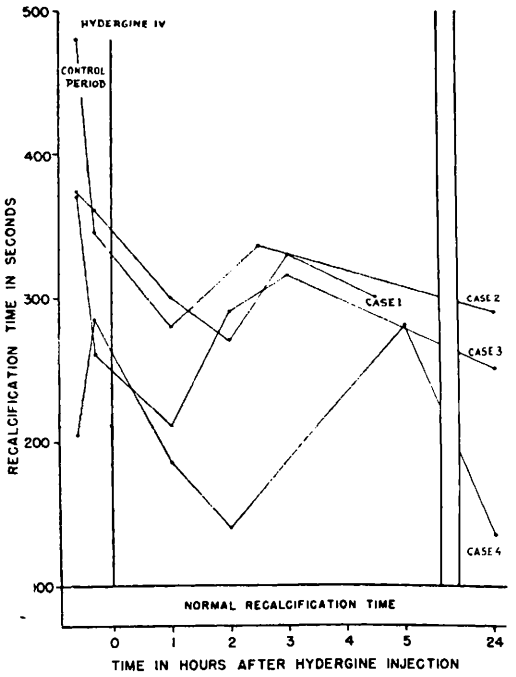

FIG. 2. Recalcification time in 4 hemophilics following intravenous administration of Hydergine. Case 1 received 0.5 mg; cases 2 and 3 received 0.6 mg; case 4 received 1.2 mg.

whom it was determined. Of the 3 patients receiving the intravenous dose of 0.5 or 0.6 mg the serum prothrombin decreased moderately in 2, though never to levels below 50%, while it was unchanged in the third. In the fourth patient, who received 1.2 mg intravenously, it changed from an original value of 60% to a final value of 20%. The decline was gradual and did not correlate with the variations in recalcification time. Lysis of the diluted plasma clot occurred in a single specimen taken after 24 hours of oral treatment in Case 1 and in specimens from Case 4 at 2 and at 5 hours after the 1.2 mg injection. In the patients receiving 0.6 mg intravenously, none of numerous clots dissolved. Using fibrinogen tagged with radioactive iodine as a substrate, samples of plasma from Cases 2, 3, and 4 showed no increase in lysis after Hydergine administration. Plasma labile factor was insignificantly altered in any patient in the first 5 hours after injection. Only the patient who was given 1.2 mg of Hydergine intravenously experienced unpleasant symptoms. These consisted of nausea and vomiting shortly after injection, severe nasal congestion, and frontal headache which lasted about 6 hours. Alterations in pulse and blood pressure were not remarkable in any patient.

Summary and conclusions. Three hemophilics were treated with Hydergine according to a regimen reported to effect a marked reduction of clotting time. A fourth patient received twice the amount of drug stated by Vodopivec to be effective upon intravenous administration. No important acceleration of coagulation was noted. Only in the patient who received an excessive dose and became ill were there any impressive changes. These consisted of a transient decrease in recalcification time and a gradual decline in serum prothrombin, as well as fibrinolysis *in vitro*. Since the Hydergine used in this study is believed to be identical to that administered by Vodopivec, we are unable to explain our inability to confirm his findings.

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Effect of Starvation on Phagocytosis *in vivo*.* (18831)

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Phagocytosis has long been known to bear

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a relationship to the nutritional status of the experimental animal. Many investigators have concluded that phagocytosis is depressed by the omission of some element of the food supply(1-4). On the other hand, others have shown that there is an increase in this activity when the food supply is decreased(5-8). In all of these reports, the animals had been depleted by long deprivation of one or more essential elements of the diet. Recent work, by one of the authors(9), carried out in tissue cultures, showed marked differences in motility, adhesiveness and preference for saccharides, between the leucocytes of starved and fed rats. These results suggested a study of the *in vivo* effect of starvation and phagocytosis.

Methods. The phagocytic activity of rats deprived of food for 36 hours was compared with that of well fed animals. Both groups were injected parenterally with a suspension of bacteria and after intervals varying from 2-3¼ hours, peritoneal samples were removed and examined for phagocytic activity. The phagocytic activity was measured in terms of the percentage of W.B.C.-containing bacteria as well as the number of bacteria engulfed by the W.B.C. This work was conducted on mature male albino rats of the Wistar strain, weighing 275-375 g each. The phagocytic response was studied by using a non-hemolytic, coagulase positive strain of *Staphylococcus aureus* obtained from human isolation. The bacteria were grown on brain-heart infusion blood agar plates for a period of 18 hours at 37°C, at which time they were harvested and washed 3 times with saline. After partial dilution they were filtered through a thin layer of sterile absorbent cotton and then further diluted with saline to give approximately 50% transmission with a 400 mμ filter on the Coleman Junior Spectrophotometer. This suspension was stored in the refrigerator and just before use the next day, was warmed to body temperature and well shaken.

Inoculation of animals. The rats deprived of food for a period of 36 hours before inoculation are referred to as the starved group. Prior to starvation all the rats had received, for a period of 4 weeks, a complete diet in the nature of a plentiful supply of Rockland Farm Rat Pellets, supplemented by greens. Water was given *ad lib.* In general, experiments were performed on 5 starved and 5 fed animals simultaneously. Each rat was injected intraperitoneally with 20 cc of the prepared bacterial suspension. (None of the rats died as a result of the inoculation with the bacteria). After a minimal interval of 2 hours 3 to 8 cc of the peritoneal fluid were removed by alternately injecting air and then aspirating. A 10 ml syringe and a 19 gauge needle were used. The collected fluid was placed in a test tube and duplicate white cell counts were immediately made. At this time a sterile platinum loop of 5 mm diameter was dipped into the fluid and a 2 cm square smear made on one end of a glass slide. Two loops full were smeared over an equal area on the opposite end. Three such slides were made from each peritoneal specimen. The smears were allowed to dry and then were stained by a modified Gram stain. The crystal-violet was applied for not more than 15 seconds; the iodine for 10 seconds; the acetone-alcohol for 10-15 seconds, and the saffarin for 10 seconds.

Counting white blood cells and bacteria. Each of the duplicate W.B.C. counts were made in the usual way with a white-cell pipette and diluting fluid. Nine sq. mm were counted on each specimen and the average of the two replicates taken. The stained smears were counted in the following manner: Areas

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TABLE I. Comparison of the Phagocytic Activity of Starved and Fed Rats.

	No. of rats	WBC/cmm of perit. fluid ×100	% of Phago./ 100 WBC (stained smear)	Avg No. of bact. in Phago./ 100 WBC (stained smear)	Avg No. of Phago./cmm	Avg No. of bact. in Phago./cmm
Starved	21	14900	38	423	4563	52855
Fed	29	13700	19	129	2205	15344

TABLE II. Comparison of Phagocytic Activity of Groups of Rats Based on Time Interval Between Injection of *Staphylococcus aureus* and Withdrawal of the Peritoneal Sample.

Group	No. of rats	WBC/cmm ×100	% of phago./ 100 WBC	Avg No. bact. in phago./100 WBC	Avg No. phago./cmm	Avg No. bact. in phago./cmm
		2 to 2½ hr group				
Starved	7	7.3	37	475	2839	31846
Fed	18	10.3	19	128	1488	10040
		2¾ to 3¾ hr group				
Starved	14	17.5	38	399	5425	63358
Fed	11	19.4	20	131	3384	24028
		Total of all animals				
Starved	21	14.9	38	423	4563	52855
Fed	29	13.7	19	129	2205	15344

were selected in which the extra as well as the intracellular bacteria were clearly distinguishable. Three hundred or four hundred consecutive W.B.C. were counted on each peritoneal sample and the number containing staphylococci as well as the number of bacteria in each cell was tabulated. The average number of phagocytosing W.B.C. was found by multiplying the percentage of phagocytosing cells by the total number of W.B.C. per cmm of peritoneal fluid. The number of bacteria engulfed was determined by multiplying the number of bacteria phagocytosed per 100 W.B.C. by the total (in hundreds) of W.B.C. per cmm.

Results. The data on the 21 starved rats and on the 29 fed animals is given in Table I. The significant features brought out by these data are the following: 1. The average number of W.B.C. per cmm of peritoneal fluid was essentially the same in both the starved and the fed groups, *i.e.*, 14900 and 13700. 2. The percentage of W.B.C. showing phagocytosis in the starved animals was twice that in the fed rats, *i.e.*, 38% and 19%. 3. The number of bacteria engulfed by the phagocytes of the starved rats per 100 W.B.C. was 3 times that of the fed group, *i.e.*, 423 and 129. Since all the rats were not aspirated exactly 2 hours after inoculation with *Staph. aureus,* it was thought that perhaps the length of time in the peritoneal cavity had some bearing on the results. Consequently, the rats were divided into two groups with regard to the time that elapsed between injection and aspiration. It may be observed (Table II) that although the 2¾-3¾ hour group had approximately twice as many W.B.C. per cmm as the 2-2½ hour animals, nevertheless, the starved groups showed twice the percentage of phagocytes, which engulfed three times the number of bacteria. That this relationship held despite the difference in time of aspiration and W.B.C. count, may be explained by assuming that at the time of the earliest aspiration, (2 hours) the peak outpouring of W.B.C. into the peritoneal cavity had been reached and that phagocytosis too was at the maximum. The subsequent rise in W.B.C. count was due to absorption of the injected liquid with consequent concentration of the cells present.

TABLE III. Distribution of Starved and Fed Rats in Relation to % of Phagocytosing WBC.

% of phagocytosing WBC	No. of starved rats	No. of fed rats
5–14	0	12
15–24	1	10
25–34	7	6
35–44	9	1
45–54	3	0
55–64	1	0
Total	21	29

TABLE IV. Distribution of Starved and Fed Rats in Relation to Avg Number of Bacteria Phagocytosed per 100 WBC.

Avg No. bacteria phagocytosed/100 WBC	No. of starved rats	No. of fed rats
24–99	0	13
100–199	2	12
200–299	4	3
300–399	5	1
400–499	2	0
500–599	3	0
600–699	3	0
700–799	2	0
Total	21	29

A comparison of the distribution of fed and starved rats on the basis of the percentage of phagocytosing W.B.C. is shown in Table III. It may be observed that 75% of the fed animals (22 of 29) fall into the lower range and 95% of the starved group (20 of 21) are found in the higher categories.

Table IV illustrates the difference in distribution between the 2 groups of rats on the basis of the average number of bacteria engulfed per 100 W.B.C. Here again the starved group far outdistanced the fed animals with 90% (19 of 21) rats showing a greater number of bacteria engulfed than 86% (25 of 29) in the fed group.

Discussion. The technics used in this report to determine *in vivo* phagocytic activity are an application of the *in vitro* methods of Wright(10), and Hamburger(10). Wright determined the number of particles engulfed by the leucocyte in a given time. The criterion used by Hamburger was the percentage of leucocytes showing phagocytosis in a definite period. In our experiments both the number of bacteria engulfed per phagocyte per unit of time and the percentage of leucocytes showing phagocytosis was determined. As the starved rats produced twice the percentage of phagocytic leucocytes which engulfed 3 times more bacteria than the phagocytes of fed animals, it can be seen that the results obtained with these *in vivo* technics are in agreement with each other and of a similar order of magnitude.

Inasmuch as there is difference of opinion with regard to the effect of starvation on phagocytic activity(1-3,9,11,12) it is not surprising that the explanations of this phenomenon are many and varied. It has been suggested that changes in temperature(13), vit. C (11,12), pH(14), complement(15), to mention just a few, were the cause of the rise or fall in phagocytic activity. More recently, Kuna and Chambers(9), as a result of their work in tissue cultures, observed marked differences in behavior between the leucocytes of rats starved for 36 hours and those from fed animals. The leucocytes of starved animals were attracted by mono, di and polysaccharides, whereas those of fed rats reacted only to polysaccharides. Starved rats showed a greater number of W.B.C. in motion as well as an increase in the rate of motion. The W.B.C. of starved rats were characterized by a greater degree of adhesiveness to particles in the media. The details of the mechanism whereby starvation induces greater phagocytic activity in rats, remains to be ascertained.

A number of observations are to be found in the literature, which emphasize the enhanced resistance to infection induced by dietary deficiency.

Rats on a B complex deficient diet survived infection with the blood flagellate (*Trypano-*

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some equiperdum) somewhat longer than control animals(6). Ascorbic acid deficiencies in monkeys markedly prolonged the course of an otherwise rapidly fatal infection with *Plasmodium knowlesi*(7). Healthy well nourished fowl were found to be more susceptible to virus chicken sarcoma than thin and ill ones(16). In experimental Murine typhus infection of guinea pig, the starved animals showed considerably less fever and scrotal reaction(8). Undernourished rabbits were less reactive to vaccine virus(17), and vitamin deficient mice failed to show characteristic signs of infection with Western equine encephalitis(18). In children, the original observation of Heine that poliomyelitis seems to attack the better fed children with relatively higher incidence has been confirmed(18).

In the above cited cases it would seem that lack of nutrition has rendered the host a relatively unsuitable culture medium for the parasite. Perhaps the increased phagocytic ability of the starved animals, as demonstrated by the rats in the experiment here reported, is a factor in rendering the host more resistant to invasion by the parasite.

Conclusions. Leucocytes obtained from rats well fed for 4 weeks prior to a 36-hour period of starvation were compared with leucocytes from continuously well fed rats. The starved group showed twice the percentage of phagocytosing W.B.C. which had engulfed 3 times the number of bacteria.

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A Rapid Method for Clinical Total Blood Volume Determination Using Radioactive Iodinated Human Serum Albumin (RIHSA)* (18832)

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This paper reports a rapid method of directly measuring blood volumes using radioactive iodinated human serum albumin as a tracer agent. Human serum albumin is iodinated with I_{131} (RIHSA) in an essentially neutral buffer. Each cc of RIHSA† contains 5 mg of human serum albumin and 2 mg of salt(4). Metabolic studies of RIHSA have been carefully done and reported elsewhere(1,3). By the end of 24 hours after administration, approximately 55-65% is still in the circulation and since it is gradually metabolized, free I_{131} is liberated, and may be taken up by the thyroid. This can be successfully prevented by "blocking" the thyroid with stable iodine in the form of Lugol's solution, 10 drops, 3 times daily for a week, starting at least 24 hours previous to the administration of RIHSA(1). RIHSA was selected as a tracer agent for blood volume determination because it fulfills better than any other substance known at present, the basic criteria as laid down by Keith and Rowntree(2).

Fig. 1 shows the design of the 3-tube Geiger counter constructed for the blood volume determinations. The 3 tubes were held in place on a steel disc by a lucite plate. A thin wall brass tube was soldered in the center to hold

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† Obtained from Abbott Laboratories.

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