. This was not marked in later experiments, due perhaps to the fact that the same animals were used repeatedly, and thereby

lost their susceptibility somewhat. The differential curve (Fig. 4), however, shows a rise in lymphocytes on the second day after exposure, although it is much less marked than the rise on the fifth day. Comparing the total curve with the differential, the polymorphonuclears are seen to remain nearly steady throughout, though a little below normal, while the total curve and lymphocyte curve run almost

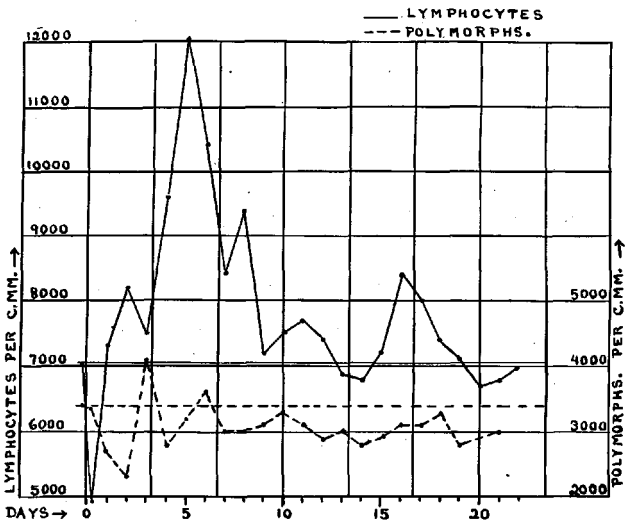

FIG. 4. Lymphocyte and polymorphonuclear counts, after exposure to unscreened iron arc (average of four rabbits). Ordinates—cells per c.mm. of blood. Abscissae—time, in days, after exposure.
———— lymphocytes — — — — — polymorphonuclear leucocytes.

parallel. The large maximum on the fifth day is followed by a return to normal on the ninth. This is followed by two small rises on the eleventh and sixteenth days. After three weeks the blood was apparently normal again, so that the experiments were not carried any further. The result of the exposure to the bare iron arc is an approximately normal polymorphonuclear count and, except for the initial drop immediately after exposure, a lymphocyte count above

normal for three weeks. The maximum rise on the fifth day amounts to an increase of almost 100 per cent. over the original count.

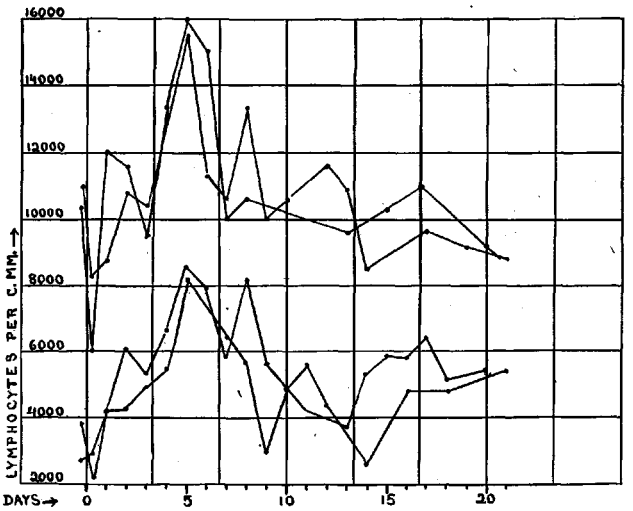

FIG. 5. Lymphocyte counts on four individual rabbits (same rabbits, averaged in Fig. 4), after exposure to unscreened iron arc. Ordinates—lymphocytes per c.mm. of blood. Abscissae—time in days after exposure.

In Fig. 5 the lymphocyte curves of four individual rabbits are plotted, in order to show how closely they agree with each other and with the average lymphocyte curve in Fig. 4.

RESULT OF EXPOSURE TO THE IRON ARC THROUGH A GLASS SCREEN FIG. 6.

Only two animals were radiated through glass, but the results were sufficiently parallel to warrant drawing conclusions from them. The total leucocyte count and the absolute lymphocyte and polymorphonuclear counts are all plotted in Fig. 6. There is practically no change in the total count immediately after radiation, but the differential count shows a large decrease in lymphocytes, which is balanced by a corresponding increase in polymorphonuclears. The

total count falls steadily until the third day, after which it fluctuates until the thirteenth day, although far below normal all the time. On the thirteenth and fourteenth days there is a marked drop, followed by a gradual rise to normal. After the initial rise and fall, immediately following the exposure, the polymorphonuclears stay approximately normal, except for a small rise on the seventh day. The decrease in total count is therefore due entirely to a fall in the num-

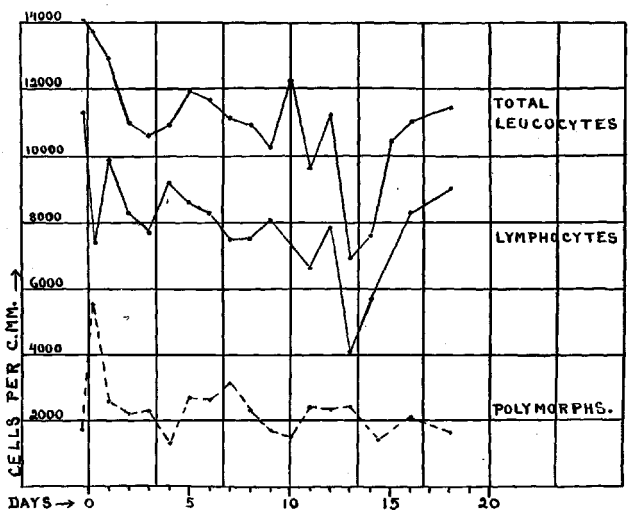

FIG. 6. Total leucocyte, and absolute lymphocyte and polymorphonuclear counts, after exposure to iron arc through glass (average of two rabbits). Ordinates—cells per c.mm. of blood. Abscissae—time, in days, after exposure——— ——— leucocytes and lymphocytes — — — — — — — polymorphonuclear leucocytes.

ber of lymphocytes. These stay far below normal for three weeks or more, and are reduced to one third of the original count on the thirteenth day after exposure.

The curves for radiation with the iron arc bare (Figs. 3 and 4), and with the iron arc through glass (Fig. 6), are strikingly different. Neither radiation produces any change in the number of polymorphonuclears after the first day, but both produce a very profound change

in the number of lymphocytes. The lymphocyte count is greatly increased by radiation with the bare iron arc while it is decreased by radiation with the iron arc through glass. As the glass transmits everything above 320 $\mu\mu$, one must conclude that the stimulating action is due to the region between 230 and 320 $\mu\mu$; i.e., the far ultra-violet. It is probable that if this region could be isolated and used separately the increase would be much greater as the depressing action of the rest of the spectrum would not have to be counteracted.

RESULT OF EXPOSURE TO THE IRON ARC THROUGH ULTRA-VIOLET GLASS (TRANSMISSION 330 TO 390 $\mu\mu$) (FIGS. 7, 8 AND 9).

In order to further analyze the action of light of wave-length longer than 320 $\mu\mu$, the effect of special regions was investigated.

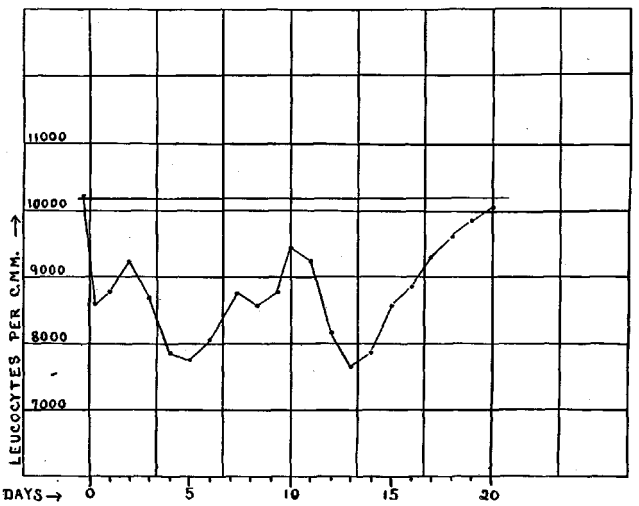

FIG. 7. Total leucocyte count, after exposure to iron arc through ultra-violet glass (average of five rabbits). Ordinates—leucocytes per c.mm. blood. Abscissae—time, in days, after exposure.

With Wood's ultra-violet glass the region between 330 and 390 $\mu\mu$ was tried. These experiments gave remarkably consistent results.

The total count (Fig. 7) shows a fall, followed by a rise on the second day. This is followed by a fall on the fifth day, a gradual rise on the tenth, another fall on the thirteenth and a gradual rise to normal. The differential (Fig. 8) shows that the initial fall is due entirely to lymphocytes, as the polymorphonuclears rise slightly. The rise on the second day is due to lymphocytes alone, but the two falls on the

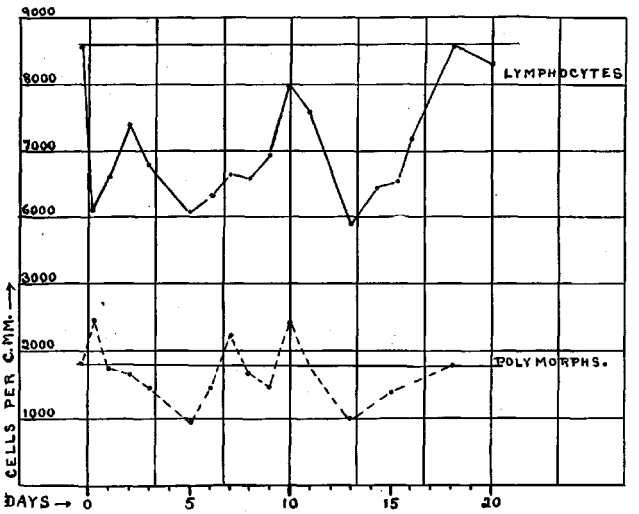

FIG. 8. Lymphocyte and polymorphonuclear counts, after exposure to iron arc through ultra-violet glass (average of three rabbits). Ordinates—cells per c.mm. of blood. Abscissae—time, in days, after exposure ———— lymphocytes — — — — — — polymorphonuclear leucocytes.

fifth and thirteenth days are due to both polymorphonuclears and lymphocytes. This region therefore causes a decrease in both polymorphonuclears and lymphocytes, but especially in the latter. Although the curves are not analogous throughout, the lymphocyte curve for radiation through ultra-violet glass (Fig. 8) is somewhat similar to that for radiation through plain glass (Fig. 4), especially as regards the large drop on the thirteenth day. This indicates that

the depressing action of the radiation through glass is largely due to the near ultra-violet in this radiation, which is not surprising as the iron arc is very rich in lines in this region.

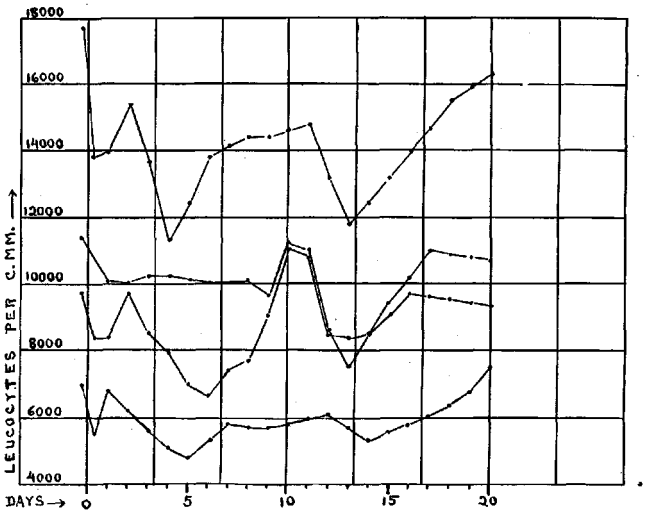

FIG. 9. Total leucocyte counts on four individual rabbits, after exposure to iron arc through ultra-violet glass. Ordinates—leucocytes per c.mm. of blood. Abscissae—time, in days, after exposure.

Fig. 9, in which the total white counts of four individual rabbits are plotted, shows how closely they agree with each other and with the average curve.

RESULT OF RADIATION WITH THE IRON ARC THROUGH A PICRIC ACID SCREEN (TRANSMISSION $450 \rightarrow 750\,\mu\mu$) (FIG. 10).

The region transmitted by this screen includes the two yellow absorption bands of hemoglobin.

There is a moderate drop in the total count immediately after radiation, due to the combination of a large drop in lymphocytes and a small rise in polymorphonuclears. After this immediate effect the

total count, the lymphocyte and the polymorphonuclear counts follow parallel curves, showing a large rise on the first and second days after

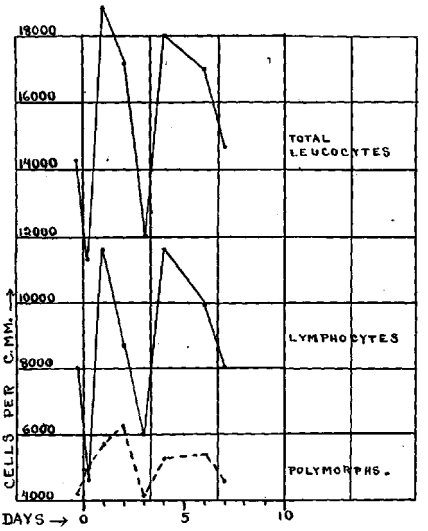

FIG. 10. Total leucocyte and absolute lymphocyte and polymorphonuclear counts, after exposure to iron arc through picric acid screen (average of two rabbits). Ordinates—cells per c.mm. of blood. Abscissae—time, in days, after exposure ———— leucocytes and lymphocytes — — — — — — polymorphonuclear leucocytes.

radiation, followed by a sharp drop on the third, an equally sharp rise on the fourth, and a return to normal on the seventh, after which no noticeable variation was found.

RESULT OF RADIATION WITH IRON ARC THROUGH RED GLASS (FIG. 11).

This resulted in an immediate fall in lymphocytes which returned to normal by the second day, and no further change was then observed. The polymorphonuclears were practically unchanged. One may conclude, therefore, that radiation of wave length longer than 650 $\mu\mu$ produces no effect other than the usual lymphocyte drop immediately after radiation.

Since there is always a drop in lymphocytes immediately after radiation, irrespective of the wave-lengths used, it seemed possible that this drop might be due to some other agency than light. A series of control experiments was performed on the animal to see

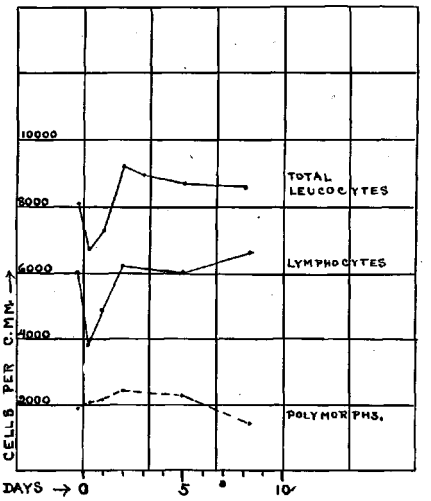

FIG. 11. Total leucocyte and absolute lymphocyte and polymorphonuclear counts, after exposure to iron arc through red glass (average of two rabbits). Ordinates—cells per c.mm. of blood. Abscissae—time, in days, after exposure. ———— leucocytes and lymphocytes — — — — — — polymorphonuclear leucocytes.

whether the mere position in the box might be responsible for this drop. They were placed in the box for an hour, and blood counts were made before and after, which showed no significant change in either total or differential counts.

EFFECT OF REPEATED RADIATION.

Radiation of one hour daily, for a week, seemed to give no greater response to the bare iron arc than a single radiation. This, however, has not been very extensively tried.

Discussion.

There is always a fall in the number of circulating lymphocytes immediately after exposure to light of any wave-length. When this is accompanied by an increase in polymorphonuclears, as in the radiation through plain glass, the total count is unchanged, but usually it results in a fall in the total count as well. Light of wave-length greater than 650 $\mu\mu$ has no effect except this usual fall in lymphocytes immediately after exposure.

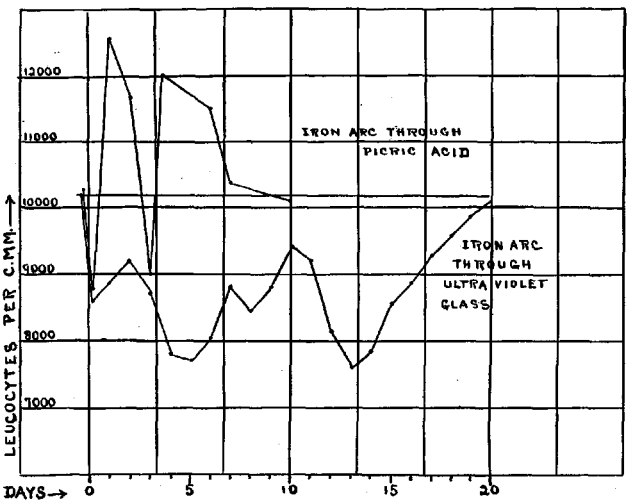

Fig. 12. Average total leucocyte counts, after exposure to iron arc through picric acid and through ultra-violet glass, showing complementary effects. Ordinates—leucocytes per c.mm. of blood. Abscissae—time, in days, after exposure.

Light of wave-length shorter than 320 $\mu\mu$, or perhaps 300 $\mu\mu$, results solely in a great increase in lymphocytes. This confirms the suggestion, made in the previous work referred to, that the ultra-violet stimulates a lymphocytosis, leaving the polymorphnuclears constant in absolute number. It is, however, only the far ultra-violet ($\lambda < 300\ \mu\mu$) which is capable of this action, as the region of ultra-violet just below the visible has a depressing effect on the lympho-

cytes (See Fig. 8). This near ultra-violet gives a rise in polymorphonuclears immediately after exposure, which is also found as a result of radiation through plain glass, but this is followed by a decrease below normal.

The region between 450 and 650 $\mu\mu$, however, increases both the polymorphonuclear and lymphocyte counts. From this fact a sufficient explanation can be found perhaps for the experimental observation that animals kept continually in sunlight do not as a rule show a different leucocyte count from those kept in darkness. The stimulating effect of the yellow light, and the small amount of far ultra-violet present in the solar spectrum, is offset by the depressing action of the blue, violet, and near ultra-violet as indicated in Fig. 12. In the solar spectrum the intensity of yellow light is greater than the intensity of the blue and violet, and the relative intensity is possibly such that this balancing action is practically complete, except when an unusual intensity of far ultra-violet (290 to 300 $\mu\mu$) is present, as may be the case in the tropics. In the spectrum of the iron arc through glass which is composed of somewhat the same range of wave-lengths as the sunlight, although it does not reach as far as 290 $\mu\mu$ in the ultra-violet, the intensity of the violet and near ultra-violet is relatively very much greater, and the total effect is more parallel to that of the near ultra-violet alone (See Fig. 6).

Cause of effect.

The cause of the action of light on blood is not known. Experiments are now in progress on this point, but as yet no satisfactory conclusion has been reached.

The invariable drop noticed in the lymphocytes immediately after radiation, furnished the suggestion that some product of the disintegration of these cells may act as a stimulus to the lymph nodes and thus account for the subsequent rise. This suggestion is not borne out by the results of radiation with the longer wave lengths, 650 $\mu\mu\rightarrow$, but it was tested directly by the following experiments, with a negative result.

An emulsion of lymphocytes in salt solution was made from the lymph glands and thymus gland of one rabbit. Half of this was injected into a second rabbit, and the rest, after being radiated in a quartz tube, with the bare iron arc, for one hour, was injected into a third rabbit. These two rabbits had had their blood counted before injection, and were then examined daily for a week. They both

showed a great and immediate increase in polymorphonuclears, due possibly to the introduction of foreign protein. This was accompanied by a decrease in lymphocytes. By the next day the blood was practically normal, and no further significant change was found.

A more probable explanation is that the absorption of light by the hemoglobin or plasma of the blood may lead directly or indirectly

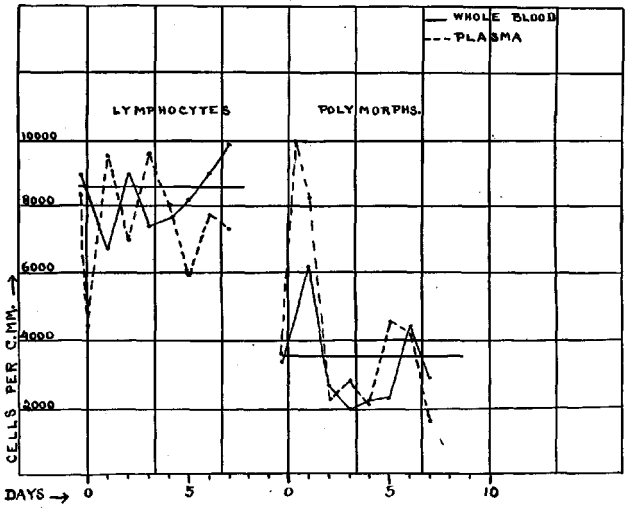

FIG. 13. Lymphocyte and polymorphonuclear counts for rabbits injected with radiated whole blood and radiated plasma. ———— injected with radiated whole blood — — — — — — injected with radiated plasma. Ordinates—cells per c.mm. of blood. Abscissae—time, in days, after injection.

to the formation of products that have a stimulating effect on lymphocyte production. In line with this suggestion the following experiments were made.

Experiment I. 3 c.c. of blood from the heart of a rabbit were mixed with 1.5 c.c. of heparin (20 mgm. in salt solution) to prevent coagulation (22), radiated in a quartz tube with the bare iron arc for one hour, and then injected back into the same animal through an ear vein.

Experiment II. 4 c.c. of blood from the heart of a rabbit were mixed with 2 c.c. of heparin (25 mgm. in salt solution), centrifugalized, and 3.5 c.c. of clear plasma separated. This plasma was radiated for one hour in a quartz tube with the bare iron arc, and was then injected into the same rabbit through an ear vein.

The results are shown in Fig. 13. The total count in the rabbit with injected plasma showed a suggestive rise in the total count on the first day after injection. The differential, however, proved this to be due entirely to a very great rise in polymorphonuclears. Apparently the radiated plasma acted like a foreign protein. Except for this increase in polymorphonuclears the injected radiated plasma had no other stimulating effect.

The injection of radiated whole blood also gave a polymorphonuclear rise, but it was less marked than in the case of the plasma. Except for this change the action was depressing, both on lymphocytes and polymorphonuclears. The stimulating effect of the far ultra-violet on lymphocyte formation is apparently not due to an action resulting directly from the absorption of light by the hemoglobin or plasma, although no general conclusion can be drawn from this single experiment.

As it is known that ultra-violet light exerts a direct effect on the skin, leading to sunburn or marked inflammatory changes, it is possible that its action on this tissue may be responsible indirectly for the lymphocytosis. As bearing upon this suggestion an attempt was made to determine the effects of radiating a bloodless ear. In three rabbits the common carotid artery on one side was exposed under local action of cocaine and clamped. The corresponding ear was then exposed for an hour to the bare iron arc. The experiments were not satisfactory. It was found as a matter of fact that clamping the carotid did not suspend entirely the circulation in the ear and, in addition, while aseptic precautions were attempted in the slight operation involved, it was not found possible to avoid opportunities for infection. Further experiments along this line will be reported in the future.

Significance of the action of light on blood.

In his work on animals with lymphocytosis, induced by exposure to dry heat, Murphy (23) has shown that mice with a lymphocytosis show a high degree of immunity to certain transplantable cancers, and also a very great increase in their resistance to doses of bovine

tubercle bacilli. The resistance to tuberculosis is increased from two to three fold. Amoss, Taylor, and Witherbee (24) also showed that monkeys with greatly reduced lymphocyte counts, as a result of exposure to x-rays, show a much increased susceptibility to an intracerebral inoculation of poliomyelitic virus filtrate. From these results it would seem that any method of inducing a lymphocytosis (heat or far ultra-violet light) would aid in maintaining a high resistance to these diseases at least. It has been suggested, in fact, by Rollier (25) and others that the beneficial effects of heliotherapy in tuberculosis may be due to the action of the actinic rays in increasing the lymphocyte count. Chamberlain, Wickline and others (11, 12, 13, 14, 15), working on the blood count of soldiers in the Philippines, and finding, as a result of the tropical climate, an increased lymphocyte and a decreased polymorphonuclear differential count, took this to indicate a poor physical condition on the part of the men. The chief phagocyte cell of the blood is the polymorphonuclear neutrophile and it is presumably of great importance in the destruction of bacteria in the blood stream. Wickline (12) quotes Cabot as saying "It would appear that the degree of health in persons not organically diseased might perhaps prove to vary directly with the percentage of polymorphonuclear cells in the blood." In Wickline's work (12) the total leucocyte count remained practically constant throughout the nineteen months of examination while the percentage of polymorphonuclears steadily fell, and the percentage of lymphocytes steadily rose. There was therefore an absolute decrease in polymorphonuclears, and this condition may signify an increased susceptibility to bacterial invasions.

If a condition may be obtained in which the absolute number of polymorphonuclears remains practically normal while the absolute number of lymphocytes is greatly increased, as after exposure of rabbits to a bare iron arc, the blood would have normal phagocytic power together with such increased resistance as may depend upon the peculiar activity of the lymphocytes.

Conclusions.

Direct radiation of rabbits' ears for short periods (one hour) by an iron arc through different filters has shown:

1. The region of far ultra-violet (wave length shorter than 300 $\mu\mu$) has practically no effect on the absolute number of polymorphonuclears, but produces a very marked lymphocytosis, lasting about three weeks.

2. The near ultra-violet (330 to 390 $\mu\mu$) has a marked depressing effect on the lymphocytes, and, to a less degree, on the polymorphonuclears.

3. The region between 450 and 650 $\mu\mu$ has a stimulating action on both lymphocytes and polymorphonuclears, particularly on the former.

4. The wave-lengths longer than 650 $\mu\mu$ (the red and infra-red), produce no effect on the blood beyond the drop in lymphocytes which occurs immediately after exposure to light of any kind.

BIBLIOGRAPHY.

1. BLESSING, H. G. Von der Norwegischen Fram-Expedition. Deutsch. med. Wchnschr., 1897, 251.
2. GROBER AND SEMPELL, O. Die Blutzusammensetzung bei jahrelanger Entziehung des Sonnenlichtes. Deutsch. Arch. f. klin. Med., 1919, CXXIX, 305.
3. BORISSOW. Zur Lehre von der Wirkung des Lichts und der Dunkelheit auf den tierischen Organismus. Zeit. f. Diätet. u. physik. Therapie, 1900, V, 337.
4. GRAFFENBERGER, L. Versuche über die Veränderungen, welche der Abschluss des Lichts in der chemischen Zusammensetzung des tierischen Organismus und dessen N-Umsatz hervorruft. Archiv. f. d. ges. Physiol., 1893, LIII, 253.
5. MARTI, A. Wie wirken die chemischen Hautreize und Belichtung auf die Bildung der roten Blutkörperchen. Verhandl. d. 15. Kongresses f. innere Medizin, 1897, 598.
6. ORUM, H. P. T. Ueber die Einwirkung des Lichts auf das Blut. Pflüger's Archiv. f. Physiologie, 1906, CXIV, 1.
7. POLITO, G. L'influenza dei Raggi Solari sul Sangue. Gazz. internaz. di Med., 1908, XI, 329.
8. MURPHY, J. B., AND STURM, E. Effect of dry heat on the blood count in animals. Jour. of Exp. Med., 1919, XXIX, 1.
9. ASCHENHEIM, E. Der Einfluss der Sonnenstrahlen auf die leukocytäre Blutzusammensetzung. Zeitschr. f. Kinderheilkunde, 1913, IX, 87.
10. TAYLOR, H. D. Effect of exposure to the sun on the circulating lymphocytes in man. Jour. Exp. Med., 1919, XXIX, 41.
11. CHAMBERLAIN, W. P., AND VEDDER, E. B. A study of Arneth's nuclear classification of the neutrophiles in healthy and adult males, and the influence thereon of race, complexion and tropical residence. Phil. Jour. Sci. (Sec. B.) 1911, VI, 405.
12. WICKLINE, W. A. The effects of tropical climate on the white race. Mil. Surgeon, 1908, XXIII, 282.
13. GUERRERO, L., AND SEVILLA, A. Medical survey of the town of Tay Tay. Phil. Jour. Sci. (Sec. B.) 1909, IV, 273.
14. PHALEN, J. M. An experiment with orange red underwear. Phil. Jour. Sci. (Sec. B.) 1910, V, 525.

15. CHAMBERLAIN, W. P., BLOOMBERG, AND H. D. KILBOURNE, E. D. Examinations of stools and blood among the Igorots at Baguio, Philippine Islands. Phil. Jour. Sci. (Sec. B.) 1910, V, 505.
16. SIMON, C. E. Clinical diagnosis. Lea and Febiger, Philadelphia, 1918, 31–39.
17. FREER, P. C. The tropical sunlight. Phil. Jour. Sci. (Sec. B.) 1910, V. 1,
18. MIETHE, A., AND LEHMANN, E. Uber das ultraviolette Ende des Sonnenspektrums. Sitzungsber. d. k. Pr. Akad. d. Wissensch., 1909, VIII, 268.
19. PFLÜGER, A. Die Anwendung der Thermosäule im Ultraviolett und die Energieverteilung in den Funkenspektren der Metalle. Ann. d. Physik., 1904, XIII, 890.
20. JESIONEK, A. Lichtbiologie. Vieweg u. Sohn, Braunschweig, 1910, 137–145.
21. KLIENEBERGER, C., AND CARL W. Die Blut-Morphologie der Laboratoriums-Tiere. Barth, Leipzig, 1912, 30–31.
22. HOWELL, W. H., AND HOLT, E. Two new factors in blood coagulation, heparin and pro-antithrombin. Amer. Jour. Physiol., 1918, XLVII, 328.
23. MURPHY, J. B., AND STURM, E. The lymphocytes in natural and induced resistance to transplanted cancer. Jour. Exp. Med., 1919, XXIX, 25.
Effect of stimulation of the lymphocytes on the rate of growth of spontaneous tumors in mice. Jour. Exp. Med., 1919, XXIX, 31.
Experiments on the rôle of lymphoid tissue in the resistance to experimental tuberculosis in mice. Jour. Exp. Med., 1919, XXIX, 35.
24. AMOSS, H. L., TAYLOR, H. D., AND WITHERBEE, W. D. Effects of large doses of X-rays on the susceptibility of the monkey to experimental poliomyelitis. Jour. Exp. Med., 1919, XXIX, 115.
25. ROATTA, G. B. L-Elioterapia nella pratica medica e nell' educazione. Milan, 1914.