Biological Properties of a Transplantable Islet-cell Tumor of the Golden Hamster

II. Insulin Content of the Tumor and Some Metabolic Characteristics of the Tumor-bearing Animals

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SUMMARY

Hamsters bearing a transplantable pancreatic tumor developed marked hypoglycemia upon fasting. Glucose assimilation constant and fasting plasma insulin levels were statistically higher in these animals than in the controls. The insulin content of the tumor (expressed in "human-insulin equivalent") was 0.072 ± 0.014 U./gm. wet weight. Insulin could be extracted with acid-alcohol and inactivated with anti-insulin serum or cysteine. The possible significance of these findings is discussed. DIABETES *16*:415-17, June, 1967.

In the first paper of this series, Grillo et al.[1] described some histological and histochemical characteristics of a transplantable pancreatic tumor obtained by Kirkman[2] from a golden hamster. This paper describes the results of some biochemical and metabolic studies performed on tumor-bearing animals.

MATERIALS AND METHODS

Inbred golden hamsters (Rega Institute, Louvain) were kept in a temperature-controlled animal room (23 ± 0.5° C.), and fed a standard diet ad libitum. The tumor was transplanted subcutaneously as described by Grillo et al.,[1] and the animals were studied between the tenth and twelfth week after transplantation. The effects of fasting were studied in animals kept in individual metabolic cages without food for thirty-six hours. Water was given ad libitum. Blood samples were taken from the cavernous sinus[3] every twelve hours. Intravenous glucose tolerance tests (GTT) were performed under light sodium pentobarbital anesthesia in animals fasted for sixteen to twenty-four hours. The jugular vein was catheterized, and glucose was injected rapidly (0.5 gm./10 ml. of water per kg.). Blood samples were obtained every five minutes for thirty to forty minutes. Sugar was determined according to Huggett and Nixon,[4] and the glucose assimilation constant (k) was calculated as well as determined graphically.[5]

Tumor extracts. Pooled tumor tissue was homogenized in two volumes of acid-ethanol (absolute ethanol 750 ml., 12 N HCl 15 ml., distilled water to 1,000 ml.). After centrifugation at 4,000 rpm for three hours, the supernatant was recovered, dialyzed overnight at 4° C. and lyophilized. The dried lyophilized extract was dissolved in 0.001 N HCl (10 mg./ml.) and the solution injected intravenously into normal hamsters (2 ml./animal). In some experiments extracts treated either with cysteine, glycine or trypsin[6] were used.

Insulin content and characterization in the tumor. Acid ethanol extracts of small nonhemorrhagic fragments of the tumor were diluted 1 to 100 in phosphate buffer (0.1 M, *p*H 7.5) and tested for insulin using a modification of the radioimmunologic technic of Hales and Randle.[7]* In some experiments, insulin determinations were performed on lyophilized extract prepared as described above and, on this material, comparisons were made between the results of the radioimmunological (IRI) and biological (ILA)[8] technics for insulin determination. The influence of anti-insulin se-

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*Human insulin is used as standard in the immunoassay. The results must, therefore, be understood as "human-insulin equivalents." The validity of the procedure has been established by the parallelism of the dilution curves of human insulin and of hamster tumor extracts.

rum (AIS) on the ILA activity of lyophilized tumor extracts was studied, using the method of Froesch et al.[9] The cysteine-, glycine- and trypsin-treated extracts were also tested for insulin.

Insulin content of plasma. Plasma IRI levels were determined in plasma samples diluted 1:3 or 1:5.[7]

RESULTS

As shown in figure 1, the mean blood sugar of normal animals remained fairly constant during the thirty-six-hour fasting period. On the other hand, hypoglycemia developed rapidly in the tumor-bearing animals, reaching an average blood sugar level of 15 mg. per 100 ml. after thirty-six hours. In some fasted animals no blood glucose could be detected. The glucose assimilation constant was higher in the hypoglycemic animals than in the normal hamsters (table 1). The intravenous injection of tumor extract into a normal hamster caused a marked fall in blood sugar (figure 2). The hypoglycemic activity of the extract was destroyed by cysteine, but not by glycine treatment. The trypsin-treated extract was not tolerated by the animals, who died during the course of the injection. The average insulin content of acid-ethanol extracts of portions of nine tumors obtained from different animals was 0.072 U./gm. wet weight ± 0.014 (S.E.). The average IRI and ILA contents of lyophilized material were practically identical: 1.012 and 1.015 U./gm. respectively.* The non-AIS-suppressible ILA in this material was less than 16 per cent. After treatment with glycine, cysteine or trypsin, the IRI was 40.6 per cent, 1.21 per cent and 0.13 per cent of their original values, respectively. After a twenty-four-hour fast, the average plasma IRI was 92 μU. eq./ml. ± 11 (S.E.: fifteen determinations) in normal animals and 145 μU. eq./ml. ± 18 (S.E.: twelve determinations) in tumor-bearing animals. The difference is statistically significant ($t = 2.47$; $p = 0.02$). One IRI value of 650 μU. eq./ml. was found in one animal and verified twice, but was not used in the above calculations.

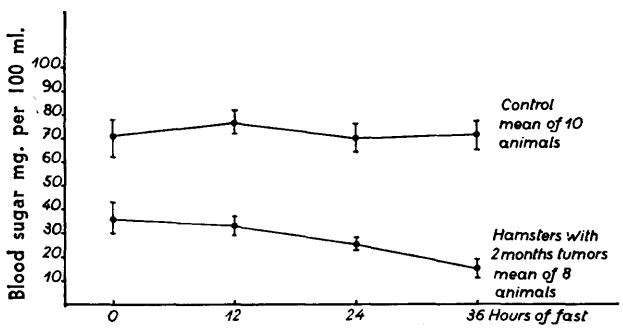

FIG. 1. Mean blood sugar ± S.E. during a thirty-six-hour fast in normal and tumor-bearing hamsters.

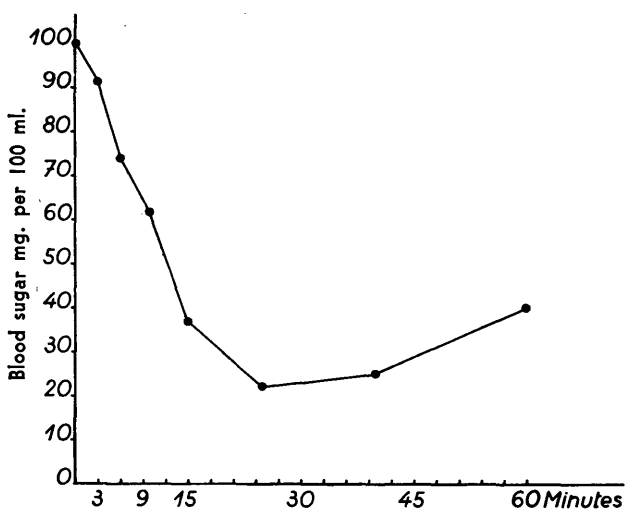

FIG. 2. Typical hypoglycemia induced in a normal hamster by the intravenous injection of 20 mg. of lyophilized tumor extract dissolved in 2 ml. of 0.001 N HCl.

TABLE 1

Intravenous glucose tolerance test (k values [$\times 10^{-2}$])

	Control hamsters (12)		Tumor-bearing hamsters (2-mo.-old transplants) (10)	
	Graphically determined	Calculated	Graphically determined	Calculated
Mean±S.E.	1.35±0.21	1.36±0.22	2.65±0.41*	2.62±0.42*

*$p < 0.01$

DISCUSSION

The results described herein suggest that the transplantable hamster tumor contains a hypoglycemic substance with some of the chemical, biologic and immunologic properties of insulin. The concentration of this substance in the tumor, expressed as human insulin equivalent, is 0.072 U./gm. wet weight, a value much lower than the ILA concentration found in normal hamster pancreas using the epididymal fat pad.[10] Although these data cannot be compared directly, it seems likely that the tumor tissue either produces insulin at a relatively low rate or cannot store it or both. Nevertheless, since the tumor may reach a very large

*These values cannot be compared on the basis of tissue water content as the lyophilized powder was not obtained from whole tissue, but from supernatant of acid-alcohol extracts.

size compared to that of a normal hamster pancreas (20 gm. or more vs. about 100 mg.), the total amount of insulin available to the tumor-bearing animal may be significantly higher than normal. Indeed, the mean fasting plasma insulin was statistically increased in the tumor-bearing animals, reaching very high values in some instances. It should be pointed out that the insulin released by the tumor is secreted into the general circulation and collected by the inferior vena cava, reaching the peripheral tissues before entering the liver. This abnormal situation, completely different from that of pancreatic tumors in situ, may increase the peripheral utilization of glucose in tumor-bearing animals and decrease their tolerance to fasting. In addition, the persistently high plasma insulin levels in the fasting state may decrease hepatic glucose output. Under these circumstances, the animals must depend upon their food intake for the maintenance of a normal blood sugar concentration and profound hypoglycemia is the unavoidable result of fasting.

REFERENCES

[1] Grillo, T. A. I., Whitty, A. J., Kirkman, H., Foà, P. P., and Kobernick, S. D.: Biological properties of a transplantable islet-cell tumor of the golden hamster. I. Histology and histochemistry. Diabetes *16*:409-14, June, 1967.

[2] Kirkman, H.: A preliminary report concerning tumors observed in Syrian hamsters. Stanford Med. Bull. *20*:163, 1962.

[3] Lapeyrac, F.: Le prélèvement du sang dans le sinus caverneux. Rev. Franç. Etud. Clin. Biol. *8*:195, 1963.

[4] Huggett, A., and Nixon, D. A.: Enzymic determination of blood glucose. Biochem. J. *66*:12p, 1957.

[5] Conard, V.: Mesure de l'Assimilation du Glucose. Brussels, Acta Medica Belgica, 1955.

[6] Unger, R. H., Lochner, J. de V., and Eisentraut, A. M.: Identification of insulin and glucagon in a bronchogenic metastasis. J. Clin. Endocr. *24*:823, 1964.

[7] Hales, C. N., and Randle, P. J.: Immunoassay of insulin with insulin-antibody precipitate. Biochem. J. *88*:137, 1963.

[8] Renold, A. E., Martin, D. B., Dagenais, Y. M., Steinke, J., Nickerson, R. J., and Sheps, M. C.: Measurement of small quantities of insulin-like activity using rat adipose tissue. I. A proposed procedure. J. Clin. Invest. *39*:1487, 1960.

[9] Froesch, E. R., Bürgi, H., Ramseier, E. B., Bally, P., and Labhart, A.: Antibody-suppressible and nonsuppressible insulin-like activities in human serum and their physiologic significance. An insulin assay with adipose tissue of increased precision and specificity. J. Clin. Invest. *42*:1816, 1963.

[10] Macchi, I. A., Beaser, S. B., and Sak, M. F.: Tentative identification of insulin from hamster pancreatic homografts and pancreas of other vertebrates. Endocrinology *78*:873, 1966.

High Resolution Screening of Aminoacidurias

While specific screening tests have been developed for some of the aminoacidurias, such as phenylketonuria, it has been difficult to study large population groups in relation to possible abnormal amino acid excretion. The type of test used for phenylketonuria, utilizing *Bacillus subtilis,* allows for the study of large numbers of infants in a one-day period (Nutrition Reviews 22:196, 1964).

Recently, it has been suggested by S. Samuels (Arch. Neurol. *10*:322, 1964) that the use of high voltage paper electrophoresis combined with a rapid chromatographic solvent can give good resolution of amino acid compounds found in tissue or urine specimens. Samples containing from 1 to 10 μg. of the component amino acids can be spotted on a paper 2 cm. apart, utilizing a paper 46 by 57 cm. Twenty samples can be run simultaneously using a current of 3,000 volts. One hour gives optimum migration.

A variation is also suggested by the author in which the initial separation is expanded by the use of a rapid chromatographic solvent procedure using a sixteen-hour separation with a solvent system of n-propanol-methylethylketone—formic acid. Separated amino acids can be located using a ninhydrin solution. Using standards of normal urine plus urine samples from phenylketonuric patients and from individuals with maple syrup disease, several satisfactory qualitative separations are reported.

Following the initial screening evaluation of certain amino acids can be carried out. The use of this system could make possible large-scale screening of patients in a reasonable length of time where other methods, such as complete amino acid analysis, would require considerable time and expense. It is pointed out that, in some cases, rapid desalting using some solvent extraction has been necessary; but, for most samples, separation sufficient to distinguish the general amino acid pattern can be carried out in approximately one hour.

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