

of galactin daily beginning immediately after hypophysectomy, while in the other 2 the testis was removed. In addition, 3 cryptorchid intact males were given 5 mg. daily as controls. One of the injected hypophysectomized animals died on the second day after the first injection and the other two 6 days later. The untreated animals were sacrificed after 10 days. Lactation did not occur in any of the hypophysectomized animals. Lactation was well established after 3 daily injections in the intact cryptorchid males.

Summary. Hypophysectomy in lactating guinea pigs is immediately followed by a rapid decline in lactation and a complete cessation within 2 to 3 days after operation. Galactin was not able to initiate lactation in hypophysectomized experimentally cryptorchid males or to re-initiate and maintain lactation following hypophysectomy at any stage during the first week of lactation. The injection of a suspension of ground fresh whole sheep pituitary gland tissue was able to maintain lactation in 2 cases.

The failure of galactin to initiate or maintain lactation in the hypophysectomized guinea pig is believed to emphasize the importance of the products of the other endocrine glands controlled by the hypophysis in lactation. The importance of the thyroid⁷ and of the adrenal cortex⁸ has been indicated. Carbohydrate metabolism is also intimately concerned in milk secretion.

8641 P

Maintenance of Carbohydrate Levels in Fasted Hypophysectomized Rats Treated with Anterior Pituitary Extracts.*

JANE A. RUSSELL AND LESLIE L. BENNETT. (Introduced by Herbert M. Evans.)

From the Institute of Experimental Biology, University of California.

It has previously been reported by one of us¹ that hypophysectomized rats, while they have normal carbohydrate levels in

⁷ Graham, W. R., Jr., *Biochem. J.*, 1934, **28**, 1368.

⁸ Gaunt, R., and Tobin, C. E., *Anat. Rec. (Supp.)*, 1936, **64**, 18.

* Aided by the Presnell Fund for the study of the metabolic relations of the anterior hypophysis contributed by Robert R. Presnell, Frank Tuttle, Arthur Stebbins and Mrs. Gordon Kahn, of Los Angeles.

¹ Russell, J. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 279.

TABLE I.
Carbohydrate Levels* in Fasted Hypophysectomized Rats Treated with Anterior Pituitary Extracts.

	No. of Animals	Blood Glucose mg. %		Liver Glycogen mg. %		Muscle Glycogen mg. %	
		Aver.	Range	Aver.	Range	Aver.	Range
I. Fasted 8 hours							
1. Unoperated controls	6	100 ± 3	89-113	1210 ± 210	420-2620	490 ± 16	440-566
2. Untreated hypophysectomized control	7	60 ± 2	48-71	17 ± 1	15-18	445 ± 15	369-558
3. W6027 (alk. extract) .5 cc. in 24 hrs.	7	99 ± 4	74-119	541 ± 130	71-1470	617 ± 8	554-700
4. Treatment† over 3 days	8	87 ± 5	44-147	620 ± 150	15-1940	611 ± 16	502-700
II. Fasted 24 hrs.							
1. Unoperated controls	10	71 ± 2	62-83	26 ± 3		529 ± 4	513-550
2. Untreated hypophysectomized controls	7	42 ± 2	30-50	13 ± 1		354 ± 10	302-418
3. W6027 .5 cc. in 24 hrs.	8	53 ± 3	36-75	13 ± 2		578 ± 15	496-651
4. W6027 .1 cc. per day, 4-6 days.	4	54	45-73	15		566	542-592
III. Adrenalectomized-hypophysectomized.							
Fasted 24 hrs.							
W6027 .5-7 cc. in 48-72 hrs.	6	35 ± 2	28-45	17 ± 3	5-26	545 ± 21	427-653

*Details of determinations as reported previously.
†In most cases 0.7 = 1.0 cc. of W6027 total dosage

blood, liver, and muscle when fed, do not maintain these levels during fasting as well as do normal animals. With the administration of anterior pituitary extracts, we have been able to prevent this abnormal fall in carbohydrate levels in short fasting periods.

The results of the most important series in these experiments have been summarized in Table I.

The extract (W 6027) used in most cases in this work was prepared routinely in the laboratory.† It contained 17 mg. of organic matter per cubic centimeter, each cubic centimeter being equivalent to about 130 mg. of fresh tissue.

It was found necessary to give the extracts in divided doses, spread over 18 to 24 hours for the best effects. When the same amount of total material was given in one dose 8 or 24 hours before the determinations of carbohydrate levels were made, no effect was obtained. Subcutaneous injections, however, were found to be no more effective than the intraperitoneal route usually used. Shorter periods of treatment than 18 hours were less effective, and more chronic treatment (3 to 6 days) was not found more effective than the 24-hour period.

In addition to the extract W6027, which contains all the known anterior lobe hormones, 2 fractions high in growth hormone§ and one high in adrenotropic effect|| but also containing fairly large amounts of growth hormone were used on 8 hypophysectomized rats and found to be very active in preserving glycogen stores. The 2 growth preparations were free of gonadotropic and mammatropic effects as shown by immature pigeon tests, and one of these preparations as well as the adrenotropic fraction appeared to contain no thyrotropic hormone. These hormones would thus appear to be ruled out in this action of the anterior pituitary. Although this "glycostatic" factor appears to accompany the growth hormone, its relation to this hormone is not established.

In 6 cases the right adrenal was removed a few days prior to hypophysectomy and the left adrenal 11 to 19 days after hypophysectomy. Treatment with the standard alkaline extract, W6027, was instituted immediately after the removal of the second adrenal

† A given amount of frozen, ground beef hypophyses is taken up in 7½ volumes of 0.04 N Ba(OH)₂, extracted in the ice box 18 hours and then passed 4 times through a brine-cooled Sharples centrifuge. The supernatant is neutralized to pH 8.0 with 0.2 N H₂SO₄ and any excess barium precipitated out by the addition of a solution of Na₂SO₄. The solution is again centrifuged and the supernatant is ready for use.

§ Prepared in this laboratory by Doctors N. Uyei and Q. Bartz.

|| Prepared in this laboratory by Mr. Henry Moon.

and the animal sacrificed in 48 or 72 hours after a 24-hour fast. The glycogen stores in these animals do not differ significantly from those in the other treated 24-hour fasted, hypophysectomized rats. It would thus seem that this effect cannot be exerted through the adrenal cortex.

Two preparations, W6027 and the adrenotropic fraction, when heated to boiling for one hour, were without activity of any sort in 4 rats.

The method by which these anterior pituitary preparations act to preserve carbohydrate stores is unknown. New formation of carbohydrate was not demonstrated when, in a few animals, attempts were made to raise fasting levels already low; nor when the extract was given to unfasted animals were abnormally high levels found. A possible gluconeogenetic action of the anterior lobe is not ruled out, however, by the experiments.

It may be significant that the muscle glycogen content seems to be primarily affected in this action of the anterior lobe and that the effects on blood and liver do not necessarily follow those on the muscle. Although there is a general correspondence between the 3 values, in many individual cases marked hypoglycemia and very low liver glycogen levels have been observed accompanied by muscle glycogen figures between 650 and 700 mg. % in 8-hour and 550-620 mg. % in 24-hour fasted treated rats, values well above those encountered in normal controls.

Summary: The abnormal fall in body carbohydrate suffered by hypophysectomized rats during fasting can be prevented by the administration of anterior pituitary extracts, and the muscle glycogen values can be raised to supernormal levels for the fasted conditions. This action of the anterior pituitary occurs in the absence of the adrenal glands.