case the peak is reached at the polychromatic stage (94%), and higher activity is revealed in the basophilic erythroblasts. These data should be considered in relation to relatively earlier sampling (3 hours), the higher radioiron dosage (100 μc), and the stimulation of bone marrow by previous bleeding.

The present investigation then shows that in the rat and with the doses used, there is a definite turnover of radioiron even by the earliest erythroid elements, namely pro-erythroblasts and basophilic erythroblasts. This was not known before, and it can be assumed as indirect evidence of the earliest stages of hemoglobin formation in the marrow.

Summary. The presence of iron-59 in the earliest stages of the erythroid series of the rat bone marrow was demonstrated by means of stripping film autoradiography after intraperitoneal injection of labeled ferric chlorides. Pro-erythroblasts and basophilic erythroblasts take up a detectable amount of radioiron after 3 to 6 hours. The maximum uptake, however, occurs in the polychromatic and orthochromatic stages.

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1. Thorell, B., *Studies on the formation of cellular substances during blood cell production,* H. Kimpton, London, 1947.

2. London, I. M., Shemin, D., and Rittenberg, D., *J. Biol. Chem.,* 1948, v173, 797.

3. Altman, K. J., Casarett, G. W., Masters, R. E., Noonan, T. R., and Salmon, K., *ibid.,* 1948, v176, 319.

4. Boyd, G. C., Casarett, G. W., Altman, K. J., Noonan, T. R., and Salomon, K., *Science,* 1948, v108, 529.

5. Elmlinger, P. J., Huff, R. L., Tobias, C. A., and Lawrence, J. H., *Acta Haematol.,* 1953, v9, 73.

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A Metabolic Study of Hydrocortisone in Rats. (20782)

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It has been demonstrated that slices of rat liver, kidney and spleen all effectively inactivate cortisone(1). Blood 17-hydroxycorticosteroid levels that have been elevated by administration of a single dose of cortisone or hydrocortisone to humans or experimental animals quickly decline to pretreatment values (2,3). Therefore, it seemed desirable to investigate the participation of the liver, kidney and spleen in the removal of exogenous 17-hydroxycorticosteroids from the blood of the rat *in vivo*.

Materials and methods. Male Sprague-Dawley rats weighing from 190 to 270 g were used without fasting. The removal of organs was carried out through a midline abdominal incision under light ether anesthesia. "Physiological hepatectomy" was performed by a modification of the method of Russell(4). The portal vein, hepatic artery and common bile duct were ligated after securing the gut from pylorus to descending colon in a mass ligature. This operation will be referred to henceforth as hepatectomy. Nephrectomy and splenectomy were accomplished after ligating and cutting the major blood vessels to these organs. The abdominal incision was closed with sutures. Control animals were subjected to laparotomy and a handling of the viscera. It was estimated that hepatectomy removed about 3 ml of blood from the circulation. To evaluate the effect of reduced blood volume upon 17-hydroxycorticosteroid levels, 3 ml of blood were withdrawn from the inferior vena cava in a group of rats through an abdominal incision, and absorbable gelatin (Gelfoam) was applied for hemostasis. Gelfoam was also placed in the peritoneal cavity of control animals for this group. Immediately following each operation, 0.5 ml of a hydrocortisone solution was injected into a tail vein. Solutions were prepared in concentrations of

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TABLE I. Plasma Levels of Hydrocortisone after Intravenous Injection of a Given Dose Following Hepatectomy, Nephrectomy, Splenectomy, or the Removal of 3 ml of Blood.

	Operation	No. of rats	Avg body wt, g	Dose, mg	Time after inj., min.	Plasma hydrocortisone conc. (μg/10 ml)	S.E.	p
A.	C*	6	212	.4	2	21	2	
	H	7	223	"	"	34	3	<.01
	N	6	216	"	"	16	2	<.2
	S	6	226	"	"	17	2	<.2
B.	C	7	206	"	10	4	1	
	H	7	200	"	"	22	2	<.001
	C	7	197	"	30	1	1	
	H	7	195	"	"	26	2	<.001
C.	C	7	191	"	"	3	1	
	B	7	195	"	"	2	1	>.5
	C	7	198	.0	"	0.7	0.2	
	H	6	206	"	"	0.7	0.5	
D.	C	6	269	1.6	10	24	1	
	N	6	272	"	"	28	4	>.5
	H	7	266	"	"	71	2	<.001
E.	C	6	233	"	"	50	4	
	S	7	242	"	"	48	3	<.5

* C = Control; H = Hepatectomy; N = Nephrectomy; S = Splenectomy; B = Blood removal.

0.8 or 3.2 mg per ml by dissolving a saline suspension of hydrocortisone† in 8 or 20% ethanol, respectively. A sample of aortic blood was drawn 2, 10 or 30 minutes later and analyzed for 17-hydroxycorticosteroids by the method of Nelson and Samuels(5).

Results. Controls. Considerable day to day variation was encountered in the plasma 17-hydroxycorticosteroid level of control animals injected with hydrocortisone under similar conditions. For this reason, the experiments in each section of Table I were all performed on the same day, using a single solution of hydrocortisone, and littermates for controls.

Preliminary studies in rats weighing an average of 230 g disclosed that, half a minute after the intravenous injection of 0.4 mg of hydrocortisone, the blood level rose from an average concentration of less than 1 μg per 10 ml to approximately 87 μg per 10 ml of plasma. At one minute after injection, the blood level had again fallen precipitously to about 37 μg per 10 ml of plasma, and thereafter declined at a much slower, fairly steady rate.

† Kindly supplied by Dr. Elmer Alpert of Merck and Co.

Hepatectomy. Two minutes after injection of 0.4 mg of hydrocortisone into hepatectomized animals the plasma level was found to be significantly higher than in the controls (Table IA). Ten and 30 minutes after injection the concentration fell to very low values in control animals, but remained high in hepatectomized animals (Table IB). In fact, there was no significant difference between the 10-minute and 30-minute determinations in the hepatectomized animals. The animals appeared to be weakened by hepatectomy, but were not in circulatory collapse. Simple removal of 3 ml of blood, approximating the amount that might be cut off from the circulation by the operation, was not found to interfere with the rapid fall in plasma hydrocortisone concentration following injection (Table IC). In the absence of injected hydrocortisone, there was no detectable accumulation of endogenous 17-hydroxycorticosteroids in hepatectomized rats within 30 minutes (Table IC).

Splenectomy and nephrectomy. Removal of the spleen or kidneys just prior to injection of 0.4 mg of hydrocortisone did not prevent the rapid fall in plasma concentration in 2 minutes (Table IA). Moreover, the concentration of hydrocortisone was not significantly

different from that of control animals 10 minutes after the injection of 1.6 mg in nephrectomized (Table ID) or splenectomized (Table IE) animals; this larger dose was used in order to keep blood levels in a readily detected range at the longer time interval. Hepatectomized animals given the 1.6 mg dose for comparison showed much higher levels than the controls.

Discussion. Hepatectomized rats showed a fall in plasma hydrocortisone level within the first 10 minutes but not in the next 20 minutes. This suggests that the initial decline may have been produced by a distribution of the hormone throughout the body tissues, after which there was negligible removal of the compound from the circulation.

The stress of physiological hepatectomy could conceivably accelerate endogenous secretion of adrenal cortical steroids to a degree that this could in itself elevate blood levels. However, no significant rise in plasma 17-hydroxycorticosteroids was detected in hepatectomized animals that did not receive hydrocortisone.

Hepatectomy involved the simultaneous exclusion of the gastrointestinal tract and spleen as well from the circulation. However, splenectomy alone was without effect on the blood hydrocortisone level. The known high metabolic activity of the liver in degrading and excreting steroids(6,7) makes it highly likely that it, rather than the intestinal tract, plays the major role in producing the effects observed. The data do not distinguish between biliary excretion and transformations of hydrocortisone which would make it no longer detectable by the analytical procedure used.

The results with nephrectomized animals corroborate the findings of others(6,8) that very little exogenous 17-hydroxycorticosteroid is removed from the circulation by the kidneys.

Though the influence of the liver might be relatively less at physiological levels of hydrocortisone, the data presented indicate that this organ has a definitely greater capacity than other tissues in the metabolism of administered corticoid.

Summary. 1. Employing the analytical procedure of Nelson and Samuels for plasma 17-hydroxycorticosteroids, it has been shown that the intravenous injection of hydrocortisone in rats produces high concentrations of hormone, followed by a swift return to very low levels. 2. The rapid disappearance of exogenous hydrocortisone from the circulation is prevented by hepatectomy, but nephrectomy and splenectomy exert no appreciable effect.

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1. Louchart, J., and Jailer, J. W., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 393.

2. Nelson, D. H., Sandberg, A. A., Palmer, J. G., and Tyler, F. H., *J. Clin. Invest.*, 1952, v31, 843.

3. Samuels, L. T., and West, C. D., *Vitamins and Hormones*, New York, Academic Press, 1952, v10, 251.

4. Russell, J. A., *Am. J. Physiol.*, 1942, v136, 95.

5. Nelson, D. H., and Samuels, L. T., *J. Clin. Endocrinol. and Metab.*, 1952, v12, 519.

6. Nelson, D. H., *Adrenal Cortex*, E. P. Ralli, Editor, Trans. Third Conf., New York; Josiah Macy, Jr. Foundation, 1952, p89.

7. Hechter, O., Solomon, M. M., and Caspi, E., *Endocrinology*, 1953, v53, 202.

8. Burstein, S., Savard, K., and Dorfman, R. I., *ibid.*, 1953, v53, 88.

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