

sensitive indicator of a deficiency or unbalance of Ca and P. Most of the control rats developed a considerable degree of baldness. This was still more accentuated in Group B, which points to a further damage caused by the change in the Ca/P ratio from 1:11 to 1:13.6. It also appeared markedly in Group K, receiving calcium gluconate without additional P, but was absent in E, where the gluconate was balanced with P. None of the other groups showed loss of hair. By comparing the Ca/P ratio in Group K (1:0.32) with those of the other groups showing baldness (Ca/P, 1:11; 1:13.6) it may be concluded that the disproportion in Ca/P ratio to either extreme has a similar effect. The non-appearance of baldness in Group I may point to some assimilation of hypophosphite P, since otherwise this group would have the

same Ca/P ratio as K. More data will be required to make a positive statement on this point.

Summary. Calcium hypophosphite is well suited to serve as a supplement for dietary calcium. To what extent the hypophosphite phosphorus is utilized by the organism remains uncertain. The ratio Ca/P in the diet may be varied within a wide range without affecting the calcification of the bones, provided the absolute quantities of each constituent are adequate. Extreme disproportion of Ca and P in either direction seems to cause loss of hair, as was observed in rats receiving either very low or very high relative levels of calcium.

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17073. Extra-Renal Removal of Hemoglobin from Circulation in the Rat. 1. Effect of Parenteral Bovine Albumin.

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Hemoglobin in the circulation, whether resulting from hemolysis of erythrocytes *in vivo*, or introduced by parenteral injection, is removed at least in part by excretion through the kidney. Hemoglobin filtered through the glomerulus may appear in the urine, although part is reabsorbed by the proximal tubule cells.¹⁻³ That hemoglobin is removed by extra-renal routes, as well, may be inferred from

the accumulation of hemosiderin in reticulo-endothelial cells, when erythrocytes are destroyed at an abnormally rapid rate.

Parenteral injections of albumin might be anticipated to alter the rate at which hemoglobin is removed from the circulation by extra-renal routes. Since injected albumin is itself removed rapidly from the circulation, a nonspecific increase in protein removal might occur. On the other hand, the mechanism for protein homeostasis might be so saturated that the rate of hemoglobin removal might diminish. Overloading the reabsorption mechanism with bovine albumin is known⁴ to affect the renal tubule cells so as to diminish reabsorption of hemoglobin from tubular urine.

Our interest in the extra-renal removal of hemoglobin from the circulation arose during an investigation into the mechanism of proteinuria. Using hemoglobin as an indicator,

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² Gerard, P., *J. Anat. and Physiol.*, 1936, **70**, 354.

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⁴ Lippman, R. W., *Am. J. Physiol.*, 1948, **154**, 532.

we wanted to know the speed with which it left the circulation by extra-renal routes, so that the rate of urinary excretion could be better understood.

The rate of urinary hemoglobin excretion is the resultant of two opposed rates: filtration at the glomerular membrane, and reabsorption by tubular cells. Neither of these may be measured directly. However, the rate at which hemoglobin is removed from the circulation may be measured by determining total circulating hemoglobin (the product of plasma hemoglobin concentration and plasma volume) at suitable intervals. If the kidneys are surgically removed, then hemoglobin can leave the circulation only by extra-renal routes, so that the total rate of hemoglobin removal, which can be measured, becomes equal to the extra-renal rate.

Methods. In this study 48 male rats were used, each weighing about 150 g, with little variation. One group of rats received two 16 cc intraperitoneal injections of 6% bovine albumin in 0.85% sodium chloride solution at 9:00 A.M. and 4:30 P.M. on the day preceding the experiment. At 9:00 on the day of the experiment they received another such injection, and 5½ hours later they were subjected to bilateral nephrectomy under light ether anesthesia. The operative procedure was performed rapidly through 2 flank incisions. The kidney was delivered through the incision, a silk ligature was placed around the renal pedicle, and the pedicle severed. The wound was closed with Michel clips. Very little blood was lost. Previous experience has shown that rats endure this procedure with no immediate evidence of ill effects. Within a few minutes the rats are awake again, and show normal behavior.

Immediately after the completion of nephrectomy, and while still anesthetized, the rats received an intravenous injection of 141.0 mg of hemoglobin,⁵ in a volume of 1.7 cc. Groups of rats were killed at intervals after injection by exsanguination from the abdominal aorta. By previous experience we know

that no hemolysis occurs under the conditions of collection. Determinations were made of the serum hemoglobin concentration, total serum protein concentration, and Wintrobe hematocrit value. The serum protein concentration other than hemoglobin was calculated by difference. Another group of rats was treated in exactly the same manner, but, in place of the injections of bovine albumin, received intraperitoneal injections of 0.85% sodium chloride solution in equivalent volume, without the added protein.

Total hemoglobin concentration was measured by the method of Evelyn and Malloy.⁶ Plasma volumes were calculated from the serum hemoglobin concentration and the known amount of hemoglobin injected, in the groups killed at 2 minutes. The small error introduced by the short mixing time has been neglected, as it is of smaller magnitude than other causes of error in such measurements.⁷ If we assume that the circulating erythrocyte mass remains constant over short periods of time, the hematocrit value may be taken as an index of plasma volume changes at the 30 and 60 minute intervals, at which times plasma volumes were calculated from the mean initial plasma volume.

Total protein determinations were made by a modification of the Kingsley biuret method.⁸

Results. Hemoglobin was removed from the circulation with relatively great rapidity. In 60 minutes, approximately 1/3 of the quantity injected intravenously had disappeared from the circulation (Table I). The injections of bovine albumin did not appear to affect significantly the rate at which hemoglobin left the circulation.

Discussion. The speed at which physiological functions involving proteins occur is not generally appreciated. Thus, only recently, Stoerk, John, and Eisen⁹ reported that the half-life of serum protein in the rat is about

⁶ Evelyn, K. A., and Malloy, H. T., *J. Biol. Chem.*, 1938, **126**, 655.

⁷ Lippman, R. W., *Proc. Soc. Exp. Biol. and Med.*, 1947, **66**, 188.

⁸ Kingsley, G. R., *J. Biol. Chem.*, 1939, **131**, 197.

⁹ Stoerk, H. C., John, H. M., and Eisen, H. N., *Proc. Soc. Exp. Biol. and Med.*, 1947, **66**, 25.

⁵ Pennell, R. B., Smith, W. E., and Werkheiser, W. C., *Proc. Soc. Exp. Biol. and Med.*, 1947, **65**, 295.

TABLE I.
Rate of Removal of Hemoglobin from the Circulation of Nephrectomized Rats.

Min. after inj.	Final serum conc. of Hb, mg/100 cc	Wintr. hematocrit	Plasma vol., cc	Total circ. Hb, mg	Hb removed mg	Rate of Hb removal mg/min./100 g BW	Serum Prot.-Hb conc., %	Total circ. Prot.-Hb, mg
Sodium Chloride injections								
2	1492	35.3	9.5	141	0		3.54	336
30	1398	37.9	8.9	124	17	0.41 ± .19	4.53	400
60	1078	38.5	8.8	95	46	0.50 ± .06		
Bovine albumin injections								
2	1403	33.2	10.0	141	0		6.40	640
30	1179	33.5	10.1	119	22	0.49 ± .16	6.56	650
60	1135	37.8	8.8	100	41	0.44 ± .08	7.54	664

36 hours. Control rats in this series had a total circulating protein value (other than hemoglobin) of approximately 350 mg. As the result of injections which introduced approximately 2500 mg of albumin into the circulation during a period of 30 hours (about half of the last injection remained unabsorbed at the time of autopsy), the total circulating protein rose to about 650 mg, nearly twice its previous level. Consequently a minimum of 2200 mg entered and left the circulation during the period of observation. In spite of this, no appreciable change in the rate of hemoglobin removal was detected.

Comparison of the extra-renal rate of removal with the renal excretion of hemoglobin at comparable serum concentrations indicated that, in the rats injected with sodium chloride solution, the renal excretion rate⁴ was less than twice the extra-renal removal rate, while in

the rats injected with bovine albumin the renal excretion rate was relatively somewhat greater. The difference in ratio depended upon the effect of bovine albumin injections, increasing the rate of renal excretion.

Summary. 1. During the hour following intravenous injection of hemoglobin in the rat, about 1/3 of the quantity injected left the circulation by extra-renal routes.

2. Intraperitoneal injection of bovine albumin, with the associated rapid passage of albumin from the circulation, did not affect the rate at which hemoglobin left the circulation.

3. The rate at which hemoglobin leaves the circulation by extra-renal routes is of comparable magnitude to, though less than, the rate of renal excretion.

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17074. Experimental Toxoplasmosis. II. Effect of Sulfadiazine and Antiserum on Congenital Toxoplasmosis in Mice.

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Although toxoplasmosis has been known as an infectious disease of man for over a decade, therapy of the acute condition has remained unsatisfactory. Sabin and Warren,^{1,2} testing

a wide variety of antimicrobial agents, reported that only the sulfonamides have any beneficial effects on the course of the disease in laboratory infected mice and rabbits. Weinman and Berne³ extended these observa-

¹ Sabin, A. B., and Warren, J., *Proc. Soc. Exp. Biol. and Med.*, 1942, 51, 15.

² Sabin, A. B., and Warren, J., *Proc. Soc. Exp. Biol. and Med.*, 1942, 51, 19.

³ Weinman, D., and Berne, R., *J.A.M.A.*, 1944, 124, 6.