conut oil, which is composed largely of shorter chain saturated triglycerides.

The observation that sodium chloride and protamine can effect the removal from the system of fatty acid released by 'lipoprotein' lipase in the absence of other fatty acid acceptors indicates the care that must be taken in interpreting studies of this kind. If the appropriate conditions are chosen sodium chloride or protamine can be shown to stimulate, inhibit or have no effect on 'lipoprotein' lipase activity.

Summary. The effect of sodium chloride and protamine on the activity of postheparin 'lipoprotein' lipase was studied. Chylomicrons, low density lipoproteins and coconut oil emulsions stabilized with various emulsifying agents were used as substrate. Inhibition, stimulation, or no effect could be observed depending on the system used. The results are interpreted as indicating that where sodium chloride or protamine inhibit 'lipoprotein' lipase the site of the action is more likely to be at the oil-water interface of the substrate emulsion than on the enzyme molecule.

The emulsions used in this investigation were prepared with the assistance of Mr. J. W. Hadgraft, Chief Pharmacist, and his assistant Mr. C. W. Barrett.

1. Korn, E. D., *J. Biol. Chem.,* 1955, v215, 1.
2. Hollet, C., Meng, H. C., *Am. J. Physiol.,* 1956, v184, 428.
3. Brown, R. K., Boyle, E., Anfinsen, C. B., *J. Biol. Chem.,* 1953, v204, 423.
4. Robinson, D. S., Jeffries, G. H., French, J. E., *Quart. J. Exp. Physiol.,* 1954, v39, 165.
5. Robinson, D. S., French, J. E., *ibid.,* 1953, v38, 233.
6. Cottafavi, M., *Arch. Int. Pharmacodyn.,* 1953, v95, 1.
7. Nikkila, E. A., *Scand. J. Clin. Lab. Invest.,* 1953, v5, Suppl. 8.
8. Levy, S. W., Swank, R. L., *J. Physiol.,* 1954, v123, 301.
9. Anfinsen, C. B., Boyle, E., Brown, R. K., *Science,* 1952, v115, 583.
10. Robinson, D. S., *Quart. J. Exp. Physiol.,* 1955, v40, 112.
11. Datta, D. V., PROC. SOC. EXP. BIOL. AND MED., 1963, v112, 1006.
12. Dole, V. P., *J. Clin. Invest.,* 1956, v35, 150.
13. Korn, E. D., in *Methods of Biochemical Analysis,* ed. by David Glick, New York Interscience Publishers Inc., 1959, v7, 145.
14. Sparton, L. M., *Pharmaceutical emulsions and emulsifying agent, Chemist and Druggist,* London, 1959.
15. Brown, W. D., *Science,* 1953, v118, 46.
16. Laurell, C. B., *Acta Physiol. Scand.,* 1954, v30, 289.

Received November 4, 1963. P.S.E.B.M., 1964, v115.

Leukokinetic Studies. VIII. A Search for an Extramedullary tissue Pool of Neutrophilic Granulocytes.* (29040)

D. R. BOGGS, J. W. ATHENS, O. P. HAAB, S. O. RAAB, G. E. CARTWRIGHT AND M. M. WINTROBE
Department of Medicine, University of Utah College of Medicine, Salt Lake City

Considerable controversy has revolved about the anatomic location of storage reservoirs of mature granulocytes. Craddock and co-workers demonstrated a reserve supply of mature granulocytes in the bone marrow from which cells are released into the blood as needed(1). Their work has been amply confirmed in this(2) and other(3) laboratories. Debated, however, is the question of whether there is or is not a second, and perhaps even larger(4), reserve of mature granulocytes located in extramedullary tissue. Such an extramedullary granulocyte reserve might serve merely as a source of supply from which cells would return to the blood(5) or it might represent a source of supply of cells needed in the tissues(6). The latter hypothesis, that mature neutrophils leave the blood and enter an extramedullary reserve from which

* This investigation was supported by grants from Nat. Inst. of Arthritis and Metab. Dis., Bethesda, Md.

they migrate into areas of functional need in the tissues without reentering the blood, was tested in the present study.

The model of neutrophil function in tissues which was studied was their migration into induced inflammatory exudates. Whole blood, approximately 400 ml, was incubated *in vitro* with diisopropylfluorophosphate (DFP^{32}) and returned to the circulation of the donor. Infusion of such *in vitro* labeled granulocytes insures that blood granulocytes only are labeled; any cells in a hypothetical tissue pool are not labeled at the beginning of the study(7). As the labeled cells were infused an inflammation was produced(8). The specific activity (SA) of blood granulocytes and of granulocytes in the developing inflammatory exudate was then determined simultaneously. By comparing the SA of granulocytes in these two sites, the blood and the exudate, the proportion of exudate granulocytes that came directly from the blood was calculated.

In 1843 William Addison deduced from morphologic observation of an inflammation in a living frog's foot that the white corpuscles viewed therein were derived from the blood(9). In recent years, his observations have been confirmed by other morphological techniques(10) and have been further confirmed by observing the P^{32}(1) or histochemically labeled cells(11) in the blood migrate into inflammatory exudates. The question asked in the present study is therefore, not whether some of the cells come from the blood but rather whether all or only a part of the cells come from the blood.

Materials and methods. Volunteers from the Utah State Prison, age 21 to 38 years, served as subjects for the experiments. The methods for labeling blood granulocytes *in vitro* with DFP^{32}, isolation of blood granulocytes and determination of their specific activity have been described(7,12). The technique of producing inflammatory exudates used herein and the cellular content of these exudates in normal subjects has been described(8). In brief, blisters were produced on the skin of the volar surface of the forearms by the application of cantharidin and the relatively acellular contents of these blisters were transformed into purulent exudates by addition of a heat-killed culture of staphylococci. Enough granulocytes accumulated in the developing exudates by 4 hours after the addition of staphylococcus to determine their SA. One to 3 blisters were studied in each subject. When 2 or 3 blisters were placed on a single subject, the mean value was used in calculating the results in that subject.

Exudate samples were obtained by puncturing the thin blister wall, gently expressing all exudate and collecting it in a siliconized pipette. The exudate was transferred to a siliconized 10 × 75 mm test tube, centrifuged for 10 minutes at 500 × *g* and the supernate removed. The cell button was dispersed in 3 ml of 0.6% saline containing 0.1 ml of 1% saponin, allowed to stand for 3 minutes and centrifuged. The resulting cell button was washed with isotonic saline, suspended in 3 drops of absolute alcohol and its SA determined, as for blood granulocytes (12). It was observed that by the time staphylococci were added to the blisters a small number of leukocytes was present(8). The error in calculation of SA introduced by these unlabeled cells was corrected by the following formula: exudate granulocyte nitrogen (N) = determined N × [(T_4 cell count − T_0 cell count)/(T_4 cell count)]. "T_0 cell count" refers to the concentration of leukocytes in the exudate when staphylococci were added to the blister and "T_4 cell count" is the concentration of leukocytes in the blister 4 hours later. The magnitude of the increase in granulocytes by 4 hours was usually so great that this correction factor introduced little change in the calculated exudate SA.

Erythrocytes are labeled with DFP^{32}(12) but the rare erythrocyte present in the exudate was removed by the saponin-hypotonic saline hemolysis. The exudate supernate did contain radioactivity and nitrogenous substances, but the cell button was washed and since the SA of the exudate supernate was only 10% of that of the cells and the nitrogen content only 5% per volume of that of the cells, it was concluded that there was no

significant contamination of the neutrophil button from this source.

Results. Four experiments were performed. The only difference between each experiment was the time at which labeled blood granulocytes were infused in relation to the start of the inflammation. The start of inflammation (T_0) was the time at which staphylococci were added to the blisters. All inflammations were sampled when 4 hours old (T_4). Blood granulocyte SA was measured 5 minutes after labeled granulocytes were infused, usually hourly thereafter and always at the time an exudate was sampled. The results of each experiment are illustrated in Fig. 1.

Eight subjects were infused with labeled granulocytes at T_0 (Exp. 1). The mean exudate SA at T_4 was 75% of that of blood granulocyte SA at T_0. The granulocyte SA of all T_4 exudates was higher than that of granulocytes remaining in the blood at T_4.

Three subjects were infused with labeled granulocytes at T_1 (Exp. 2). The SA of exudate granulocytes in 2 subjects was higher and in one it was lower than that determined from a simultaneous sample of blood granulocytes.

Four subjects were infused with labeled granulocytes at T_2 (Exp. 3). The SA of granulocytes in all exudates was less than the SA of the blood granulocytes.

Two subjects were infused with labeled granulocytes at T_3 (Exp. 4). The SA of exudate granulocytes was considerably less than that determined simultaneously for blood granulocytes.

Discussion. Since blood granulocytes only were labeled initially(7), any granulocytes entering an exudate by direct migration from a hypothetical tissue pool would be unlabeled and only cells recently derived from the blood would be labeled. Thus, an estimate of the proportion of granulocytes that entered the inflammatory exudates from the blood could be made.

In Exp. 1 the maximal theoretical SA of exudate cells would be an SA equal to that of blood granulocytes at T_0. The observed exudate SA was 75% of the blood at T_0 which means that at least 75% of the exudate granulocytes were derived from the blood. The remaining 25% of exudate granulocytes could theoretically have been derived from an unlabeled, tissue granulocyte pool. However, blood neutrophil SA was falling rapidly during the course of the 4 hour experiment and exudate granulocyte SA could not be expected to equal granulocyte SA initially found in the blood, unless all granulocytes destined to enter the exudate left the blood within a few minutes after T_0, a hypothetical possibility not supported by our data.

TABLE I. Summary of Data Used in Calculating Percentage of Granulocytes in a 4 Hour Exudate Which Were Derived from the Blood.

	Mean blood SA† at:				Mean exudate SA† at:
Exp	$T_{0.5}$*	$T_{1.5}$*	$T_{2.5}$*	$T_{3.5}$*	$T_{4.0}$
1	1320	1050	830	670	1095
2	—	1090	740	500	574
3	—	—	1670	1310	530
4	—	—	—	1240	108

* $T_{0.5, 1.5}$, etc. refers to 0.5 hr, 1.5 hr, etc. after the start of inflammation.

† SA refers to specific activity expressed as cpm/mg leukocyte nitrogen.

Exp. 2, 3, and 4 were designed to supplement the data from Exp. 1 in such a manner that the approximate percentage of cells in an exudate of 4 hours which had left the blood during each preceding hour could be calculated. The basis for such a calculation is as follows. If any cells in a 4 hour exudate were in the blood during the preceding hour, then when labeled blood was infused into a subject 3 hours after inflammation was begun and the exudate was evacuated one hour later, the exudate should contain labeled cells. Exp. 4 (Table I, Fig. 1) demonstrated that some exudate granulocytes did leave the blood during the fourth hour of inflammation. The proportion leaving the blood during the fourth hour is determined approximately by the formula, (exudate SA)/(blood SA at $T_{3.5}$). The data needed to make this and subsequent calculations are summarized in Table I. Thus, approximately 9% of cells in a 4 hour old exudate had left the blood during the preceding hour.

The proportion of cells leaving the blood during the third hour and reaching the exu-

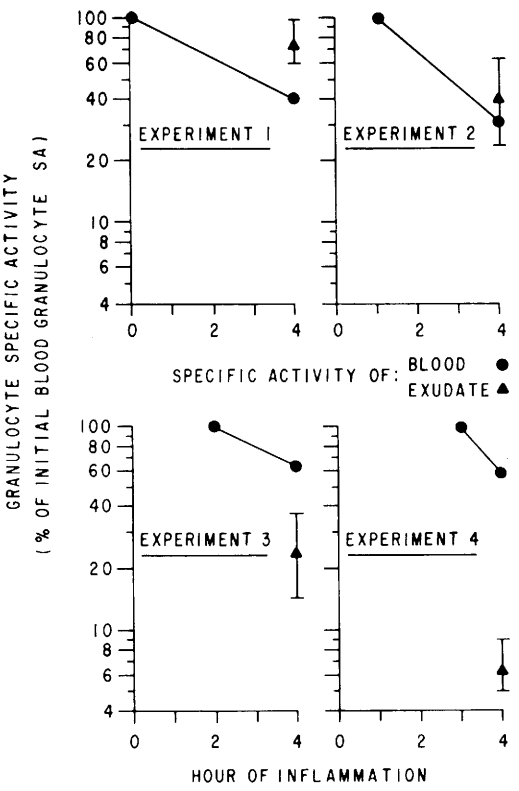

FIG. 1. Specific activity of blood and exudate granulocytes. Labeled granulocytes were infused as inflammation was begun (Exp 1); 1 hr later (Exp 2); 2 hr later (Exp 3); and 3 hr later (Exp 4). Solid line connecting the dots indicates mean SA of blood neutrophils. Triangle indicates mean SA of neutrophils from exudates. Range of exudate values is indicated by bar above and below triangle.

date by the end of the 4th hour (Exp. 3) is determined by: (exudate SA — 9% of blood SA at $T_{3.5}$)/(blood SA at $T_{2.5}$) and yields a figure of 25%. A similar calculation indicates that 36% and 32% of the cells present in the exudate at 4 hours left the blood, respectively, during the first and second hours.

The summation of these percentages is 102%. If a significant number of unlabeled cells had come into the exudate from a tissue pool a figure less than 100% should have been derived. These studies therefore fail to demonstrate the existence of a tissue pool of granulocytes.

These data and calculations are not so exact as to exclude the possibility that a very small percentage of exudate granulocytes may come from the tissues rather than the blood. The possibility is not ruled out that there exists a tissue pool of granulocytes with functions other than that of supplying granulocytes to an exudate.

Certain assumptions are implicit in this study. First, it is assumed that cells labeled with DFP^{32} are functionally identical to unlabeled cells. The evidence supporting this assumption has been presented in detail(7, 12). It would also seem justifiable to assume that DFP^{32} labeling does not significantly damage the granulocytes since labeled granulocytes readily left the blood and entered an inflammatory exudate. Secondly, for purposes of calculation it was assumed that there was no turnover of granulocytes in the exudate during the first 4 hours. However, if there were a rapid turnover of granulocytes through the exudate, this would tend to lower the exudate SA and would thus prejudice our results in favor of rather than against the existence of a tissue pool of granulocytes. It was also assumed that exudate granulocytes had acquired no signficant amounts of radioactivity or unlabeled nitrogenous substances through phagocytosis of exudative substances. Other than a few intracellular staphylococci, no phagocytosed materials were observed in smears of the exudate.

Summary. This study was designed to determine whether inflammatory granulocytes are derived solely from the blood or from a hypothetical extramedullary tissue pool of granulocytes, in addition to the blood. Blood granulocytes were labeled *in vitro* with DFP^{32}, infused, and their subsequent appearance in induced inflammatory exudates studied. From a comparison of the maximum blood granulocyte specific activity and the exudate granulocyte specific activity it was evident that at least three-fourths of the exudate neutrophils were derived directly from the blood. To determine if more than three-fourths of the exudate neutrophils were from the blood the proportion of granulocytes in an exudate 4 hours old which had left the blood during each of the preceding 4 hours was calculated. It was found that approximately 36% of the granulocytes in a 4 hour

exudate had left the blood during the first hour of inflammation. The figures for the 2nd, 3rd and 4th hours of inflammation were, respectively, 32%, 25% and 9%, making a total of 102%. If significant numbers of unlabeled granulocytes from the tissues had entered the exudate, the summation of these percentages should have been substantially less than 100%. Consequently, these studies provide no evidence for the existence of a tissue pool of granulocytes.

We are indebted to Miss Helen Ashenbrucker for technical assistance. The cooperation of Dr. William Knott, Warden John Turner and the inmates of the Utah State Prison is sincerely appreciated. Dr. Paul Hoeprich kindly maintained the culture of staphylococci used.

1. Craddock, C. G., Jr., *Am. J. Med.,* 1960, v28, 711.

2. Athens, J. W., Haab, O. P., Raab, S. O., Mauer, A. M., Ashenbrucker, H., Cartwright, G. E., Wintrobe, M. M., *J. Clin. Invest.,* 1961, v40, 989.

3. Patt, H. M., Maloney, M. A., Jackson, E. M., *Am. J. Physiol.,* 1957, v188, 585.

4. Osgood, E. E., *Blood,* 1954, v9, 1141.

5. Patt, H. M., Maloney, M. A., *Brookhaven Nat. Lab. Symp.,* 1957, v474, 75.

6. Osgood, E. E., in discussion of ref. 5, p82.

7. Mauer, A. M., Athens, J. W., Ashenbrucker, H., Cartwright, G. E., Wintrobe, M. M., *J. Clin. Invest.,* 1960, v39, 1481.

8. Boggs, D. R., Athens, J. W., Haab, O. P., Raab, S. O., Cartwright, G. E., Wintrobe, M. M., *Am. J. Path.,* 1964, v44, 61.

9. Addison, W., Tr., *Provinc. Med. Surg. Assn.,* 1843, v11, 233.

10. Allison, F., Smith, M. R., Wood, W. B., *J. Exp. Med.,* 1955, v102, 655.

11. Brecher, G., Cronkite, E. P., Bond, V. P., Dutcher, T. F., *Acta Haemat.,* 1958, v20, 179.

12. Athens, J. W., Mauer, A. M., Ashenbrucker, H., Cartwright, G. E., Wintrobe, M. M., *Blood,* 1959, v15, 303.

Received November 9, 1963. P.S.E.B.M., 1964, v115.

Distribution of Mutarotase in Subfractions of Rat Intestinal Mucosa and Rat Kidney.* (29041)

J. MARTYN BAILEY AND PETER PENTCHEV

Department of Biochemistry, George Washington University School of Medicine, Washington, D.C.

An enzyme with the ability to accelerate mutarotation of glucose was first demonstrated in extracts of the mold *P. notatum* by Keilin and Hartree(1). Presence of the enzyme was first noted by the fact that action of notatin (glucose oxidase) from the same mold upon glucose was accelerated by the presence of an enzymatic impurity. It was subsequently shown that glucose oxidase was specific for the β-form of glucose, and that mutarotase accelerated the conversion of α-glucose to the form utilized by notatin. This property has been used to assay for mutarotase(2).

Keston(3) detected large amounts of a similar enzyme in extracts of rat kidney. It was proposed that the enzyme functioned in tubular resorption and membrane transport of glucose by accelerating conversion of glucose to a preferentially diffused form. The theory fell into disrepute when it was shown that 1-deoxyglucose(4) and α-methylglucoside(5), compounds containing no mutarotable hydroxyl group, were, however, actively absorbed.

It has been suggested recently, however(6), that mutarotase activity is only a coincidental function of the enzyme protein. In support of this proposal it was shown that both 1-deoxyglucose and α-methylglucoside interact with the mutarotase active center. Both compounds were efficient competitive inhibitors of the enzyme acting on α-glucose.

It has been shown(7) that during intestinal absorption of sugars the concentrative gradient for actively transported sugars is established at the epithelial cells lining the villi. Crane(8) has also demonstrated recently a

* This investigation was supported by grants from U. S. Public Health Service.