cinogenic activity of these products is now under investigation.

Summary. The stability of 3-hydroxyanthranilic acid (3-HOA) in buffer solutions was investigated as it relates to pH, oxygen tension, and catalytic ion concentrations. The data show that 3-HOA is unstable under certain simulated physiologic conditions, and suggest that the amounts found in some samples of voided urine are not necessarily the true quantities formed, but rather may be the amounts remaining after decomposition of the metabolite in the interim between formation and analysis.

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Leukokinetic Studies. XII. Kinetic Studies of Normal Isologous Neutrophilic Granulocytes Transfused into Normal Subjects.* (30600)

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The clinical usefulness of erythrocyte transfusions is well established. In addition, understanding of the pathogenesis of the many types of anemia has been materially advanced by the development of methods for measuring red cell mass and red cell survival. Thus, infusion of labeled erythrocytes into a patient's own circulation (autologous study) or into the circulation of a compatible donor (isologous study) has made it possible to determine whether erythrocytes with a shorter than normal survival are themselves defective (intracorpuscular defect) or are subjected to an abnormal environment (extracorpuscular defect). Recently, granulocyte transfusions have also been employed both with the hope of clinical benefit and to study mechanisms of granulocytosis and granulocytopenia. For example, Yankee *et al* have reported clinical improvement in infected neutropenic patients following transfusion of granulocytes from normal donors(1). Similar results have been obtained in neutropenic patients transfused with granulocytes from donors with chronic myelocytic leukemia(2). Krill *et al*(3) have attempted to define intracorpuscular and extracorpuscular granulocyte defects in a patient with idiopathic neutropenia by comparing the kinetic results of isologous and autologous granulocyte infusions. We(4) and others(5) have attempted similar studies in patients with chronic myelocytic leukemia.

In an earlier study(6) 10 normal male subjects were transfused with isologous blood and it was observed that in 2 of the subjects the granulocytes disappeared at an accelerated rate. The present study was designed to extend these earlier studies by comparing transfused isologous and autologous granulocytes labeled with radioactive diisopropylfluorophosphate (DFP^{32}) with respect to their distribution in the vascular system at completion of the transfusion, their subsequent disappearance from the blood and their ability to migrate from the blood and enter an inflammatory exudate. A preliminary report of a portion of these data has been published(7).

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Materials and methods. The subjects were hematologically normal inmates of the Utah State Prison who had received no previous injections of blood or blood products. Eight pairs of subjects were selected for cross transfusion studies on the basis of compatible erythrocyte ABO and Rh antigens. In 6 pairs approximately 400 ml of blood was withdrawn from each subject into plastic transfusion bags, incubated for 1 hour with DFP^{32}, and then infused rapidly (15 minutes or less) into a compatible recipient. Upon completion of the infusion the size of the total blood granulocyte pool (TBGP), the circulating granulocyte pool (CGP) and the marginal granulocyte pool (MGP) were measured and rate of disappearance of the labeled granulocytes from the blood was followed. In 2 pairs of subjects, exudates were produced by adding heat killed staphylococci to cantharidin induced skin blisters at the same time that labeled isologous granulocytes were infused into the blood. Data for the half-disappearance time (T ½) of the labeled cells from the circulation and for distribution of the cells within the circulation from these 4 subjects are not recorded in the tables since in the presence of such exudates these two measurements of granulocyte kinetics are grossly disturbed(8).

In each subject, exudate granulocyte radioactivity was determined at 4, 7, 10 and 24 hours in 3 exudates. Blood granulocyte specific activity was determined at 0, 2, 4, 7, 10 and 24 hours. Granulocyte radioactivity was calculated as counts per minute per mg granulocyte nitrogen from both blood (BGSA) and exudate (EGSA). Details of the methodology used in these studies have been published(6,9,10).

Results. The size of the TBGP and the distribution of granulocytes between the CGP and MGP in 12 isologous transfusions (6 pairs of subjects) studied without inflammation were the same as in previous studies on normal subjects given autologous granulocytes (Table I). In 11 of the 12 isologous transfusions the subsequent decrease in BGSA followed a single exponential curve as was the case in 56 of 59 normal autologous studies. However, in 5 of the isologous studies the half-disappearance time of the BGSA curve was considerably less (faster rate of fall) than the lowest value encountered after the infusion of autologous granulocytes (Table I). To be certain that the faster than normal decrease in BGSA in these 5 studies was related to the infusion of isologous cells these 5 recipients were subsequently infused with labeled autologous granulocytes, on one or more occasions. In each instance the T ½ of autologous granulocytes was within the normal range (Table II).

To compare the appearance of labeled isolo-

TABLE I. Total Blood Granulocyte Pool (TBGP), Circulating Granulocyte Pool (CGP) and Granulocyte Half-Disappearance Time (T ½) After Isologous and Autologous Transfusion.

Code No.	TBGP ($\times 10^7$/kg)	CGP (as % of TBGP)	T ½ (hr)
VII-164	142.8	42.8	.6
VII-156	66.6	32.2	1.0
VIII-19	94.1	22.2	1.5
VIII-31	121.8	39.4	1.8
VII-154	34.5	91.0	2.0
VII-168	70.9	40.4	4.0
VII-170	85.3	58.6	4.4
VII-158	70.4	41.4	5.5
VIII-13	50.2	70.5	6.3
VIII-17	86.1	38.4	6.5
VII-166	76.5	62.8	8.3
VII-160	51.2	71.0	*
Isologous studies	79.2	50.9	3.8
(Mean & 95% limits)	(21.2-137.2)	(12.9-88.9)	(0 -8.8)
(Determined range)	(34.5-142.8)	(22.2-91.0)	(.6-8.3)
109 autologous studies	70.0	56.0	6.7†
(Mean & 95% limits)	(14 -160)	(5.5- 96.3)	(4 -10)
(Determined range)	(16.7-149)	(19.1-127.7)	(3.5-10.3)

* Complex curve. † Based upon 56 studies.

TABLE II. Comparison of Half-Disappearance Time (T ½) of Autologous and Isologous Granulocytes in 5 Subjects.

Code No.	T ½ (hr) following Isologous	Autologous
VIII-19	1.5	4.0
VIII-31	1.8	5.0, 6.0
VII-154	2.0	3.5
VII-156	1.0	3.8
VII-164	.6	4.5
Mean	1.4	4.5
(Determined range)	(.6-2.0)	(3.5-6.0)

TABLE III. Comparison of Exudate Granulocyte Specific Activity (EGSA) and Blood Granulocyte Specific Activity (BGSA) After Infusion of Labeled Isologous or Autologous Granulocytes.

	BGSA - 4 hr / BGSA - 0 hr	EGSA - 4 hr / BGSA - 0 hr		Observed / Expected
		Expected	Observed	
Column	(1)	(2)	(3)	(4)
Autologous studies (8)				
Mean and range	.36 (.15–.50)	.68 (.31–.89)	.67 (.42–.90)	.99 (.60–1.34)
Isologous studies				
Subject: VI-76	.18	.50	.07	.14
VI-78	.07	.46	.16	.35
VI-102	.06	.43	.12	.28
VI-104	.30	.61	.22	.36

gous and autologous blood granulocytes in exudates, 2 pairs of subjects were infused with isologous labeled cells at the same time that exudates were induced. The results of these studies are compared with results of similar studies in 8 additional subjects previously reported(10) who were infused with autologous labeled granulocytes (Table III). The exudate granulocyte specific activity (EGSA) was determined at 4 hours and compared with the BGSA at several points during the first 4 hours, an interval during which granulocytes leave the blood and migrate into the exudate.

The ratio of EGSA at 4 hours to the BGSA at 0 hours (Column 3 in Table III) was less with isologous than with autologous granulocytes. The 4-hour EGSA was less than the 4-hour BGSA in 2 of 4 isologous studies (compare Columns 1 and 3 in Table III), a situation not seen in autologous studies. However, since the 4-hour EGSA, relative to initial BGSA, is a function of the rapidity with which BGSA declines during the first 4 hours(10) direct comparison of EGSA (Column 3, Table III) in isologous and autologous studies is not applicable unless the rate of decline in BGSA was the same in both studies.

A rapid decline in BGSA followed induction of exudates in both isologous and autologous studies, but there was a tendency for the 4-hour BGSA to be lower in the isologous studies than after autologous infusions (Column 1 in Table III). Although the difference was not statistically significant ($p = > 0.30$) in the small group studied, a correction for the influence of the slightly more rapid decline in BGSA in isologous studies upon EGSA was made. In autologous studies(10), it was calculated that approximately 36% of neutrophils in a 4-hour-old exudate had left the blood during the first hour after infusion of labeled cells, 32% during the second hour, 25% during the third hour and 9% during the fourth hour. From these percentages and the determined curve of BGSA decline during the first 4 hours an expected EGSA (Column 2, Table III) was calculated for comparison with the observed EGSA (Column 3, Table III). The ratio of observed to expected EGSA (Column 4, Table III) was significantly less ($p = < .01$) in isologous studies than in autologous studies and the highest ratio observed in any isologous studies was but 60% of the lowest ratio observed in an autologous study.

Values for EGSA and BGSA beyond 4 hours were consistent with the difference between autologous and isologous studies observed at 4 hours. There was no significant difference in the concentration of cells in exudates of subjects infused with isologous cells as compared to those infused with autologous cells.

Discussion. Of the 4 measures of kinetic behavior of transfused isologous granulocytes studied herein, only 2, the size of TBGP and the distribution of granulocytes between circulating and marginal sites, were consistently normal. If an excessive number of isologous granulocytes had been removed from the blood during the transfusion, then the size of TBGP and the proportion of cells in the

MGP should have been measured as abnormally large. In therapeutic transfusion of neutrophils from either normal donors(1) or donors with chronic myelocytic leukemia(2), less than 10% of the transfused cells were reported to be in the neutropenic recipients CGP at completion of the infusion. Whether the rapid rate of granulocyte removal during the transfusion observed in those studies as compared to the normal rate observed in this study reflects differences in numbers of cells damaged during *in vitro* manipulations preceding transfusion or differences in the recipients is undetermined. However, if normal autologous cells are kept *in vitro* for but a few hours, the majority are removed from the blood during transfusion(4).

Following completion of the transfusions, the half disappearance time of labeled isologous granulocytes was significantly shorter than that of labeled autologous granulocytes in 5 of 12 studies. This observation together with the previously reported finding of a short T ½ in 2 of 10 similar studies(6) indicates that approximately one-third (7/22) of normal subjects remove normal isologous cells from their circulation at a faster than normal rate.

Although labeled isologous granulocytes entered exudates in considerable numbers it is evident that labeled autologous cells did so more readily. Brecher *et al*(11) demonstrated that rat granulocytes transfused into the blood of mice would enter peritoneal exudates induced in the mice. They calculated that the majority of transfused rat granulocytes were removed very rapidly from mouse blood but that most cells leaving the blood did not enter the peritoneal exudate.

In all 4 subjects transfused isologous cells failed to appear in exudates as readily as did autologous cells and perhaps left the blood more rapidly. Therefore it seems likely that a significant proportion of the isologous cells was removed from the blood *via* an abnormal route. Thus, the possibility must be entertained that even in subjects who exhibit an entirely normal blood granulocyte kinetic pattern (TBGP, CGP/TBGP ratio, and T ½) at least some of the infused isologous cells behave in an abnormal manner.

These observations are of practical significance in two areas. Firstly, since normal isologous cells unequivocally behave abnormally in a significant proportion of normal subjects, interpretation of the kinetic significance of "cross-transfusion" data for neutrophils is rendered difficult if not impossible. Secondly, this abnormal behavior adds further limitations to the already difficult therapeutic maneuver of granulocyte transfusion. However, assuming that the transfused cells which do find their way into exudates are still efficient phagocytes, these data do provide some evidence for the therapeutic efficacy of such transfusions.

The observation that about one-third of isologous transfusions resulted in an abnormally rapid removal of neutrophils is compatible with recently published observations on the incidence of certain leukocyte antigens(12). It is possible that the rapid removal of neutrophils after isologous transfusion which we have observed in some subjects is due to naturally occurring granulocyte incompatibilities. If so, with granulocyte "blood group" typing it may be possible to select compatible types for kinetic studies and for therapeutic granulocyte transfusion.

Naturally occurring isoagglutinins might also be responsible for the failure of isologous granulocytes to enter exudates as readily as autologous cells. However, this relative failure to enter exudates was not a function of rapid removal of isologous cells from the blood. Therefore, if isoagglutinins explain this observation, the antibody must injure the circulating isologous cell in a subtle manner so that migration of the cells into exudates is hampered but the injury does not lead to immediate removal from the blood.

Summary. To determine if DFP^{32}-labeled isologous granulocytes behave kinetically in a fashion similar to autologous granulocytes, the size of the total blood granulocyte pool, the distribution of cells in the circulating and marginal compartments and the half-disappearance time of isologous granulocytes were determined in 6 pairs of normal subjects. In 2 pairs of normal subjects the entry of labeled isologous granulocytes into experimentally induced inflammatory exudates was investi-

gated. The results were compared with previously reported studies in which autologous blood was infused.

The size of the total blood granulocyte pool and the distribution of cells in the circulating and marginal compartments were normal in all 12 of the subjects studied. However, in 5 of the 12 subjects the half-disappearance time was more rapid than normal. It is suggested that in these 5 subjects, naturally occurring granulocyte incompatibilities may have accounted for the rapid disappearance of cells from the circulation.

Labeled isologous granulocytes migrated into inflammatory exudates less readily than did labeled autologous granulocytes. This observation may be of significance in regard to the therapeutic efficacy of isologous leukocyte transfusion.

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Erythropoietic Stimulating Factor (ESF) Associated with an Oncogenic Virus-Host System *in vitro*.* (30601)

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The existence of an erythropoietic stimulating factor or factors (ESF) or erythropoietin has been established beyond reasonable doubt(1,2,3). Recently ESF has been reported to stimulate cell division or growth both *in vivo*(4) and *in vitro*(5). The former report was based on experiments with Novikoff hepatomas in rats. The latter report concerned cultures of bone marrow cells from a human monocytic leukemia patient and human synovial membrane cells.

Several workers have reported the association of increased concentrations of ESF or situations compatible with an increased level of ESF with different types of cancer. Remission of associated polycythemia has been reported to occur following surgical removal of 2 malignancies(6,7). An ESF has been reported in fluid from a cystic cerebellar hemangioblastoma(8). Tumor growth was also reported to be related to the process of

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