Blood Glucose and Liver Glycogen Following Intravenous Injection of Amylase.* (30266)

NATHAN HIATT (Introduced by A. L. Sellers)
Department of Surgery, and Institute for Medical Research, Cedars-Sinai Medical Center, Los Angeles, Calif.

In a previous investigation in this laboratory, it was observed that intravenously administered amylase is rapidly cleared from the serum of dogs by a means other than urinary excretion. It was found that as the level of serum amylase falls there is a sharp rise in amylase activity of the liver (Fig. 1). Amylase is a powerful glycogenolytic agent. If the observed increase in amylase activity of the liver is due to deposition of the injected enzyme in the parenchymal cells, the glycogen content of liver might be affected.

In the present study, it will be shown that liver glycogen falls following intravenous injection of hog amylase.

Method. Eleven dogs weighing between 10 and 20 kg and 6 pigs weighing between 10 and 13 kg were used in these experiments. Hyperamylasemia was produced in both species by intravenous injection of 2000 units of crystalline hog amylase (Worthington) per kilo body weight. Pigs were used in order to determine whether any observed effects could be reproduced in animals injected with homologous amylase.

The dogs were anesthetized with intravenous pentobarbital after obtaining a specimen of venous blood for determination of the levels of amylase and glucose. The pigs were anesthetized with propiopromazine hydrochloride (Tranvet) and pentobarbital, administered by the intraperitoneal route. The external jugular veins were then exposed and a polyethylene catheter placed in each, one for administration of fluids and the crystalline hog amylase, the other for withdrawal of blood specimens. The first venous blood samples for determination of the levels of serum amylase and blood glucose were drawn at the time of insertion of the catheters.

After induction of anesthesia, the abdomen of each animal was opened and a specimen of liver taken for determination of its amylase activity and glycogen content. The amylase was then injected intravenously over a period of 2-3 minutes into 7 of the dogs and 4 of the pigs. A venous blood sample for determination of the serum amylase and blood glucose was drawn 5-10 minutes after completion of the injection and, thereafter, at half-hour or hourly intervals for 5-6 hours. Simultaneously, specimens of liver were taken for determination of the amylase activity. The glycogen content was also determined on the final specimen of liver. The animals received only physiological saline solution intravenously during the experimental period and most were given less than 1000 ml. All the urine excreted during the experimental period was collected by catheter or cystostomy, the volume was measured and an aliquot was taken for amylase determination.

The control animals, 4 dogs and 2 pigs, were treated exactly like the experimental

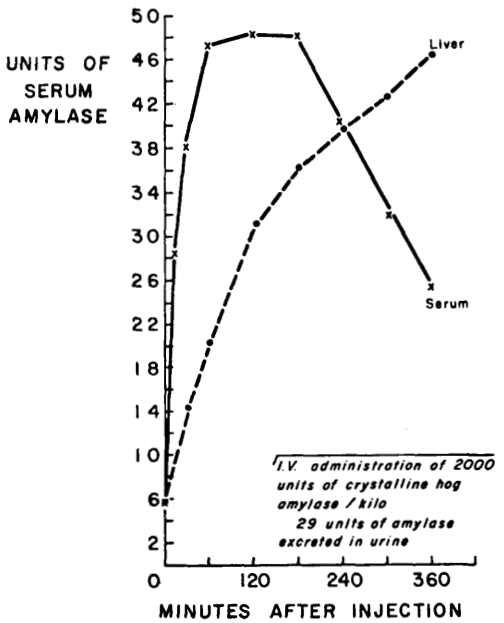

FIG. 1. Typical clearance of amylase in dogs.

* Supported by Grant AM 3672-05A1, USPHS, Nat. Inst. Health.

TABLE I. Dog—Intravenous Injection of 2000 Units Crystalline Hog Amylase per Kilo.

Time	Blood		Liver	
	Serum amylase	Blood glucose*	Amylase activity	Glycogen*
Pre-inj	6.0 (4.9– 7.0)	91 (73–112)	3.3 (2.6– 4.6)	89 (68–122)
5	20.8 (12.6–31.7)	— —	— —	
30	26.3 (17.9–36.5)	100 (84–114)	— —	
60	31.3 (23.7–39.6)	102 (77–127)	14.9 (12.8–16.4)	
120	36.0 (28.1–45.6)	116 (84–144)	19.3 (16.9–20.4)	
180	39.0 (26.2–44.7)	123 (94–160)	26.3 (22.2–28.2)	
240	35.9 (21.3–40.7)	135 (98–192)	31.9 (28.1–35.2)	
300	28.8 (19.1–36.1)	131 (101–170)	35.6 (25.4–39.4)	
360	21.8 (16.6–27.3)	140 (97–206)	36.2 (21.7–41.1)	58 (36– 79)

Mean values—pre-injection and minutes post injection; serum amylase—units per ml; blood sugar—mg per 100 ml; liver amylase—units per g; liver glycogen—mg per g; values—arithmetic mean and range.

* $P < .005$ when compared to controls.

TABLE II. Pig—Intravenous Injection of 2000 Units Crystalline Hog Amylase per Kilo.

Time	Blood		Liver	
	Serum amylase	Blood glucose*	Amylase activity	Glycogen*
Pre-inj	8.9 (8.3– 9.6)	96 (84–108)	6.8 (6.3– 7.3)	65 (58–71)
5	22.3 (19.8–24.6)	— —	— —	
30	27.0 (26.1–28.0)	107 (104–110)	— —	
60	32.3 (30.3–34.3)	111 (104–118)	20.6 (20.6–20.7)	
120	37.6 (35.6–39.6)	116 (108–126)	27.3 (26.3–28.4)	
180	42.0 (41.2–42.7)	120 (119–132)	37.0 (36.6–37.3)	
240	33.4 (33.1–33.6)	129 (123–138)	40.2 (40.1–40.3)	
300	25.4 (24.2–26.7)	132 (120–140)	37.5 (36.9–38.1)	
360	22.4 (21.3–23.6)	127 (120–134)	30.0 (29.4–30.6)	42 (35–49)

Mean values—pre-injection and minutes post injection; serum amylase—units per ml; blood sugar—mg per 100 ml; liver amylase—units per g; liver glycogen—mg per g; values—arithmetic mean and range.

* $P < .005$ when compared to controls.

animals except that they were not given amylase intravenously.

Amylase levels of the serum and urine were determined by the method of Van Loon (1). Blood glucose was determined by the Glucostat method(2). Liver glycogen was determined by a modification of the method of Good *et al*(3). The procedure for determining amylase activity of the liver is that of McGeachin *et al*(4).

Results. In both the dog and pig, intravenous injection of 2000 units per kilo of crystalline hog amylase results in similar changes in the blood and liver. As previously observed, the level of the serum amylase rises sharply following the intravenous injection and then falls, more gradually. Simultaneously, the amylase activity of the liver increases steadily. In addition, the level of the blood glucose rises and there is a significant fall in glycogen content of the liver during the experimental period (Tables I, II). Less than 10% of the amylase appears in the urine.

Control animals show no significant changes in levels of serum amylase or blood glucose; nor does the amylase activity of glycogen content of the liver change significantly (Tables III, IV).

Discussion. Dogs, unlike humans, have a high level of amylase in the serum and a very low one in the urine(5). The function of this high level of serum amylase is not known. It has been proposed that it is one of the sources of the salivary amylase(6), but dogs have no amylase in the saliva or salivary glands(7). In rats, an animal whose liver is capable of synthetizing amylase, the amylase appears to have some function in the metabolism of carbohydrates(8).

In dogs, the liver apparently does not synthesize amylase(9). But the association of serum amylase clearance with an increase in blood glucose and a fall of liver glycogen

TABLE III. Dog—Control.

Time	Blood		Liver	
	Serum amylase	Blood glucose	Amylase activity	Glycogen
Pre-inj	5.8 (4.3–6.8)	98 (94–102)	3.9 (2.9–4.4)	106 (96–112)
30	5.8 (4.4–6.6)	97 (96– 98)	4.0 (3.2–4.4)	
60	5.9 (4.4–6.6)	93 (92– 95)	4.1 (3.2–4.4)	
120	5.9 (4.3–6.6)	93 (90– 96)	4.1 (3.2–4.2)	
180	6.0 (4.3–6.8)	96 (93– 99)	4.0 (3.2–4.2)	
240	5.9 (4.3–6.8)	92 (90– 96)	4.0 (3.2–4.2)	
300	6.0 (4.4–6.6)	88 (86– 94)		
360	5.8 (4.3–6.8)	91 (89– 95)	4.3 (3.8–4.6)	98 (88–103)

Mean values—pre-injection and minutes post injection; serum amylase—units per ml; blood sugar—mg per 100 ml; liver amylase—units per g; liver glycogen—mg per g; values—arithmetic mean and range.

TABLE IV. Pig—Control.

Time	Blood		Liver	
	Serum amylase	Blood glucose	Amylase activity	Glycogen
Pre-inj	9.8 (8.3–11.2)	104 (94–114)	6.5 (4.9–8.1)	68 (63–73)
30	9.8 (8.9–10.6)	104 (90–118)	—	
60	9.9 (8.9–11.0)	105 (96–114)	6.4 (5.2–7.6)	
120	9.8 (9.2–10.3)	108 (98–117)	6.1 (4.6–7.6)	
180	9.8 (9.0–10.6)	99 (90–109)	5.6 (4.4–6.9)	
240	9.3 (8.6–10.1)	90 (90)	6.0 (4.1–8.0)	
300	10.0 (10.0)	110 (110)	6.5 (6.5)	
360	9.1 (8.8– 9.4)	102 (92–113)	5.8 (4.8–6.8)	71 (70–72)

Mean values—pre-injection and minutes post injection; serum amylase—units per ml; blood sugar—mg per 100 ml; liver amylase—units per g; liver glycogen—mg per g; values—arithmetic mean and range.

as is demonstrated in this investigation, strongly indicates that this animal can deposit intravenously injected amylase in the parenchymal cells of the liver. Thus, the present experiment suggests that in these animals serum amylase may be deposited in the liver and cause glycogenolysis with a rise of the blood glucose. Of course, the result of injecting a large amount of crystalline hog amylase into a dog, need be no true indication of what happens in the normal or diseased animal. The possibility that the serum amylase plays some part in the carbohydrate metabolism of the dog, by its ability to mobilize liver glycogen, is enhanced by the fact that almost identical results are obtained when a homologous amylase (crystalline hog amylase) is injected intravenously into pigs.

All the pigs given crystalline hog amylase intravenously, in this series of experiments and in others, show a fall in the level of amylase activity of the liver after the fourth hour. This may represent destruction of the amylase, and is being investigated with crystalline hog amylase labeled with radioactive iodine.

Summary. Dogs and pigs were injected intravenously with crystalline hog amylase. Clearance of the amylase from the serum was associated with a rise in the level of the blood glucose. Simultaneously, the amylase activity of the liver rose and its glycogen content fell. The results in both species of animals were almost identical.

I would like to express by appreciation to Mr. George Bonorris for chemical analyses, and to Drs. A. Sellers, J. Katz and H. Sobel for review of the manuscript. I would also like to thank Mr. Miles Coverdale and Mr. Howard Mack for invaluable technical assistance.

1. Van Loon, E. J., Likens, M. R. Segar, A. J., Am. J. Clin. Path., 1952, v22, 1134.

2. Teller, J. D., Abst. of Papers, 130th Meeting, A.C.S. September 1956.

3. Good, C. A., Kramer H., Somogyi, M., J. Biol. Chem., 1953, v100, 485.

4. McGeachin, R. L., Gleason, J. R., Adams, M. R., Arch. Biochem. Biophys., 1958, v75, 403.

5. Somogyi, M., Arch. Int. Med., 1941, v67, 665.

6. Rutter, W. J., Arnold, M., Brosemer, R. W., Miller, J. A., J. Biol. Chem., 1961, v236, 1259.

7. Mendel, L. D., Underhill, F. P., ibid., 1907,

v3, 135.

8. Brosemer, R. W., Rutter, W. J., ibid., 1961, v236, 1253.

9. Flock, E. V., Block, M. A., Bollman, V. L., Mann, F. L., Am. J. Physiol., 1952, v170, 467.

Received March 8, 1965. P.S.E.B.M., 1965, v119.

In vitro Formation of Free Iodotyrosines and Iodothyronines by Rat Thyroid Lobes or Isolated Follicular Cells.* (30267)

SHIN-ICHI SHIMODA† AND MONTE A. GREER

Division of Endocrinology, Department of Medicine, University of Oregon, Medical School, Portland

Roche *et al*(1) have reported that free thyroid hormones can be detected in the medium 18 to 24 hours after addition of radioactive iodine to tissue cultures of young rat thyroid tissue. Kerkof *et al*(2) have also noted that isolated sheep thyroid follicle cells in monolayer tissue culture can release free amino acids, including thyroxine, into the culture medium after prolonged incubation with radioiodine. However, no studies have been reported on the formation and liberation of free iodinated amino acids during short-term *in vitro* incubations of thyroid tissue.

In studies of iodine metabolism of rat thyroid lobes and isolated beef thyroid cells during short-term *in vitro* incubations we have found that free iodothyronines can be detected both in the medium and in the thyroid tissue itself.

Materials and methods. 1. Rat thyroids. Male Sprague-Dawley rats weighing 150-300 g were fed a low iodine diet (LID) containing 30 μg ^{127}I/kg for up to 60 days before autopsy. Two groups were additionally fed 0.15% propylthiouracil (PTU) in the low iodine diet for 10 days. One PTU group was killed at the end of this period; the other was fed LID without PTU for 6 days after autopsy (PTU-withdrawal). At autopsy the thyroid lobes were carefully removed and individually incubated in 50 ml Erlenmeyer flasks containing 4 ml of Earle's balanced salt solution containing 25 μc carrier-free $^{131}I^-$ at 37°C in a shaking water bath. Room air was used as the gas phase. One lobe from each rat was incubated 3 hours and the opposite lobe was incubated 6 hours. At least 2 lobes from different animals were used for the analysis at each time interval. The lobes from animals fed propylthiouracil until autopsy were pre-incubated in 2 fresh changes of Earle's solution for 15 minutes each before being placed in the radioiodine solution.

2. Isolated beef thyroid follicle cells. The isolated thyroid cells were prepared by the method of Tong(3). Approximately 20 μl of cells were suspended in 3 ml of Earle's solution containing 100 μc $^{131}I^-$. Incubations were carried out as with the rat thyroid lobes for a period of 3 hours. At the end of incubation the cells were separated from the medium by centrifugation at 50 *g* for 10 minutes.

3. Analysis of thyroid tissue and media. After separation from the media, the thyroid tissue was homogenized in 1 ml of pH 8.5 phosphate buffer containing 100 μg thiouracil. Chromatography of the homogenates and of the medium before and after digestion with 1% pancreatin for 20 hours was performed in both n-butanol-absolute alcohol-0.5% ammonia (5:1:2) and in butanol-acetic acid-water (4:1:5). The identification of radioactive components was determined by comparing their location with suitable standards added to the chromatographed material at time of application to the paper. The amount of radioactivity corresponding to each iodinated material was determined with a windowless gas-flow strip scanner and quantified by planimetry.

Results. As documented in Table I, no organic radioiodine-labeled materials were found in the medium at the end of 3 hours incubation in any of the groups fed a low

* Supported by Research Grants from USPHS.

† Postdoctoral Trainee in Endocrinology of NIAMD.