which seems sufficient to account for the decreased percentage of saturation. There seemed to be no decrease in oxygen uptake by the normal cells in the presence of malarial plasma; nor were the malarial cells benefitted by the normal plasma excepting the increase in saturations resulting from the rise in CO_2 combining power due to the presence of normal plasma.

Discussion. During the course of malarial infection in the duck an anemia develops which is accompanied by a fall in pH, a marked decrease in CO_2 combining power and a decrease in pK. There is also a shift of the oxygen dissociation curve to the right and a decrease in saturation of the blood *in vitro*. This decrease in saturation bears a linear relationship with the pH and the CO_2 combining power with the exceptional case of the blood taken from ducks on the 6th and 7th day of the infection. During this time young red cells make up from 60 to 80% of the total number of cells present. These cells have been demonstrated to have an abnormal behavior with respect of O_2 capacity, acid base binding power and dissociation curve. It is the authors' opinion that the young cells present on the 6th and 7th days after inoculation are responsible for the abnormal decrease in O_2 saturation with the decreased CO_2 combining power which occurs at this time.

When the effect of temperature is superimposed on the effect of acidosis the percentage of saturation is greatly lowered at the higher oxygen tensions of the equilibrating gas. There seems to be no need for the assumption of a "factor," such as suggested by Wong, which prevents the O_2 saturation of blood other than the decreased CO_2 combining power, the fall in pH, the presence of young cells and increased temperature in the case of *P. lophurae* infected duck blood to account for the decrease in oxygen saturation of the blood *in vitro*.

It should be pointed out here that Cullen *et al.*[7] have investigated arterial oxygen saturation in patients whose body temperatures have been raised in the fever box and have observed decreases in arterial saturation down to 70% of the normal value. It should be noted also that the data obtained by Wong are correlated absolutely with the degree of fever. It is quite possible that the decreased arterial saturation in severe febrile condition has a physiological basis. Various investigators are not in agreement as to the mechanisms involved in the cause of decreased O_2 saturations in febrile conditions but the phenomenon does not seem to be peculiar to the fever of malarial infection.

Summary. Data have been presented which indicate that the decreased arterial saturation, *in vitro*, of the blood of malarial-infected ducks may be caused by the state of acidosis and the presence of great numbers of young red cells during the terminal stage of *P. lophurae* infection. The combined effect of increased temperature and acidosis on the *in vitro* oxygen saturation is sufficient to reduce the percentage of saturation to 70%. No evidence was obtained for the existence of a "factor" in malarial duck blood which would prevent the saturation of the hemoglobin of normal red cells obtained from uninfected birds.

[7] Cullen, S. C., Weir, E. F., and Cook, E., *Anesthesiology*, 1942, **3**, 123.

15405

Effects of Reduced Caloric Intake on Leucocyte Count of the Rat.*

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The purpose of the present communication is to report the effects of reduced caloric intake on the leucocyte count of the rat. It

* The subject matter of this paper has been undertaken in cooperation with the Quartermaster Corps Committee on Food Research.

TABLE I.
Effects of Reduced Caloric Intake on the Body Weight, Hemoglobin, and Total Erythrocytes of the Rat.

Exp. group	No. of animals	Initial body wt	Body wt on 56th day	Avg R.B.C. (in millions)	Avg Hb mg/100 cc
Diet A—6 g	6	171	125	9.0 (8.3-10.4)	13.9
" B—6 "	6	167	128	8.5 (8.2- 9.1)	14.3
" A—9 "	6	174	172	8.6 (7.1- 9.9)	13.8
" B—9 "	6	168	188	9.8 (9.3-10.5)	14.6
" A—*ad lib**	6	165	173	8.8 (8.2- 9.4)	14.3
" B—" "*	6	171	208	9.6 (9.2-11.4)	14.6

* Average food consumption per day for the 8-week period in the *ad lib* series was 10.9 g on diet A and 11.9 g on diet B.

TABLE II.
Leucocyte Count of Rats Fed a Reduced Caloric Intake.

Group	Total leucocyte count		Granulocytes	
	Avg*	Range	%*	Total*
Diet A—6 g	7,400± 540	(5,800- 9,800)	11.7±1.5	866±111
" A—9 "	10,340± 860	(8,000-13,200)	9.2±0.5	952± 52
" A—*ad lib*	13,350± 650	(11,000-15,800)	15.0±2.5	2025±338
" B—6 g	8,100± 600	(6,200- 9,800)	36.8±2.4	2980±194
" B—9 "	11,000± 960	(7,800-14,200)	24.8±1.6	2728±176
" B—*ad lib*	15,600± 820	(12,700-18,000)	20.8±3.0	3245±468
Effects of folic acid therapy				
" A—6 g	21,900±3800	(13,800-33,200)	28.2±4.4	6175±964
" A—*ad lib*	13,900± 770	(12,300-16,100)	16.7±3.1	2321±431

* Including standard error of the mean calculated as follows:

$\sqrt{\frac{\Sigma d^2}{n}} \Big/ \sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of observations.

has been demonstrated by a number of investigators that diets adequate for apparently normal growth and development cease to be adequate when animals are subjected to various toxins, infection, drugs, lactation and other "stress factors." This inadequacy is reflected in the blood picture to the extent that leucopenia and granulocytopenia develop on rations which support a normal blood picture in the absence of these factors.[1-4] In the present experiment we were interested in determining whether the adjustment to reduced caloric intake would constitute a "stress factor" sufficiently pronounced to manifest itself in changes in the leucocyte count.

Procedure and Results. Female rats of the U.S.C. strain were raised to maturity on a stock ration and were selected for the present experiment at approximately 110 days of age and an average body weight of 169 g (range 151 to 200 g). Two basal rations were employed: diet A and diet B.† Diet A was a

[1] Sebrell, W. H., and Daft, F. S., *Pub. Health Rep.*, 1943, **58**, 1542.

[2] Goldsmith, E. D., Gordon, A. S., Finkelstein, G., and Charipper, H. A., *J. Am. Med. Assn.*, 1944, **125**, 847.

[3] Daft, F. S., Kornberg, A., Ashburn, L. L., and Sebrell, W. H., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 154.

[4] Nelson, M. M., Van Nouhuys, F., and Evans, H. M., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 74.

† Diet A consisted of the following: Vitamin Test Casein 30, Sure's Salt Mixture No. 15 4.5, cottonseed oil 10 and sucrose 55.5. To each kg of the above were added 2 mg thiamine HCl, 4 mg riboflavin, 2 mg pyridoxine HCl, 3 mg calcium pantothenate, 1200 mg choline chloride and 5 mg 2-methyl-napthaquinone. Diet B consisted of Vitamin Test Casein 30, Sure's Salt Mixture No. 1 4.5, cottonseed oil 10, sucrose 45.5 and

purified ration containing the B complex factors in synthetic form; diet B was similar in composition but contained yeast in place of the synthetic B factors. Both rations were administered at 3 levels of caloric intake: (1) 6 g daily, (2) 9 g daily and (3) *ad lib*. Animals were kept in individual cages with screen bottoms to prevent access to feces; body weight was recorded weekly; and food intake was determined daily for the *ad lib* series. Thirty-six rats were employed in the present experiment, consisting of 6 groups of 6 animals each.

After 8 weeks of feeding total and differential white cell counts, hemoglobin determinations and total red cell counts were made on the tail blood of all rats. Differential counts were made on smears stained with Wright's stain, 100 cells on each of 2 slides being employed for each analysis. All blood counts were made in duplicate.

Results are tabulated in Tables I and II. No significant differences in total erythrocytes nor hemoglobin levels were observed in any of the rations at any of the levels fed. Significant reductions in total leucocytes however were observed when animals were fed a reduced caloric intake, the highest value for the 6 g per day series being smaller in every instance than the lowest when these rations were fed *ad lib*, values for the 9 g per day series being intermediate between the 2. Differences were similarly noted in per cent and total granulocytes. On Diet B a marked increase in per cent granulocytes was observed in the 6 g per day series so that total granulocytes per cc of blood were equal to levels present in the *ad lib* series; no such increase occurred with reduced caloric intakes on diet A with the result that total granulocytes in this series were reduced by more than 50%. Inasmuch as diets A and B differed only in their content of yeast, these findings suggest that yeast may contain some factor essential for the maintenance of a normal granulocyte count under conditions of reduced caloric intake.

A number of reports have appeared concerning the curative effects of folic acid on granulocytopenia induced by sulfonamide feeding,[1,6] diet alone,[7] deficiency of pantothenic acid[8] or riboflavin,[9] or administration of thiourea and thyroxin.[3] Accordingly folic acid was administered to animals on diet A on both the 6 g per day and *ad lib* levels. Crystalline folic acid‡ was fed for 4 days at a level of 50 γ daily, and on the 5th day total white cell and differential counts were determined. Results are summarized in Table II. A significant increase both in total leucocytes and granulocytes occurred on the 6 g per day level. Total leucocytes ranged from 13,800 to 33,200 per cc after 4 days of folic acid feeding with an average increase of 300%; while granulocytes averaged 28% (range 2208 to 13,944 per cc). No significant differences in total leucocyte or granulocyte count were observed subsequent to folic acid administration in animals fed *ad lib*.

These findings suggest that the low granulocyte counts observed on diet A when fed at reduced caloric intakes may have been due in part to a deficiency of folic acid, while sufficient amounts of this nutrient were present in yeast to meet body requirements for this factor on diet B under similar experimental conditions. Results indicate further that under conditions of the present experiment caloric restriction on diet A interfered either with the synthesis or utilization of folic acid or increased body requirements for this factor to an amount greater than that being synthesized by intestinal microorganisms. Rats on a reduced caloric intake of diet A thus

yeast (Hi-Ribo-24, Anheuser-Busch, Inc.) 10. To each kg of the above were added 1200 mg choline chloride and 5 mg 2-methyl-napthaquinone. Each rat on diet A or B also received a weekly supplement containing 100 U.S.P. units of vitamin A and 10 U.S.P. units of vitamin D.

5 Sure, B., *J. Nutrition*, 1941, **22**, 499.

6 Endicott, K. M., Daft, F. S., and Ott, M., *Arch. Path.*, 1945, **40**, 364.

7 Kornberg, A., Daft, F. S., and Sebrell, W. H., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 46.

8 Daft, F. S., Kornberg, A., Ashburn, L. L., and Sebrell, W. H., *Pub. Health Rep.*, 1945, **60**, 1201.

9 Kornberg, A., Daft, F. S., and Sebrell, W. H., *Arch. Biochem.*, 1945, **8**, 431.

‡ We are indebted to Dr. Stanton M. Hardy of Lederle Laboratories, Pearl River, N. Y., for the folic acid employed in the present experiment.

responded to folic acid administration inasmuch as they were presumably deficient in this factor; whereas animals fed the same ration *ad lib* had sufficient amounts of folic acid available presumably through intestinal synthesis for an optimal effect.

Summary. A significant reduction in total granulocytes was observed in female rats fed a synthetic ration at reduced caloric intake. Such reduction was not observed in animals fed a similar ration *ad lib* or yeast in place of the synthetic B vitamins. The low granulocyte counts were corrected by treatment with folic acid.

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Influence of Streptomycin and Promin* on Proliferation of Tubercle Bacilli in the Tissues of Albino Rat.

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It has been shown[1] that streptomycin and promin when administered together, the one intramuscularly and the other orally, to guinea pigs infected with a human strain of tubercle bacilli, exerted a chemotherapeutic action greater than what might have been anticipated from simple summation of effects. No information is as yet available on the mechanisms that might be involved in this synergistic action. The effect might conceivably be a direct one, on the invading microorganism, or it might be an indirect effect on the defense mechanisms of this normally susceptible host.

In the present work experiments were undertaken to study this phenomenon in the albino rat, normally a highly resistant host to tubercle bacillus infection. It was shown in 1926[2] and amply confirmed since then[3-8] that intraperitoneal or intravenous inoculation of a virulent strain of human or bovine tubercle bacilli in the white rat results within a month in progressive proliferation of monocytes, forming aggregates of epithelioid cells, with some multinucleated, and occasionally a few giant cells. These cellular aggregates are found predominantly in the lungs and to a lesser extent in the liver and spleen. Tubercle bacilli multiply at the same time in great numbers in these cells without producing necrosis, caseation, or other serious tissue damage. The whole process appears to present a simple host-parasite symbiosis having little effect on the general health of the animal until much of the normal lung tissue is replaced by the epithelioid masses which ultimately so impair the normal respiratory functions by reducing the available alveolar space that the animal dies of asphyxia.

In an attempt to learn more about the manner of action of streptomycin and promin it was aimed in the present experiments to ascertain the influence of these drugs, when used individually or in combination, on the proliferation of tubercle bacilli in the tissues of the rat, their viability when planted on culture media, and their pathogenicity when inoculated into guinea pigs.

Experimental. Four groups of rats, 12 each, weighing 100 to 150 g, were inoculated intraperitoneally with 5 mg tubercle bacilli, human

* Sodium p-p'diaminodiphenylsulfone N-N'didextrose sulfonate.

1 Smith, M. I., and McClosky, Wm. T., *Pub. Health Rep.*, 1945, **60**, 1129.

2 Smith, M. I., and Hendrick, E. G., *J. Lab. Clin. Med.*, 1926, **11**, 712.

3 Steinbach, M., *Am. Rev. Tub.*, 1932, **26**, 52.

4 Long, E. R., and Vorwald, A. J., *Nat. Tub. Ass. Tr.*, 1930, **26**, 205.

5 Vorwald, A. J., *Am. Rev. Tub.*, 1933, **27**, 270.

6 Hehre, E., and Freund, J., *Arch. Path.*, 1939, **27**, 289.

7 Oswald, N. C., *Arch. Path.*, 1940, **29**, 678.

8 Wessels, C. C., *Am. Rev. Tub.*, 1941, **43**, 449, 637.