consistent with the observations of Recant and Fischer(6) that pretreatment of rats with tolbutamide causes a greater incorporation of glycine into protein by their livers. Therefore, as determined by these 2 studies, tolbutamide when effective in producing hypoglycemia, has an action parallel to that of insulin on amino acid metabolism(7). This similarity of action adds support to the evidence that tolbutamide acts by increasing the output of insulin and speaks against the possibility that depression of gluconeogenesis from amino acids could be a factor in production of hypoglycemia.

Summary. 1) In normal subjects sodium tolbutamide given intravenously in dosage of 40 mg/kg of actual body weight produced hypoglycemia comparable to that produced by insulin (0.1 unit/kg body weight) intravenously. In 3 of 5 subjects the fall in blood glucose took place within 5 minutes following injection of tolbutamide. 2) In 7 patients with stable diabetes the decrease in blood glucose following tolbutamide was slower than that with insulin and no fall occurred in 2 patients with unstable diabetes. 3) In normal persons and in the 7 diabetic patients who experienced a fall in blood glucose following tolbutamide, there was likewise a decrease in serum amino acids comparable to that following insulin. No fall in serum amino acids occurred in the 2 patients with unstable diabetes.

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Pituitary and Plasma Bioassay for Trophic Hormones in the Alloxan-Diabetic Rat.* (24237)

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Among endocrine disturbances reported in experimentally induced diabetes mellitus have been impairment of reproductive physiology and hypoactivity of the thyroid(1-6). In the present study the gonadotrophic, thyrotrophic, and growth-promoting potencies of the pituitary glands and blood plasma of diabetic rats were determined by bioassay in hypophysectomized immature female rats.

Materials and methods. Female rats of the Long-Evans strain maintained on an adequate natural food diet‡ were injected with alloxan at 50 days of age and sacrificed at 100 days of age. Pituitaries and plasma were assayed in hypophysectomized rats, 28 days old at operation, used 14 days later. Hypophysectomy was performed by the parapharyngeal approach. Completeness of ablation was checked by observation of the sella turcica at autopsy. Atrophy of target organs furnished

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‡ All diabetic rats were maintained on a modified McCollum Diet I consisting of ground whole wheat 67.5%, casein 15%, skim milk powder 7.5%, sodium chloride iodized 0.75%, calcium carbonate 1.5%, melted fat 6.75%, fish oil (Vit. A and D concentrate) 1%. KI solution added to 1 μg of iodine/g of diet (450 mg KI/liter, 300 cm^3/270.5 lb of diet). Hypophysectomized rats were offered daily a wet mash of this diet. All rats received a supplement of lettuce 2-3 times a week and were maintained at 74° ± 1°F temperature.

confirmatory evidence. To induce diabetes a 5% solution of alloxan (recrystallized) in isotonic saline was made immediately prior to injection. Prior to alloxan administration, the rats were fasted for a period not exceeding 72 hours. A dose of 5 mg/100 g body weight was injected rapidly into the tail vein. Access to food was allowed immediately after injection. The diabetic status was allowed to stabilize for 7 weeks and rats with blood sugar levels greater than 350 mg% were used as donors (controls, 112 mg %). Blood glucose levels were determined by Nelson's modification of the Somogyi procedure(7). Assays of plasma from diabetic and control rats for presence of trophic hormones were performed as follows. Whole blood from etherized donors was collected from the abdominal aorta into an heparinized syringe. Blood was quickly transferred to a conical centrifuge tube, covered with a layer of mineral oil, and centrifuged 12 minutes at 3000 rpm. The plasma was then aspirated into a syringe and injected into recipient hypophysectomized rats within less than 30 minutes after withdrawal. Recipient hypophysectomized animals received 4 daily injections of plasma. Assays of pituitaries of donor rats for trophic hormone content was conducted as follows. Pituitaries were dissected immediately after exsanguination, posterior and intermediate lobes were separated, and anterior lobes were quickly frozen on dry ice. Suspensions for injection were prepared immediately before the assay period. The glands were weighed and ground by motor-driven ground glass rod inside a test tube, known amounts of isotonic saline being added. Butanol (1%) was added and suspensions were stored at 0-4°C during the 4-day injection period. Recipients were autopsied 24 hours after the last injection. Ovaries and uteri with oviducts were weighed, fixed in Bouin's solution, sectioned and stained. The tibias were split, fixed in 10% formalin, and stained with silver nitrate. Eighteen hours prior to autopsy all assay animals received intraperitoneally a tracer dose of I^{131} (0.5-1 μc). Thyroids were placed in fixative in a small vial suitable for counting radioactivity(8). Differences between counts/minute of thyroids and of a leg muscle fragment of comparable size, expressed as percent of injected dose, represented the I^{131} uptake by thyroid gland. Gonadotrophic potency of pituitaries and plasma was determined by microscopic structure of ovaries of recipients (9, 10).§ Thyrotrophic hormone content of pituitary glands and plasma was appraised on the basis of iodine-concentrating capacity and histology of thyroids from assay animals. An I^{131} uptake greater than 1% of injected radioactivity has been adopted as indicating minimal thyrotrophic stimulus. The cytological criteria adopted for determining minimal effective dose for thyrotrophic stimulation were an increase in height of follicular cells to low cuboidal, associated with some loosening of nuclear chromatin and beginning vacuolation of colloid. Evaluation of growth-stimulating activity was based on ocular-micrometer measurements of the uncalcified proximal epiphyseal cartilage of the tibia(11). A width increment of 50 μ above hypophysectomized control level, or an absolute value of 200 μ was regarded as a significant response. Bioassays were repeated at least once for each dose level.

Results. Bioassay of anterior pituitary hormone content. Dose levels between 1/32 gland and 4 anterior lobes from diabetic rats were assayed in hypophysectomized recipients (Table I). A total dose of 2 glands was required to increase ovarian weight; a dose of 4 glands also increased uterine weight. Minimal stimulation to follicular growth resulted

§ Minimum effective dose for follicle stimulating hormone was judged by appearance of healthy small-medium follicles with beginning antrum formation. Degree of follicular growth was determined by measurement of diameter of health (non-atretic) follicles by the aid of an ocular micrometer. The following gauge is used throughout this study: small follicles, sF = 0.375 mm; small-medium follicles, smF = 0.375-0.5 mm; medium follicles, mF = 0.5-0.65 mm; medium-large follicles, mlF = 0.65-0.75 mm; large follicles lF = 0.75-1.0 mm. Minimum effective dose for interstitial-cell-stimulating hormone was judged by beginning repair of ovarian interstitial tissue. This was noted by an increase in vesicularity of the nuclear pattern, accompanied by an increase in size and depth of staining of these cells.

mal. (Assayed in hypophysectomized rats, 8 rats/group.)

Type of donors	No. of rats	Total dose: No. of glands	Total dose: Wt, mg	Uterus wt, mg	Ovaries: Histology: Follicles	Ovaries: Histology: Interstitial cells	Ovaries: Wt, mg	Thyroid: I^{131} uptake, %	Thyroid: Histology	Tibial cartilage width, μ
Diabetic	21	0	0	24 ± 2	sF	Atrophic	9 ± 1	.50 ± .1	—	154 ± 2
	8	1/32	.15	—	—	—	—	.92 ± .1	—	153 ± 4
	8	1/16	.3	—	—	—	—	1.25 ± .1	+ (MED)	159 ± 3
	8	1/8	.6	—	—	—	—	2.34 ± .2	+	164 ± 3
	8	1/4	1.1	—	—	—	—	3.07 ± .3	++	161 ± 3
	8	1/2	2.2	26 ± 3	sF	Atrophic	10 ± 2	4.52 ± .5	++	191 ± 2
	8	1	4.5	24 ± 2	sF	Partial repair (MED)	10 ± 1	5.68 ± .9	++	203 ± 4 (MED)
Normal	8	2	9.0	30 ± 3	s, smF (MED)	Repair	17 ± 1	9.61 ± 2.5	++	245 ± 5
	8	4	18.0	98 ± 2	sm, m, lF, CL	„	39 ± 7	16.68 ± 1.8	++(+++)	263 ± 6
	15	1/32	.3	—	—	—	—	.95 ± .2	—	193 ± 4
	22	1/16	.6	—	—	—	—	1.53 ± .3	+ (MED)	202 ± 5 (MED)
	22	1/8	1.2	—	—	—	—	2.62 ± .5	+	232 5
	12	1/4	2.4	—	—	—	—	3.94 ± .6	+++	248 4
	16	1/2	4.8	24 ± 2	sF	Atrophic	9 ± 1	5.72 ± .4	+	286 5
	14	1	9.7	27 ± 1	sF	Repair (MED)	11 ± 1	7.85 ± 1.1	++	297 8
	12	2	19.4	35 ± 4	s, smF (MED)	Repair	15 ± 2	10.95 ± 1.5	++(+++)	308 8
	6	4	38.8	124 ± 4	sm, m, lF, CL	„	35 ± 3	18.50 ± 1.7	+++	316 ±12

after administration of the 2 glands (9 mg) and corpora lutea were present after 4 glands. Beginning repair of the interstitial tissue was detectable at the 1 gland level (4.5 mg). The minimal effective doses did not therefore differ from those of pituitaries of normal female rats of the same age (Table I)(12). In this connection attention is called to the disparity between pituitary weights in diabetic and normal rats; glands from diabetic rats were approximately half normal weight, 4.5 mg compared with 9.7 mg. The body weight of diabetic rats was also half normal, 146 g compared with 233 g so that pituitary weight remained the same percent of body weight. A dose of 1/16 pituitary gland from diabetic rats elicited a minimum response in the thyroid of hypophysectomized recipients, judged both by histology of the thyroid and I^{131} uptake. Comparison with assay of normal pituitary glands, shows that 1/16 anterior lobe was also minimal for thyrotrophic hormone activity (Table I). A dose of 1 anterior pituitary from diabetic rats caused a significant increase in width of the epiphyseal plate. Pituitares of normal female rats of the same age gave minimal growth stimulation at 1/16 of an anterior lobe. The growth hormone content of anterior pituitary glands from diabetic female rats was therefore markedly reduced (Table I).

Bioassay of blood plasma. Table II shows that growth-promoting potency of blood serum was reduced in alloxan-induced diabetic female rats, as compared with normal rat plasma. At a daily dose of 3.5 to 4 cc the plasma of normal donors gave a significant increase in tibial epiphyseal width(13), where-

TABLE II. Decreased Growth Promoting Potency of Blood Plasma of Diabetic Rats.

Donor group	Daily dose, ml	No. of rats per assay	Body wt, g: Initial	Body wt, g: Final	Width of proximal epiphyseal cartilage of tibia, μ
Diabetic	4.0	4	66	68	190 ± 2
	3.5	3	70	75	186 ± 4
Normal	4.0	4	67	73	210 ± 9
	3.5	4	66	72	214 ± 5
Uninj. recipients	0.0	4	64	68	169 ± 3

as plasma of diabetic donors gave only a marginal response. No other hormones were detected in plasma of diabetic and normal rats at doses tested.

Discussion. Houssay and Foglia reported a decrease in gonadotrophic content in the pituitaries of alloxan injected diabetic rats (14), but no other differences were detected between trophic hormone potencies of pituitaries of diabetic and normal rats. Diabetic rats used in this study were 7 weeks post-alloxan treatment, whereas in studies of Houssay and Foglia the rats were sacrificed 48 hours after administration of alloxan. In the present study no significant differences were found between diabetic and normal adult female pituitary gonadotrophic and thyrotrophic potency whereas a 16 fold reduction in growth hormone content was found in pituitaries from diabetic female rats. Growth hormone potency of pituitaries was reduced, whether measured in fractions of anterior lobe, or in mg of pituitary tissue. There was also a reduction in growth-promoting activity of plasma of diabetic rats, as judged by tibial cartilage width.

Pituitary trophic hormone content should always be considered in relation to hormone production and release. The similarity in gonadotrophic and thyrotrophic content of pituitaries from normal and diabetic rats does not necessarily indicate production of these hormones at the same rate. Differences in secretion rate and storage might result in the same final content. The atrophic status of the gonads observed in diabetic donors(1-4) indicates reduced secretion of gonadotrophic hormones, unless the response of these target organs to the trophic hormones is different in diabetic animals. The reduced growth promoting potency of plasma can not be attributed solely to decreased growth hormone release from the pituitary without consideration of changes of titers of other hormones.

Summary. The gonadotrophic, thyrotrophic, and growth hormone content of anterior pituitaries from female rats rendered diabetic by alloxan was determined by bioassay in hypophysectomized female rats. Trophic hormones in blood plasma were also determined. The number of glands required to evoke minimum follicle stimulating, interstitial cell stimulating, and thyrotrophic activity were the same in diabetic and normal rats, whereas growth hormone content of the pituitary was reduced 16-fold. The titer of circulating growth promoting factors was also reduced.

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