

OBSERVATIONS ON THE NATURE OF POST-OPERATIVE LEUCOCYTOSIS IN THE DOG

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IN a previous work on the blood picture at various periods after splenectomy,¹ mention was made of the constant occurrence of a polymorphonuclear leucocytosis immediately after removal of the dog's spleen. This was considered to be different from the ordinary post-operative leucocytosis because of the fact that although the operation was done under complete aseptic precautions, it was practically bloodless and lasted only fifteen or twenty minutes, nevertheless the leucocyte count always passed 30,000 per cubic millimetre (an increase of over 200 per cent.), and continued in a lesser degree above normal for several days or even two or more weeks. This comparatively long duration of the leucocytosis is even more striking in some human blood counts after removal of the spleen (Musser² and others). Since the above work was completed, however, leucocyte counts on dogs have shown that equally high figures may be obtained shortly after other aseptic operations. Furthermore, Dr. N. M. Percy³ has found that a transient lymphocytosis occurs while the spleen is being removed for pernicious anæmia. It has therefore seemed advisable to study post-operative leucocytosis more intensively, and as the effect of etherization and of hemorrhage on the leucocyte count also has remained undecided, studies on these two features have also been included.

Numerous studies, dating mostly from fifteen or twenty years ago (White,⁴ Da Costa and Kalteyer,⁵ Cabot, Blake and Hubbard,⁶ and others), have resulted in the establishment of fairly definite figures for the average height and duration of post-operative leucocytosis in man. Not only have such figures been wanting for the dog, however, but also it has seemed as if counts at more frequent intervals than has hitherto been attempted might throw more light on the mechanism involved.

Method.—To this end, total leucocyte and differential counts have been made on 20 dogs, either normal or in the leucopænic stage of trypanosome anæmia, before and after ether narcosis, hemorrhage and various surgical operations. One or more counts having been taken previous to the experiment, in most cases subsequent counts were made every ten or fifteen minutes for the first hour, then hourly for the rest of the day, and finally every day or two until the count returned to normal. For the leucocyte counts, 2 square millimetres were counted

from drops from different parts of the Thoma pipette, according to the usual method; and for the differential count 200 to 500 leucocytes, stained by Wright's method, were examined. It is of course recognized that no significance can be attached to small changes that may be within the factor of error, and also that the individual response to a given procedure may vary greatly. The leucocytes were classified in the four customary groups: (1) polymorphonuclear leucocytes; (2) small mononuclears or lymphocytes; (3) large and transitional mononuclears, and (4) eosinophiles. (Mast cells occur rarely if ever in normal dog's blood.) As will be noted later, various forms were found that varied from the typical description of these four classes; but for the

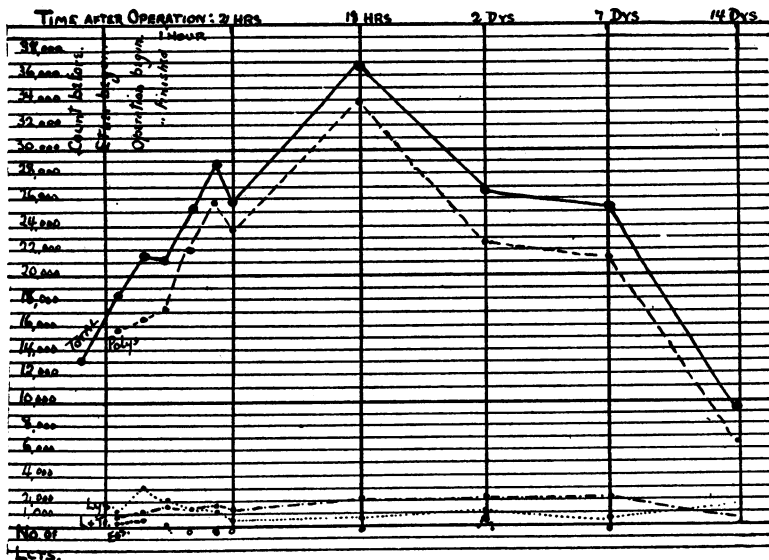


FIG. 2.—No. 17.19. Post-operative leucocytosis. Bile-duct-ureter anastomosis.

sake of clarity in the charts these have not been given separate recognition.

Most of the counts were made on dogs after removal of a kidney, spleen or eye, or after anastomosis of the common bile-duct with the ureter. Two experiments were on dogs etherized for an hour, using about 3 ounces of ether by intratracheal anæsthesia. Two other experiments were on dogs that had had 75 c.c. of blood withdrawn from the jugular vein in three minutes without anæsthesia; in one other this fresh blood was reinjected into the peritoneum, to simulate internal hemorrhage; and in another an accidental fatal internal hemorrhage occurred after nephrectomy from slipping of a ligature. In none of the dogs considered in this work were any signs of distemper or other acute infection visible. A temporary post-operative rise of temperature was of course present.

Post-operative Results.—From the accompanying charts it will be

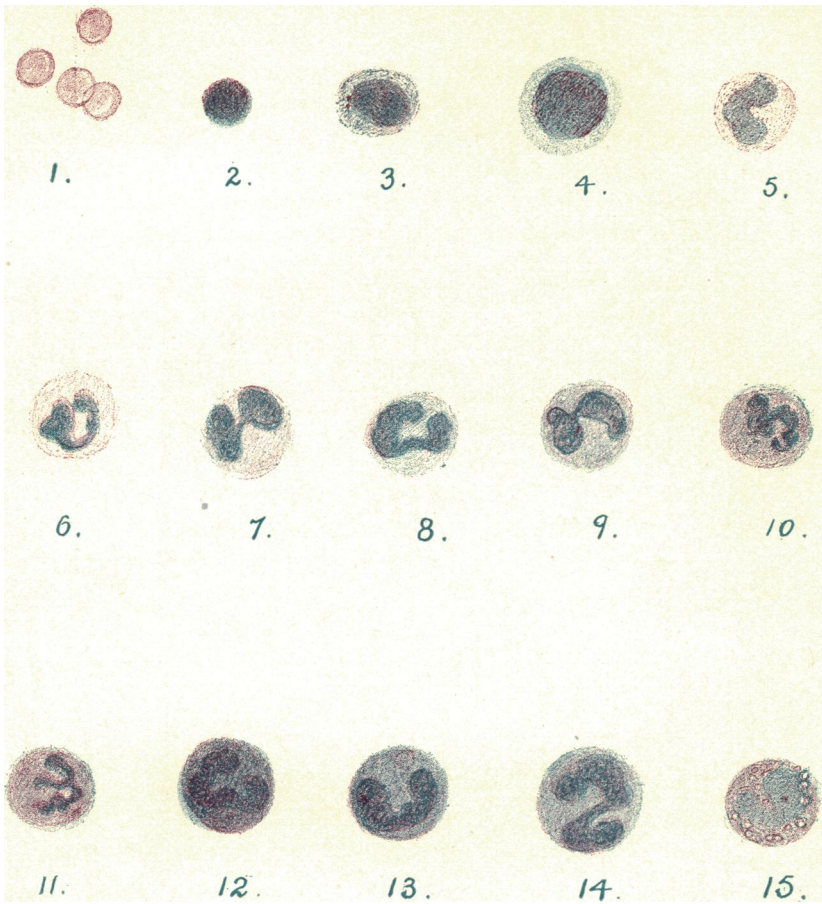


FIG. 1.—Appearance of cells in post-operative blood smears from the dog. 1, normal erythrocytes; 2 and 3, small lymphocytes; 4, basophilic lymphocyte (irritation form, Turk?); 5 and 6, young and adult polymorphonuclear leucocytes (Arneth); 7 to 11, intermediate leucocytes, showing different grades between polymorphonuclear and transitional forms (form of nucleus, staining reaction of protoplasm); 12 to 14, transitional and large mononuclear cells; 15, eosinophilic leucocyte. Note vacuoles and absence of definite eosinophilic granules. To increase ease of selection and reproduction, specimens were selected from two smears. Wright's stain. Zeiss, Homog. imm. $\frac{1}{2}$. Eye piece 4.

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seen that the post-operative leucocytosis of splenectomy is essentially the same as that of other operations, so that the four post-operative charts may be considered together. Starting almost immediately after etherization (noted in one instance within three minutes of beginning etherization), a leucocytosis becomes evident, and after a slight pause (to be considered later) reaches a maximum in about eighteen hours. This great rise is almost entirely due to the polymorphonuclear elements, although the large and transitional group is also affected, especially in the latter half of the leucocytosis. Although the numerical changes in the latter group appear insignificant on the chart as compared with the extreme polymorphonuclear change, the figures in the ordinate column show that these forms have sometimes doubled or trebled in number. Furthermore, qualitative changes of two kinds are observed in the polymorphonuclear group. The nuclei of a distinctly greater number possess only one or two lobes (a shift of the Arneth scale to the left); and in other cells (between 2 and 10 per cent. in different animals at different times) the general appearance of the cell approaches to a greater or less degree that of the so-called transitional cell. This cell, which for the sake of convenience I have called an "intermediate" cell, is slightly larger, the protoplasm more opaque and without definite neutrophile granules and the nucleus of a more compact shape and taking a less brilliant purple stain. In fact, so many gradations of these changes (approaching the polymorphonuclear cell at one extreme and the transitional at the other) are sometimes found, that their proper classification in one or other of these groups becomes entirely arbitrary.

The small lymphocytes or small mononuclear cells, except for an early transient rise (which will be considered later), show practically no quantitative change. A qualitative change is noted, however, in that one of the subdivisions of this group is represented more frequently. In the normal differential count of dog's blood, a few of the lymphocytes (two or three per cent.), when stained by Wright's method, show a protoplasm of Prussian blue, resembling Turk's irritation forms, and are considerably larger than the ordinary small lymphocyte. These are often increased to 10 or more per cent. of the small mononuclear group after operation. As the reaction of these and the "intermediate" cells to other stains has not been investigated, no attempt can be made to determine their true position in the leucocyte family. Attention should be called, however, to their appearance in the blood stream after operation.

The eosinophiles, as has been noted in human post-operative leucocytosis, are either diminished or in many cases disappear entirely from the peripheral blood for many hours after operation.

The leucocytosis following splenectomy differs only from other post-operative leucocytoses in that it tends to last longer. Thus in

Fig. 3 it will be seen that the leucocyte count is still above normal when the last count was made eight days after operation. In Fig. 4 the quicker return to normal is probably to be accounted for by the youth of the animal. The longer duration of the leucocytosis is in accord with Bernheim, King and Jones' ⁷ recent observations that the rise in the count of the various blood-corpuscles after blood transfusion is longer maintained in splenectomized than in normal animals.

Opportunity was offered by coincident work in this laboratory on trypanosome anæmia in dogs to study as well the leucocytic response to operation of leucopænic animals. For several weeks after infection,

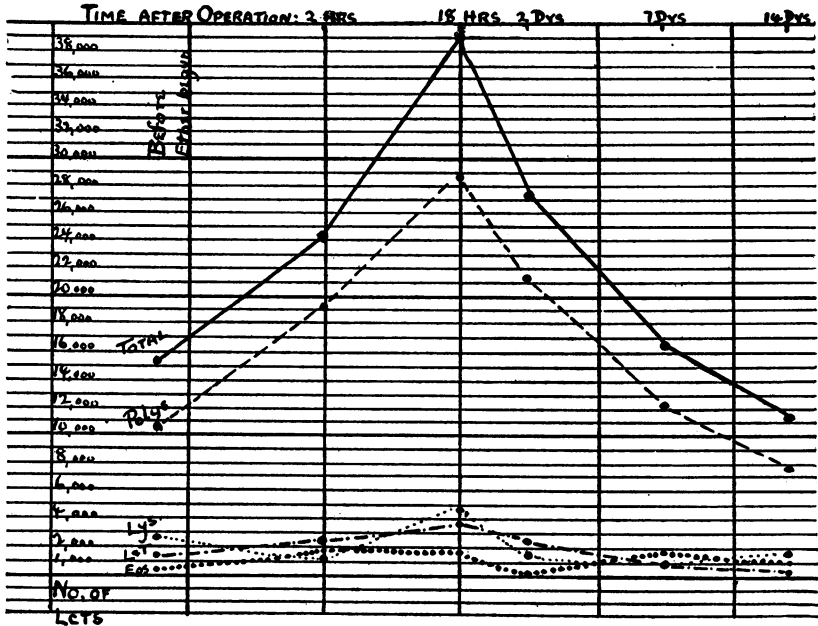


FIG. 3.—No. 16.71. Post-operative leucocytosis. Bile-duct-ureter anastomosis.

T. equiperdum causes a progressive leucopænia, reaching as low as 5000 or 6000 cubic per millimetre. When dogs are operated upon at this stage, whether for splenectomy (16.69) or other surgical procedures, such as removal of an eye (16.93), a post-operative leucocytosis of the same nature as in normal dogs occurs. It often fails, however, to attain as high a level, and may take three or four days to reach its maximum. As the bone marrows of these animals are definitely hyperplastic (chiefly leucoblastic), this delayed response, like the Arnetz shift to the left and the appearance of intermediate cells, would indicate that post-operative rise is chiefly, at least, due to the summoning of new leucocytes from the bone marrow, which in the leucopænic trypanosome dogs cannot be so well or so quickly supplied.

Narcosis.—Charts of the two dogs etherized for one hour show that a definite leucocytosis is produced, and that it characteristically

disappear before the end of the hour's etherization, is due to an increase in small mononuclears as well as in the polymorphonuclear cells. Its influence can be observed in those post-operative charts in which a sufficient number of early counts were made, as the small peak occurring in the first hour after operation, before the larger and later rise. The second phase is due almost entirely to polymorphonuclear cells, and like the post-operative rise shows to a less degree an increase in transitional cells, a slight shift to the left of the Arneht scale, and the appearance in small numbers of the "intermediate" cells previously described. In the post-operative charts it is buried in the greater post-operative rise.

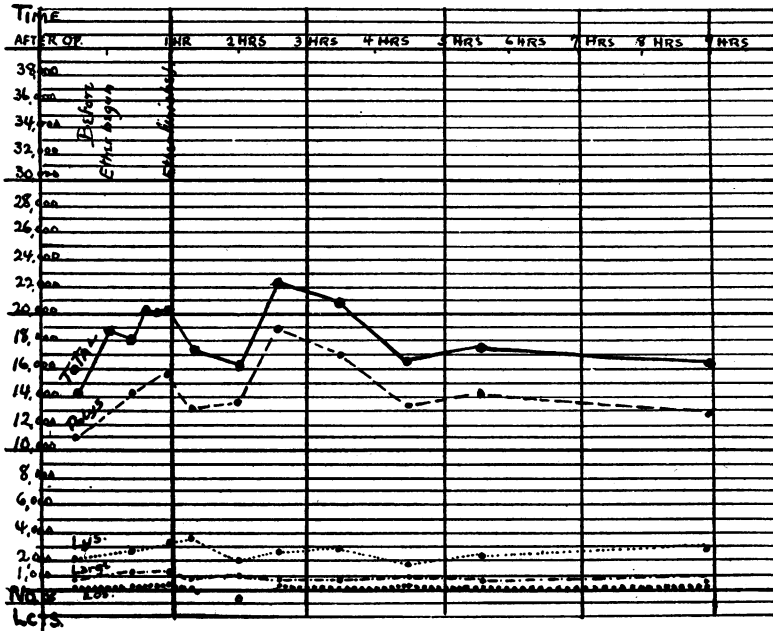


FIG. 6.—No. 17.30. Narcosis leucocytosis. Ether one hour.

Hemorrhage.—The leucocytosis that occurs after the withdrawal of 75 to 100 c.c. from the jugular vein of a medium-sized dog (10 kilos) is definite, but of different character from those previously described. Except for slight initial fluctuations, the count remains unchanged for several hours. As post-hemorrhagic hydræmia was not controlled, it is possible that an earlier rise could have been masked by such a factor. The red cell count in the two dogs examined was only slightly reduced (less than 1 million per cubic millimetre) for the first hour and then remained normal throughout the experiments. The leucocyte count begins to rise after four or five hours, almost doubling in the first thirty hours, and gradually declines to normal during several days. In one dog the rise was due entirely to poly-

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morphonuclears, in the other both polymorphonuclears and small mononuclears were increased. In a third experiment (No. 17.34) an equivalent amount of blood (100 c.c.) was withdrawn from the jugular vein during a period of five minutes. As fast as it was withdrawn it was reinjected into the same dog's peritoneal cavity, thus attempting to simulate the conditions of an internal hemorrhage. One needle was left in the jugular vein during the transferece and another in the peritoneal cavity. The whole fresh blood was transferred by the alternating use of 20 c.c. syringes, without defibrinating. As

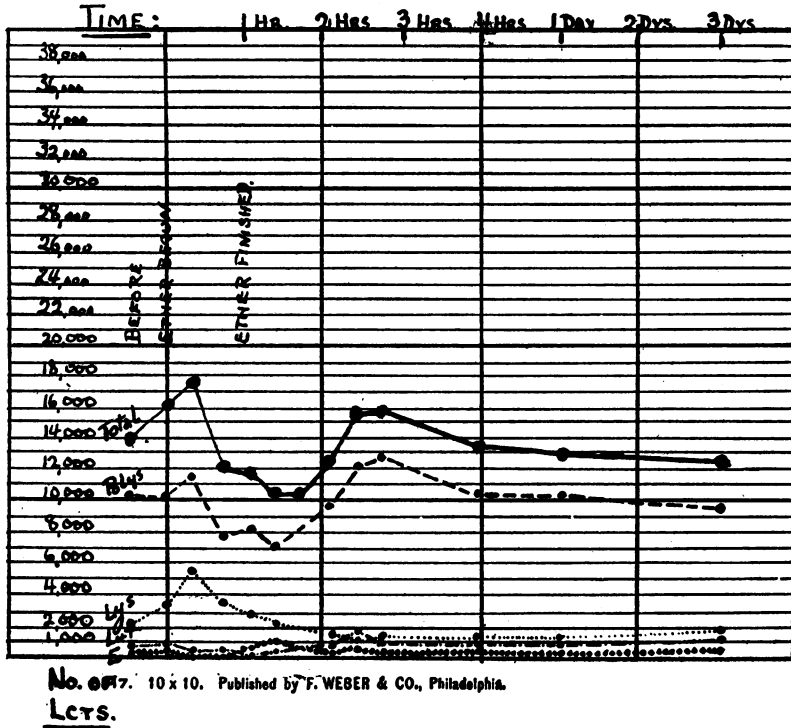


FIG. 7.—No. 17.28. Narcosis leucocytosis. Ether one hour.

Sweet⁹ has recently shown that fresh blood in the peritoneal cavity is rapidly taken up in large quantities by the thoracic duct and thus reintroduced into the circulation, it was thought that the stimulus to leucocytosis caused by the loss of blood from the body might thus be overcome. It will be seen that there was no noteworthy change for eight hours, and then only two counts (at twenty and thirty hours) showed a rise in leucocytes, similar to those previously described. It is of course impossible to know whether any of the injected blood underwent coagulation. Such coagulation would not only impede reabsorption but on disintegrating would afford more than sufficient stimulus for the late rise that was noted. It will also be noticed that

an extreme leucocytosis, such as Dold¹⁰ considers of value in the diagnosis of internal hemorrhage, was not found in this dog. In another animal (17.35), whose leucocytes were being followed after removal of a kidney, a fatal internal hemorrhage followed slipping of a ligature. Although the final count was made as the animal was dying, the leucocyte counts maintained an almost constant level, showing on the one hand that copious hemorrhage may continue for at least five hours without leucocytosis; but on the other hand, that the leucocyte count, unlike the red cell count, may be maintained until the moment of death.

Summary.—Splenectomy, like other aseptic operations, causes in the dog a marked leucocytosis that reaches its maximum in about 18 hours. In normal dogs the highest count, if frequent enough counts

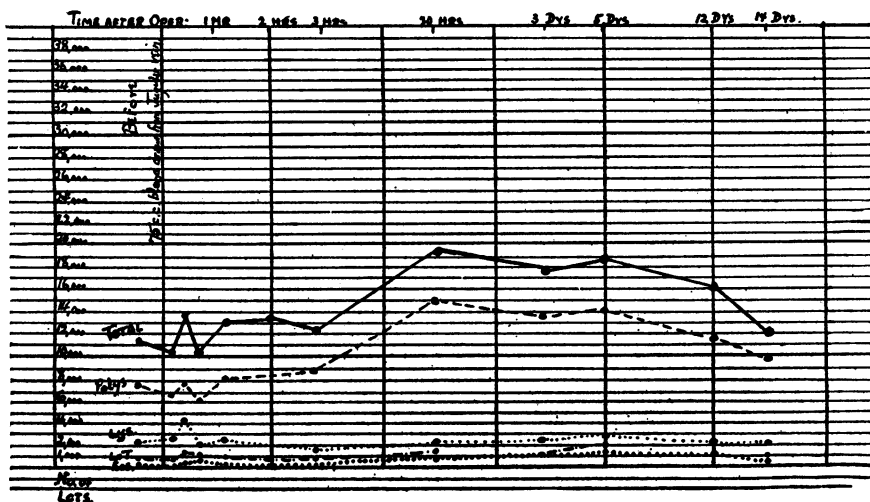


FIG. 8.—No. 17.27. Post-hemorrhagic leucocytosis. 75 c.c. bled from jugular vein.

are made, is always over 30,000 and usually over 40,000 per cubic millimetre. Post-operative leucocytosis in dogs lasts from four to seven days, but post-splenectomy leucocytosis is usually more protracted.

The leucocytosis is almost entirely due to the polymorphonuclear leucocytes. The proportion of younger forms is increased (Arneth shift to the left) and "intermediate" forms appear, that partake of some of the characteristics of so-called transitional cells. The later group is increased and eosinophiles are diminished or disappear from the peripheral blood.

In dogs in the leucopænic stage of trypanosome anæmia, surgical operation does not cause such an extreme leucocytosis and it may take several days instead of hours to reach its maximum.

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Uncomplicated narcosis (ether) produces a rise of from 3 to 10,000, occurring characteristically in two phases. The first of these phases reaches its maximum in fifteen to sixty minutes and is due to

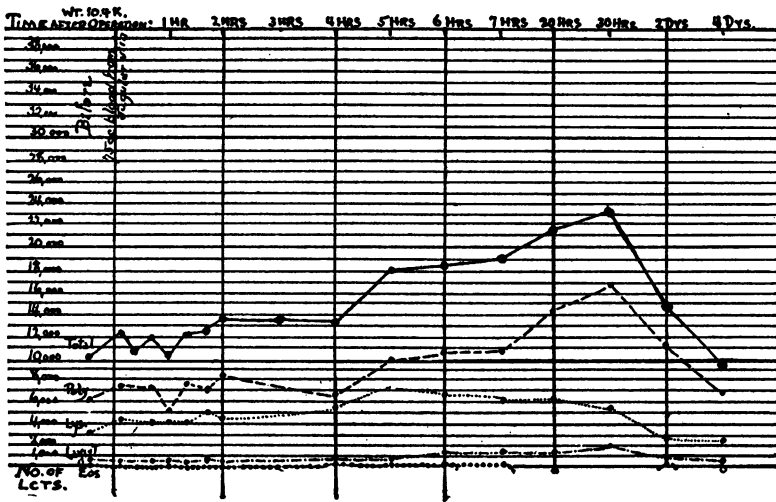


FIG. 9.—No. 17.29. Post-hemorrhagic leucocytosis. 75 c.c. bled from jugular vein. Red blood-cell was lowered less than 1 million in the one-hour count, was normal from two-hour on.

an increase both in the polymorphonuclear and small mononuclear elements. The second rise usually begins in about two hours and is practically finished in another two hours. The first of these is prob-

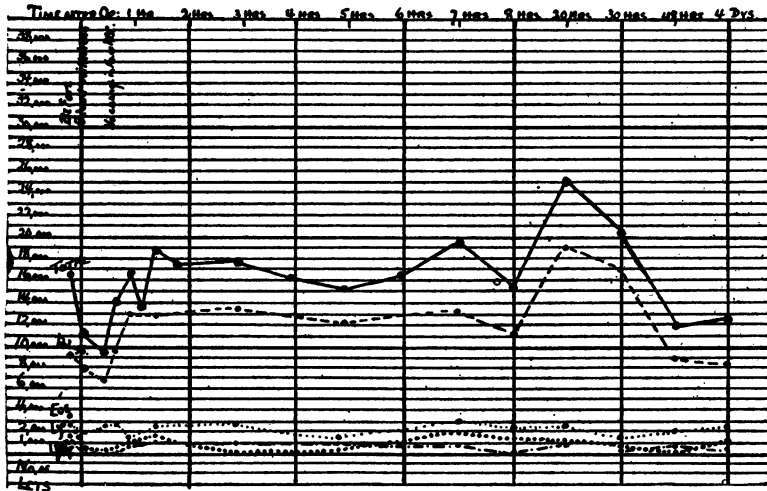


FIG. 10.—No. 17.34. Post-hemorrhagic leucocytosis. Internal. 100 c.c. from jugular vein, reinjected intraperitoneally.

ably due to a direct irritant effect of the ether (positively chemotactic to the lymphocytes), the second to a summoning of new polymorphonuclears from the bone-marrow, as in post-operative leucocytosis.

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Hemorrhage of moderate amount causes a leucocytosis of 100 per cent. or more, that typically does not become apparent for four or five hours, reaches its maximum in the first twenty-four hours, but may take more than a week before it returns to normal. These changes are more marked in external than in internal (intraperitoneal) hemorrhage of equal amounts of blood. In one animal an internal hemorrhage that proved fatal in five hours failed to cause any noteworthy change in the leucocyte count up to a few minutes before death. It is difficult to understand why withdrawal of an amount of blood, insufficient to cause more than a fleeting disturbance in the red blood count, and in the absence of ether or operative disturbance, should cause a distinct leucocytosis lasting several days. As such occurs, however, a bone-marrow stimulant must be sought.

CONCLUSIONS

1. In dogs, the leucocytosis that followed splenectomy only differs from other post-operative leucocytoses in the somewhat slower return to normal.
2. Post-operative leucocytosis, and the lesser degrees of leucocytosis that follow narcosis and hemorrhage, are chiefly due to the summoning of new and younger forms of polymorphonuclear cells from the bone-marrow into the peripheral blood stream.
3. The appearance of "intermediate" forms in the blood stream after operation suggests that a relationship exists between the polymorphonuclear leucocyte and so-called transitional cells.

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