Increase in Circulating Red Cell Volume after Oral Administration of Pituitary Anterior Lobe

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Flaks, Himmel and Zlotnik[1, 2] reported that the oral administration of fresh anterior lobe of beef pituitary or extracts of anterior lobe resulted in a significant increase in the erythrocyte count in both normal and hypophysectomized rats. Although it is not generally accepted that pituitary hormones exert their specific effects when administered orally, it has recently become evident that some of the pituitary hormones will withstand digestive enzymes.[3-6] This, as well as the fact that no laboratory appears to have reinvestigated the effects of oral administration employing modern and sensitive assay methods, seems to justify a reinvestigation of the problem. Meyer et al.[7] have reported that they were unable to obtain the results reported by Flaks, et al. with orally administered pituitary, but have not described in detail how the experiments were repeated. Because the reports of Flaks, et al. are still influencing the discussions of present investigators, the original experiments have been repeated, using not only beef but also sheep pituitaries and following as closely as possible the procedure described by them. Reticulocyte counts, hemoglobin concentrations, bone marrow smears and red blood cell counts were made and, in addition, determinations of the circulating red cell volumes were made rather than relying only on peripheral erythrocyte counts. Erythrocyte counts are subject to error when changes occur in plasma volume, whereas determinations of circulating red cell volume are not subject to this error. The pituitary target organs were also examined at autopsy and in section to determine whether there was any effect of orally administered pituitary hormones on the adrenal, thyroid or gonad.

Materials and Methods

Normal as well as hypophysectomized rats were used in these experiments. The normal animals were male rats 47 days of age at the onset of the experiments. Female rats of the Long-Evans strain were hypophysectomized at 28 days of age and maintained for 40 days to allow selection of completely hypophysectomized animals and to allow the development of the characteristic anemia.[8] Fresh beef and sheep pituitaries were collected daily for each day's feeding and were kept on ice for a few hours (3 to 5) until they were dissected and fed. Groups of rats were fed either supplements of fresh beef or sheep anterior pituitary, beef pituitary extract* or fresh beef liver and two groups received no supplement. The supple-

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* Beef pituitary extracts were prepared as described by Flaks, Himmel and Zlotnik. Dissected anterior lobes of beef were ground with solid CO_2. The powdered glands were then extracted with 0.001 N HCl, stirred for 2 hours and centrifuged. The precipitate was discarded. An equal volume of 5 per cent sulfosalicylic acid was added to the supernatant. This mixture was allowed to stand at 3 C. overnight, then was centrifuged and the precipi-

ments were fed for different periods of time and in different daily amounts. Small amounts of beef pituitary were given for long periods of time (2.5 to 3.0 Gm. per day for 40 days) in order to repeat exactly the work of Flaks et al. Large amounts were given for a shorter period of time (5 Gm. of beef pituitaries for 19 or 29 days). Another group was fed fresh anterior sheep pituitaries (10 Gm. per day) daily for 19 days. All supplementary gland feeding was given in addition to an adequate diet.* Food was removed from the rats each afternoon for 2 hours; they were then offered the supplement during the night. In the morning the regular diet was given again. After a few days of training the supplements were accepted.

The total circulating red cell volume was determined by the labeled cell dilution method.[9] The labeled cells were obtained from a donor of the same strain previously injected with Fe^{59}. The experimental animals were injected with 0.1 ml. of donor blood which contained 0.03 microcuries Fe^{59} in the erythrocytes. After allowing 6 minutes for the blood to mix, the abdomen was opened and a sample of blood was drawn from the vena cava into a heparinized syringe. A known volume of this blood was pipeted into a vial and its activity counted directly in a scintillation counter.[10] The total blood volume was calculated from the fraction of the injected activity found in the sample of known volume. The hematocrits were determined in Wintrobe tubes. The total blood volume thus calculated, multiplied by

TABLE 1.—*Hematologic Data on Normal Rats*

Group Fed	Body Weight	Hematocrit	Hemoglobin	Red Blood Cell Volume/100 Gm. Body Weight	Reticulocytes	Nucleated Red Blood Cells in Marrow	Red Blood Cells × 10^6
	Gm.	%	*Gm./100 ml.*	*ml.*	%	%	*per cu. mm.*
Fresh pituitary (Group I)	294 ± 12	45.3 ± 2.9	12.2 ± 1.8	2.32 ± 0.07	4 ± 1	40 ± 10	
Pituitary extract (Group II)	284 ± 15	44.7 ± 0.5	13.4 ± 0.7	2.27 ± 0.05	3 ± 1	37 ± 11	
Liver (Group III)	276 ± 24	45.1 ± 3.2	13.3 ± 0.7	2.32 ± 0.30	5 ± 3	40 ± 12	
Maintenance diet	297 ± 29	45.7 ± 1.9	13.5 ± 1.1	2.34 ± 0.11			6.93 ± 0.25

the hematocrit gave the total circulating red cell volume. The values for the total circulating red cell volume were then divided by the values for the body weights of the animal and the results are presented in tables 1, 2 and 3 in terms of ml. of red blood cells per 100 Gm. body weight. The hemoglobin concentration was determined by the method of Turner.[11] Reticulocyte counts were made from unheparinized blood from the tail vein. The percentage of reticulocytes was based on a count of 500 cells. Red blood cell counts were made with the aid of a Levy-Hausser counting chamber. Marrow smears were made from the shaft of the femur and the percentage of nucleated red cells was obtained by counting 500 cells. The completeness of hypophysectomy was determined by examination at autopsy of the pituitary site and in untreated animals by histological examination of the target organs.

tate was discarded. The supernatant was extracted three times with acid ether (10 ml. of 1.0 N HCl per liter of ether) and the ether fraction was discarded. The resulting solution (pH 1 to 2) was adjusted to pH 7.0 with 5 N NaOH, brought to boiling three times and then dried by lyophilization. The yield was 0.2 Gm. from each gram of fresh glands.

* Ground whole wheat 67.5 per cent, casein 15.0 per cent, skim milk powder 7.5 per cent, sodium chloride iodized 0.75 per cent, calcium carbonate 1.5 per cent, melted fat 6.75 per cent, fish oil (vitamin A and D concentrate) 1.0 per cent.

Experimental Groups

I. Five normal rats were given fresh beef anterior lobe daily for 35 days. They were given 2.5 Gm. daily for the first 10 days, 3.0 Gm. daily for the next 15 days and 4.0 Gm. daily for the last 10 days. This made a total of 110 Gm. fresh gland per rat.

II. Five normal rats were given the anterior lobe extract for 35 days. The amount of extract given was equivalent to the amount of fresh gland given in group I.

III. Six normal rats were fed fresh beef liver for 35 days. The weight of liver given was the same as that of fresh pituitary fed in group I.

IV. Seven hypophysectomized rats were fed fresh beef anterior lobe daily for 40 days in amounts of 2.5 to 3.0 Gm. The total amount eaten was from 100 to 120 Gm. per rat.

V. Six hypophysectomized rats were fed fresh beef anterior lobe for 19 days in amounts of 4.0 to 5.5 Gm. daily. The total amount eaten was from 76 to 105 Gm. per rat.

VI. Five hypophysectomized rats were fed fresh beef anterior lobe for 29 days in amounts of 4.5 to 6.5 Gm. daily. The total amount eaten was from 130 to 188 Gm. per rat.

TABLE 2.—*Hematologic Data on Hypophysectomized Rats Fed Beef Pituitary, Beef Liver, or Maintenance Diet*

Group Fed	Body Weight	Hematocrit	Hemoglobin	Red Blood Cell Volume/100 Gm. Body Weight	Nucleated Red Blood Cells in Marrow	Red Blood Cells × 10^6
	Gm.	%	*Gm./100 ml.*	*ml.*	%	*per cu. mm.*
Fresh beef pituitary 75–110 Gm. in 40 days (Group IV)	85 ± 5	36.4 ± 0.6	11.4 ± 0.2	1.80 ± 0.05	45 ± 8	
Fresh beef pituitary 78–110 Gm. in 19 days (Group V)	76 ± 3	36.6 ± 1.1	11.4 ± 0.2	1.71 ± 0.13		6.18 ± 0.31
Fresh beef pituitary 130–180 Gm. in 29 days (Group VI)	78 ± 6	39.0 ± 1.3	11.8 ± 1.0	1.91 ± 0.21		6.32 ± 0.59
Beef pituitary extract (Group VII)	83 ± 3	34.5 ± 2.4	10.6 ± 0.8	1.67 ± 0.07	53 ± 8	
Beef liver 80–110 Gm. in 40 days (Group VIII)	85 ± 7	35.8 ± 2.7	11.0 ± 1.1	1.77 ± 0.15	47 ± 8	
Maintenance diet (Group X)	83 ± 10	36.4 ± 2.1	10.9 ± 1.0	1.68 ± 0.10		5.61 ± 0.49

VII. Seven hypophysectomized rats were given the beef anterior lobe extract for 40 days. The amount of extract given was equivalent to the amount of fresh gland given in group IV.

VIII. Seven hypophysectomized rats were fed fresh beef liver for 40 days. The weight of liver given was the same as that of fresh pituitary fed in group IV.

IX. Five hypophysectomized rats were fed fresh sheep anterior lobe for 19 days in amounts of 10 Gm. daily. The total amount eaten was 190 Gm. per rat.

X. Two groups of hypophysectomized control rats were kept on a complete maintenance diet without supplementary feeding.

XI. Six normal rats were kept on a complete maintanance diet without supplementary feeding.

RESULTS

An effort has been made to repeat the experiments of Flaks, et al. as exactly as possible. Fresh beef pituitary and fresh beef liver and pituitary extract were

fed in amounts from 75 to 180 Gm. as compared to 75 to 150 Gm. reported to be effective by Flaks. The procedure given by Flaks for the preparation of the pituitary extract was not complete but was followed as closely as possible. Their criteria for erythropoietic stimulation were reticulocyte counts, hemoglobin concentration, red cell counts and percentage of nucleated erythrocytes in marrow smears. These same criteria were used in this work with the addition that the more meaningful total circulating red cell volumes were determined.

The hematologic data for the groups fed beef pituitary, beef pituitary extract or beef liver are kept separate in tables 1 and 2 because they represent the exact repetition of the work of Flaks, et al. It can be seen that in these experiments no increase was found either in reticulocyte count, red blood cell counts, total circulating red cell volume, hemoglobin, hematocrit or marrow nucleated red

TABLE 3.—*Hematologic Data on Hypophysectomized Rats Fed Sheep Pituitary or Maintenance Diet*

Group Fed	Body Weight	Hematocrit	Hemoglobin	Red Blood Cell Volume/ 100 Gm. Body Weight	Hemoglobin/ 100 Gm. Body Weight	Red Blood Cells $\times 10^6$
	Gm.	*%*	*Gm./ 100 ml.*	*ml.*	*Gm.*	*per cu. mm.*
Anterior lobe of sheep pituitary (Group IX)	75	47.3	14.4	2.50	0.760	6.84
	75	47.7	14.2	2.44	0.726	6.50
	70	47.8	13.4	2.64	0.740	6.75
	73	48.8	14.0	2.64	0.757	6.48
	70	47.5	13.4	2.90	0.820	6.72
Average	73	47.8	13.9	2.62	0.761	6.66
Maintenance diet (Group X)	72	40.4	12.4	2.00	0.615	6.32
	69	33.0	9.8	1.68	0.507	6.50
	79	31.5	10.0	1.68	0.540	5.48
	76	44.7	12.6	2.32	0.655	6.80
	69	37.3	11.0	2.10	0.626	5.85
	72	39.5	11.6	2.01	0.610	6.40
Average	73	37.7	11.2	1.97	0.592	6.22

cells. The average total circulating red cell volumes in all experimental groups were the same as those of their controls.

The hematologic data for the group of hypophysectomized rats fed whole anterior lobe of sheep pituitary are given in table 3 (group IX). There is shown a marked increase (33 per cent) over the controls in the circulating red cell volume and a corresponding increase in hemoglobin concentration and hematocrit. It will be seen that the daily dose of sheep anterior pituitary fed this group was twice that of any of the beef fed groups.

At autopsy there was no increase in body weight, tail length or tibial epiphyseal cartilage width in the hypophysectomized rats fed either beef or sheep pituitary or pituitary extract as compared with the controls.

The pituitary target organs were examined to determine whether any of the

known pituitary hormones had withstood gastro-intestinal digestion. The weights of the various pituitary target organs are shown in tables 4 and 5. There was no significant increase in weight of thyroid, gonads or reproductive accessories in either normal or hypophysectomized rats fed beef or sheep anterior pituitary at any dose levels or duration of feeding. The thyroid in both the pituitary fed and the control groups showed flattened epithelium and compact colloid typical of hypophysectomy. The ovaries of the hypophysectomized animals in both the pituitary fed and control groups showed a few very small follicles and deficient interstitial tissue.

TABLE 4.—*Weight of Pituitary Target Organs*

Group Fed	Body Weight	Adrenals	Thyroid	Thymus	Seminal Vesicle	Testes	Prostate
	Gm.	*mg.*	*mg.*	*mg.*		*mg.*	*mg.*
Fresh pituitary (Group I)	294 ± 12	41 ± 4	25 ± 1	337 ± 55	1124 ± 174	3102 ± 205	651 ± 187
Pituitary extract (Group II)	284 ± 15	35 ± 2	22 ± 3	336 ± 74	970 ± 184	2980 ± 180	631 ± 41
Liver (Group III)	276 ± 24	40 ± 5	22 ± 4	308 ± 93	991 ± 193	3018 ± 180	713 ± 147

TABLE 5.—*Weight of Pituitary Target Organs*

Group Fed	Body Weight	Adrenals	Thyroid	Ovaries
	Gm.	*mg.*	*mg.*	*mg.*
Fresh beef pituitary 75–110 Gm. in 40 days (Group IV)	85 ± 5	8 ± 1	7 ± 1	3 ± 1
Fresh beef pituitary 78–108 Gm. in 19 days (Group V)	76 ± 3	11 ± 1	7 ± 1	4 ± 1
Fresh beef pituitary 130–180 Gm. in 19 days (Group VI)	78 ± 6	10 ± 1	7 ± 1	4 ± 1
Beef pituitary extract (Group VII)	83 ± 3	6 ±	8 ± 1	3 ± 1
Beef liver 80–110 Gm. in 40 days (Group VIII)	85 ± 7	8 ± 1	7 ± 1	4 ± 1
Fresh sheep pituitary 190 Gm. in 19 days (Group IX)	73 ± 2	13 ± 1	7 ± 1	5 ± 1
Maintenance diet (Group X)	73 ± 3	8 ± 1	6 ± 1	5 ± 1
Maintenance diet (Group X)	83 ± 10	8 ± 1	8 ± 1	4 ± 1

However, the adrenals of the hypophysectomized rats fed either beef or sheep pituitary showed evidence of stimulation. The cortex was homogeneous and opaque as compared to the darker, mottled cortex of the hypophysectomized controls with or without the liver supplement. The average weight of the adrenals of the hypophysectomized rats fed either beef or sheep anterior pituitary was increased from an average of 8 mg. for hypophysectomized controls to 12 mg. (table 5). Microscopic examination of the adrenals from the hypophysectomized rats fed anterior pituitary showed evidence of stimulation. The width of the cortex was increased. The lipid was evenly distributed through the zones of the cortex and droplets were fine (fig. 1a). The hypophysectomized controls fed laboratory diet or supplements of liver showed a narrow cortex with coarse lipid droplets restricted to the glomerulosa and outer part of the fasciculata (fig.

1b). Thus, some of the adrenocorticotropic hormone ingested was absorbed through the gastro-intestinal wall.

In an attempt to estimate how much of the ingested ACTH was absorbed, groups of hypophysectomized rats were injected intraperitoneally with various doses of an homogenate of fresh beef or sheep anterior pituitary. The lowest dose given (50 mg. per day for 11 days) showed an adrenal response slightly better than that obtained by feeding 5 Gm. anterior pituitary per day. This would indicate that less than 1 per cent of the ingested adrenocorticotropic hormone was absorbed from the gastro-intestinal tract.

Because injection of adrenocorticotropic hormone has been reported to repair the anemia after hypophysectomy[12] it was necessary to determine the im-

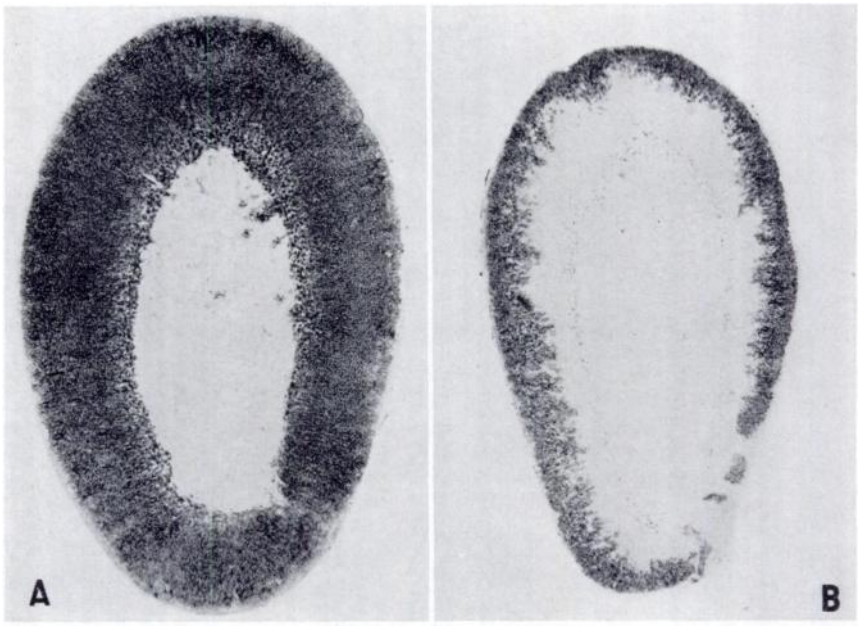

FIG. 1a—Frozen section of adrenal of an hypophysectomized rat fed dietary supplement of fresh beef anterior pituitary, 96 days old, 68 day postoperative. 5 Gm. daily for 19 days. Sudan black × 55.

FIG. 1b—Frozen section of adrenal of an hypophysectomized control rat, 96 days old, 68 day postoperative. Fed an adequate diet. Sudan black × 55.

portance of even this small amount of absorbed adrenocorticotropic hormone in producing the erythropoietic stimulation observed in this experiment. For this reason the erythropoietic effect of orally administered pituitary was tested in adrenalectomized rats. A group of rats was adrenalectomized at 30 days of age. Half of the group was fed fresh sheep pituitary anterior lobe daily and the other half was fed fresh sheep liver. A group of normal rats of the same age, fed a complete maintenence diet, was included for comparison. The gland feeding was started the day after adrenalectomy and continued for 30 days. The amount given was 15 Gm. for the first 5 days, increased to 25 Gm. for the next 10 days and to 30 Gm. for the remainder of the experiment (total of 775 Gm. given).

Both groups of adrenalectomized rats were maintained on 1 per cent salt in the drinking water and because of the high potassium content of the meat diet were given 1 mg. desoxycorticosterone-acetate in oil subcutaneously every 4 days. The blood volumes of these animals were determined by the same method as described previously with the exception that blood was drawn from the tail vein (2 cc.) rather than from the vena cava in order to keep the animals alive to determine the completeness of adrenalectomy. After the blood volume determinations all animals were put on a salt free, high potassium diet.* Death within 14 days was taken as evidence of completeness of adrenalectomy. None of the normal animals put on this same diet died within this or a much greater time.

TABLE 6.—*Hematologic Data on Adrenalectomized Rats*

Group Fed	Body Weight	Hematocrit	Hemoglobin	Red Blood Cell Vol. / 100 Gm. Body Wt.	Hemoglobin / 100 Gm. Body Wt.	Red Blood Cells × 10^6
	Gm.	%	*Gm./100 ml*	*ml.*	*Gm.*	*per cu. mm.*
Anterior lobe of sheep pituitary (775 Gm. in 30 days)	163	51.8	16.0	2.85	0.870	9.25
	198	50.2	15.0	2.97	0.875	8.70
	186	44.8	12.6	2.50	0.699	—
	197	47.7	13.4	2.82	0.793	7.39
	179	46.9	14.8	2.47	0.777	7.25
	178	54.6	17.0	3.17	0.987	8.15
Average	184	49.3	14.8	2.80	0.834	8.15
Liver (775 Gm. in 30 days)	160	44.0	12.2	2.29	0.634	6.75
	197	42.0	12.2	2.04	0.599	6.30
	182	43.8	13.8	2.21	0.699	6.10
	198	48.5	14.8	2.43	0.741	6.85
	162	40.3	11.8	2.18	0.622	6.39
	206	43.0	12.6	2.39	0.703	6.62
Average	184	43.6	12.9	2.26	0.668	6.50
Laboratory diet (ad lib.)	184	41.9	11.4	2.28	0.619	7.10
	192	42.3	12.6	2.30	0.686	6.95
	196	45.0	14.6	2.35	0.745	7.00
	175	45.3	13.6	2.39	0.718	6.81
	195	42.6	13.4	2.30	0.723	6.98
	173	44.3	13.0	2.42	0.712	6.00
Average	186	43.6	13.1	2.34	0.701	6.64

The hematologic data are given in table 6. It can be seen from the table that the values for the adrenalectomized rats fed liver and the normal rats fed a complete maintenance diet are not significantly different. A comparison of tables 1 and 6 will show the consistency of results obtained with untreated normal controls. However, the adrenalectomized rats fed fresh anterior lobe of sheep pituitary showed significant increases in all hematologic values with a 20 per

*Alcohol extract casein 24.0 per cent, sucrose 63.6 per cent, Crisco 8.0 per cent, NaCl free salts and K_2CO_3 4.4 per cent. Crystalline vitamins per Kg. of diet were: 300 μg. d-biotin, 5 mg. 2-methyl-1,4 naphtoquinone, 5 mg. thiamine HCl, 5 mg. pyridoxine HCl, 5.5 mg. pteroylglutamic acid, 10 mg. riboflavin, 10 mg. p-aminobenzoic acid, 20 mg. nicotinic acid, 50 mg. d-calcium pantothenate, 400 mg. inositol, 1.0 Gm. choline chloride. K in the normal diet 0.616 per cent, K in this diet approximately 1.3-1.4 per cent.

cent increase in circulating red cell volume above those of either normal or adrenalectomized controls.

Discussion and Conclusions

Normal and hypophysectomized rats were given dietary supplements of fresh beef and sheep anterior pituitary in order to re-evaluate the work of earlier investigators who reported stimulation of erythropoiesis by oral administration of pituitary. No evidence was obtained to confirm the reports that oral administration of beef anterior pituitary resulted in stimulation of erythropoiesis.

However, the oral administration of fresh sheep anterior pituitary (group IX) not only repaired the anemia of the hypophysectomized rats but brought the circulating red cell volume to higher than normal levels (table 1). This degree of stimulation suggests the presence in sheep pituitary of an erythropoietic factor which is at least to some degree active by mouth. In this respect these results confirm Flaks, Himmel and Zlotnik who contended that the anterior lobe of the pituitary contains a specific erythropoietic hormone with the bone marrow as its target organ.

Of the known pituitary target organs, no evidence was obtained to suggest that growth, follicle-stimulating, interstitial cell-stimulating or thyrotropic hormones were effective when given by mouth. However, the adrenals of the hypophysectomized rats fed fresh beef or sheep anterior pituitary were stimulated. It was estimated that less than 1 per cent of the ingested adrenocorticotropic hormone was absorbed from the gastro-intestinal tract.

In spite of the fact that it had been found that a large amount of ACTH must be injected to influence erythropoiesis[12] it was necessary to rule out the possibility that the small amount of ACTH absorbed from the gastro-intestinal tract was responsible for the erythropoietic effect. It has been reported here that adrenalectomized rats fed anterior lobe of sheep pituitary develop a polycythemia with hematologic values significantly above those of either adrenalectomized or normal controls. If then one is willing to assume that ACTH acts only through its target organ, the adrenal, then the increase in the circulating red cell volume in adrenalectomized animals rules out the identity of the erythropoietic factor with ACTH. With no evidence obtained that any target organ other than the adrenal was stimulated by oral administration of pituitary one must postulate the presence in sheep anterior pituitary of a specific erythropoietic factor.

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