Failure of Cirrhotic Sera to Inhibit Renal Tubule Hippurate Transport *in Vitro** (34013)

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(Introduced by H. V. Murdaugh)

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Late in the course of cirrhosis, renal failure is often a complication (1, 2). In many cases, histologic examination of the kidneys fails to reveal specific lesions to explain progressive azotemia and oliguria (2, 3). While renal tubule function seems well preserved, reductions of glomerular filtration rate (GFR) and renal plasma flow (RPF) have been observed (4, 5). Baldus *et al.* (1) and Shear *et al.* (5) noted reduced paraaminohippurate (PAH) extraction ratios (E_{PAH}) in patients with cirrhosis. The latter authors attributed this decreased (E_{PAH}) to possible intrarenal shunting of blood as seen in other circulations (5). Recently Schroeder *et al.* (6) evaluated 22 cirrhotic patients and suggested that decreased E_{PAH} was not a consequence of decreased GFR or RPF and suggested renal cortical vasoconstriction with relative increase in medullary flow as a possible explanation of changes in renal function in cirrhotic patients. An alternative explanation for the reduced E_{PAH} suggested by Schroeder *et al.* was the possible impairment of renal tubule transport of PAH either by an altered transport mechanism or a circulating substance that inhibited transport. Preuss *et al.* (7) have shown recently that a substance or substances present in azotemic sera inhibits PAH transport in an *in vitro* preparation of renal tubules. Whether such substances, which could alter hippurate transport, were also present in the sera of cirrhotics has not been reported. The purpose of this study was to determine if a substance or substances exist in cirrhotic sera that alter renal hippurate transport and thus could effect the interpretation of PAH data in *in vivo* studies performed on cirrhotic patients.

Materials and Methods. Bood was obtained from patients admitted to the ward service of the Oakland Veterans Administration Hospital, Pittsburgh, Pennsylvania, with a diagnosis of Laennec's cirrhosis. The diagnosis was based on history, physical examination, and laboratory data. Three of the patients had the diagnosis confirmed by liver biopsy. No patient with a blood urea nitrogen exceeding 20 mg/100 ml was included in the cirrhotic study. Control blood was obtained from normal volunteers who had no history or physical evidence of liver or renal disease. Both patients and control subjects were fasted overnight prior to the blood collection, and both sets of bloods were drawn at the same time.

The blood was allowed to clot and the separated sera were kept on ice for 1–2 hr until incubation with the kidney slices were begun. Experiments were performed on male albino rats weighing 200–300 g. Animals were killed by cervical dislocation, and the kidneys were removed, decapsulated, and placed in cold isotonic saline. Renal cortical slices, 0.3–0.4 mm thick were prepared with a Stadie-Riggs microtome. The weight of tissue, after blotting on filter paper, was between 75 and 150 mg. Less than 10 min elapsed from removal of kidneys to the start of the incubation. Incubation of slices were perfomed on a Dubnoff shaker at 25° shaking at a frequency of 100/min with a 100% oxygen gas phase. The slices were placed in 3.0 ml of Cross and Taggart medium (8) with a final pH between 7.3 and 7.5. Sera, control or cirrhotic, was added to a final volume of 10%. ^{131}I labeled hippurate (Hippuran-Abbott Laboratories) was added to the medium in a concentration of approximately 2 ×

* Supported in part by Grant J-50 from the Health Research and Services Foundation and by Grant 67-875 from the American Heart Association.

[1] Performed during tenure as an Established Investigator of the American Heart Association.

TABLE I. Clinical Data for the 12 Cirrhotic Patients Studied. SM_L and SM_C Refer to the Slice/Medium Ratios of ^{131}I Hippurate.[a]

Case no.	Age (yr) and sex	Diagnosis[b]	Ascites	BUN (mg/100 ml)	Total bilirubin (mg/100 ml)	SGOT (Karmen units)	LDH (Wacker units)	Alk. phos. (King-Armstrong units)	S/M_L or L/C	S/M_L	Mean L/C
1 R.K.	67 M	L; F[c]	+2	10	1.2	55	150	15	.86		.86
2 A.F.	48 M	L[c]	+3	7	4.6	238	215	27	1.36		1.36
3 F.J.	57 M	L	+1	13	1.0	78	350	34	1.23		1.23
4 J.S.	54 M	L	+2	13	2.4	105	175	34	0.93	.68	.81
5 L.K.	57 M	L	+2	6	6.4	95	170	18	0.90	.40	.65
6 C.K.	55 M	L	+3	12	5.0	120	160	21	2.05	1.60	1.83
7 V.B.	43 M	L	+3	3	3.7	73	115	20	0.64	1.04	.87
8 J.M.	67 M	L[c]	+1	16	1.0	30	100	12	2.00		2.00
9 A.C.	43 M	L	±	6	1.0	60	130	15	1.33	.89	1.11
10 F.S.	43 M	L	+3	12	11.0	80	85	19	.82		.82
11 S.M.	47 M	L	+2	17	1.1	80	100	23	1.22		1.22
12 F.B.	41 M	F	0	5	6.8	65	165	9	1.02		1.02
											$\bar{X} = 1.15$
											± .42 SD

[a] See text.

[b] L = Laennec's cirrhosis; F = fatty liver.

[c] Histologically confirmed.

10^{-5} *M*. The hippurate content in the slices (S) and medium (M) was determined by isotope counting using a well-type gamma scintillation counter, and a slice/medium (S/M) ratio was determined from the isotopic counts calculated for 1 g of tissue and the counts in 1 ml of medium. In order to eliminate a variable, tissue from the same animal was used when the effects of each cirrhotic sera was compared with its normal control. Results are expressed as the ratio of the S/M for the cirrhotic sera (S/M_L) divided by the S/M for the control serum (S/M_C) run simultaneously with the tissue from the same animal; *i.e.*, $S/M_L/S/M_C$ or the L/C ratio.

Results and Discussion. Table I shows the patients studied, their diagnosis, blood urea nitrogen values, and the ratios between the S/M of these bloods divided by the S/M for the paired controls expressed as the L/C ratio. None of the patients had been on antibiotic therapy for at least 1 month prior to the study. Many of the patients were jaundiced and the majority of them had grossly abnormal liver-function tests. In 5 of the 12 patients the L/C ratio was determined in duplicate using a different paired control, and a mean L/C ratio was calculated. It can be appreciated that there were great varibilities in the L/C ratios among the patients with a mean of 1.15 ± 0.42 SD and a range of 0.65 to 2.00. The data show that using paired cirrhotic and control sera there was no consistent depression of hippurate uptake in the kidney slices incubated with sera from patients with Laennec's cirrhosis. The mean S/M ratio was 10.69 ± 4.67 SD for the cirrhotic group vs. 10.21 ± 2.50 SD for the controls, $t = .3408$, $p = .78$. In order to validate that there is no effect *in vitro* on hippurate transport in the presence of cirrhotic sera as there is with azotemic sera, six studies were performed comparing again the effects of pooled azotemic sera (blood urea nitrogen > 100 mg/100 ml) in this system. Once more, azotemic sera significantly decreased hippurate transport when added as 10% volume to the system (decreased by 50%, $p<.001$). The lack of a demonstrable depression in hippurate transport in rat renal slices by sera from cirrhotic patients as compared to the depression from azotemic sera may be due to the lack of a method sensitive enough to pick up subtle changes in hippurate transport or to the absence of metabolic substances in cirrhotic sera which actively inhibits renal hippurate transport. Unlike *in vitro* studies with azotemic sera, these *in vitro* studies suggest that alterations in E_{PAH} seen in *in vivo* studies in cirrhotic patients are the consequence of factors other than circulating substances which inhibit renal hippurate transport.

Summary. In order to evaluate possible depression of renal hippurate transport by cirrhotic sera, hippurate transport into rat kidney slices incubated in sera from cirrhotic and normal patients was investigated. Unlike studies with uremic sera, there was no significant difference in hippurate uptake in rat renal slices incubated with cirrhotic sera when compared to normal sera. It is suggested by this *in vitro* technique that a metabolic inhibitor of hippurate transport is not present in cirrhotic sera and that other factors, such as renal tubule function *per se* or changes in renal hemodynamics, may contribute to the decreased PAH uptake observed *in vivo* in the cirrhotic patient.

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Received April 7, 1969. P.S.E.B.M., 1969, Vol. 131.