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Note

The effects of puromycin and actinomycin D on the serum and liver amylase levels in the mouse, rabbit, and rat*

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Amylase synthesis by the rat liver has been demonstrated both in this laboratory[1,2] and that of Rutter[3]. Both laboratories have also shown that synthesis by the rat liver can be inhibited or blocked completely by puromycin[4–6]. Data from the puromycin studies indicate that the turnover of liver amylase is relatively rapid (half-life about 4 h).

This report describes the effects of actinomycin D on amylase in rats *in vivo*, on rat liver *in vitro*, and in mice *in vivo*. Effects of puromycin on amylase levels in both rabbits and mice are also described. In addition, there is a commentary on the validity of the arguments in two recent reports[7,8] that question the synthesis of amylase in liver.

EXPERIMENTAL

Male Sprague–Dawley white rats (400–450 g) used as liver donors in the isolated perfusion studies were obtained from Rolfsmeyer Laboratory. Rats used for *in vivo* studies were also Sprague–Dawley males weighing 300–350 g. Rabbits were male New Zealand whites obtained locally, all weighing about 2 kg, and the white mice were males weighing 25–30 g. Puromycin dihydrochloride was obtained from Nutritional Biochemical Corporation and the actinomycin D was a gift from Merck, Sharp and Dohme.

The liver-perfusion experiments were carried out as described previously[1,2,4]. Details of the *in vivo* experiments are given in notes immediately below each of the Tables.

Amylase was determined in plasma and liver by Van Loon's amyloclastic method[9], following procedures previously described[2,10]. The Van Loon amylase unit

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is numerically equivalent to the Somogyi unit. Liver samples were routinely frozen, kept overnight at −18°, and thawed before homogenization. Homogenates were centrifuged at 12,000*g* for 15 min in an SS-1 Sorvall centrifuge and the supernatant fluids were analyzed.

RESULTS

Although amylase has been found in the serum and livers of several species[10,11], liver-perfusion studies have been carried out only in the rat. Likewise, puromycin[4,6] has been shown to decrease liver and serum amylase only, in the rat. The data in Table I show that in mice, whose amylase levels in the serum and liver are high like those of the rat, puromycin in divided doses decreases the serum amylase by 40% and liver amylase by 60% in 4 h. These results are comparable to those found for the rat[5], where puromycin decreased serum amylase by 45% and liver amylase by 60% in 4 h.

Puromycin induced a different result in rabbits. Even though large doses of puromycin were used (200 mg in 2-kg animals), there was little change in either serum or liver amylase levels after 4 h (Table II). However, as the turnover time of serum amylase in the rabbit may be longer than that in the rat or the mouse, it is possible that in a prolonged experiment (such as 16 instead of only 4 h) a significant decrease might have been seen. On the other hand, serum and liver levels in the rabbit are an order of magnitude lower than those of the rat and mouse.

To investigate further the amylase-producing system in rat liver, the effects of actinomycin D were determined, both *in vitro* and *in vivo*. John and Miller[12] had previously shown that both actinomycin D and puromycin would inhibit the production of plasma albumin in the isolated, perfused, rat liver. Table III reveals that actinomycin D does not inhibit the synthesis of amylase by the isolated, perfused liver, even when large doses of the drug (500 μg) are used or when the donor rats are preinjected with large doses (1 mg). Actinomycin D administered to the whole animal by intraperitoneal (i.p.) injection (Table IV) produced little effect on serum or liver amylase levels in either rats or mice, even with the injection of very large

TABLE I

EFFECT OF PUROMYCIN ON SERUM AND LIVER AMYLASE LEVELS IN THE MOUSE[a]

Tissue	*Amylase levels (units/100 ml or 100 g)*			
	Controls		*Puromycin-treated*	
			One dose (4)	Divided dose (5)
	0 h	4 h	4 h	4 h
Serum	5500 ±430	5200 ±390	4000 ±210	3200 ±150
Liver	4400 ±290	4500 ±310	3000 ±170	1800 ±120

[a]Control animals were injected i.p. with 0.5 ml of 0.9% sodium chloride at time zero. Puromycin (5 mg) was injected into some animals in a single dose at time zero; other animals received the same amount in divided doses; 2 mg at time zero and 1 mg at 1, 2, and 3 h. The numbers in parentheses indicate the number of mice used in each category. All amylase levels in all Tables are given as the mean, ± standard error.

TABLE II

THE EFFECT OF PUROMYCIN ON SERUM AND LIVER AMYLASE LEVELS IN THE RABBIT[a]

Time (h)	*Control* (3)	*Puromycin-treated*	
	Serum amylase (units/100 ml)		
		I.p. (5)	I.v. (3)
0	270 ±25	340 ±30	240 ±15
1	280 ±30	340 ±25	250 ±19
2	270 ±26	350 ±24	220 ±13
3	310 ±29	350 ±31	210 ±11
4	280 ±19	350 ±27	200 ±12
	Liver amylase (units/100 g)		
4	300 ±21	210 ±20	330 ±25

[a]Control animals were injected at zero, 1, 2, and 3 h with 2 ml of 0.9% sodium chloride. Puromycin was injected into each animal either i.p. or i.v. on the following schedule: 50 mg at zero time and 50 mg at 1, 2, and 3 h. All animals were sacrificed by bleeding at 4 h. The numbers in parentheses indicate the number of animals used.

TABLE III

EFFECT OF ACTINOMYCIN D ON AMYLASE PRODUCTION IN THE ISOLATED, PERFUSED RAT LIVER[a]

Drug added to perfusing blood	*Net gain in amylase (units/g liver)*
0 (10)	38.7 ±4.1[a]
500 μg (3)	45.2 ±6.2
100 μg (4) (donor rats injected with 100 μg 4 h before sacrifice)	42.7 ±4.9
100 μg (3) (donor rats injected with 1 mg 16 h before sacrifice)	47.1 ±4.7

[a]See ref. 2. In each of these experiments, the actinomycin D was added to the perfusing blood in divided doses, 0.4 of the total dose at zero time and 0.2 portions of the dose at 1, 2, and 3 h. This was in addition to the drug injected into the donor rats prior to time of sacrifice in some groups.

TABLE IV

EFFECT OF ACTINOMYCIN D ON SERUM AND LIVER AMYLASE LEVELS IN THE RAT AND THE MOUSE[a]

Drugs administered i.p.	*Amylase levels (units/100 ml or 100 g)*	
	Serum	*Liver*
	Rats	
0 μg (5)	4490 ±300	3660 ±240
500 μg (5)	3870 ±310	3670 ±250
	Mice	
0 μg (6)	4520 ±320	3730 ±300
100 μg (10)	3590 ±290	3620 ±270

[a]In these experiments, control animals were injected with 0.9% sodium chloride. Drug-treated animals were sacrificed at the following times: rats, 16 h; mice, 8 h. The numbers in parentheses are the number of animals used in each category.

doses. In a few experiments whose results are not noted in Table IV, the animals were sacrificed at the end of 4 h instead of 8 or 16 h. Here too, no lowering of amylase levels was seen.

DISCUSSION

Our experiments (Tables I and II) on the effects of puromycin in the mouse and the rabbit seem to indicate that the mouse does produce an amylase in the liver, whereas the rabbit probably does not. As noted earlier, the effects of puromycin on amylase in the rat *in vivo*[5] are quite similar to those found in the mouse. Comparison of the normal low levels of liver and serum amylase in the rabbit with the very high levels in the mouse support this interpretation. MacKenzie and Messer[13] agreed that amylase is produced in the liver of the mouse.

Amylase produced in rat liver is secreted from the liver into the plasma quite rapidly, in somewhat the same manner as albumin[12,14]. Thus, it was thought that actinomycin D, which inhibits albumin production in the rat liver, might very well have the same effect on amylase production in the liver. Table III shows that there is no such inhibition. In any of the several combinations of administration for actinomycin D, amylase production by the isolated, perfused rat-liver was normal or perhaps even slightly elevated. Therefore the mRNA template for production of amylase must be stable for periods up to 16 h, even though the turnover rate for the amylase itself is quite rapid. The half-life of rat-liver amylase, as calculated in the earlier studies with puromycin[4], is about 4 h. In intact animals, actinomycin D also had very little effect on liver and serum amylase, even with fairly high doses of the drug. The lack of an effect of actinomycin D on the synthesis of liver proteins has been noted in other systems; thus, although the estrogen-induced synthesis of lipovitellin in *Xenopus laevis* liver was blocked, the basal rate for synthesis of soluble protein was not significantly altered[15].

Recent papers have cast doubt on the reality of rat-liver amylase[7,8], and the validity of using an amyloclastic amylase method has been questioned. We should like to comment briefly on the objections raised. It has been stated that the apparent increase in amylase activity in the plasma of the blood used in liver-perfusion experiments is an artifact arising from production and release of plasma albumin. However, when rat plasma (a 1:100 dilution with 0.9% sodium chloride) having an amylase activity of 2500 units/100 ml was heated for 1 h at 60°, with no discernible coagulation of plasma proteins, the amylase activity was completely lost. Also, a solution of bovine plasma albumin containing 7 mg/ml had no amylase activity when measured by the Van Loon method. It should be noted that consumption of iodine by proteins, in the solution being tested for amylase activity, is carefully compensated for in the Van Loon method, in which the same amount (1 ml) of the amylase solution is added to the control flask *after* addition of the iodine–fluoride mixture. Thus it seems certain that the 100% increases in plasma-amylase levels seen during liver-perfusion experiments[1,2] are not artifacts, even though the production of amylase by the liver may be very

small when compared to the pancreas and salivary glands. Puromycin has been shown in earlier studies[16] to promote glycogenolysis in the liver. Another objection suggests that the apparent synthesis of amylase by rat liver was only due to release of stored amylase from a glycogen–amylase complex. If this argument were valid, administration of puromycin, which promotes liver glycogenolysis, should lead to an increase in amylase activity. In reality, a marked decrease is seen.

It may well be, however, that the amylase produced in the liver is not immunologically distinct from pancreatic and salivary amylases of the same species, and that our earlier observations[17,18] were in error in this respect. Indeed, our later experiments using goat antisera to hog-pancreatic amylase[19] show inhibition of liver amylase. Furthermore, the rat, and perhaps the mouse and guinea pig, may produce amylase in the liver, whereas man and other species do not. Recent reports from Messer's laboratory[20] suggest that rat liver is the main source of rat-serum amylase.

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More than twenty years ago our mutual research interests brought Dexter and me (R.L.M.) together, and at the time I marveled at how much this young-looking man (with a crew-cut then) had already contributed to the field of starch and its degrading enzymes. Over the years I have valued his friendship and helpful advice and I am happy to dedicate this paper to my still youthful (with slightly longer hair now) comrade in amylase.

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