and virus-neutralization tests with acute and convalescent phase measles sera.

1. Enders, J. F., and Peebles, T. C., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 277.

2. Enders, J. F., Peebles, T. C., McCarthy, K., Milovanovic, M. V., Mitus, A., and Holloway, A., *Am. J. Pub. Health,* 1957, v47, 275.

3. Enders, J. F., *Am. J. Med. Sci.,* 1956, v231, 622.

4. Zitcer, E. M., Fogh, J., and Dunnebacke, T. H., *Science,* 1955, v122, 30.

5. Youngner, J. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 202.

6. Peebles, T. C., McCarthy, K., Enders, J. F., and Holloway, A., *J. Immunol.,* 1957, v78, 63.

7. Fulton, F., and Dumbell, K. R., *J. Gen. Microbiol.,* 1949, v3, 97.

8. Svedmyr, A., Enders, J. F., and Holloway, A., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 296.

9. Enders, J. F., in *Virus and Rickettsial Diseases,* Harvard Univ. Press, 1940, pp. 237-267.

10. Rake, G., and Shaffer, M. F., *Nature,* 1939, v144, 672.

11. ———, *J. Immunol.,* 1940, v38, 177.

12. Taniguchi, T., Okuno, Y., Aoyama, A., and Kusumoto, K., *Med. J. Osaka Univ.,* 1956, v6, 1013.

13. Zhdanov, V. M., and Fadeyava, L. L., *Voprosy virusologii* (Problems of Virology), No. 2, March-April 1956, pp. 47-51, Moscov.

14. Reissig, M., Black, F. L., and Melnick, J. L., *Virology,* 1956, v2, 836.

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Decreased Oxygen Need as a Factor in Anemia of Hypophysectomized Animals.* (23141)

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Hypophysectomy induces an anemia in all animals studied, including the human. Various therapies have been shown to prevent this anemia in the rat, success being obtained with thyroxin and androgen(1), thyroxin, androgen, and a high protein diet(1,2), thyroxin and cortisone(3,4), adrenocorticotropic hormone(5), "erythropoietic hormone"(6,7), and cobalt(8,9). In spite of these results, the reason hypophysectomy induces an anemia is still under discussion. Some have claimed that loss of a pituitary erythropoietic factor explains it(6,7), while others have claimed that hypothyroidism and hypoadrenocorticalism, which inevitably follow hypophysectomy, are playing a role(10,11,3,4), although the pituitary seems to be involved in some manner which is in addition to its role in regulating thyroid and adrenal glands(11). Recent work whereby a combined therapy of growth hormone, thyroxin and cortisone returned all aspects of post-hypophysectomy anemia to near normal values‡ has indicated that growth hormone may be involved as well as thyroid and adrenal hormones.

These findings, however, do not explain the underlying mechanism of post-hypophysectomy anemia. The fact that oxygen consumption is decreased after hypophysectomy (12,13,14) and that anoxic anoxia is still considered to be the fundamental stimulant for erythropoiesis induced us to attempt to determine whether this anemia was correlated in any way with a decreased need for oxygen in hypophysectomized rats.

Methods. Unless otherwise indicated, adult female rats of the Wistar strain were used. All rats were fed Purina Chow *ad libitum* supplemented once a week with lettuce. Completeness of hypophysectomy was checked by observing the sella turcica with a binocular dissecting microscope at autopsy. Erythrocyte counts were done in duplicate using U.S. certified blood pipettes and the improved Neubauer counting chamber. Hemoglobin deter-

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TABLE I. Blood Picture and Oxygen Consumption in Hypophysectomized Adult Female Rats before and after a Blood Transfusion. Average amount of blood transfused was 4.5 cc.

	Normal controls	Hypophysectomized rats			
		Before transfusion		After transfusion	
	13 rats	7 rats	% of normal	7 rats	% of normal
Erythrocyte count in millions/mm³	8.21 ± .16	6.99 ± .17	86	9.39 ± .36	114
Hematocrit, %	46.3 ± .75	36.3 ±1.10	79	48.1 ±1.60	103
Hemoglobin, g/100 cc	14.9 ± .23	13.2 ± .28	89	16.3 ± .46	109
Oxygen consumption, l/m² body surface/hr	7.79 ± .22	5.26 ± .17	68	4.71 ± .24	61

± = Stand. error.

minations were made on a Klett-Summerson photoelectric colorimeter, and hematocrit readings were made with Van Allen hematocrit tubes (no diluent) and spun for 1 hour at 2000 rpm. Oxygen consumption was measured as follows: rats were placed in the lower chamber of a 200 mm Frühling and Schultz desiccator; the upper chamber contained wire baskets of Ascarite and Dehydrite for absorption of Co_2 and water respectively. This desiccator was connected with a 500 ml linear or volume recording spirometer which, in turn, was attached to the oxygen supply. Oxygen was placed in the spirometer; the amount used by the rat was recorded directly on a kymograph placed beside the spirometer. Rats were allowed a 30 minute stabilizing period before measurements were started, the oxygen consumed being recorded for 30 minutes with the animal at rest; the figure obtained was then corrected to standard temperature and pressure, and the final result expressed as liters consumed/m^2 of body surface/hour according to the following formula

$$(15):\ \text{l/sq. meter/hr} = \frac{v}{t} \times \frac{P}{760} \times \frac{273}{T} \times \frac{10{,}000}{(wt)^{2/3} \times 10} \times \frac{1}{1000},$$

where $\frac{v}{t}$ = ml of oxygen used over time in hours, P = barometric pressure, T = temperature in the desiccator, $\frac{10{,}000}{(wt)^{2/3} \times 10}$ a means of converting weight to surface area, and $\frac{1}{1000}$ simply a means of converting to liters. The temperature inside the desiccator showed very little change. As fasting hypophysectomized animals for 12 hours occasionally induced a hemoconcentration, fasting had to be avoided. These results are, therefore, subject to the error that the process of digestion might induce.

Procedure and results. The experiment was designed (1) to establish that hypophysectomy actually induces a decrease in oxygen consumption, (2) to determine whether such a decrease, if confirmed, was the result of the anemic condition of the animals, and if not, (3) to attempt to elevate oxygen consumption to see what effects it would have on the blood picture. To confirm that hypophysectomized animals consume less oxygen than normal animals, adult female rats were hypophysectomized and left unmolested for 60 days. At this time the rats were anesthetized with ether and blood removed from the tail for analysis; this was followed by an oxygen consumption determination.

The results are shown in Table I. Hypophysectomy induced an anemia exhibiting a 14% decrease in erythrocyte count, 21% decrease in hematocrit, and an 11% decrease in hemoglobin; the oxygen consumption was 5.26 l/hour in contrast to the value for normal rats of 7.79 l, a 32% decrease.

To determine whether this decrease in oxygen consumption was due to the anemia, these same hypophysectomized rats were given a transfusion of an average of 4.5 cc of whole blood via tail vein. This was followed by an oxygen consumption determination and blood study. Table I shows that the erythrocyte count, hematocrit reading and hemoglobin values were returned to normal by the transfusion; in spite of this, the oxygen consumption remained at a level which was only 61% of normal. These results showed that the

TABLE II. Effects of Various Treatments on Blood Picture and Oxygen Consumption of Adult Female Rats.

Treatment	# rats	Erythrocyte count, millions/mm³	% of normal	Hematocrit, %	% of normal	Hemoglobin, g/100 cc	% of normal	Oxygen consumption, l/m²/hr	% of normal
Normal controls—no treatment	52	7.97 ± .13	100	43.8 ± .50	100	15.0 ± .31	100	8.74 ± .26	100
Thyroidectomy + adrenalectomy 60 days after surgery	6	6.56 ± .21	82	33.2 ±1.24	76	11.3 ± .21	75	5.28 ± .44	60
Hyp. for 50 days followed by .005 mg thyroxin + .6 mg cortisone for 60 days	8	8.44 ± .14	106	46.2 ± .74	105	15.5 ± .25	103	8.44 ± .72	97
*Hyp. for 75 days followed by growth hormone for 50 days	15	6.67 ± .12	84	37.1 ± .5	85	12.4 ± .2	83	6.43 ± .20	74

* Sprague-Dawley rats. Growth hormone (Armour's lyophilized lot #R377237) given in gradually increasing doses starting with 0.2 mg and ending with 0.8 mg.

± = Stand. error.

hypophysectomized rat does not consume less oxygen because of the anemia for it continued to utilize a decreased amount of oxygen when the erythrocyte picture was normal.

Extensive work was done using dinitrophenol to elevate oxygen consumption without affecting general metabolism; it was hoped that this would furnish direct evidence that oxygen need on the part of the hypophysectomized animal was regulating the rate of erythropoiesis. This material proved to be very toxic to hypophysectomized rats when injected chronically; our experiments with this substance were failures in that small doses were ineffective and large doses lethal.

Since the idea of a possible correlation between post-hypophysectomy anemia and oxygen need has been in mind for some time, oxygen consumptions were determined in several recent experiments. These results are shown in Table II. The dual surgery of thyroidectomy and adrenalectomy induced an anemia which was similar to that found after hypophysectomy, and the oxygen consumption was reduced to 60% of normal. Table II also shows that a treatment such as daily injections of 0.005 mg of thyroxin and 0.6 mg of cortisone for 60 days will eliminate post-hypophysectomy anemia; at the same time oxygen consumption was also returned to normal levels. In addition, daily injections of gradually increasing doses of growth hormone (0.2 mg to 0.8 mg) over a 50-day period had no effect on the peripheral blood picture and no effect on oxygen consumption.

Discussion. The hypophysectomized animal becomes anemic and this anemia is accompanied by a decrease in oxygen consumption. Is this decrease in oxygen consumption a result of the anemia or is the anemia simply a reflection of the decreased need for oxygen by the hypophysectomized rat which, in turn, is reflected in a decreased erythropoiesis? The fact that the hypophysectomized rat does not utilize a normal amount of oxygen when its blood picture is restored to normal supports the theory that the hypophysectomized animal is anemic simply because fewer erythrocytes provide an adequate oxygen and Co_2 transport. In addition, procedures such as combined thyroidectomy and adrenalectomy

induce a concurrent decrease in oxygen consumption and erythrocyte count; therapy which had no effect on the peripheral blood picture, such as growth hormone, had no effect on oxygen consumption and a combined thyroxin-cortisone therapy, which eliminated the anemia, elevated oxygen consumption to normal levels. The above evidence is admittedly indirect in character; direct evidence, which might be obtained by elevating oxygen consumption (without increasing general metabolism) and concurrently stimulating erythropoiesis, is difficult to obtain in hypophysectomized animals.

Other features of post-hypophysectomy anemia are in agreement with this theory. Erythropoiesis does occur in hypophysectomized animals, as can be shown in several ways. (1) The erythrocyte count, hematocrit, and hemoglobin do not show a continued decrease after hypophysectomy; 30 to 40 days after surgery the blood picture plateaus, remaining approximately at this level for as long as one year after hypophysectomy. (2) Although Meyer, *et al.*(16) could not stimulate erythropoiesis in bone marrow of hypophysectomized rats by decreasing oxygen tension, Feigin and Gordon(17) were able to do so by decreasing oxygen tension to a greater degree. (3) Querido and Overbeek (18), Finkelstein, *et al.*(19), and Silbergleit (20) have all shown that the hypophysectomized rat can respond to bleeding by an increased rate of erythrocyte formation and subsequent increase in reticulocytes, the counts returning to a level typical of unbled hypophysectomized animals. (4) Baker, *et al.*(21) have reported that hypophysectomized rats treated with total x-radiation became more anemic than untreated hypophysectomized rats, but soon recovered to such a degree that the blood picture was typical of the untreated hypophysectomized animal. All of these experiments indicate that erythropoiesis does occur in the marrow of the hypophysectomized rat; in addition, several of these studies indicate that the bone marrow in these rats will respond to alterations in oxygen supply and demand if the stimulus is severe enough.

This theory that a decreased oxygen need reduces rate of erythropoiesis in the hypophysectomized rat has been used by Fried, *et al.* (22) to explain erythropoietin production. The amount of plasma factor may very well be the intermediary between oxygen need on the part of the tissues and the bone marrow response.

The present experiment does not prove that decreased oxygen need is the sole cause of the anemia which follows removal of the hypophysis but the evidence is plentiful and cannot be ignored. There is no doubt but that erythropoiesis can occur in the absence of the pituitary gland. If this theory is correct, the endocrine glands do not influence erythropoiesis directly, as is suggested by sponsors of the erythropoietic hormone, but merely influence the rate of red cell production by their control over general metabolism and the concomitant changes in oxygen need by the tissues.

Summary. Adult female rats were hypophysectomized and studied 60 days later; they exhibited the usual post-hypophysectomy anemia. The theory that the anemia in the hypophysectomized animal is simply a reflection of a decreased need for oxygen is supported by the finding that oxygen consumption was decreased in these animals and that it remained at a low level even when the peripheral blood picture was restored to normal via transfusion of whole blood. The theory is further supported by the finding that procedures which altered the erythrocyte level caused a similar change in oxygen consumption, and by much evidence indicating that the bone marrow can function normally in the absence of the pituitary gland.

1. Crafts, R. C., *Endocrinology,* 1954, v54, 542.
2. ———, *ibid.,* 1949, v45, 159.
3. ———, *ibid.,* 1954, v54, 84.
4. Meineke, H. A., and Crafts, R. C., *Blood,* 1957, v12, 11.
5. Garcia, J. F., Van Dyke, D. C., Huff, R. L., Elmlinger, P. J., and Oda, J. M., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 707.
6. Flaks, J., Himmel, I., and Zlotnik, A., *Presse Med.,* 1938, v46, 1506.
7. Contopoulos, A. N., Ellis, S., Simpson, M. E.,

Lawrence, J. H., and Evans, H. M., *Endocrinology,* 1954, v55, 808.

8. Crafts, R. C., *Blood,* 1952, v7, 863.

9. Garcia, J. F., Van Dyke, D. C., and Berlin, N. I., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 472.

10. Crafts, R. C., *Endocrinology,* 1953, v53, 465.

11. Meineke, H. A., and Crafts, R. C., *Anat. Rec.,* 1956, v124, 47.

12. Foster, G. L., and Smith, P. E., *J. Am. Med. Assn.,* 1926, v87, 2151.

13. Teague, R. S., *Endocrinology,* 1939, v25, 953.

14. Christensen, B. G., *Acta Physiol. Scan.,* 1946, v12, 11.

15. Holtkamp, D. E., Ochs, S., Pfeiffer, C. C., and Heming, A. E., *Endocrinology,* 1955, v56, 93.

16. Meyer, O. O., Stewart, G. E., Thewlis, E. W., and Rusch, H. P., *Folia Haematologica,* 1937, v57, 99.

17. Feigin, W. M., and Gordon, A. S., *Endocrinology,* 1950, v47, 364.

18. Overbeek, G. A., and Querido, A., *Arch. internat. de pharmacodyn. et de therap.,* 1939, v61, 475.

19. Finkelstein, G., Gordon, A. S., and Charipper, H. A., *Endocrinology,* 1944, v35, 267.

20. Silbergleit, A., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 170.

21. Baker, B. W., Pliske, E. C., Kent, J. F., Van Dyke, J. G., and Bethell, F. G., *Endocrinology,* 1955, v57, 472.

22. Fried, W., Plzak, W. F., Jacobson, L. O., and Goldwasser, E., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 237.

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Estimation of Amount of Oxytocin Released as Result of Nursing Stimuli in Lactating Rat.* (23142)

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There seems to be little doubt that the posterior lobe of the pituitary gland plays an important role in milk let-down(1). It is also generally accepted(2-4) that oxytocin from this gland is responsible for contraction of myoepithelial elements surrounding alveoli and small ducts of the mammary gland thus making available milk for withdrawal. Amount of oxytocin required by various species for milk let-down has been investigated to some extent for the rabbit(5-8), sheep(9), goat(9,10), sow(11) and human(12). However, the amount required by the rat has not been adequately determined. Folley(10), reporting upon unpublished work by Cowie, found that young of anaesthetized lactating rats were able to obtain milk, as determined by test weighing, after a single i.p. injection of .025 I.U. oxytocin into the mother. Grosvenor and Turner(13) found that .2 I.U./kg subc. restored milk let-down in lactating rats in which normal let-down had been inhibited by ergotamine treatment.

In the present study the amount of oxytocin required for normal milk let-down in lactating rats has been determined by injecting various levels of oxytocin i.v. into anaesthetized mothers and comparing weight of milk obtained by the young with normal values previously obtained(14).

Materials and methods. Forty-one lactating rats, each with its first litter and weighing 190-320 g, were housed in individual cages and fed Purina Lab Chow and water *ad libitum.* Shortly after birth each litter was reduced to 6 young and when 14 days old was isolated from the mother for 10 hours. Each mother was weighed and injected with Nembutal (3 mg/100 g) i.p. 10-20 minutes prior to end of isolation period. When completely anaesthetized, she was laid on her side and her litter replaced. Twenty-seven animals received oxytocin‡ in dose of .02, .05 or .1 USP/kg injected i.v., in a volume not exceeding .06 ml, into the superior epigastric vein a few minutes after the young were replaced and while they were actively sucking.

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