MtTW5 tumors are chronically exposed to large amounts of prolactin, their thyroid inhibition seems of a different nature than the one induced by prolactin in the frogs because the histology of the gland and the response to TSH are different.

In humans with acromegaly, an increased clearance of iodine, a decreased ^{131}I uptake by the gland and slightly decreased thyroid hormone formation has been reported (17). The possibility of increased renal clearance of iodine decreasing the share of iodide trapped by the thyroid in the MtTW5-implanted rats has been excluded, as the thyroid inhibition persists in nephrectomized tumor-bearing rats compared to nephrectomized controls (1).

Summary. Implantation of rats with growth hormone- and prolactin-secreting pituitary tumors cause weight reduction of the host's pituitary gland. This change was accompanied by a decrease in the pituitary content of TSH and a fall in ^{131}I uptake by the thyroid gland in rats with prolactin- and growth hormone-secreting pituitary tumor MtTW5. These results correlated well with the finding that the proportion of organified iodine and the intrathyroid to serum iodine ratio, in the presence of high concentrations of stable iodine, were higher in the tumor bearing rats. The amount of circulating free thyroxine was measured and found to be slightly lower in tumor-bearing rats. It is suggested that the pituitary tumor hormones have a direct suppressive effect on the host's pituitary gland production of TSH and thus indirectly decrease thyroid function.

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Hepatic Deposition of ^{131}I Labeled Amylase in Dogs: Comparison of Enzymatic and Isotope Measurements* (32959)

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When ^{131}I labeled amylase (α-1, 4-glucan 4-glycanohydrolase) preparations are injected into dogs, the enzyme is first rapidly bound to blood cells and then slowly released to the plasma. As a result, it takes about 3 hours after injection for the plasma amylase-^{131}I to reach maximum values. After these are attained, the plasma concentration of the labeled enzyme declines (1). It has been found

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that the injection of unlabeled amylase is followed by an increase of amylase activity in the liver, hepatic glycogenolysis and hyperglycemia; and that there are no such responses in animals with Thorotrast blockade of the reticuloendothelial system (RES) (2,3).

In the present study, we were interested in comparing the serum and tissue distribution of amylase after ^{131}I labeled enzyme was injected into dogs which had either an intact or thorium dioxide blockaded RES. Both isotopic and enzymatic measurements were employed because iodometric modification of enzymatic procedures has been reported to produce unreliable results when applied to tissue analyses (4). This comparison of assay methods was also important in determining if the labeled enzyme was handled in the same way as the unlabeled amylase. In addition, the effects of injected ^{131}I amylase on tissue glycogen and plasma glucose were also examined. The results indicate that labeled and unlabeled enzymes are treated in a similar manner and that there is an exclusive deposition of injected amylase in the liver which is accompanied by hepatic glycogenolysis and plasma hyperglycemia.

Methods. Each of 15 mongrel dogs (9–13 kg) was fasted for 24 hours before an experiment. In 6 animals, the RES was blockaded by the intravenous injection of 3 ml of 25% ThO_2 (Thorotrast)/kg, 7 hours before the experiment (5). After venous blood specimens were obtained for plasma glucose and amylase analyses, the animals were anesthetized with sodium pentobarbital (30 mg/kg i.v.). The abdomen was then opened and small biopsies of the rectus abdominis muscle and liver were taken for amylase and glycogen assay. Two thousand enzyme units of a hog amylase preparation (approx. 0.6 mg) labeled with ^{131}I (0.012–0.014 μC/enzyme unit) were then rapidly injected intravenously. The crystalline amylase preparations (Worthington Biochemical Corp.) had been labeled by the method of Schramm (6) and 1 μC of ^{131}I assayed about 300,000 cpm with a background of 10 cpm (1). After injection of amylase-^{131}I, venous blood, urine, and biopsy specimens of liver and other tissues (see later) were obtained for analysis at intervals up to 6 hours. The animals received about 500 ml of saline during the experimental period. As controls, 2 untreated dogs and 2 dogs whose RES was blockaded received saline intravenously instead of labeled amylase.

Amylase was extracted from tissues by a modification of the method of McGeachin *et al.* (7). The biopsy specimens were weighed immediately after excision and 1 ml of 0.15 *M* NaCl/100 mg of tissue was added. This was then homogenized and clarified by centrifugation at 5°C. The sediment was then resuspended in half its original volume of 0.15 *M* NaCl, homogenized, and centrifuged again, and the combined supernatants were assayed. For the measurement of the enzymatic activity of amylase in tissue extracts, serum, and urine the method of Van Loon *et al.* was used (8). These specimens were also assayed for amylase-^{131}I concentration by an isotopic procedure previously described and calculated from trichloroacetic acid precipitable protein counts and specific activity (1). This permitted the expression of ^{131}I labeled amylase counts in terms of enzyme units (1). Tissue glycogen was extracted by the method of Good *et al.* (9) and quantitated by the anthrone method (10). Glucose was determined by a glucose–oxidase procedure (11).

Results. In all of the untreated dogs (those with an intact RES) injection of ^{131}I labeled amylase resulted in a rise of the plasma amylase to a maximum at 3 hours, followed by a gradual fall (Fig. 1). This is due to the rapid binding of the enzyme to blood cells and subsequent slow release to the plasma (1). There was a parallel correspondence between the enzymatic and isotopic amylase assay value; the difference between them was due to the endogenous plasma amylase measured by the former and not by the latter assay method. The amylase lost in the urine during this 6-hour experimental period was negligible (less than 100 units) by both assay procedures. Figure 2 shows that the liver amylase rose steadily after the injection of labeled enzyme to a concentration over 1.5 times greater than the corresponding increase in the plasma value and almost 13 times more than its preinjection value. Again, the correspondence between the enzymatic and isotop-

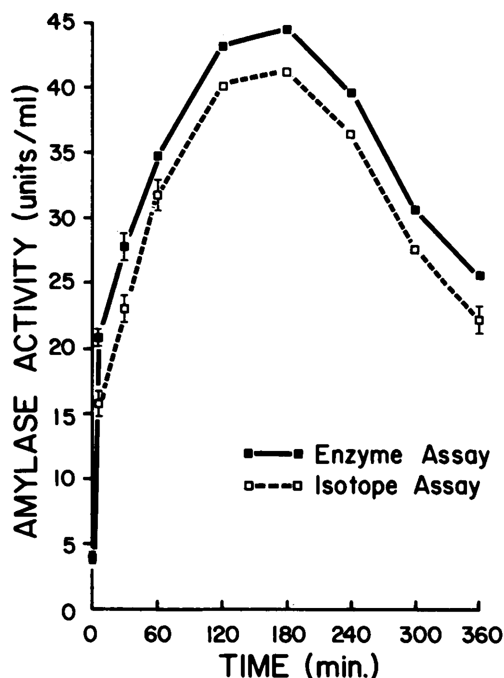

FIG. 1. Plasma amylase following the intravenous injection of ^{131}I labeled amylase into dogs. Vertical bars indicate $\pm$ 1 SE of the mean (not calculated where range was less than 10% of the mean); $N =$ 9.

ic procedures is reasonably good taking into consideration the endogenous liver amylase. With respect to other tissues, these showed a rise that was not significant when the elevated amylase level of the entrapped serum was taken into account. Thus the amylase concentration of the rectus abdominis muscle rose by 1 unit/gm, the calculated amount for a tissue with 3–5% of serum by weight (Table I, Column C). Similarly, no significant increase in isotopic or enzymatic activity could be found in other tissues and fluid examined. These included the spleen, lung, kidney, femoral bone marrow, intestinal lymph nodes, and thoracic duct lymph. The thyroid contained a considerable number of ^{131}I counts above background but almost all were nonprotein and there was no appreciable increase of enzymatically measurable amylase. The pancreas had such a high amylase content that small enzymatic changes were difficult to detect, but on isotope assay there was no significant increment of amylase.

Figure 3 shows that in RES blockaded dogs injected with labeled enzyme there is also a parallel correspondence between enzymatic and isotope assay values for the plasma and liver amylase. However, the results were different from those observed in untreated animals (Figs. 1 and 2). The serum amylase reached its maximum in just 1 hour and the liver amylase rose to only 3 times the preinjection level; this corresponded to about one third the change in the serum value. Similar to the untreated dogs, the increase in the amylase content of other tissue was negligible and reflected only the increased amylase activity of entrapped serum. No significant amount of amylase was found in the urine.

In untreated dogs (postinjection) the plasma glucose rose almost 168% (Table I, Column D), the liver glycogen fell about 35% (Table I, Column A) and the muscle glycogen increased almost 37% (Table I, Column B). On the other hand, the RES blockaded dogs showed essentially no change in their liver glycogen (Table I, Column A), muscle glyco-

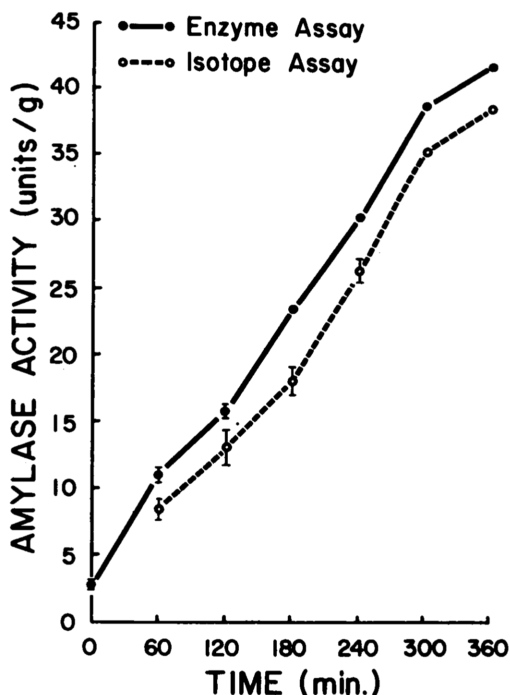

FIG. 2. Liver amylase of dogs following the intravenous injection of ^{131}I labeled amylase into dogs; $N = 9$.

TABLE I. Liver and Muscle Glycogen, Muscle Amylase, and Plasma Glucose Following the Injection of Amylase-^{131}I or Saline in Untreated and RES Blockaded Dogs.

Injection	Time (hours)	Liver (A) Glycogen (mg/gm)	Muscle (B) Glycogen (mg/gm)	Muscle (C) Amylase (units/gm[c])	Plasma (D) Glucose (mg/100 ml)
		Untreated dogs			
Amylase-^{131}I (9)[a]	0	83.6 ± 2.1[b]	6.6 ± 0.51	0.6 ± 0.1	88 ± 4.2
	6	53.8 ± 1.6	9.1 ± 0.8	1.6 ± 0.1	148 ± 4.8
Saline (2)	0	96 (94–98)[d]	8.6 (8.0–9.2)	0.4 (0–0.8)	92 (90–94)
	6	91 (89–94)	7.3 (6.3–8.3)	0.2 (0–0.4)	90
		RES blockaded dogs			
Amylase-^{131}I (6)	0	88 ± 0.83	6.5 ± 0.29	0.6 ± 0.1	87 ± 4.6
	6	88 ± 1.30	7.5 ± 0.58	1.8 ± 0.2	95 ± 3.8
Saline (2)	0	83 (79–87)	7.6 (6.9–8.2)	0.9 (0.8–1.0)	79 (78–80)
	6	88 (85–92)	8.2 (7.6–8.7)	0.6 (0.2–1.0)	88 (86–90)

[a] No. of animals in each group is in parentheses.
[b] Mean ± SE of the mean.
[c] Enzyme units calculated from isotope measurements.
[d] Range given in parentheses.

gen (Table I, Column B), or plasma glucose level (Table I, Column D) over the same experimental period. Similar measurements on control animals also demonstrated insignificant changes.

Discussion. Data presented in Figs. 1 and 2 show that the majority of the isotopically labeled amylase injected into dogs is deposited in the liver. All other tissues examined were devoid of a significant increase of amylase activity. If one calculates the relative distribution of isotope based on the liver weight of the dog (approx. 34 gm/k) (12) and its average plasma volume (38 ml/k) (1), almost all of the administered amylase-^{131}I can be accounted for in these two body compartments during the 6-hour experimental period. The results are essentially the same when unlabeled amylase is injected (2).

As noted earlier, the differences between the enzymatic and isotope assay presented in Figs. 1 and 2 are probably due to endogenous plasma and liver amylase. Plasma entrapped in the liver is approximately 10% of the weight of that organ and probably contributes a corresponding percentage of the "endogenous" liver amylase.

With respect to the RES blockaded dogs in which the injected amylase was handled diff-

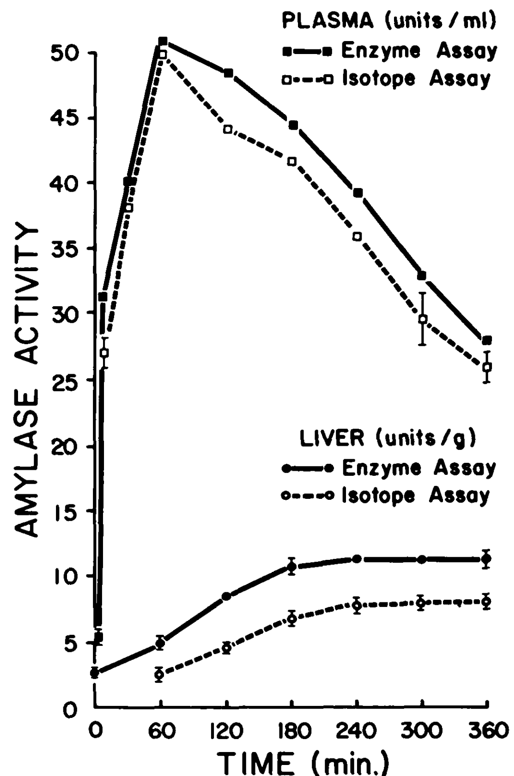

FIG. 3. Plasma and liver amylase following the intravenous injection of ^{131}I labeled amylase into dogs with Thorotrast blockade of the RES; $N = 6$.

erently (Fig. 3), the plasma and liver isotope distribution accounts for only 60% of the administered enzyme. With the exception of the relatively small amount of amylase deposited in the liver no significant isotope or enzymatic activity was detected in other tissues. Blockaded animals almost always lose moderate amounts of blood late in the experimental period because of a bleeding dyscrasia induced by ThO_2 on certain clotting factors (13). Very possibly the missing 40% of administered amylase is lost by hemorrhage. Despite the loss, the failure of liver amylase to rise is significant. For in the occasional *unblockaded* dog in which bleeding causes a comparable serum amylase deficit, the hepatic amylase rises (2). In those blockaded dogs that do not bleed, the serum amylase does not change significantly after the maximum level is reached (2).

Heterologous proteins when injected into animals are known to be deposited in the liver and kidneys (14). The deposition of labeled hog amylase in the liver of the dog (approx. 12.0×10^{-3} mg of enzyme/gm of liver) noted in these experiments does not necessarily indicate an identical nonspecific response. There is similar hepatic deposition when unlabeled *homologous* hog and canine amylases are injected into hogs (3) and dogs (2), respectively; this also occurs in dogs made hyperamylasemic by ligation of the main pancreatic duct (2). From these studies and the present work it would appear that in dogs and pigs excess serum amylase is removed by deposition in the liver. In contrast, humans apparently excrete the majority of excess amylase through the kidney.

The significance of the deposition of amylase and its effect on muscle glycogenesis (Table I, Column B), liver glycogenolysis (Table I, Column A), and plasma glucose (Table I, Column D) remains unclear at the moment. The increase of muscle glycogen following the injection of amylase is probably due to the action of insulin in the presence of hyperglycemia. This would tend to obscure any glycogenolytic activity of the exogenous enzyme if it occurred at all. The deposition of the exogenous labeled enzyme in the liver may stimulate the normal processes for the conversion of liver glycogen to glucose, a possiblity suggested by the hyperglycemia. For example, it was suggested long ago that amylase may offer an alternate pathway to the phosphorylosis mechanisms for glycogen degradation which might explain the insulin resistance in certain pancreatic and diabetic diseases of humans (15). This would seem to be a more likely possibility in dogs which, in contrast to humans, have a high serum maltase activity which would favor the production of hyperglycemia from liver glycogen breakdown. Since we did not measure the liver maltose concentrations in the present experiments, however, we have no indication that amylase per se acts on intracellular glycogen (16). Whatever the explanation, the liver glycogenolysis and plasma hyperglycemia observed in these studies were relatively minor. From the data presented, it can be calculated that 1 unit of amylase was associated with the loss of less than 1 mg of glycogen/gm of liver over the 6 hour experimental period. Amylase is 10–20 times more active on purified glycogen or on the glycogen in liver mash.

The data presented for the RES blockaded dogs suggests that exogenous amylase may be deposited in the Kupffer cell of the liver rather than in the parenchymal cells where glycogen is stored (17). Alternatively, the labeled enzyme may have become inactivated *in vivo* in the parenchymal cells due to the presence of an amylase inhibitor or the lack of intracellular chloride activation (18).

Summary. After the intravenous injection of amylase ^{131}I into dogs, the results are similar to those observed after the injection of unlabeled enzyme. With amylase ^{131}I, isotopic and enzymatic measurements indicate that the enzyme is deposited in the liver. This is accompanied by a decrease in liver glycogen, an increase in muscle glycogen, and hyperglycemia. These changes are not observed in dogs whose reticuloendothelial system (RES) is blockaded by thorium dioxide. This and other evidence suggest that the RES plays an important role in the regulation of the serum concentrations of amylase and in its deposition in the liver. The role of amylase in liver glycogenolysis remains obscure for

reasons which are discussed.

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Influence of Age and Diet on the Induction of Hexobarbital-Metabolizing Enzymes in the Mouse (32960)

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The rate of hexobarbital metabolism in the liver increases steadily from birth to adulthood (1). The microsomal enzymes responsible for such metabolism can be activated in the newborn or their activity can be increased in the adult by the administration of enzyme inducers such as phenobarbital or chlorcyclizine (2). The enzyme-inducing activity of such agents has been correlated with the synthesis of new enzyme protein in the liver (3). Factors which tend to decrease protein synthesis, such as starvation or the short-term feeding of a diet low in proteins, are known to decrease the rate of drug metabolism in the adult male rat (4). The weanling mouse maintained on a similar low protein diet, however, continues to synthesize drug-metabolizing enzymes until it reaches early maturity (5). The stimulus for protein synthesis present in such animals apparently overrides the influence of the low protein diet. Agents like phenobarbital which also stimulate protein synthesis have not been widely studied in such weanling animals. In the present study we have therefore examined the ability of such agents to induce the development of hexobarbital-metabolizing enzymes in adult and weanling mice maintained on various dietary intakes.

Materials and Methods. Adult and 21-day-old weanling CD-1 male mice obtained from the Charles River Mouse Farms, Inc. were used for this study. The induction of hexobarbital-metabolizing enzymes was promoted by the daily intraperitoneal administration of equimolar doses (0.01 ml/gm) of one of the following agents for 3 consecutive days: phenobarbital (25.4 mg/kg), chlorcyclizine (37.3 mg/kg), hexobarbital (25.8 mg/kg), antipyrine (18.8 mg/kg), metronidazole (17.2 mg/kg) or 3,4-benzpyrene (100 μg/kg). All inducers were given in H_2O except 3, 4-benzpyrene which was given in corn oil. Control groups were similarly injected with H_2O or corn oil (0.01 ml/gm). A hypnotic dose of hexobarbital (100 mg/kg) was injected 24 hours after the last injection of the inducer and the sleeping time was estimated by determination of the duration of the loss of the righting reflex.