Increase in Circulating Red Cell Volume of Normal and Hypophysectomized Rats after Treatment with ACTH.* (18601)

JOSEPH F. GARCIA, DONALD C. VAN DYKE, REX L. HUFF, PAUL J. ELMLINGER,† AND JOHN M. ODA. (Introduced by J. H. Lawrence)

From the Division of Medical Physics, and the Institute of Experimental Biology, University of California, Berkeley.

The presence of low hemoglobin concentrations in hypophysectomized animals was first noted in hypophysectomized dogs by Aschner (1). That this low concentration actually reflects a decrease in red blood cell volume per unit body weight has been shown by the P^{32} tagged cell method(2). The repair of this anemia has been the subject of many papers(4,8,11,12). Some authors(8) conclude that the alterations in red cell concentration following hypophysectomy are due to "general metabolic changes" rather than the absence of a specific hemopoietic hormone. On the other hand, a specific hemopoietic hormone has been postulated as existing in the hypophysis(5,6,9).

The general subjects of red blood cell production, destruction and volume in relation to endocrine secretions have received little attention because of lack of methods for their determination. However, concentrations of red cells and hemoglobin in large vessels and changes in these concentrations subsequent to or concomitantly with clinical and experimental endocrine changes have been studied extensively. The points of immediate interest are total red cell volumes, marrow volumes, and in particular, the manner in which these volumes change. This report concerns only the changes in total circulating red cell volume per unit mass of tissue in normal rats and hypophysectomized rats with and without treatment with ACTH.

Methods. Ninety-day-old female Long-Evans rats were studied in 4 groups of 10 animals each: Group (1) hypophysectomized control, group (2) hypophysectomized receiving 1 mg ACTH daily, group (3) normal control, group (4) normal receiving 1 mg ACTH daily. The animals were hypophysectomized by the parapharyngeal approach on the first day of the experiment. The ACTH was given in ½ mg intraperitoneal doses twice daily for 34 days to half the members and for 116 days to the rest of the members of the groups receiving the hormone. The total red cell volumes were determined with P^{32} tagged cells using the method of Hevesy (7) as modified by Berlin *et al.*(3). Each animal was anesthetized with ether and the tagged cells were injected into the saphenous vein and allowed to mix for 5 minutes, at which time the abdomen was opened and as much blood as possible was drawn from the inferior vena cava into a heparinized syringe. The P^{32} was assayed on an aliquot of this blood with the aid of an end window Geiger-Muller counter. Hematocrits were done with calibrated capillary tubing. The pituitary

* This work was supported by grants from the Atomic Energy Commission and the U. S. Public Health Service, Grant C-1098.

† Post Doctorate Research Fellow of the Atomic Energy Commission.

1. Aschner, B., *Pflüg. Arch. f. d. ges. Physiol.*, 1912, v146, 1.

2. Berlin, N. I., Van Dyke, D. C., Siri, W. E., and Williams, C., *Endocrinology*, 1950, v47, 429.

3. Berlin, N. I., Huff, R. L., Van Dyke, D. C., and Hennessy, T. G., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 176.

4. Crafts, R. C., *Am. J. Anat.*, 1946, v79, 267.

5. Flaks, J., Himmel, I., and Zlotnik, A., *Presse Med.*, 1938, v46, 1506.

6. Flaks, J., Himmel, I., and Zlotnik, A., *Presse Med.*, 1937, v45, 1261.

7. Hevesy, G., and Zerahn, K., *Acta physiol. Scand.*, 1942, v4, 376.

8. Meyer, O. O., Thewlis, E. W., and Rusch, H. P., *Endocrinology*, 1940, v27, 932.

9. Moehlig, R. C., and Bates, G. S., *Arch. Int. Med.*, 1933, v51, 207.

10. Sundstroem, E. S., and Michaels, G., *Memoirs of the University of California*, 1942, v12, 42.

11. Vollmer, E. P., Gordon, A. S., and Charipper, H. A., *Endocrinology*, 1942, v31, 619.

12. Vollmer, E. P., and Gordon, A. S., *Endocrinology*, 1941, v29, 828.

TABLE I. Total Circulating Red Cell Volume in Normal and Hypophysectomized Rats Treated with Adrenocorticotropic Hormone.

	Exp. terminated after 34 days				Experiment terminated after 116 days				
	Rat No.	Terminal body wt, g	Hct., %	Red cell vol., cc/100 g body wt	Rat No.	Terminal body wt, g	Adrenal wt (2 adrenals) mg	Hct., %	Red cell vol., cc/100 g body wt
Hypophysectomized controls	1	182	41.4	1.71	21	died	—	—	—
	2	155	37.5	1.44	22	died	—	—	—
	3	148	35.0	1.44	23	152	10	29.5	1.64
	4	178	27.2	1.31	24	153	13	28.9	1.44
	5	153	32.8	1.64	25	died	—	—	—
	Avg		34.8	1.51	Avg		11.5	29.2	1.54
Hypophysectomized receiving .5 mg ACTH twice daily	6	190	39.0	1.87	26	165	62	46.9	2.37
	7	184	38.6	2.21	27	128	56	43.6	2.54
	8	132	35.5	2.38	28	164	56	41.6	2.41
	9	146	39.2	2.15	29	died	—	—	—
	10	153	40.3	2.34	30	173	54	39.4	2.21
	Avg		38.5	2.19	Avg		57	42.9	2.38
Normal controls	11	235	46.3	2.42	31	290	52	47.6	2.25
	12	222	45.7	2.80	32	277	42	48.3	2.35
	13	210	44.2	2.76	33	242	62	49.0	2.66
	14	187	43.6	2.73	34	247	61	45.9	2.33
	15	204	40.7	2.50	35	253	67	45.5	2.32
	Avg		44.1	2.62	Avg		57	47.3	2.38
Normal rats receiving .5 mg ACTH twice daily	16	236	47.0	2.58	36	225	137	55.2	3.27
	17	225	40.0	2.43	37	Accidental blood loss			
	18	224	50.3	3.01	38	255	114	49.2	2.93
	19	213	45.3	2.95	39	278	106	55.2	3.24
	20	190	45.3	2.35	40	233	106	48.5	2.96
	Avg		45.6	2.66	Avg		116	52.0	3.10

site was examined under a binocular dissecting microscope to check the completeness of hypophysectomy.

Results. The data are presented in Table I. The red cell volumes are given in terms of cubic centimeters of packed red cells per hundred grams of body weight. In both of the hypophysectomized control groups the total red cell volumes were considerably below the values obtained for the normal animals, and in agreement with the total red cell volumes in hypophysectomized rats as shown by Berlin *et al.*(2). However, the group of hypophysectomized animals receiving 1 mg ACTH daily for 34 days showed a significant increase in total red cell volume as compared to the untreated hypophysectomized controls. The average total red cell volume for these ACTH treated hypophysectomized animals was 2.19 cc/100 g body weight as compared to 1.51 cc/100 g body weight for the untreated hypophysectomized animals sacrificed at this same time period. The normal animals receiving 1 mg ACTH daily for 34 days showed no significant increase in their total red cell volume when compared with their controls. The average red cell volume for the ACTH treated normals was 2.66 cc/100 g body weight and for the untreated normal controls was 2.62 cc/100 g body weight.

The total red cell volumes in hypophysectomized animals after 116 days of treatment with 1 mg ACTH daily increased greatly (2.38 cc/100 g body weight) over the value for total red cell volume in hypophysectomized controls 116 days after hypophysectomy (1.54 cc/100 g body weight), and in fact were brought to within the range of the total red cell volumes (2.38 cc/100 g body weight) in normal animals of the same age.

Although no significant change in total red cell volume was seen in normal animals after 34 days of treatment with ACTH, there was a significant polycythemia produced after 116 days of ACTH treatment. An average total

red cell volume of 3.10 cc/100 g body weight was produced in these animals as compared to the average of 2.38 cc/100 g body weight for normal controls at this same age.

The average total red cell volumes for the 2 groups of normal controls differ slightly; however, when significance tests were applied, this difference was shown not to be significant.

The adrenal weights were measured in the animals autopsied at 206 days of age. As can be seen from the table, these weights are directly related to the red cell volume data. The untreated hypophysectomized rats had an average adrenal weight of 11.5 mg (2 adrenals). In the hypophysectomized rats treated with ACTH the adrenal weights gave the same average (57 mg) as did the normal controls. The normal rats treated with ACTH showed an increase in adrenal weight with an average of 116 mg.

These data are interesting in view of the fact that Sundstroem(10) found, along with the well known polycythemia, greatly increased adrenal weights present in rats placed in an atmosphere of reduced oxygen tension.

Conclusions. 1. Administration of ACTH prevents the decrease in the total circulating red cell volume which is normally found in hypophysectomized rats. 2. Administration of ACTH for 116 days to normal rats elevates the total circulating red cell volume to approximately 1.3 times that of the normal untreated controls.

ACTH was supplied through the courtesy of Professor Choh Hao Li, Department of Biochemistry, University of California, Berkeley.

Received February 26, 1951. P.S.E.B.M., 1951, v76.

P^{32} Localization in the Intestine. (18602)

MARGARET W. HOLT AND SHIELDS WARREN.

From the Cancer Research Institute, New England Deaconess Hospital and Laboratory of Pathology, Harvard Cancer Commission, Boston, Mass.

Radioautographic and histochemical observations have been made on sections of frozen-dried rat and mouse intestine containing P^{32}. The tissue sections were prepared and mounted and radioautographs were made using procedures reported previously(1,2). These procedures are designed toward qualitative and quantitative preservation and retention at the original sites of tissue constituents. The radioautographs of rat and mouse duodenum and small intestine have shown a more striking concentration and localization pattern with P^{32} than has been observed for other soft tissues. This localization occurs in the glands of the mucosa with the greater concentration at the more basal groups of cells (Fig. 1).

Studies of the solubility of localized P^{32} in frozen-dried sections of rat intestine have been reported(3). The localized P^{32} exhibits the same general solubilities as the background P^{32} in other regions of the tissue, indicating a similar chemical form. The P^{32} is not in phospholipid form as it does not dissolve in organic solvents; other solubility tests have indicated the presence of free phosphate ions and at least a partial protein linkage. The use of formalin or Bouin's solution as a fixative results in a radioautograph showing the same general type of localization pattern, but of diminished intensity as compared to the results with frozen dried tissues. This would be expected on the basis of solubility data.

Observations made at varying time intervals from isotope administration to killing the animal have shown that the typical localization pattern is present in the intestine at 30

1. Holt, M. W., Cowing, R. F., and Warren, S., *Science*, 1949, v110, 328.

2. Holt, M. W., and Warren, S., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 545.

3. Holt, M. W., and Warren, S., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 4,