Reduction in Plasma Desoxyribonuclease Activity on Circulation through the Liver: Index of Liver Function.* (20731)

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In a recent report(1), reference was made to our observation that serum desoxyribonuclease (DNase) is regularly considerably elevated in parenchymatous liver disease but not in obstructive icterus in man. The following preliminary report is based upon studies to elucidate the role of the liver in serum DNase metabolism.

Methods. Serum DNase activity was measured at pH 7.5 (trismethylolamino-methane buffer) by our methyl green method(2). This is approximately the optimum pH for serum DNase in the rat, and no other optima are found between pH 3.0 and 9.5(3). Wistar albino rats weighing approximately 250 g (except where the weight is noted in the table) were used. All were fed a Purina Dog Chow Checkers diet and water *ad lib* up to the time of sacrificing. Under sodium pentobarbital anesthesia (approximately 3 mg/100 g; in some experiments ether alone or ether supplementation of pentobarbital was used), the abdomen was opened widely. By separating the lobes of the liver, a large hepatic vein draining the left lobe is exposed. From this hepatic vein approximately 2 ml of blood were drawn into a syringe moistened with heparin (Heparin sodium, Upjohn, 10 mg/ml). A hemostat was then applied to the portal vein close to the liver and 1.5 to 2 ml of blood were drawn into a heparinized syringe from the portal vein. The plasma was promptly separated by centrifugation, and sodium ethyl mercuri-thiosalicylate (Merthiolate, Lilly) added to a final concentration of 1:100,000. The plasma was kept frozen (−20°C) until used. One-half ml was used for the DNase analysis. In several cases blood was also collected from the abdominal aorta and similarly treated. Since the rat has no gall bladder, it is necessary to cannulate the common bile duct to collect bile. A No. 22 stainless steel cannula was used. The duct was cannulated just below the liver so as to exclude the pancreatic secretion, which enters the lower four-fifths of the bile duct(4). Approximately 1 ml of bile can be collected over a 3-hour period. The DNase activity was determined as for serum.

Several rats were given a single intraperitoneal injection of 0.05 ml carbon tetrachloride per 100 g body weight in 1 ml sesame oil 24 hours or 48 hours before sacrificing. Another group was rendered anoxic under anesthesia by respiring air bubbled through alkaline pyrogallate solution through a tracheotomy. Anoxia was maintained for several minutes after the organs, including the liver, had become intensely cyanotic. Blood was drawn during the continuation of anoxia (respiration was generally considerably depressed by this time). Due to the poor condition of the anoxic rats, portal and hepatic vein blood were not obtainable in every case. In another group, the common bile duct was ligated close to the liver, well above the entrance of the pancreatic ducts. These rats were sacrificed at intervals of 24 to 96 hours.

Results. Table I demonstrates that in the normal, fed rat, the portal vein plasma DNase activity is considerably higher than that of the hepatic vein and of the aorta. Circulation through the liver results in the extraction of 73 ± 4% of the portal vein plasma DNase.

In the normal rats, the bile demonstrated DNase activity similar to or higher than the portal vein plasma. For the 5 cases where both were examined, statistical test indicates that the portal vein and bile DNase do not differ significantly from each other.

Rats given CCl_4 24 hours before operation appeared ill. The livers were mottled and diffusely pitted. Ecchymoses occurred readily on handling. The pancreas appeared grossly

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TABLE I. DNase Activities in 16 Normal Rats.

	DNase activities in units × 10^{-4}/ml					
	Portal vein	Hepatic vein	% extraction*	Aorta	% extraction*	Bile
	600	30	95			
	296	48	84	61	79	
	233	76	67			
	222	8	96	30	86	
	209	38	82			
	198	15	92			
	171	61	64			
†	114	27	76	49	57	
	114	42	63			
	108	53	51			
	105	38	64			
	104	30	71	57	45	144
	89	53	40			62
	76	8	89			159
‡	69	33	52			66
	59	15	75			96
Mean	173 ± 35	36 ± 5	73 ± 4	49 ± 7	67 ± 9	105 ± 20

* Compared to portal vein.
† Rat wt 500 g.
‡ " " 535 g.

TABLE II. DNase Activities in Rats Injected with Carbon Tetrachloride.

	DNase activities × 10^{-4} units/ml			
	Portal vein	Hepatic vein	% extraction	Bile
24 hr				
	472	453	4	
	418	433	−4	
	342	318	7	19
	129	122	5	
*	—	152	—	
	130	133	−2	48
Mean			2 ± 2	
48 hr				
	157	103	34	
	276	194	30	
Mean			32 ± 2	

* Rat wt 452 g.

atrophic. After 48 hours, the general condition of the animal appeared better, but the livers were pale, yellowish, with minute red indentations over the surface.

The rats treated with CCl_4 showed markedly diminished capacity of the liver to "extract" DNase (Table II). Twenty-four hours after injection, the portal vein and hepatic vein DNase activities did not differ significantly from each other. There appears to be slight hepatic extraction of DNase 48 hours after CCl_4 injection. In the 2 cases examined, there is 34% and 30% extraction respectively. This probably represents a significant amount of extraction, and therefore improvement over the 24 hours function ($P < 0.05$). However, the percentage extraction is still definitely below normal ($P < 0.01$). The DNase concentration in the bile of CCl_4 treated rats was markedly reduced.

Anoxia also resulted in marked reduction of the capacity of the liver to "extract" DNase (Table III). The mean hepatic vein DNase of asphyxiated rats is significantly higher than that of normal rats ($P < 0.001$) and is not significantly different from the normal portal vein DNase level ($P > 0.2$). There is, thus, no significant hepatic extraction of DNase during anoxia. Likewise, the mean aortic blood DNase of asphyxiated rats is significantly higher than normal ($P = 0.001$); and the difference between the paired portal vein and aortic plasma DNase activities is not significant in the asphyxiated rat, unlike the normal. Comparison of the percentage hepatic extraction of the paired aortic plasma DNase activities before and after asphyxia confirms the significant reduction in this hepatic function during anoxia ($P < 0.05$).

Rats with bile duct ligation showed intense icterus of foot-pads, ears, and viscera. The urine contained bile and the bile duct proximal to the ligature was distended. As noted in Table IV, hepatic "extraction" of DNase even after 96 hours obstruction remains very considerable: 68 ± 7%, which does not differ

TABLE III. Plasma DNase Activities in Asphyxia.

DNase activities × 10^{-4} units/ml						
Portal vein	Hepatic vein after asphyxia	% extraction	Aorta before asphyxia	% extraction	Aorta after asphyxia	% extraction
357	—	—	99	72	190	47
296	—	—	61	79	144	51
226	181	20	—	—	—	—
105	—	—	40	62	89	15
—	111	—	—	—	—	—
—	537	—	—	—	—	—
—	486	—	—	—	—	—
—	177	—	—	—	—	—
89	93	−4	—	—	102	−15
Mean 215 ± 52	264 ± 80	8 ± 12	67 ± 17	71 ± 12	131 ± 32	25 ± 15

TABLE IV. Plasma DNase Activities in Rats Following Hepatic Duct Ligation.

Hr after ligation	DNase activities × 10^{-4} units/ml Portal vein	Hepatic vein	% extraction
24	464	106	77
72	105	47	55
96*	504	144	71
		Mean	68 ± 7

* Rat wt 430 g.

significantly from the normal mean ($P > 0.7$).

Discussion. It is apparent from the data presented that the intact rat liver depresses DNase activity in the plasma which is brought to it by the portal vein. This is a hitherto unrecognized hepatic function. In part this extraction appears to be accomplished by excretion of DNase in the bile, since bile contains DNase at a fairly high concentration. However, other mechanisms may also be active. Indeed, biliary excretion is very likely not the principal cause for the reduction in DNase on hepatic perfusion. Biliary obstruction for 24 to 96 hours resulted in marked icterus without loss of the capacity of the liver to "extract" DNase from the portal blood. The obvious possibilities are that the liver contributes an inhibitor of DNase(5) to the blood, that it inactivates the enzyme by destroying it, and that it sequestrates it (possibly for its own metabolic use). Experiments which appear to exclude the first possibility, but make the last plausible because of the very high content of DNase active at pH 7.5 in liver homogenates, amounting to several times the concentration in serum (contrary to the results of Webb(6)), will be reported later.

Hepatic damage, due to carbon tetrachloride or anoxia, results in the loss of the capacity to "extract" DNase from the portal blood. This may be compared to the effect of hepatitis in human patients. In the latter we have observed an increase in peripheral blood serum DNase. The results presented here suggest that this is due to failure of the diseased liver to "extract" the DNase brought to it in the portal circulation.

The source of the serum DNase is not demonstrated by these studies. It may be pancreatic in origin, since it has the same pH optimum as pancreatic DNase(7). The absorption into the portal circulation may be directly from the pancreas or from the pancreatic secretion in the bowel. "Recirculation" from the bile to the portal veins draining the bowel may also contribute. Other sites of serum DNase production are not excluded. Studies are in progress to determine this.

A more detailed report, including histological observations, effect of diet, prolonged biliary obstruction and stimulation and suppression of pancreatic secretion will be presented later.

Summary. 1. DNase activity was determined by the methyl green method at pH 7.5 in the portal vein, hepatic vein and aortic plasma and bile of fed, normal (except for the anesthesia and terminal surgery) albino rats, and of rats rendered anoxic, injected with carbon tetrachloride, or made icteric by hepatic duct ligation. 2. The hepatic vein plasma regularly demonstrated lower DNase activity than did that of the portal vein in normal rats (73% hepatic extraction). 3. In normal rats, the

bile demonstrated DNase activity similar to that of the portal vein plasma. 4. Hepatic damage by anoxia or carbon tetrachloride injection suppressed the capacity of the liver to "extract" DNase from the blood. This hepatic function is abolished 24 hours after the injection of CCl_4; after 48 hours there appears to be partial recovery. 5. Hepatic duct obstruction for as long as 96 hours did not impair the liver's capacity to "extract" DNase. Thus, excretion of DNase in the bile is probably not the principal means by which it is "cleared" from the portal blood.

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1. Kurnick, N. B., Carrera, Ana E., Schwartz, L., and Pariser, S., unpublished, referred to in (2).
2. Kurnick, N. B., *Arch. Biochem. and Biophys.*, 1953, v43, 97.
3. ———, to be published.
4. Colwell, A. R., *Am. J. Physiol.*, 1951, v164, 812.
5. Kurnick, N. B., Schwartz, L. J., Pariser, S., and Lee, S. L., *J. Clin. Invest.*, 1953, v32, 193.
6. Webb, M., *Nature*, 1952, v169, 417.
7. Kunitz, M., *J. Gen. Physiol.*, 1950, v33, 363.

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Modification of Thyroid Activity in Absence of the Anterior Pituitary Gland.* (20732)

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It is generally stated that the principal, if not the only, regulatory factor governing the metabolic activity of the thyroid gland is hypophyseal thyrotrophic hormone (TSH). However, it is well known that thyroid function does not altogether cease after ablation of the hypophysis, for example, Morton *et al.*(1) noted that although the thyroids of hypophysectomized rats concentrated far less radioiodine than did those of intact animals, conversion of iodide to diiodotyrosine readily occurred. Synthesis of thyroxine did, in fact, proceed in the hypophysectomized rat, albeit markedly decreased.

Factors influencing this apparent "intrinsic activity" are, however, unclear. It has been reported that a diet low in iodine content exerts a stimulatory effect on the thyroid gland of the hypophysectomized rat. Chapman(2) stated that increased thyroid gland weight, acinar cell height, and vascularity occurred in hypophysectomized rats placed on an iodine-deficient diet. Because of the importance of this observation, relative to the elucidation of factors other than pituitary TSH affecting thyroid activity, it was deemed of interest to reinvestigate this problem, utilizing additional methods of study.

Experimental. Six-week-old male Sprague-Dawley rats, raised on Purina Lab Chow, were hypophysectomized by the standard parapharyngeal approach and maintained post-operatively on non-raised cages (following I^{131} injection, rats were placed on raised cages to prevent access to urine and feces). Five per cent sucrose in the drinking water was supplied *ad lib*. One week post-operative the rats were placed on Remington(3) diet No. 342 (containing 2% desiccated liver) which was (with the ingredients used here) found to contain 0.1 γ iodine/g by chemical analysis. Addition of iodine to the diet was carried out by preparation of an aqueous solution of NaCl and NaI; this was evaporated to dryness, pulverized to a fine powder with mortar and pestle, added to the basic diet, and distributed with a mechanical mixer. Final iodine content of the diet was further checked by analyses of random samples. In Exp. I, hypophysectomized rats

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