WHITE CELL MORPHOLOGY IN RABBITS WITH INDUCED PERITONEAL EXUDATES*

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When a peritoneal exudate is induced in the rabbit by the method of Mudd, Lucké, McCutcheon, and Strumia (1), it can be shown that the number of white cells appearing in the exudate is greater than the number of such cells in the animal's circulating blood. This withdrawal of cells results in a stimulation of the bone marrow, and by inducing several exudates the rabbit's marrow can be thrown into a state of extreme stress. This paper is concerned with the morphology of the young cells which appear in the circulation, and with the morphology of the white cells contained in the exudates.

Production of Exudates

The method used for the production of the peritoneal exudates was similar to that of Mudd, Lucké, McCutcheon, and Strumia (1). About 300 cc. of sterile 1 per cent NaCl, or of Ringer's solution,[1] are injected intraperitoneally, and 18 hours afterwards the fluid is drawn off without further injection of saline. This fluid is very rich in leucocytes, usually containing approximately 0.25 cc. of cells for each 15 cc. of exudate, and in the case of a single exudate at least 90 per cent of the cells are polymorphonuclear leucocytes.

The procedure can be repeated at intervals, and we have used the same animals repeatedly for a period of several months. In order to produce extreme marrow stress, it is necessary to produce the exudates frequently, and most of the work with which this paper is concerned was carried out on a colony of twelve mature rabbits whose weights ranged from 2.5 to 4 kilos, four successive exudates being

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[1] We have injected both 1 per cent NaCl and a balanced buffered Ringer's fluid into the peritoneal cavity, without noticing any difference in the exudates which result. One would expect toxic effects to result from the injection of such large quantities of NaCl, but we have never observed such.

produced at intervals of 10 days, and thereafter more frequently, so as to bring about the death of the animal in some cases.

The blood picture in the circulation was followed by making total white cell counts, differential counts, and polynuclear counts (Cooke and Ponder, 2) at frequent intervals. Red cell counts and reticulocyte counts were made as required, and supravital counts were carried out on the cells of the exudates.

Effects of Producing Single Peritoneal Exudates

For some reason, at present obscure, the only cells which leave the circulation and appear in the peritoneal exudates induced by the injection of isotonic NaCl or Ringer's solution are the polymorphonuclear leucocytes, as can be shown by supravital staining. The sequence of events can be seen from the figures of Table I, which show the total

TABLE I

Time after injection	Total count per c.mm.	Polymorphonuclear leucocytes	Polymorphonuclear leucocytes per c.mm.	Polynuclear mean
hrs.		*per cent*		
0	7,400	36	2,660	2.06
2	8,600	50	4,300	1.53
4	3,050	59	1,800	1.14
6	3,650	61	2,200	1.10
8	5,300	68	3,600	1.13
24	11,000	32	3,460	1.22

white cell counts and percentages of polymorphonuclear leucocytes in the blood stream of a typical animal at various times after the saline injection, together with the total polymorphonuclear counts and the means of the polynuclear counts.

The simplest interpretation of these figures is that shortly after the injection of the isotonic NaCl the polymorphonuclear leucocytes start to leave the blood stream to appear in the exudate. This is followed by a marrow stimulation, so that at the end of 2 hours the total number of circulating polymorphonuclear leucocytes is increased, while the polynuclear mean has fallen from 2.06 to 1.53 because of the addition of young cells of class I to the circulation. Thereafter, the picture is one of a competition between production of young cells from the marrow, as indicated by the continued fall in the polynuclear

mean, and a passage of polymorphonuclear leucocytes from the blood stream into the exudate, as indicated by the fall in the total number of circulating polymorphonuclear leucocytes. After 24 hours the greater part of the effect seems to be over, and the polynuclear mean has begun to increase because of the development of the cells of class I into cells of class II (Ponder, 3), this increase in the mean continues, so that the original figure of 2.06 is reached in less than 7 days.[2]

In experiments of this kind, the question always arises: What is the relation between the number and kind of the cells found in the exudate and the number and kind of the cells in the circulating blood? The cells of the exudate must, of course, have at one time been cells of the blood stream, the composition of which varies from the time at which the exudate begins to be produced, until the time at which it is withdrawn. Table I, however, shows clearly that the bone marrow stimu-

[2] Our experience has been peculiar as regards the length of time required for a deflected polynuclear count to return to normal. In 1926 one of us (3) described the deflection of the polynuclear count in the rabbit after the injection of thyroid extract, and found that a complete return to the steady state took from 14 to 21 days; it was therefore concluded that this is the normal lifetime of the polymorphonuclear leucocyte in the blood stream. This result was confirmed, in its general aspects, by Ponder and Flint (4), deflection with various drugs and extracts, including nucleic acid, Kennedy and Grover (5), deflection by x-rays, Kennedy and Thompson (6), deflection by ultraviolet light, Charipper (7), deflection by thyroid extract in *Necturus*, Danzer (8), deflection by tissue extracts, and Climenko (9), deflection by ergosterol and ultraviolet light. In this series of experiments, we have found the polynuclear count to return to its steady state much more rapidly after a deflection. When the deflection occurs as a result of a single injection of nucleic acid (1 mg. per kilo), the count returns to the original steady state within 4 days and usually more rapidly; when a single intraperitoneal exudate is produced, the return may take about 7 days, but in this case the stimulus may be operative for a longer period. The only suggestion we can make for this more rapid return to an apparently steady state is that the polynuclear counts of all our rabbits were more left-handed than those of the animals used in the investigations mentioned above. This seems to be another case of either environmental or genetic variation in the count (Macleod, 10). The more left-handed the count, the more rapidly would one expect a return from a deflection, and there is the additional difficulty that when the count is left-handed, there are few classes containing cells, and so it is more difficult to follow the successive maxima in the classes, which act as a guide to the rate of development of the polymorphonuclear leucocyte.

lation, and the pouring of young cells into the blood stream, precedes the leucopenia which presumably, if not necessarily, corresponds to the passage of the greatest number of cells into the exudate; the type of cell in the exudate accordingly approximates more nearly to the type of cell in the circulation at the time when the exudate is withdrawn than to the type of cell initially present. Supravital staining of the cells of the exudate is not altogether satisfactory; one can show that the great majority of the cells are neutrophiles, but it is impossible to do an accurate polynuclear count.

The exudate is withdrawn after 18 hours, and in this particular case, 60 cc. of fluid containing 7,000 cells per c.mm. were obtained. The amount of fluid which can be withdrawn, however, is always less than the total amount in the peritoneal cavity, and the total amount can be determined only by killing the animal. This was done in several cases, and the average amount of fluid recovered was 130 cc. with an average cell content of 6,500 per c.mm.; this means that 8.5 (10^8) cells, nearly all polymorphonuclear leucocytes, passed into the exudate from the blood stream which, on an average, contains about 3 (10^8) polymorphonuclear leucocytes, and so the number of polymorphonuclear leucocytes which pass into the exudate is greater than the entire number in the circulation. The amount of intraperitoneal saline injected, however, is really enormous, being more than one and a half times the blood volume of the rabbit.

The Mechanism of the Marrow Stimulation

Without considering why only polymorphonuclear leucocytes leave the circulation to appear in the exudate, some light can be thrown on the nature of the marrow stimulation which appears shortly after the intraperitoneal injection of isotonic NaCl. Danzer (8) has shown that subcutaneous injection of saline extracts of a variety of tissues (muscle, liver, testicle, brain, etc.) produces a marrow stimulation and a deflection of the polynuclear count similar to that which follows the injection of nucleic acid, and that the stimulating substance is associated with the protein fractions. By centrifuging a portion of the intraperitoneal exudate, a supernatant fluid can be obtained, the injection of which into another rabbit brings about a marrow stimulation and an extreme deflection of the polynuclear count; a typical experi-

ment is shown in Table II, in which 10 cc. of supernatant fluid from an exudate was injected intraperitoneally into a second rabbit.

Comparing this table with Table I, it is clear that in each case there is a marrow stimulation with a deflection of the polynuclear count, although the transient leucopenia which occurs in the case of the intraperitoneal exudate does not occur in the case of the injection of the supernatant fluid, because there is no withdrawal of neutrophiles from the blood stream into the abdominal cavity. When an intraperitoneal injection of saline is given, and the leucocytes leave the blood stream to appear in the exudate, it is hard to conceive that a mere absence of cells from the circulation should result in a stimulation of the marrow; it is much more probable that disintegration products of the cells

TABLE II

Time after injection	Total count per c.mm.	Polymorphonuclear leucocytes	Polymorphonuclear leucocytes per c.mm.	Polynuclear mean
hrs.		*per cent*		
0	9,300	27	2,500	1.58
1.5	6,900	33	2,280	1.31
3.0	8,950	53	4,750	1.12
4.0	8,050	64	5,100	1.18
6.0	7,950	67	5,350	1.30
9.0	7,850	48	3,760	1.22
22.0	10,800	26	2,800	1.41

which first appear in the exudate are reabsorbed, and that these are the stimulating agents.

The Effect of Producing Repeated Exudates

The time required for the polynuclear count to return to normal after the production of a single exudate is about 4 days, and if a second exudate is produced after this time the same sequence of events follows as in the case of the first exudate, the circulation being flooded with young cells, and the polynuclear count deflecting to the left. When the exudate is drawn off 18 hours after injection of the saline, over 90 per cent of the cells are again found to be young polymorphonuclear leucocytes. After allowing an interval for recovery, the process can be repeated again and again, but after exudates have been produced several times the picture begins to present new features.

As the exudates are repeatedly produced, the marrow responds by throwing younger and younger cells into the circulation. In a colony of 12 rabbits, we produced 4 successive exudates in each animal, with an average interval of 10 days between each exudate, and after the injection of saline for the fourth exudate, the circulating polymorphonuclear leucocytes were all very young cells of class I, or even metamyelocytes. These young cells, however, are morphologically different from the normal polymorphonuclear leucocyte; the nucleus is large and single lobed, and its chromatin content is relatively poor and irregularly distributed, so that some areas in the nucleus stain deeply while others do not stain at all. The cytoplasm contains large granules which are not all oxyphile, as in the normal rabbit polymorphonuclear leucocyte, but mixed, some being oxyphile and others azurophile. The cells have also a tendency to be larger than the normal polymorphonuclear leucocyte, their characteristics, in fact, are very similar to those of the macropolycyte (Cooke and Ponder, 2) which appears under conditions of extreme marrow stress, except that the nucleus is not hyper-segmented.[3]

In the case of some of the animals, after having obtained 4 successive exudates at intervals of 10 days, we gave several intraperitoneal injections of isotonic saline at 2 day intervals, withdrawing each exudate after 18 hours, as before. Metamyelocytes and myelocytes then appear in the circulation, until after 4 or 5 exudates in rapid succession the polymorphonuclear leucocytes of the blood stream are entirely replaced by myelocytes. Finally the animal develops a severe leucopenia (average count about 1,500 cells per c.mm.), and dies shortly thereafter.

In contrast to the production of exudates at intervals of less than 3

[3] In a recent paper Jones (11) has pointed out that the macropolycyte is not necessarily multi-lobed and has given a description of the macropolycyte in pernicious anemia which is almost identical with our description of the cells found after repeated peritoneal exudates. The same type of cell is almost invariably found after an intravenous injection of sodium nucleinate (1 mg. per kilo), and they seem to be the result of a hurried maturation in a marrow under stress. On a single occasion an intravenous injection of sodium nucleinate resulted in a flooding of the blood stream with macropolycytes having as many as 5 to 10 lobes each. We have not observed this particular phenomenon again, but it is doubtless merely another manifestation of what is essentially the same process.

days, when leucopenia and death result, if exudates are produced at intervals of from 3 to 4 days the marrow seems capable of delivering polymorphonuclear leucocytes into the circulation almost indefinitely. Its response seems, indeed to be almost of an "all or none" nature; given about 2 days rest between repeated stimulations such as we use, it will respond to each new stimulus in virtually the same way; stimulated a little more frequently, however, it rapidly fails to deliver even young polymorphonuclear leucocytes and liberates metamyelocytes and myelocytes in diminishing numbers.

Examination of the red cell counts shows that the total number remains at a comparatively constant level, even when repeated exudates are produced. We have observed, however, that considerable numbers of normoblasts, amounting to as many as 10 per cent, may appear in the circulation at the height of the marrow response to a demand for new white cells. Injection of nucleic acid may also, in some cases, be followed by the appearance of normoblasts, but, as in the case of the intraperitoneal injection of saline, the appearance of these is quite irregular, and apparently dependent on some factor which we have not been able to control.

SUMMARY

1. When peritoneal exudates are produced in the rabbit by the injection of a large volume of isotonic saline, nearly all the cells in the exudate are polymorphonuclear leucocytes, and the number contained in a single exudate may exceed the entire number originally present in the circulation.

2. The migration of polymorphonuclear leucocytes from the blood stream into the exudate is followed by a stimulation of the marrow, so that the blood stream is filled with young cells, many of which also pass into the exudate. This marrow stimulation, with the resultant shift of the polynuclear count to the left, is probably produced by the absorption of breakdown products of the cells which first appear in the exudate.

3. If exudates are produced repeatedly at intervals of from 4 to 10 days, the marrow responds by throwing younger and younger cells into the circulation, so that the blood stream becomes full of very young polymorphonuclear leucocytes of class I, or even metamyelo-

cytes. If 4 or 5 exudates are produced in rapid succession, the polymorphonuclear leucocytes in the circulation are all replaced by metamyelocytes and myelocytes.

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