Insulin Secretion in Rats with Elevated Levels of Circulating Growth Hormone Due to MtT-W15 Tumor

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SUMMARY

Circulating levels of growth hormone (GH) in Wistar-Furth rats bearing the MtT-W15 tumor are twenty to thirty times higher than normal. The effect of constantly elevated levels of GH on the pancreatic islets was studied. Tumor-bearing rats (TR) and normal controls (C) were pair-fed for seven weeks. In TR, fasting serum levels of GH and insulin were 599 ± 157 ng./ml. and 142 ± 43 μU./ml., respectively, and only 18.2 ± 1.9 ng./ml. and 26.9 ± 7.9 μU./ml., respectively, in the controls. The islets of TR were enlarged with an islet/acinar ratio of 3.38 per cent (C: 0.82 per cent). The extracted pancreatic insulin was 9.84 ± 0.9 mU./mg. (C: 5.36 ± 0.4 mU./mg.). Incubated slices or isolated islets of TR pancreas released greater amounts of insulin when submitted to glucose stimulation. Incorporation of labeled leucine into insulin was increased also in TR. An exaggerated insulin response to a glucose load was found in vivo (peak value at 30 min., TR: 183 ± 42.5 μU./ml.; C: 71 ± 17.9 μU./ml.). None of the TR became diabetic, however, and two to six weeks after removal of the tumors serum GH levels had returned to normal (17.2 μg./ml.) as had insulin levels both fasting (19.0 μU./ml.) and following a glucose load (56.4 μU./mg.). Insulin output by incubated pancreas decreased 50 per cent (26 μU./mg.). The islet/acinar ratio returned to normal but the insulin content remained high. These results indicate that in animals exposed to high levels of endogenous GH there is an increased responsiveness of the islets to glucose stimulation. DIABETES 17:661-67, November, 1968.

Houssay,[1,2] Evans,[3] and Young[4] first demonstrated the diabetogenic effect of growth hormone (GH). A comprehensive review on the subject was published by Sirek and Sirek.[5] Richardson and Young[6,7] have described the sequence of morphological changes in the islets of animals with GH-induced diabetes.

In all these studies the rat is unique, contrary to the effect observed in dogs, cats, rabbits, ferrets, goats, monkeys, and men, since the administration of large doses of GH did not induce diabetes in the intact rat. An early observation indicated that in animals susceptible to the diabetogenic effect of GH, the hormone stimulates the secretion of insulin until, eventually, the islets of Langerhans undergo degeneration by "exhaustion." Investigations made on rats at that time[8] failed to demonstrate an increase of insulin secretion induced by GH. Young had found previously that GH is not diabetogenic in growing animals (puppies and kittens[9]) and that the epiphyses in the rat do not fuse and it continues to grow slowly throughout its life.[10] In view of these facts, Young postulated that GH would be diabetogenic only in those animals in which the hormone cannot promote growth, and instead, would stimulate the excessive secretion of insulin by the pancreas.[11]

Bovine GH was used in most early experiments but when Li[12] established the species specificity of the hormone, it seemed desirable to reinvestigate the response of the pancreatic islets of the rat to rat GH. Until recently, such an approach was not possible because of the limited supply of a pure preparation of rat GH. Furth has now developed, however, some strains of pituitary tumors which secrete mainly GH,[13] indistinguishable from the normal pituitary hormone either biologically or immunologically.[14,15] These tumors can be transplanted from animal to animal by subcutaneous inoculation and neither infiltrate nor metastasize in the host, Wistar/Furth* female rats, a highly inbred strain. Once the tumor develops, the concentration of GH in

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the serum increases from ten to sixty times the normal values, and the host rat becomes clearly "acromegalic."[16]

In the only study published, to our knowledge, on the effect of these GH-secreting tumors on the pancreas, Bates et al.[17] found a higher prevalence of diabetes after partial pancreatectomy in tumor-bearing rats than in the controls.

We have investigated the effect of a chronically elevated concentration of endogenous GH on the morphology of the islets, the insulin content of the pancreas, and the secretion of insulin in intact rats. The response variables were measured in normal controls, tumor-bearing rats, and in animals after the tumor had been removed. In addition to studies in vivo, the responsiveness of islets to glucose stimulation was explored in vitro on pancreas slices and on isolated islets from the same three groups of animals.

MATERIAL AND METHODS

Thirty-three Wistar-Furth female rats weighing approximately 120 gm. were inoculated subcutaneously with a fine suspension of tumor tissue in saline.* The tumors became palpable three to four weeks after inoculation and grew to maximum size during the following four to five weeks. Seven inoculated rats and seven controls, matched by age and weight were pair-fed on a food intake basis with regular Rockland rat chow. The control animal was the "leader" of the pair since we have learned from previous experiments that the tumor-bearing rat consumes more food than the normal. The fasting concentration of serum growth hormone and insulin were determined on pairs at random after the tumor became palpable, the serum concentration of growth hormone increasing from this time on. The tolerance to oral glucose was tested at various intervals in all pairs from the fourth to the seventh weeks. Each rat was given 250 mg. glucose dissolved in 1 ml. of water per 100 gm. body weight by gavage. Blood samples were obtained with a capillary tube from animals lightly anesthetized with ether from the retro-orbital plexus at 0, 30 and 120 minutes after the glucose load.

In the seventh week the animals were decapitated, blood collected for glucose and hormone determinations, and the pancreas carefully dissected and weighed. Half of the organ was immediately frozen for subsequent insulin extraction. The other half containing portions from the splenic, gastric and duodenal regions of the pancreas, was fixed in Zenker formol for histological study.

Additional groups of twelve tumor-bearing rats and twelve controls were similarly treated, but in five of the tumor-bearing animals the tumor was removed in the seventh week after inoculation. Serum growth hormone and insulin determinations and glucose tolerance tests were done on the experimental animals and the controls four, six and eight weeks after removal of the tumor. At the end of the eight-week period the animals were killed and the pancreas processed as described above. The remaining animals (seven control and seven tumor-bearing rats) served as pancreas donors for studies in vitro. Slices of pancreas or isolated islets were incubated in media with low or high content of glucose in order to assess the effect of glucose concentration on insulin release.

Growth hormone[18] and insulin[19] were determined by radioimmunoassay procedures. The purified growth hormone used in the assay system was extracted in our laboratory from rat pituitaries by the technic of Ellis,[20] and its purity assessed electrophoretically and immunologically by comparison with a preparation kindly given to us by Dr. Ellis (H-III-41-E). Details of the procedure were described elsewhere.[21]

Glucose was determined by the glucose oxidase method.[22] We extracted the insulin from the pancreas by the method of Grodsky[23] as modified by Taylor,[24] and measured it by immunoassay expressing the results in mU./mg. of tissue. The islet volume was measured by the technic of Chalkley[25] on sections of pancreas stained with aldehyde-thionine with Gomori counterstain. For the in vitro studies we incubated slices of pancreas for fifteen minutes in a medium with 60 mg. glucose/100 ml. (baseline) and then transferred them for another period of fifteen minutes to a medium with 300 mg. glucose/100 ml. (stimulation). The concentration of insulin in each incubation medium was determined by immunoassay and expressed in μU./mg. of tissue in fifteen minutes. We also incubated the isolated islets obtained by the technic of Lacy and Kostianovsky.[26] Five to seven islets were placed in each incubation flask. The incubation procedure was the same as that for the pancreas slices except for the addition of I-C-14-leucine (0.5 mM/l; specific activity 10 mc/mM) to the medium. We measured the insulin output, the insulin content, and the incorporation of labeled leucine into the extracted insulin at the end of each fifteen-minute incubation period. The results were calculated and expressed per microgram of protein. The protein content of the islets was determined by the method of

*MtT/W15 tumors from the strain developed by Dr. J. Furth were kindly supplied by Mason Research Institute, Tumor Bank, Worcester, Mass., U.S.A.

Lowry[27] on the TCA precipitate remaining after insulin was extracted with acid-alcohol. Details of the procedure have been published.[28]

RESULTS

1. *Body weight and food intake*

The control animals ate between 13.1 and 18.8 gm. of food per day. The same daily amount was offered to the tumor-bearing rats. Figure 1 shows the curves of body weight. During the first four weeks the control animals were heavier but, after the fourth week, coincident with the development of the tumor, the experimental group began to grow faster. In spite of the equal food consumption the tumor-bearing animals attained a significantly higher body weight, which was maintained for the remainder of the experiment. In addition, the tail-length was 0.5 to 1.5 cm. longer at seven weeks in the experimental group, the difference being statistically significant ($p < 0.02$). Following removal of the tumor a 12 to 13 per cent decrease in tumor-free body weight occurred during the first two weeks. Thereafter the body weight remained constant.

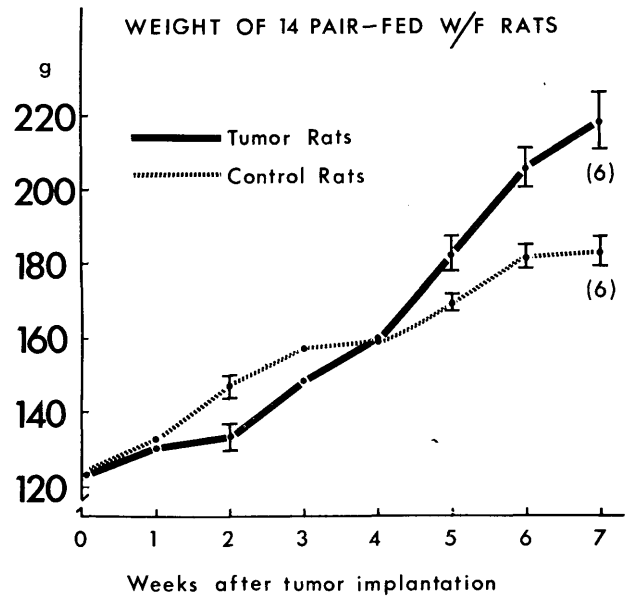

FIG. 1. Each point, except for the last, is the mean value ± standard error of seven animals.

2. *Fasting levels of serum growth hormone and insulin* (table 1)

Circulating levels of growth hormone and insulin were significantly higher in the tumor-bearing rats compared to the control group. Four weeks after the surgical removal of the tumor these values had returned to normal.

3. *Oral glucose tolerance tests* (table 2)

The glucose levels in the control group were within the normal range. The value recorded at 120 minutes was still higher than the fasting level, a phenomenon commonly observed in normal rats when this amount of glucose is given. Both the tumor-bearing and the tumor-removed groups had lower values of blood glucose than the controls throughout the test. The differences were significant at 120 minutes. The fasting concentration of glucose of the tumor-bearing group, although within normal limits, was significantly lower than in that of the controls by statistical analysis. More data should be collected in order to evaluate the importance of this observation. The highest insulin values were found in tumor-bearing rats and the lowest in the tumor-removed animals.

4. *Islet volume and mass, and pancreatic insulin content* (table 3)

In estimation of islet volume, an islet-acinar ratio of 0.82 per cent was found in pancreas of control animals. This value is in keeping with previous observations on normal rat pancreas using Chalkley's technic[29] and by the camera lucida procedure.[30] Tumor-bearing rats had a significantly larger islet volume with a ratio of 3.38. After removal of the tumor the islet/acinar ratio returned toward normal.

By correlation of the islet/acinar ratio with the weight of the total pancreas, the islet mass was cal-

TABLE 1

Fasting values of serum growth hormone and insulin

	a Normal rats (7)*	*b* Tumor rats (7)	*c* Tumor removed (5)	*P values*‡ *a* vs. *b*	*a* vs. *c*	*b* vs. *c*
G.H. (ng./ml.)	18.2 ± 1.9†	598.8 ± 157.4	17.2 ± 6.95	<0.005	NS	<0.005
Insulin (μU./ml.)	26.9 ± 7.9	142.1 ± 42.9	19.0 ± 4.37	<0.025	NS	<0.025

*Number of animals in parenthesis
†Mean values ± standard error
‡P value was calculated by Student's *t* test

TABLE 2

Concentration of circulating glucose and insulin following an oral glucose load (2.5 gm./kg.)

Experimental groups	Blood glucose (mg./100 ml.) Time in minutes			Serum insulin (μU./ml.) Time in minutes		
	0	30	120	0	30	120
Normal rats (5)* (*a*)	106.7 ± 2.5	164.7 ± 9.6	142.0 ± 7.1	55.0 ± 12.9	71.0 ± 17.9	56.0 ± 10.7
Tumor rats (5) (*b*)	94.0 ± 4.5	148.2 ± 9.0	100.2 ± 4.5	125.3 ± 52.4	212.0 ± 25.1	115.0 ± 30.6
Tumor removed (5) (*c*)	88.1 ± 10.3	132.3 ± 15.4	120.3 ± 3.9	20.4 ± 11.5	56.4 ± 31.7	8.0 ± 5.3
P values†						
a vs. *b*	<0.05	NS	<0.005	NS	<0.005	NS
a vs. *c*	NS	NS	<0.05	NS	NS	<0.005
b vs. *c*	NS	NS	<0.02	NS	<0.005	<0.01

*Number of animals in parentheses. Figures are mean ± standard error.
†P values were calculated by Student's *t* test.

culated using the formula

$$\frac{\text{islet/acinar ratio} \times \text{pancreas weight.}}{100}$$

While in control and tumor-removed animals, the calculated islet mass was 4.2 and 6.2 mg. respectively, in the tumor-bearing rats it increased significantly to 18.0 mg.

A pancreatic insulin content of 5.36 mU./mg. was found in the control animals while a higher value, 9.84 mU./mg. was found in tumor-bearing rats. After removal of the tumor the pancreatic insulin content remained high although the islet mass had returned to normal.

5. *Insulin secretion in vitro*

A basal insulin output of 13.42 ± 2.05 μU./mg. was found when slices of pancreas from normal rats were incubated in a medium with 60 mg. glucose/100 ml. (figure 2). Under the same conditions, slices of pancreas from the tumor-rats secreted 37.90 ± 3.19 μU./mg. When the same slices of pancreas were submitted to glucose stimulation, 300 mg. glucose/100 ml., the insulin secretion increased to 19.37 ± 3.19 and 52.36 ± 4.42 in normal and tumor-rat pancreas respectively. In both cases, however, the percentage increase over the base line was of the same magnitude: 43.9 per cent in normal rat pancreas, 41.0 per cent in pancreas of tumor-bearing rat.

Slices of pancreas taken from animals after removal of the tumor secreted less insulin into the incubation medium than similar slices from the pancreas of rats

TABLE 3

Islet volume, islet mass and pancreatic insulin content

	Ratio $\frac{\text{Islet}}{\text{Acinar}} \times 100$	Islet mass (mg.)	Insulin content (mU./mg. pancreas)
a) Normal rats (7)	0.82 ± 0.16	4.2 ± 0.32	5.36 ± 0.38
b) Tumor rats (7)	3.38 ± 0.63	18.0 ± 3.78	9.84 ± 0.92
c) Tumor removed (5)	1.17 ± 0.25	6.2 ± 1.54	10.63 ± 1.21
P values			
a) vs b)	<0.005	<0.005	<0.005
a) vs c)	NS	NS	<0.001
b) vs c)	<0.01	<0.02	NS

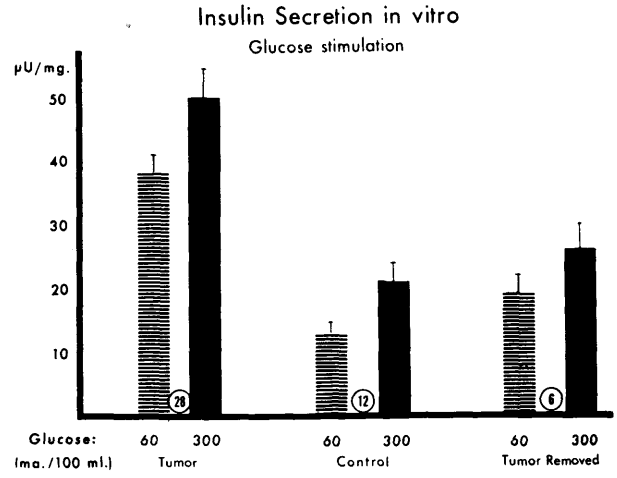

FIG. 2. Bars represent mean values ± standard error. The figure in the circle indicates the number of pancreas slices.

with tumors. The response to low and high glucose concentrations in the medium was similar to that of normal rat pancreas: 19.2 ± 2.79 and 26.0 ± 2.94 μU./mg., respectively.

The results obtained from pancreas slices were similar to those obtained from isolated islets (table 4). In addition to an increased insulin release by the islets from tumor-bearing rats, a higher insulin content per islet was demonstrated in the experimental animals compared with the controls. The incorporation of labeled leucine into the insulin extract of islet tissue was also higher in the tumor rats (when calculated in relation to islet protein). However, when expressed as specific activity of insulin it was lower, which might be due to dilution of the trace-amino acid in a larger insulin pool. Analysis of the data showed that while the baseline and the glucose stimulated release of insulin were three times higher in the islets of tumor-bearing rats than in islets from control rats, the increase elicited by the high glucose concentration was of the same magnitude, between 220 and 260 per cent, in both groups. The amount of insulin released represented 1.2 per cent of the insulin content of the islets in the tumor group, and 12.0 per cent in the control. In the latter no significant increase in insulin content was observed after glucose stimulation, although insulin synthesis increased three times. The phenomenon has been observed before,[28] and may indicate that insulin synthesis and release were equally enhanced by glucose and, therefore, the insulin content remained constant. In tumor rats, on the other hand, a two-fold increase in pancreatic insulin was found after glucose stimulation. This could result from the combined effect of a four-fold increase in insulin synthesis and the relative secretion (only 1.2 per cent) of the total insulin during the stimulation period.

DISCUSSION

Our results, both in vivo and in vitro, demonstrate an increased insulin secretion in the tumor-bearing rats. The increase in circulating insulin observed either in the fasting state or following a glucose load is similar to the response in acromegalic patients. Further support for considering these animals as an experimental model of acromegaly was provided by other results obtained in our laboratory[31,32] which demonstrated that glucose utilization by peripheral tissues (diaphragm and adipose tissue) in vitro was significantly depressed in both the presence or absence of insulin.

The increased secretion of insulin, indicated by the level of circulating insulin, was reflected in an enlargement of the islets and a significantly higher content of extractable insulin. The in vitro results confirmed the in vivo observations. The insulin secretion and the insulin content of islets were higher in tumor-bearing rats as compared with the controls, both in the presence of low and high glucose concentrations. But the relative increase in insulin secretion over the baseline in response to glucose stimulation in vitro was of the same magnitude in the two groups.

These results do not establish the mechanism by which GH induces an increased responsiveness of the islets to glucose stimulation. Our previous studies on the effect of GH on the isolated islets of hypophysectomized rats suggested that GH did not exert a direct stimulatory action on the beta cells but rather facilitated the stimulatory effect of glucose.[28] The present results reinforce

TABLE 4

Incubation of isolated islets from tumor-bearing and control rats

	Glucose concentration (mg./100 ml.)	Normal rats* (6)	Tumor rats* (8)	P
Insulin release	60	2.9 ± 0.72†	10.2 ± 1.93	< 0.01
(μU./μg. protein)	300	10.7 ± 6.49	32.5 ± 8.38	< 0.05
Insulin content	60	0.69 ± 0.07	1.39 ± 0.22	< 0.01
(mU./μg. protein)	300	0.87 ± 0.22	2.58 ± 0.42	< 0.005
C-14-leucine incorporation in extracted insulin				
(cpm/U. insulin)	60	371 ± 61.92	179 ± 26.0	< 0.02
	300	1,085 ± 291.49	677 ± 216.6	NS
(cpm/μg. protein)	60	273 ± 49.9	404 ± 149.9	NS
	300	710 ± 300.7	1,671 ± 320.1	< 0.05

*Number of observations in parentheses. Mean values ± standard error.
†Values calculated for protein content of the islet. Incubation period of fifteen minutes.

this concept since insulin release proceeded at a higher rate than normal even in the presence of a normal glucose concentration. We tested this assumption by incubating pancreatic tissue from normal and tumor-bearing rats in the absence of glucose. The negligible amounts of insulin recovered in the incubation media were not different in the two groups. The alternative, that the insular changes could be a reflection of the metabolic effect of GH on peripheral tissues, is not supported by our results unless some humoral factor (other than glucose) acts on the islets, a proposal too speculative at the present time.

Regardless of the exact mechanism of the GH effect, it is unlikely that the changes observed were a nonspecific response to variations of food intake. Aware of the importance of the nutritional state of the animal on the morphology and function of the islets,[33-35] we conducted this study under strict nutritional control. In spite of the identical food intake the tumor-bearing rats grew more than the controls, suggesting an increased food utilization brought about by GH.

None of the animals in the present series became diabetic. After removal of the tumor most parameters returned to normal within a period of four to eight weeks. The observation that in the group of animals with the tumor removed a low insulin response was obtained after a glucose load merits further investigation. Preliminary observations indicate a decreased insulin content per islet in animals that have carried the tumor for longer periods than those reported here. Probably the functional reserves of the islets of the rat are higher than in other species, and the islets must be exposed to high GH concentrations for longer periods to produce irreversible beta cell degeneration. Bates et al.[17] achieved a similar effect with partial pancreatectomy. We are now attempting in our laboratory to induce permanent damage of the islets in intact rats using successive reinoculations of the tumor.

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Plasma Growth Hormone in Childhood

Two recent articles record experiences with the use of insulin-induced hypoglycemia in evaluating children with disturbances of growth. A. W. Root, R. L. Rosenfield, A. M. Bongiovanni, and W. R. Eberlein (Pediatrics 39:844, 1967) report plasma growth hormone levels, as measured by the radioimmunoassay technic, in sixty-three children. S. L. Kaplan et al., (Pediatric Res. 2:43, 1968) present a similar study of 134 children.

After an overnight fast, insulin in a dose of 0.1 unit per kilogram body weight is given intravenously, and blood samples taken at frequent intervals during the next two hours. In twenty normal children, four to sixteen years of age, the fasting level of growth hormone ranged from less than one to 15 ng. per milliliter of plasma; eleven had levels below 5 ng. and four had levels above 10 ng. In this connection, it must be remembered that the trauma involved in giving the insulin and in taking the blood samples may in itself constitute a stress sufficient to provoke growth hormone release. This can be offered as a possible reason for the observed variation in fasting levels.

The maximum level of plasma growth hormone following insulin administration exceeded 4 ng. per milliliter in nineteen of the twenty normal children, and the twentieth child had a high fasting level. The maximum value for those who had low fasting levels ranged from 7.1 to 18.5 ng. (average 9.9); the highest recorded level was twenty-eight in a child whose fasting level was 11 ng. In adults tested in similar manner the maximum level often exceeds 30 ng. per milliliter (Roth et al., loc. cit.; F. C. Greenwood, J. Landon, and T. C. B. Stamp, J. Clin. Invest. *45*:429, 1966), although the fasting levels are comparable to those in children. This difference suggests, then, either that a child's mechanism for release of growth hormone is quantitatively different from that of an adult or that the rate of peripheral utilization or degradation of the hormone differs. However, as Kaplan et al. and Root et al. point out, there are no definitive data with which to answer this intriguing question.

The combined series included 70 children with hypopituitarism, either idiopathic or secondary to an intracranial lesion. Many showed other manifestations of pituitary dysfunction in addition to growth failure. With

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