8504 C

Serum Bilirubin Content of the Blood of Rats Consuming a Ration Deficient in Inorganic Salts.*

JAMES M. ORTEN† AND ARTHUR H. SMITH.

From the Department of Physiological Chemistry, Yale University.

That the extreme restriction of the inorganic salts of the diet of the albino rat produces striking changes in the composition of the blood has been repeatedly demonstrated in this laboratory.[1, 2, 3] A marked increase in the number of erythrocytes occurs, accompanied by a progressive decrease in the concentration of hemoglobin. As yet, no satisfactory explanation for the phenomenon has been obtained. The increase in erythrocytes cannot be due to a diminution in blood volume since the plasma and total blood volume remain within normal limits.[2] Nor is there any evidence of an increased rate of erythrocyte formation, inasmuch as no apparent reticulosis occurs during the period of the rapid increase in the number of erythrocytes.[3] It seemed desirable, therefore, to seek an explanation of the polycythemia in another direction, and the possibility that a decreased rate of destruction of erythrocytes leading to a "passive accumulation" of cells might be involved seemed worthy of investigation. As an index to the rate of erythrocyte destruction, the bilirubin content of the serum is usually employed since, given a normal liver and biliary system, there appears to be a direct relation between the two.[4, 5] If the increase in the number of erythrocytes in the blood of the "low-salt" rats is a result of a decreased rate of cell destruction there should be some detectable decrease in the concentration of bilirubin in the serum. If, however, cell destruction proceeds at a normal rate, a concentration of serum bilirubin equal to or perhaps slightly greater than normal is to be expected.

The same procedure was followed in this experiment as in a pre-

*A preliminary report was made before the Division of Biological Chemistry at the New York Meeting of the American Chemical Society, April, 1935.

Aided by a grant from the research funds of the Yale University School of Medicine, 1934.

†National Research Council Fellow in the Department of Physiological Chemistry, Yale University, 1933-34.

1 Smith, A. H., and Schultz, R. V., *Am. J. Physiol.*, 1930, **94**, 107.

2 Swanson, P. P., and Smith, A. H., *J. Biol. Chem.*, 1932, **98**, 479.

3 Orten, J. M., and Smith, A. H., *J. Biol. Chem.*, 1934, **105**, 181.

4 Magath, T. B., and Sheard, C., *Arch. Int. Med.*, 1927, **39**, 214.

5 Barron, E. S. G., *Medicine*, 1931, **10**, 77.

vious one.[5] Male albino rats of the Connecticut Agricultural Experiment Station strain weighing from 40 to 50 gm. at weaning (21 days of age) were placed in individual cages and fed the stock colony ration.[6] Those animals which attained a weight of 120 ± 4 gm. at 35 ± 2 days of age were divided into 2 groups: one, the control group (12 rats), received an "adequate synthetic" ration; the other (10 rats), received a diet extremely poor in inorganic salts. Both diets were fed *ad libitum*. The composition of these diets and of the vitamin supplements have been described in an earlier publication.[3] Quantitative analyses, recently reported,[7] of the amounts of the chief inorganic constituents present in both the "adequate" and "low-salt" diets, serve to emphasize the extreme degree of the limitation of inorganic elements in the latter ration.

At the end of a 10-week experimental period, the serum bilirubin content of the blood of the animals was determined by a modification[8] of the van den Bergh procedure. The body weights, hemoglobin concentrations, and erythrocyte counts of the various rats were likewise recorded at the end of the experimental period. The methods employed for each of the foregoing determinations, including slight modifications of the bilirubin procedure, have been described in detail elsewhere.[6, 9]

The averaged data obtained on the animals of the various groups, together with the actual limits of variation encountered, are given in Table I. The body weight, erythrocyte, and hemoglobin values of the control animals agree well with those of comparable rats consuming either the stock colony ration or the same synthetic ration.[6] The serum bilirubin values are likewise in close agreement with those obtained in stock rats[9] and rats given a similar synthetic ration (unpublished data). As has been observed in previous experiments,[1, 2, 3] the body weights and hemoglobin levels were decidedly less and the erythrocyte counts were greater in the "low-salt" animals than in the controls given the adequate ration. The bilirubin content of the serum of the low-salt rats, however, did not differ significantly from that of the control animals. In several instances, the serum bilirubin values of the low-salt animals tended to be somewhat greater than those of the controls. A slight increase of this type might be expected since there are more erythrocytes per

[6] Orten, J. M., and Smith, A. H., *Am. J. Physiol.*, 1934, **108**, 66.

[7] Smith, A. H., and Smith, P. K., *J. Biol. Chem.*, 1934, **107**, 681.

[8] Gibson, R. G., and Goodrich, G. E., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 413.

[9] Orten, J. M., *Am. J. Physiol.*, 1936, **114**, 414.

unit volume in the blood of the former animals and therefore a greater opportunity exists for the fragmentation of the cells with the subsequent formation of bilirubin.

TABLE I.
Body Weight, Hemoglobin, Erythrocyte, and Serum Bilirubin Values in Control and Low-Salt Rats.*

Group	No. of Rats	Body Weight (gm.) Aver.	Range	Hemoglobin (gm. per 100 cc.) Aver.	Range	Erythrocytes (M. per cmm.) Aver.	Range
Control	12	319	282-386	16.3	15.1-18.7	8.3	7.1- 8.9
Low-Salt	10	167	130-184	13.5	10.4-16.0	10.1	8.8-11.3

	Serum Bilirubin.					
	Total		Blank		Serum Bilirubin (mg. per 100 cc.)	
	Aver.	Range	Aver.	Range	Aver.	Range
Control	1.1	0.6-1.4	0.6	0.3-1.0	0.5	0.2-0.7
Low-Salt	1.5	1.0-2.2	0.8	0.3-1.4	0.7	0.2-1.0

*At the time these data were obtained the animals were 105±2 days of age and had been on experiment for 70 days.

The "total" bilirubin values of 4 additional low-salt rats, not included in the present report, were also determined. "Blank" readings in these cases could not be made because sufficient blood could not be obtained from the animals in question. It is of some importance, however, that the "total" bilirubin values obtained on the limited amount of serum available from these rats were practically identical with those reported in Table I.

Conclusion. The bilirubin content of the serum of rats ingesting a diet deficient in inorganic salts does not differ significantly from that of control animals fed an adequate ration. This finding indicates that "low-salt" polycythemia is not the result of a decrease in the rate of red cell destruction leading to a passive accumulation of erythrocytes.