THE LIPID CONTENT OF LEUCOCYTES FROM HEPARINIZED BLOOD*

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IN recent years a series of investigations on the lipid composition of the white blood cells has been designed by Boyd and associates with a view to provide a further quantitative means of measuring the activity and metabolism of these cells in diseases in which they form an important part of the pathological process. The basis of this study rests upon the fundamental work of Bloor, Corner, Okey, Snider, Boyd and certain continental authors, who have shown that there exists a relation between lipid composition and the "physiological activity" of a tissue. These fundamental studies demonstrated that a tissue which has been rendered more active through use, more active in the gymnastic sense of training, exhibits an increase in its phospholipid and frequently also in its free cholesterol content. This general physiological phenomenon is in agreement with and confirmed by the older observations that the most active tissues of the body, such as the various endocrine glands, the kidneys, the liver and the brain, contain large amounts of phospholipid. Conversely, inactive tissues, such as, for example, the jelly of Wharton,[1] contain small amounts of phospholipid and free cholesterol. A tissue which is degenerating, in contrast with one which is purely inactive, possesses in addition to small amounts of phospholipid and free cholesterol relatively large amounts of cholesterol esters, an example of such a tissue being the degenerating corpus luteum. The concentration of neutral fat depends upon the storage of fat, and appears to be largely independent of the physiological activity of the tissue. It has become customary thus, especially since the work of R. G. Sinclair, to divide the lipid content of a tissue into that part which is structural and bound with the active protoplasm and that part which is metabolic and taking part in the metabolism of the tissue or of the body generally.

Extending these basic studies to a consideration of the white blood cells, Boyd and associates have shown that the activity of blood leucocytes varies with their lipid content, or, vice versa, that the lipid content varies with the activity. These studies have yielded information of practical as well as theoretical value on the various leucocytes and on the rôle of the blood leucocytes in infective and potentially infective conditions. A review of this work has been given in a recent paper on leukæmia.[2] To the list of references therein contained may be added the investigation of Boyd and Stephenson[3] on the normal variations of the lipid content of the white blood cells, and of Boyd[4] on the relation of values for component lipids to increasing amounts of total lipid.

The chief technical difficulty in performing lipid analyses of the blood leucocytes lies not so much in the analytical methods as in the separation of the leucocytes from blood, and from a sufficiently small amount of blood to permit the test to be repeated at intervals if desired. In experiments on large animals, such as those of Willstätter and colleagues on the leucocytes of horses, the use of several litres of blood obviates many of the difficulties encountered when attempting to isolate leucocytes from small amounts of blood in man. More than 50 c.c. of blood cannot be conveniently obtained from patients, especially from patients who are definitely sick. This amount of blood, and often less, will, however, provide sufficient leucocoytes to permit of complete lipid analyses, providing that all or nearly all of the leucocytes can be separated cleanly from the plasma and red blood cells.

In previous studies either sodium citrate or potassium oxalate has been used as an anticoagulant. Recently the Connaught Laboratories have prepared a highly potent, standardized form of the so-called "natural" anticoagulant, heparin. In former years the price of this substance and the frequent variability of its potency rendered its routine use prohibitive. Because of its negligible effect on the volume relations of plasma and the red cells we have now introduced it as a routine anticoagulant in the

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Chemical Laboratory of the Kingston General Hospital for analyses on plasma.

Experimenting with its use in leucocytic analyses, the buffy layer of white cells was invariably found to separate in a more cohesive form than that previously obtained with the anticoagulant salts. This greatly facilitated the separation of the leucocytes, since they could be readily removed with a pair of forceps. Before comparisons could be made of the lipid content of leucocytes separated from heparinized blood with that of leucocytes separated from oxalated or citrated blood it was necessary to determine if the anticoagulant used affected the results reported, as they are, in mg. of lipid per 100 g. of moist leucocytes. The immediate effect of anticoagulant salts upon the red blood cells is to cause a shrinkage of their volume, and if the same were true of the white blood cells then one would expect that the results in oxalated blood would be relatively higher than those in heparinized blood. This was actually found to be the case.

A number of 50 c.c. samples of blood were added, one-half to a flask containing 0.1 g. of potassium oxalate, and one-half to another flask containing 3 mg. of heparin. Both samples of blood were shaken and immediately centrifuged at full speed for 1 hour. The buffy layer of leucocytes was then removed with a pair of forceps, gently rinsed in the plasma, freed of plasma on a weighed watch glass with strips of cleaned, alcohol-extracted filter paper, weighed, ground with cleaned sand, and extracted with alcohol-ether. The filtered extracts were analyzed for their content of phospholipid and free cholesterol by Bloor oxidative micromethods as modified by Boyd.[5]

RESULTS

The concentrations of phospholipid and of free cholesterol in oxalated and heparinized specimens of the same samples of blood are given in Table I. In most cases the amount of leucocytes obtained from the halved 50 c.c. portions of blood was insufficient to permit of estimation of more than phospholipid and free cholesterol.

TABLE I.

THE LIPID CONTENT OF THE WHITE BLOOD CELLS SEPARATED FROM OXALATED AND HEPARINIZED SPECIMENS OF THE SAME SAMPLE OF BLOOD. THE RESULTS ARE EXPRESSED IN MG. PER 100 G., MOIST WEIGHT, OF LEUCOCYTES.

	Phospholipid			*Free cholesterol*		
Number	*Oxalated*	*Heparinized*	*Difference*	*Oxalated*	*Heparinized*	*Difference*
1	456	302	−154	155	130	− 25
2	577	698	+121	203	225	+ 22
3	658	658	0	210	232	+ 22
4	755	328	−427	206	196	− 12
5	796	655	−141	207	201	− 6
6	833	537	−296	257	148	−109
7	1,080	698	−382	540	403	−137
8	1,183	656	−527	310	187	−123
9	1,470	868	−602	548	397	−151
10	1,520	970	−550	830	617	−213
11	1,545	1,532	− 13	405	386	− 19
12	1,590	1,290	−300	421	327	− 94
13	1,590	1,560	− 30	392	380	− 12
14	1,702	1,395	−307	465	377	− 88
15	1,762	1,790	+ 28	441	464	+ 23
16	1,920	1,020	−900	490	362	−128
17	1,980	1,700	−280	640	490	−150
18	2,020	1,990	− 30	476	467	− 9
Mean	1,302	1,035	−267	400	333	− 67

In a few cases blood was obtained at phlebotomies and sufficient leucocytes isolated to allow complete analyses, including total lipid, neutral fat, total fatty acids, total cholesterol, ester cholesterol in addition to free cholesterol and phospholipid. The results in these latter few cases indicated that changes in the concentration of phospholipid and free cholesterol could be taken as exemplary of changes in all of the lipids.

The data presented in Table I clearly demonstrate that the lipid content of leucocytes reported in mg. per 100 g., moist weight, is higher in cells separated from oxalated blood than in cells separated from heparinized blood. This

was true in 15 out of 18, or 83 per cent, of the cases investigated. The concentration of phospholipid of cells separated from oxalated blood extended from 456 to 2,020 mg. per 100 g., moist weight, and when separated from heparinized blood from 302 to 1,990 mg. The average value for phospholipid of cells obtained from oxalated blood was 1,302 mg. per 100 g., moist weight, and from heparinized blood 1,035 mg. In two cases there was slightly more phospholipid in the heparinized portion, and in one case the values were equal, but it is quite evident that one may conclude that the phospholipid values of leucocytes expressed in terms of mg. per unit moist weight are higher in oxalated than in heparinized blood. The differences varied considerably from case to case, depending upon the concentration of the anticoagulant salt and upon the time which elapsed before the extracts were made. The average difference was 267 mg. less phospholipid in the cells of heparinized blood, or approximately 20 per cent less than that found in the cells of oxalated blood.

The average concentration of free cholesterol was 400 mg. per 100 g., moist weight, of leucocytes in cells obtained from oxalated blood, and 333 mg. in cells separated from heparinized blood. The average difference of 67 mg. may be calculated to have been 17 per cent of the mean value for free cholesterol of the cells isolated from oxalated blood. In 83 per cent of cases also there was less free cholesterol in leucocytes of heparinized than of oxalated blood.

The explanation of these differences is apparently that oxalate, and presumably also other anticoagulant salts, when added to blood does not immediately diffuse across the cell wall of the leucocytes, and by increasing the osmotic tension outside draws water into the plasma. As a result there is less water and relatively more lipid left in the white cells, although there would presumably be no increase in the lipid content expressed in mg. per unit dry weight. It has been found that red blood cells on standing a day or so in contact with potassium oxalate gradually regain the volume originally lost on adding the salt, and that the lipid content returns to the initial value. Presumably a similar situation would prevail with the leucocytes, but this is largely of academic interest since extracts of the leucocytes are generally prepared immediately or within a few hours of the drawing of blood.

The practical conclusion to be drawn from these data is that one must consider the anticoagulant used in evaluating the significance of estimated values for the lipid (particularly phospholipid, since this is the lipid of most prognostic significance) content of the white blood cells. This is especially true when only one estimation is made, since with repeated estimations the significance lies in whether the values are increasing or decreasing. One may, however, draw certain conclusions prognostically in infected cases from a single estimation of the phospholipid content of the blood leucocytes and the prognosis to be drawn from various phospholipid values has been indicated by Boyd[6] for cells separated from oxalated or citrated blood. If heparinized blood be used, then the various ranges given before[6] should each be reduced by approximately 20 per cent.

SUMMARY

The concentration of phospholipid and of free cholesterol per 100 g., moist weight, of leucocytes was found lower in white blood cells separated from heparinized than from oxalated blood, the average decrease being 20 per cent.

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AUDI ALTERAM PARTEM

When quacks, as quacks may, by good luck, to be sure,
Blunder out at hap-hazard a desperate cure,
In the prints of the day, with due pomp and parade,
Case, patient, and doctor are amply display'd:—
All this is quite just—and no mortal can blame it;
If they save a man's life, they've a right to proclaim it:
But there's reason to think they might save more lives still,
Did they publish a list of the numbers they kill.—Samuel Bishop.