EFFECT OF DRY HEAT ON THE BLOOD COUNT IN ANIMALS.

III. Studies on Lymphoid Activity.

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Plates 1 to 3.

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The study of the function of the lymphoid tissue has, in the past, been productive of few leads of importance, due undoubtedly to the lack of methods for approaching the subject. It has not been possible, owing to the wide distribution of the lymphoid tissue throughout the body, to employ the method so productive in the study of deficiency of function in the glands of internal secretion brought about by partial or complete removal. Extensive investigations on animals and man after removal of the spleen, the chief lymphoid depot, has added little to our understanding of the function of this type of tissue. The loss under these conditions is so rapidly compensated for by hyperplasia of the lymphoid tissues that a diminished function cannot be detected. By utilizing the well known fact that lymphoid tissue is extremely sensitive to x-rays, we were able to develop a method by which practically the entire lymphoid tissue of the body can be destroyed with a minimum destruction of other tissue and slight, if any, effect on the general health of the animal.[1] The method consisted of small, repeated doses of x-rays distributed over from 7 to 21 days, depending on the size and resistance of the animal. The experiments carried out on the delymphocytized animals led to the following results: (1) destruction of the mechanism of resistance against implants of foreign tissue;[1] (2) lowered resistance to inoculated cancer grafts;[2] (3) destruction of established immunity to cancer;[3]

[1] Murphy, Jas. B., *J. Am. Med. Assn.*, 1914, lxii, 1459.

[2] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 204.

[3] Murphy, Jas. B., and Taylor, H. D., *J. Exp. Med.*, 1918, xxviii, 1. Mottram, J. C., and Russ, S., *Proc. Roy. Soc. London, Series B*, 1917–18, xc, 1.

(4) lowered resistance to tuberculosis in mice,[4] guinea pigs,[5] and monkeys;[6] (5) lowered resistance to poliomyelitis in monkeys.[7]

From the experimental side we have been able to get suggestive results after the stimulation or apparent increased activity of the lymphoid tissue. The chick embryo, normally with no resistance against transplants of heteroplastic tissue, can be rendered resistant by suitable transplants of adult lymphoid tissue.[8] A single small dose of x-rays, sufficient to stimulate somewhat the circulating lymphocytes, increased the resistance of mice to autotransplants of spontaneous cancers.[9] Mice with high lymphoid counts resulting from splenectomy or from the reaction engendered in a cancer-immune animal after inoculation with a cancer graft show a marked increase in resistance to bovine tuberculosis.[4,10] All the results given above suggest, rather than prove, that the lymphocyte is an active agent in these varied processes. It seems necessary to test more completely the effect of stimulation by other means.

Many methods have been tested to produce an extensive and enduring increase in the lymphocyte and lymphoid tissue. Among these were the use of a number of drugs and dyes, ultra-violet light, and sunlight.[11] Except in the cases of the two latter agents, the results were unsatisfactory. Either the increase was too slight to be of experimental value, or not of long enough duration, or the incidental disturbance to the general health of the animal was too great. One highly promising method has been found; namely, the use of intense dry heat.

Method.

The source of the heat in these experiments was an electric bulb of frosted glass, giving the maximum heat for the amount of light generated. The method of application varied with the size of the

[4] Murphy, Jas. B., and Ellis, A. W. M., *J. Exp. Med.*, 1914, xx, 397.
[5] Morton, J. J., *J. Exp. Med.*, 1916, xxiv, 419.
[6] Taylor, H. D., unpublished observation.
[7] Amoss, H. L., Taylor, H. D., and Witherbee, W. D., *J. Exp. Med.*, 1919, xxix, 115.
[8] Murphy, Jas. B., *J. Exp. Med.*, 1914, xix, 513.
[9] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800.
[10] Taylor, H. D., and Murphy, Jas. B., *J. Exp. Med.*, 1917, xxv, 609.
[11] Taylor, H. D., *J. Exp. Med.*, 1919, xxix, 41.

animal and its ability to stand the higher temperatures. For mice an ordinary iron tripod 9 inches high and $5\frac{1}{2}$ inches at the base was covered on the sides with cardboard. A space was left at the bottom for free circulation of air. This was placed over an electric "Sunbeam" heating lamp of 110 volts, 80 watts direct current. A small circular cage of the same size as the opening was placed on top of the tripod. This was made of galvanized iron with a wire mesh bottom ($\frac{1}{4}$ inch mesh). The distance from the top of the lamp to the bottom of the cage was 3 inches. A thermometer was introduced parallel to and about $\frac{1}{4}$ inch below the bottom of the cage, directly over the light. After the light was turned on, the temperature was allowed to rise to 55°C. before the mouse was put into the cage. The animal was allowed to remain 5 minutes, this being about the time required for the temperature to rise from 55–65°C. If the temperature rose too rapidly it was found advisable to shut off the light until the thermometer registered between 57° and 59°C. and then allow it to increase again to 65°. For mice the temperature should never exceed 65°, as they can survive only 1 or 2 minutes at a higher temperature than this. A small piece of blotting paper was placed in the bottom of the cage just large enough for the mouse to rest on. In our experiments the loss was less than $\frac{1}{2}$ of 1 per cent, and only occasionally were burns encountered.

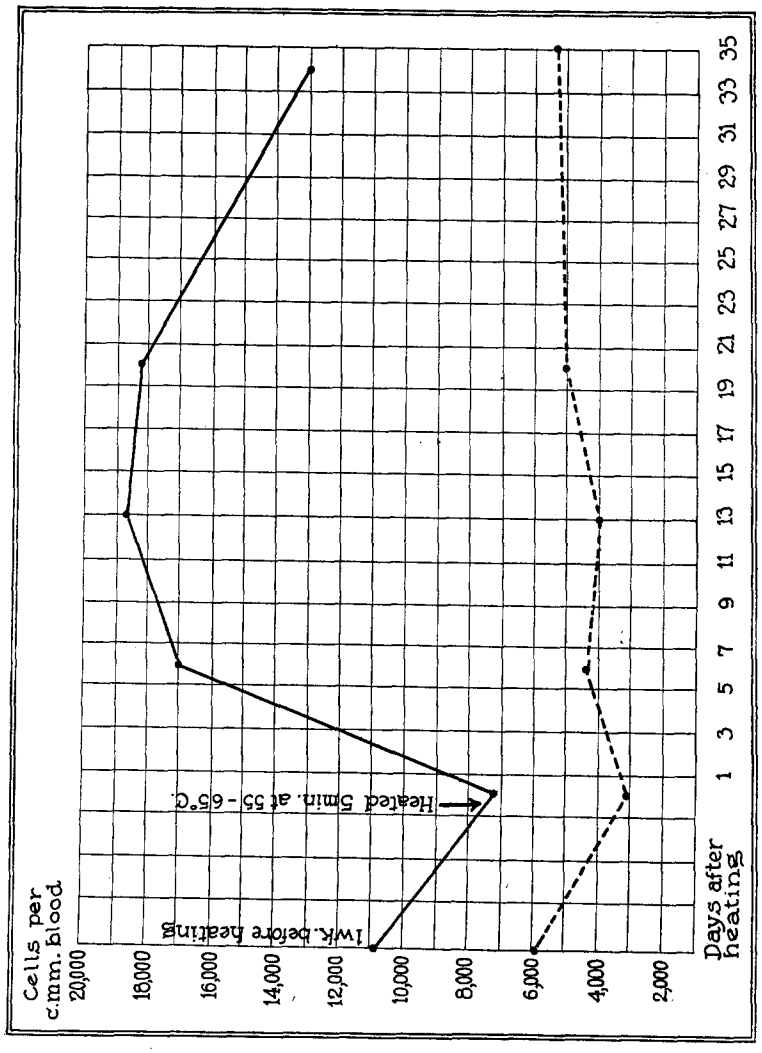
For heating larger animals, such as rats or guinea pigs, a wooden box 9 by 6 inches and $4\frac{1}{2}$ inches high was used with an electric carbon heating lamp 25 volts, 100 watts direct current, suspended directly over it. This lamp was surrounded by a metal reflecting cup. The distance from the lamp to the top of the box was 10 inches. The thermometer was placed at the level of the body of the animal. Rats and guinea pigs were found much more susceptible to higher temperatures than mice. A temperature of 60°C. or more caused extreme discomfort. The animals can, however, stand a more prolonged exposure at the lower temperature than can mice. For this reason it was found advisable to reduce the maximum temperature and increase the time of exposure for these animals.

Effect of Dry Heat on the Leucocytes in Mice.

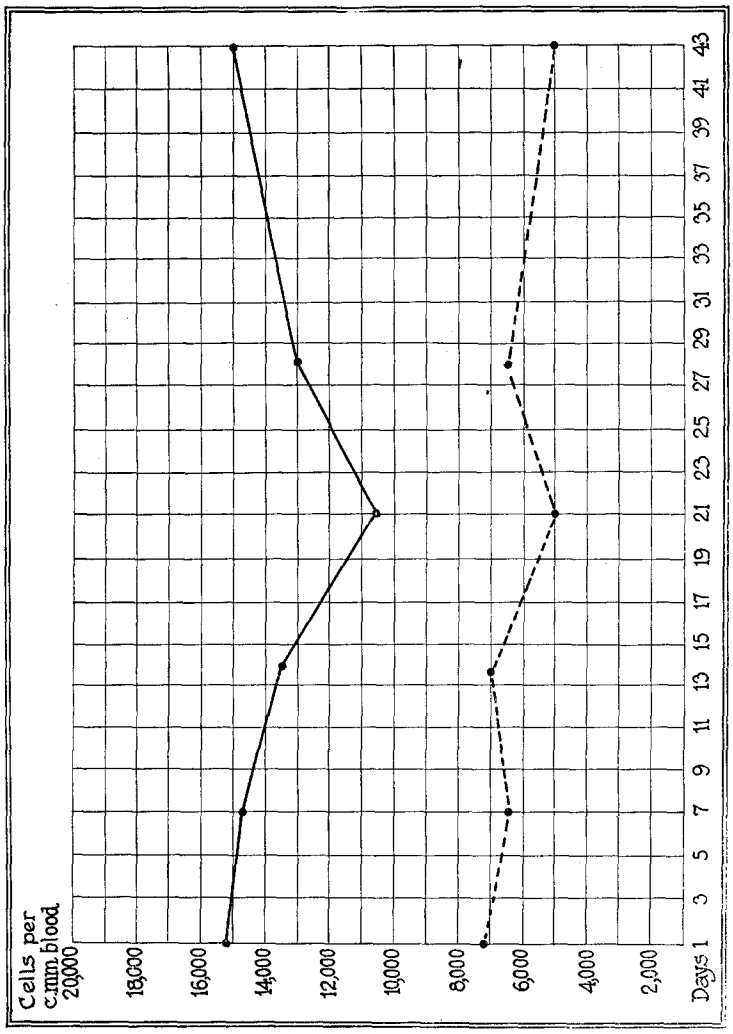
Experiment 1.—Total and differential leucocyte counts were made on fifteen healthy mice of about the same age and size. 1 week later ten of these animals were heated over an electric lamp at a temperature ranging from 55–65°C. for 5 minutes. White cell and differential counts were made immediately after this procedure, and counts were also made on five remaining animals as controls. All the fifteen animals were counted again, 7, 14, and 21 days after heating. On charting the results of the counts it was found that immediately after heat exposure there was a uniform decrease in both principal types of white cells, the lymphocytes and the polymorphonuclear leucocytes, which averaged about 3,000 cells per c.mm. of blood. The count on the five unheated mice showed no appreciable change. 7 days after heating it was found that there was an increase of from 5,000 to 10,000 cells per c.mm., this increase being almost entirely in the lymphoid group. The polymorphonuclear leucocytes remained lower than they were in the original count. The blood picture in the five control mice was similar to that at the previous determinations. 14 days after heating, the lymphocytes had increased to a figure above the original count in some of the heated animals to the extent of from 12,000 to 14,000 cells per c.mm. of blood, while the polymorphonuclear leucocytes still remained below the original determination in practically all the animals. The control animals showed no change beyond that well within the bounds of experimental error. 21 days after heating, the lymphocytes, although several thousand cells above the original count, had fallen off somewhat from that of the previous week. The polymorphonuclear cells at this point showed a tendency to rise. These results are graphically recorded in Text-fig. 1 for the heated animals and Text-fig. 2 for the controls.[12]

Experiment 2.—White blood counts were made on ten healthy mice of approximately the same age and size. A week later five of these (Group I) were heated for 5 minutes at a temperature of from 55–65°C. Immediately afterward blood counts were made on these and the five unheated animals (Group II). A week later the control mice (Group II) were heated in the same manner as that employed with Group I the previous week. Counts were made on both groups at this period and repeated 7 and 21 days later. Group I showed an average decrease of 2,500 lymphocytes per c.mm. of blood after heating, while the polymorphonuclear leucocytes dropped more than 6,000 cells. Group II, which was used as the control up to this point, showed little change in the blood picture. Counts a week later on Group I, 1 week after heating, showed an average increase of over 6,000 lymphocytes per c.mm. above the normal level. The polymorphonuclear leucocytes had recovered somewhat from the effect of the heat, but were still below the normal. Group II at this time, after having been counted twice with

[12] From past experience it was found that if counts were made more often than once a week it caused too great a fluctuation in the count.

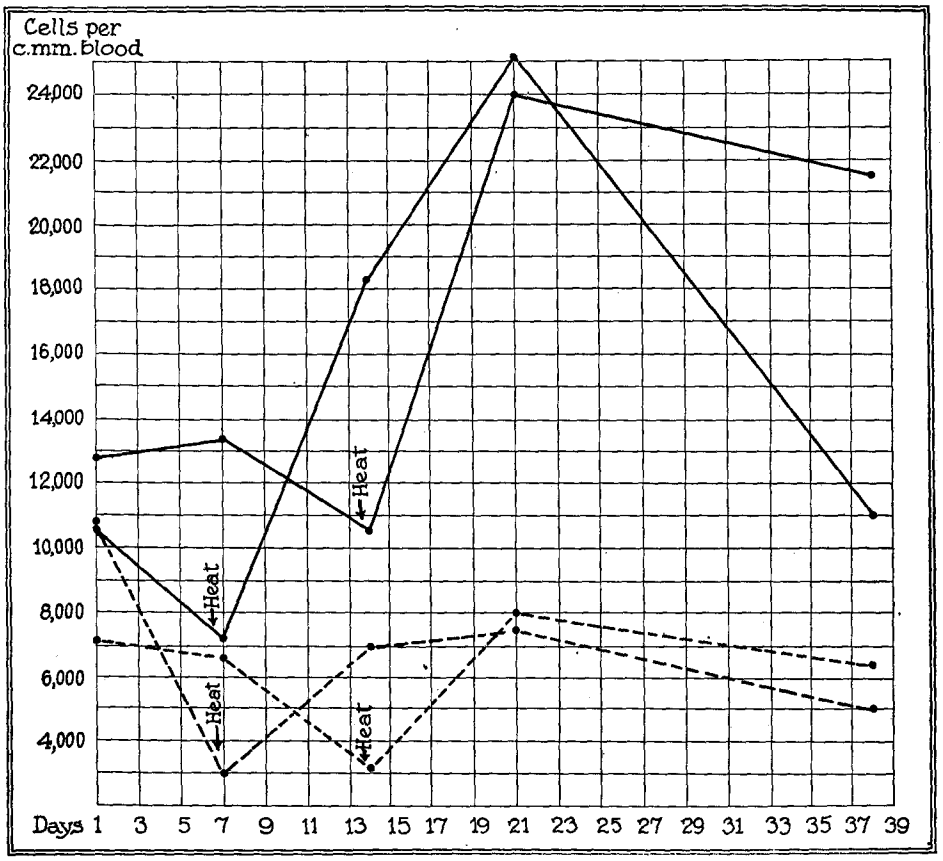


TEXT-FIG. 1. Composite curve of the white blood cell counts on ten mice before and after exposure to dry heat. ————Lymphocytes. .....Polymorphonuclear leucocytes.



TEXT-FIG. 2. Composite curve of the white blood cell counts on five normal mice of the same strain as those in Text-fig. 1. The animals were kept under the same conditions, and counts were made at the same time. ——— Lymphocytes. ..... Polymorphonuclear leucocytes.

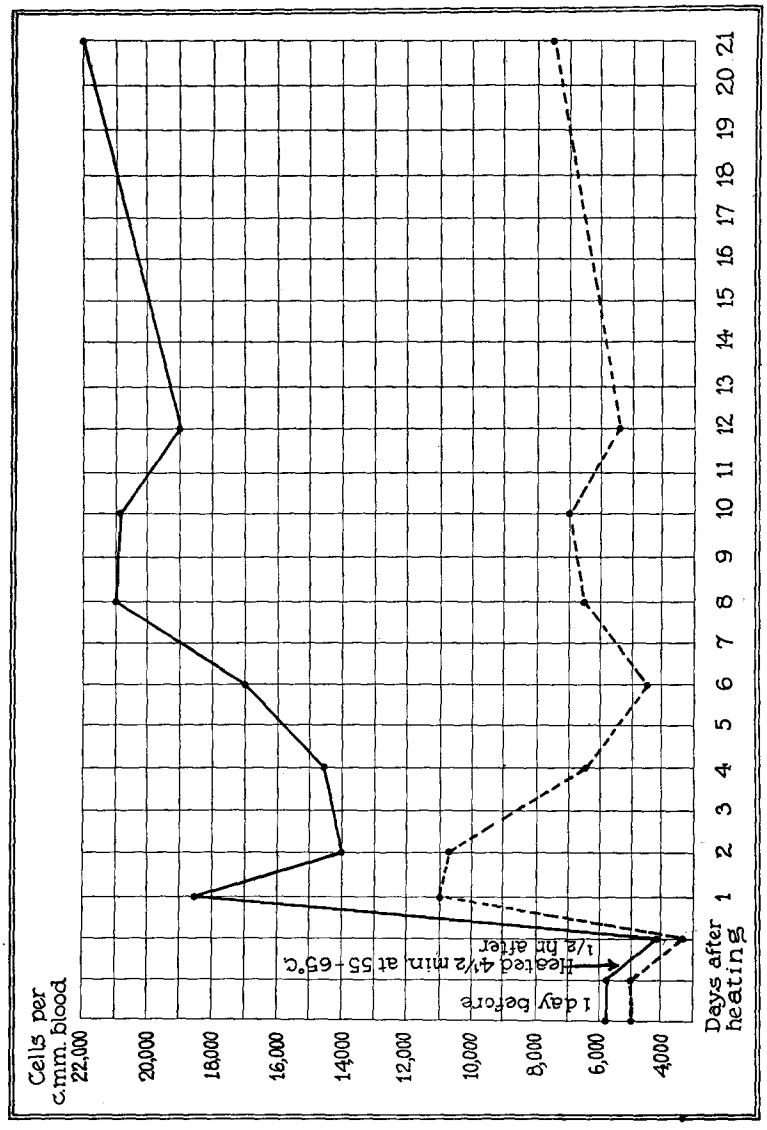
slight variation, showed the same characteristic drop in the blood count after heat was applied. After another 7 day interval the counts on Group I, 14 days after heating, showed an increase of 14,000 lymphocytes per c.mm. over the original count. Group II, 7 days after heating, showed an increase of 12,000 lymphocytes over the two previous counts. 14 days later Group I, 28 days



TEXT-FIG. 3. Composite curve of the white blood cell counts on two groups of mice, five in each group. One lot was heated a week after the first count, the others were heated 2 weeks after the first count with one intervening count. ————Lymphocytes. .....Polymorphonuclear leucocytes.

after heating, showed a count practically returned to its normal level, while Group II, 21 days after heating, was still several thousand cells above the normal count. This experiment is graphically illustrated in Text-fig. 3.

Experiment 3.—White blood counts were made on twenty healthy mice. 1 week later the entire twenty animals were heated for 5 minutes from 55–65°C.



TEXT-FIG. 4. Composite curve of the white blood cell counts on twenty healthy mice before and at intervals after heating. The first count represents the average for the whole lot. After heating they were counted in groups of five animals, the first group immediately after heating, the next 24 hours later, and then at intervals indicated on the chart. The last count represents the average of all twenty mice.
————Lymphocytes. .....Polymorphonuclear leucocytes.

and were then divided into four groups of five animals. Leucocyte counts were made on the first group immediately after heating, on the second 24 hours after heating, on the third 48 hours, and on the fourth 4 days after heating. Counts were made again on each group 6, 8, 10, and 12 days respectively after heating and again on all the animals 3 weeks after heating. The average of the counts for each day is shown graphically in Text-fig. 4, giving a continuous curve for the whole group.

Experiments 4, 5, and 6.—Three other experiments were carried out on mice in the same manner as those described above, with no variation in the result. These will not be given in detail.

In six experiments on mice it was found that after exposure of the animals to dry heat at 55–65°C. for 5 minutes there was a distinct fall in the total white blood cell count, both the polymorphonuclear leucocytes and the lymphocytes. The polymorphonuclear leucocytes recovered rather slowly, while the lymphocytes increased so rapidly that by the 2nd week after heating the count often reached a point 200 to 300 per cent above the normal figure.

The rectal temperature of mice after exposure to heat as described in the above experiments varies considerably, as shown by Table I.

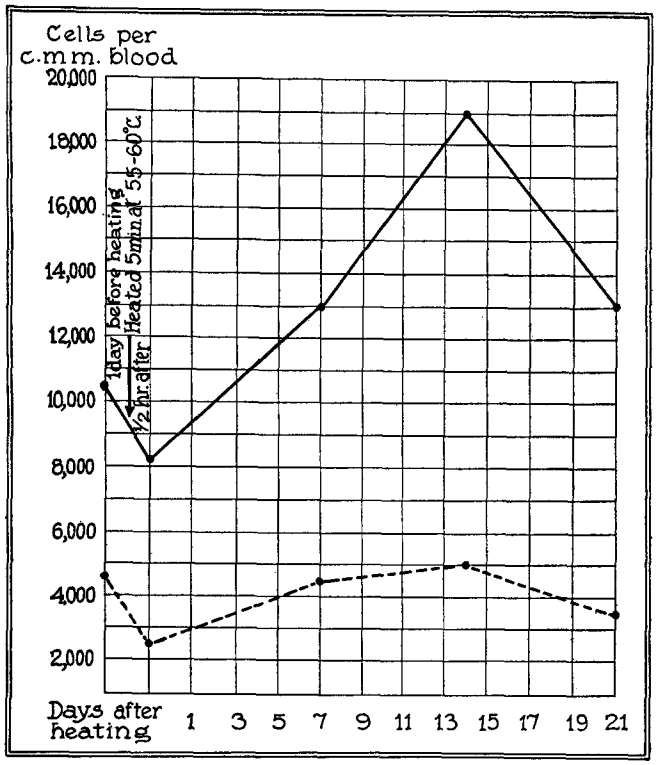
TABLE I.

Experiment No.	Before heat.	Directly after heat.	½ hr. after heat.	1 hr. after heat.
	°C.	°C.	°C.	°C.
1	36.2	39.1	38.3	37.7
2	36.7	39.0	37.2	37.0
3	36.6	39.7	37.1	35.1
4	36.0	41.6	35.7	35.2
5	37.2	40.8	38.3	37.7

Morphology of Circulating Lymphocytes after Heating.

No attempt has been made in this study to differentiate the large and small lymphocyte, as it is practically impossible to establish a satisfactory dividing line between the two types. In general it may be said that the larger type predominates in the earlier stages of the stimulation, while later the smaller ones increase. The cells of both types are for the most part normal, healthy looking lymphocytes, and do not differ in appearance from those observed in the

normal animal. At a period of from 6 to 10 days after heating it was noted that a proportion of the lymphocytes were in the process of what appeared to be amitotic division. Every stage (Figs. 1 and 2) of this division could be observed. In some of the more extreme cases there were found from three to five cells in one microscopic field (Fig. 3).

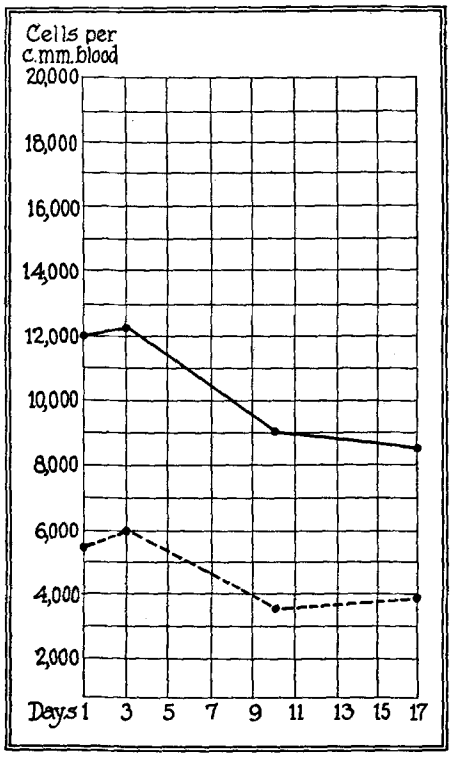


TEXT-FIG. 5. Composite curve of the white blood cell counts on five rats before and after heating. ————Lymphocytes. .....Polymorphonuclear leucocytes.

Effect of Heat on the Blood Picture in Rats and Guinea Pigs.

Experiment 7.—Blood counts were made on ten healthy rats of about the same age and size. The following day five of these animals were heated for 5 minutes at 55–60°C., counts being made immediately afterward both on these and on five control animals. The heated rats showed a decrease in both types

of cells similar to that observed in mice. The controls showed no marked change in their count. 7 days later counts showed the lymphocytes in the heated animals to be markedly increased, with the polymorphonuclear cells practically returned to their normal level. Control animals at this period showed a slight falling off in the count. 14 days after heating there was an average rise of over

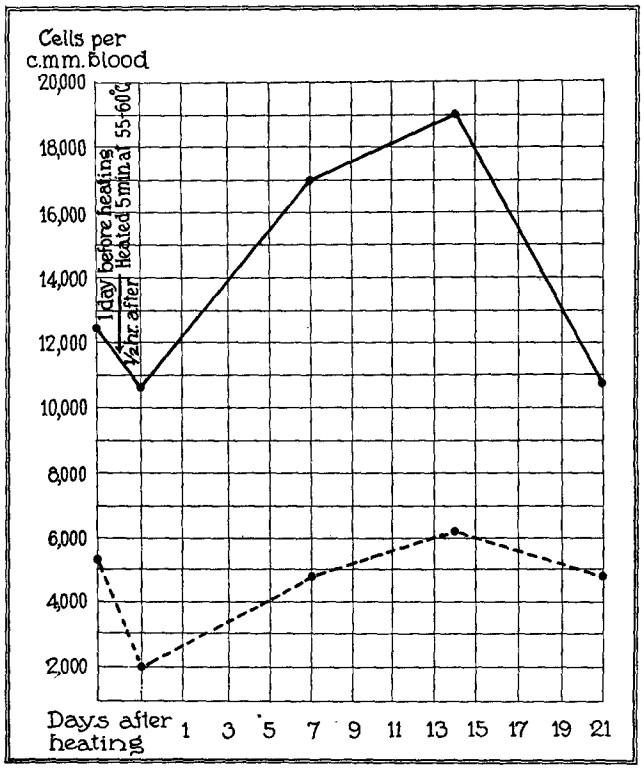


TEXT-FIG. 6. Composite curve of the white blood cell counts on five normal rats of the same lot as those in Text-fig. 5, kept under the same conditions, with counts made at the same time. ————Lymphocytes. .....Polymorphonuclear leucocytes.

8,000 cells per c.mm. of blood, while the polymorphonuclear count remained about the same. 21 days after heating, the lymphocytes showed a tendency to decrease, but still remained well above the normal level. Text-fig. 5 is the composite curve of the heated animals and Text-fig. 6 that of the controls. Text-fig. 7 shows the curve of a typical rat after heating.

Several other experiments of a similar character on rats gave identical results, the changes being similar in every way to those observed in mice.

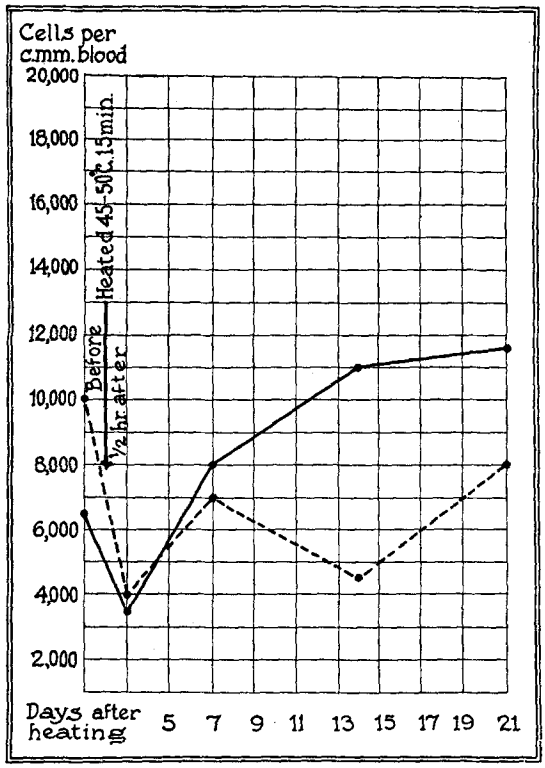
Experiment 8.—A total and differential leucocyte count was made on a fairly large guinea pig. The following day the animal was heated for 15 minutes at



TEXT-FIG. 7. Curve of the white blood cell counts of an individual rat after heating. ————Lymphocytes. .....Polymorphonuclear leucocytes.

45–50°C. and a count made shortly afterward. The lymphocytes showed only a slight decrease, while the polymorphonuclear leucocytes were reduced by 6,000 cells per c.mm. After this, counts were made at 7 day intervals for 3 weeks. They showed a steady increase in the lymphocytes, while the polymorphonuclears slowly regained their normal level and remained at that point (Text-fig. 8).

Several other experiments on guinea pigs gave identical results.



TEXT-FIG. 8. The white blood cell counts of the guinea pig after exposure to heat. ————Lymphocytes. .....Polymorphonuclear leucocytes.

DISCUSSION.

Some years ago Wickline[13] made a study of the blood cells in American troops incidental to the complete physical examination made at intervals during residence in the Philippines. He showed that there was a marked increase in the relative and absolute number of mononuclear blood units, the increase being at the expense of the polymorphonuclear leucocytes. There was no marked change in the total white cell count. Chamberlain,[14] later, incidental to the study of the Arneth count in the tropics, confirmed and extended

[13] Wickline, W. A., *Mil. Surg.*, 1908, xxiii, 282.
[14] Chamberlain, W. P., *Mil. Surg.*, 1909, xxv, 48.

this interesting observation. There is not sufficient data, however, in the literature to determine either seasonal variation, the effect of altitude, or the various climatic conditions, to enable us to discuss our present results in relation to these observations in man. Neither are we prepared to discuss the underlying mechanism which brings about these remarkable changes in the blood picture from a single exposure to dry heat, nor will we attempt to offer an explanation for this at the present time. For purposes of further investigation it offers a method by which we can produce a marked and durable increase in the circulating lymphocytes, thus affording a further opportunity for the study of the function of these cells.

The second point of interest is the large number of lymphocytes found in the circulation in the process of what appears to be an amitotic division. The majority of biologists consider that amitotic division is a degenerative process, and they are inclined to cast doubt on the possibility of the development into normal functioning cells. In either case we know that after heating there is a large increase in what appear to be normal circulating lymphocytes of both the large and small type. At present it is impossible to say positively where they have arisen. It seems probable that there is a sufficient stimulation of the lymphoid centers to account for the increase.[15]

SUMMARY.

Animals subjected to dry heat for a short period show a sharp fall in the total white blood count, both the polymorphonuclear leucocytes and the lymphocytes taking part in the fall. Following this there is a slow recovery on the part of the polymorphonuclear leucocytes, which generally require several weeks to regain their normal number. The lymphocytes rise rapidly after the initial fall and continue to rise for 2 or 3 weeks. This increase often amounts to a gain of over 200 to 300 per cent above the normal count for the animal. The observations were made on mice, rats, and guinea pigs.

The circulating lymphocytes during the more active stage of stimulation after heating show numerous examples of amitotic division.

[15] Nakahara, W., *J. Exp. Med.*, 1919, xxix, 17.

EXPLANATION OF PLATES.

PLATE 1.

FIG. 1. Microphotograph from a blood film of a mouse 6 days after an exposure to heat, showing various stages of amitosis in the lymphocytes.

PLATE 2.

FIG. 2. Drawing of various stages of amitosis seen in the lymphocytes of the blood of a mouse 8 days after exposure to heat.

PLATE 3.

FIG. 3. A single microscopic field from a blood film of a mouse 8 days after exposure to heat, showing four irregular lymphocytes.

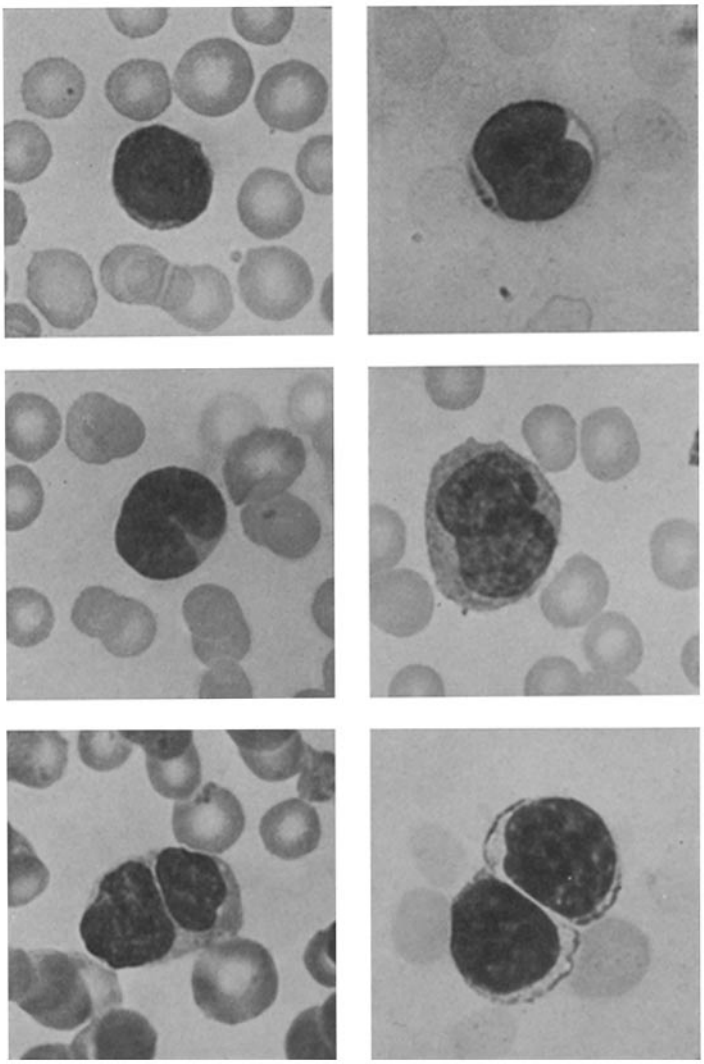


FIG. 1.

(Murphy and Sturm: Effect of dry heat on blood count. III.)

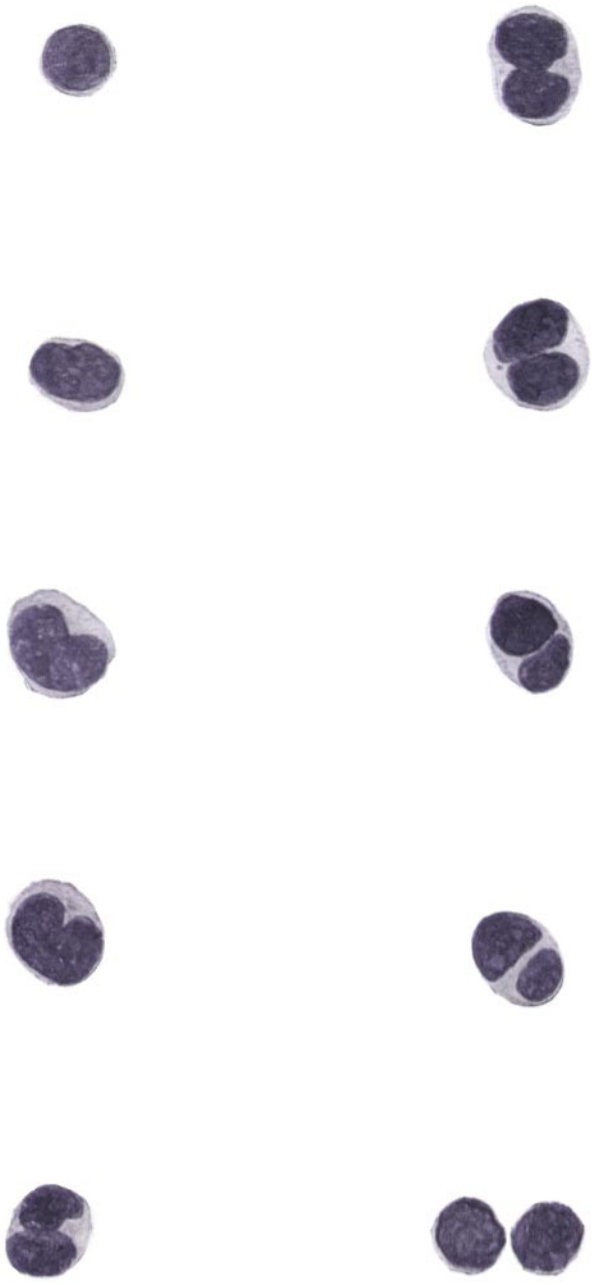


FIG. 2.

(Murphy and Sturm: Effect of dry heat on blood count. III)

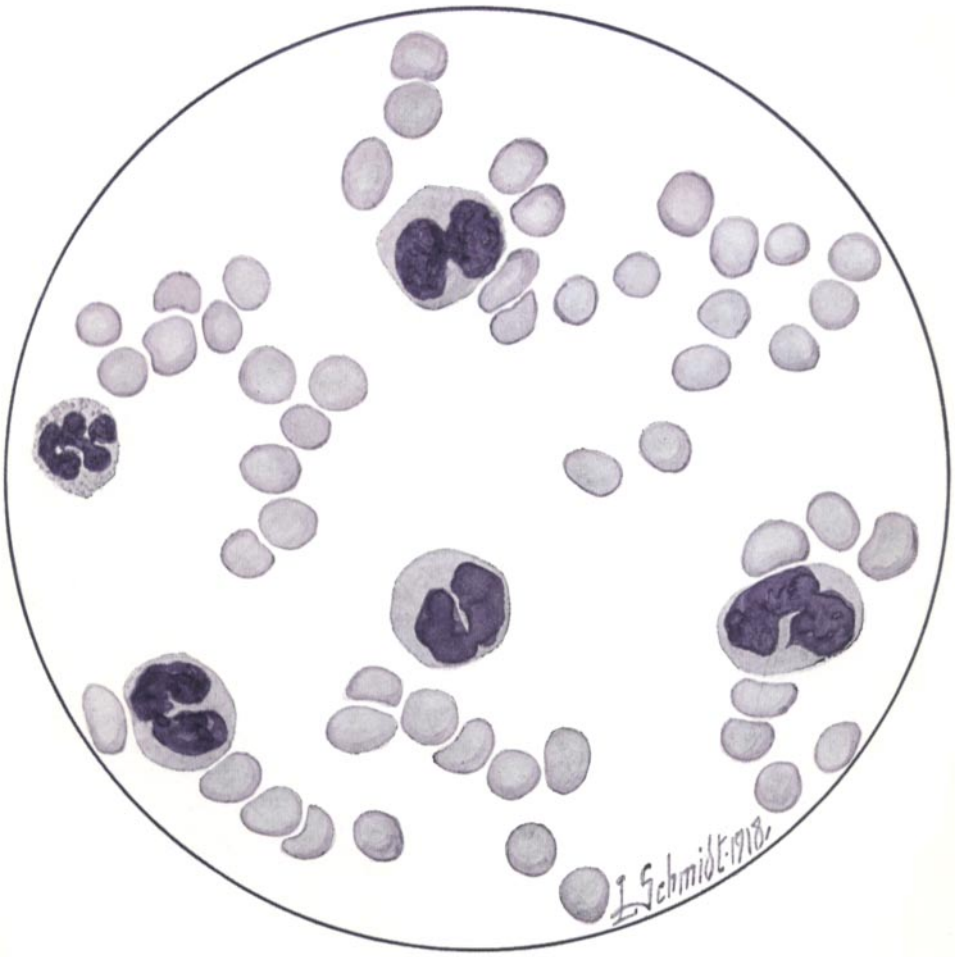


FIG. 3.

(Murphy and Sturm: Effect of dry heat on blood count. III.)