maximum amount of triolein which could be altered by this amount of extract was reached, and increasing the amount of triolein added merely increased the unaltered triolein which could be visualized in the lower of the 2 triglyceride spots. Fig. 2 illustrates the effect of varying the amount of plasma and keeping the triolein concentration constant. Serum obtained after blood clotting produced changes in the added triolein which were similar to plasma.

The triolein "shifting" activity could not be found in any of the protein fractions isolated by starch block electrophoresis and it was assumed that the factor causing the shift either migrated to an electrode bath or diffused into the PVP, either of which would have diluted the activity so that it could not be demonstrated.

Ultrafiltration clearly demonstrated the activity in the ultrafiltrate and its absence in the residue remaining within the cellophane bag (Fig. 3). The factor, therefore, is not protein-bound and is readily diffusible. It is also evident from this experiment that the activity is not present in the plasma lipoproteins, which remain within the bag.

Discussion. The fact that the activity occurs in non-aqueous organic solvents, and is present in protein-free ultrafiltrates suggests that the phenomenon is non-enzymatic. The increase in Rf value of the triglyceride may represent a dehydrogenation reaction, although to our knowledge, this has been described only with fatty acids in the presence of rigidly controlled substrates(3). A more likely mechanism may be the formation of an optical or geometric isomer. Geometric isomers have been described after ozonization of fatty acids(4). Naturally occurring triglycerides possessing optical activity in the glycerol moiety capable of resolution on thin-layer plates have been described recently(5).

Further characterization of the factor producing the shift, the exact nature of the change produced in the triglyceride molecule, and quantitative variations in shifting ability of plasma of different subjects are as yet unknown, but are under study.

Summary. A hitherto unknown factor has been found in human blood plasma capable of producing an increase in the Rf value of triglyceride in thin-layer chromatography. It has been shown that the factor is not protein-bound, appears in ultrafiltrates of plasma, and shows quantitative dose-response relationships. The activity is considered non-enzymatic.

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"Masked" Granulocytosis.* (29960)

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Blood granulocytosis, not reflected by an increased granulocyte concentration in venous blood samples, is described herein.

In 1843, Addison observed leukocytes in a marginal position along the walls of small venules(1). In 1938, Vejlens reported experiments designed to evaluate possible mechanisms leading to this margination(2). The concept that all granulocytes within the confines of the vascular system are not freely circulating was affirmed in this laboratory by labeling granulocytes *in vitro* with radioactive diisopropylfluorophosphate (DFP^{32}) and returning them to the circulation of the donor

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(3). Labeled granulocytes distributed rapidly in an intravascular pool which was approximately twice the size of the circulating granulocyte pool calculated from the granulocyte count and the blood volume. The granulocytes unaccounted for were shown to be in a pool from which granulocytes freely and rapidly exchanged with those which were circulating. Thus, the total blood granulocyte pool (TBGP) consists of 2 compartments, the circulating granulocyte pool (CGP) and the marginal granulocyte pool (MGP). After infusion of DFP^{32}-labeled granulocytes, the blood granulocyte specific activity (BGSA) in the TBGP declines due to influx of unlabeled granulocytes from the marrow and random loss of labeled granulocytes from the blood(4). Granulocyte input is equal to output under normal conditions and the BGSA decline in normal subjects follows a single exponential curve with a half disappearance time (T ½) ranging from 4 to 10 hours(5).

An abnormal BGSA disappearance curve was noted in normal subjects in whom inflammatory exudates were produced experimentally by adding killed staphylococci to cantharidin-induced blisters on the skin of the forearm(6,7). In this report, the explanation for this abnormal curve has been demonstrated to be a rapidly expanding MGP.

Materials and methods. Normal subjects were inmates of the Utah State Prison. The techniques of labeling granulocytes *in vitro* with DFP^{32}(3,4), inducing exudates by adding killed staphylococci to cantharidin-induced blisters(6) and isolating blood or exudate granulocytes and determining their specific activity (counts/minute per mg leukocyte nitrogen)(3,7) have been reported.

Results. Nine normal subjects were infused with autologous, DFP^{32} labeled granulocytes at the same time that inflammations were initiated. During the first 4 hours of exudate formation, the BGSA decreased at a faster than normal rate (Fig. 1). This rapid decrease in BGSA could be explained if exudate formation was associated with an increased flow of unlabeled granulocytes from marrow to blood. However, the blood granulocyte concentration did not change (Fig. 1), indicating that if excess cells were released to the blood, they were not within the CGP.

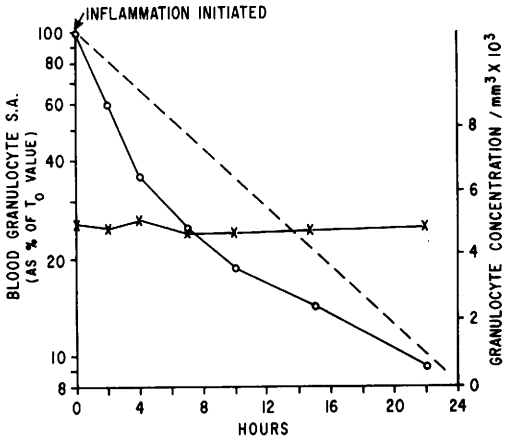

FIG. 1. The mean disappearance curve in 9 subjects in whom inflammation was initiated as labeled blood was infused (solid line with dots) is compared to the mean curve from 56 subjects without inflammation(5) (broken line). Note that concentration of granulocytes in the blood of the subjects with inflammation (solid line with ×'s) did not change significantly during the study.

Therefore, to determine if the MGP was enlarged, its size was measured at intervals following the induction of inflammations and compared to MGP size in normal subjects without inflammation. The results are shown in Table I. The mean MGP size was slightly although not significantly ($p = <.2$) larger than normal in the group of subjects in whom pool size was measured 15 minutes after inflammations were begun. The mean MGP size was greatly expanded (3 times normal, $p = <.001$) in the group of subjects in whom pool size was measured 2 to 3 hours after inflammations were begun.

Discussion. The induction of small inflammatory exudates precipitated an increased rate of inflow of granulocytes to blood from marrow. The resultant increase in TBGP size was not reflected in the circulating granulocyte concentration since only the MGP was increased in size. The degree of increase in MGP size was out of proportion to such a seemingly minor increase in peripheral demand for granulocytes. In normal subjects, the mean number of granulocytes leaving the blood each day is 163×10^7/kg(5). The maximum number of granulocytes which entered the induced exudates on any subject reported herein was less than one percent of the above figure(6). Perhaps the increase in

TABLE I. Effect of Beginning Inflammation upon Blood Pool Sizes in Normal Subjects.

	Blood pool size ($\times 10^7$ cells/kg)		
	TBGP*	CGP*	MGP*
Pool size measured:			
Without inflammation	70	31 (11-46)†	39 (0- 85)
15 min after inflammation was initiated (9 subjects)	91	34 (14-72)	57 (26-122)
2-3 hr after inflammation was initiated (8 subjects)	144	34 (28-49)	110 (59-177)

* Abbreviations refer to: Total blood granulocyte pool (TBGP), circulating granulocyte pool (CGP), and marginal granulocyte pool (MGP).

† Parenthetic numbers refer to the determined range of values in each group of subjects with inflammation and to 95% limits of confidence for values in the group of 109 subjects without inflammation(5).

number of marginated granulocytes attending these inflammations represents the build-up of a readily available "blood granulocyte reserve."

During the course of granulocytosis which follows endotoxin administration, MGP enlargement precedes CGP enlargement(8). Craddock and coworkers(9) suggested that the lag period between cessation of leukapheresis and increase in granulocyte count reflected preferential replenishment of a depleted MGP. However, "masked" granulocytosis in which enlargement of the MGP occurs without concurrent or subsequent enlargement of the CGP has not been described previously.

Summary. The effect of induced inflammatory exudates upon the distribution and absolute number of granulocytes in the blood has been studied in human subjects. In normal subjects without inflammation, the mean size of the total blood granulocyte pool (TBGP) was 70×10^7 cells/kg with 31×10^7 cells/kg in the circulating granulocyte pool (CGP) and 30×10^7 cells/kg in the marginal granulocyte pool (MGP). The TBGP increased to 144×10^7 cells/kg in 8 subjects studied 2 to 3 hours after initiation of inflammation. The CGP did not change in size. All of the additional granulocytes in the blood were within the MGP. This type of blood granulocytosis has been termed "masked" granulocytosis since it is undetectable by enumeration of granulocytes in venous blood samples.

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