

virus has been given the name Bussuquara.

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Comparative Response of Normal and Cirrhotic Rats to Intravenously Injected Bacteria.* (24910)

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During study of role of intestinal bacteria in genesis of experimental dietary cirrhosis in rats(1), we noted abscesses in the lung or liver of rats which developed cirrhosis and, by contrast, the absence of such infection in those protected from developing cirrhosis. This suggests that the cirrhotic rats were less resistant to common bacterial invaders. In this communication we report results of a study of antibacterial resistance of these cirrhotic rats.

Method. The experiments were designed to compare ability of normal and cirrhotic rats(1) to clear the circulation of bacteria injected into the portal vein; and (2) to lyse the ingested bacteria. *E. coli*, labelled with I^{131} by method previously reported(2), were washed in saline (0.85%) and then dialyzed against saline at room temperature until almost all radioactivity was removed from supernatant. Assay of the supernatant of the bacterial suspension for radioactivity at various intervals during 48 hours after dialysis, demonstrated that the iodine taken up remained tightly bound. All radioactivity determinations were done with deep well scintillometer. The different bacterial suspensions injected were adjusted by dilution to yield approximately the same concentration of bacteria and were about equal in terms of radioactivity. The viability of these labelled bacteria was tested by subculture every 12 hours. It was estimated from colony counts that all remained viable for duration of experiment.

Normal and cirrhotic† male albino rats of

the Wistar strain (wt. 250-300 g) received 10 drops of saturated solution of KI in their drinking water each day for 30 days prior to experiment to saturate the thyroid as well as other tissues with iodine, and thus preclude binding of liberated I^{131} . Through a small midline abdominal incision made under light ether anesthesia, 0.5 ml of a bacterial suspension (10^8 bacteria) of I^{131} labelled *E. coli* was injected into the splenic vein of each rat. The same volume of this suspension was used as standard to measure total radioactivity injected. Smaller injectates gave poorly reproducible curves; larger injectates were rapidly fatal to cirrhotic rats.

Each rat was isolated in individual metabolic cage and all excreta collected. At various intervals after injection the rats were killed by exsanguination. One gram samples of blood, liver, spleen, lung, and urine were planted in various media for cultures of aerobic and anaerobic bacteria. Samples of cirrhotic livers were taken for microscopic confirmation of cirrhosis. Liver, lungs, spleen, kidneys, gastrointestinal tract, thyroid, heart, and a portion of muscle (hamstring) were removed, weighed and homogenized. Appropriate aliquots (2 ml) of the homogenates were then assayed in duplicate for gamma radiation by deep well scintillometer. Remainder of homogenate was then dialyzed at room temperature against running tap water for 24 hours, after which 2 ml aliquots were measured for gamma radioactivity. The total, bound and dialyzable radioactivity

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† Cirrhosis was induced by feeding choline deficient diet for 300 days(1).

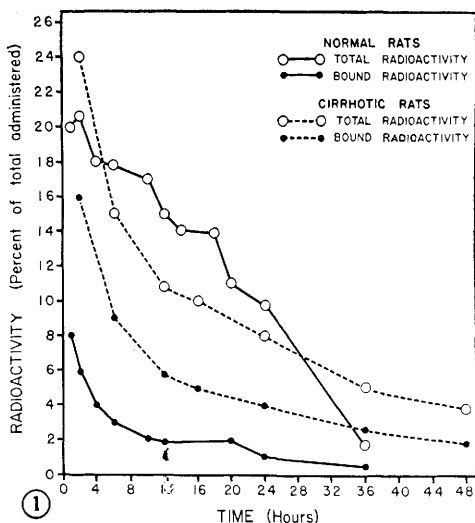


FIG. 1. Total and bound (non-dialyzable) radioactivity in blood of normal and cirrhotic rats at intervals after inj. of I^{131} labelled *E. coli* into the splenic vein.

of blood and each tissue was then calculated,[‡] and with the aid of the standard, the results expressed in terms of percent of total radioactivity injected.

That bound and free radioactivity could be taken as a measure of unlysed and lysed bacteria respectively in samples of tissues and fluid was demonstrated by results of experiments in which KI^{131} in saline§ (1 ml) was infused intravenously into normal and cirrhotic rats prefed iodine (KI) as described above. Sixty-five to 78% of the KI^{131} was excreted within 24 hours by both normal and cirrhotic rats, and all radioactivity measured in aliquots of blood and in tissue homogenates was dialyzable. Because nearly all recoverable infusate of I^{131} was rapidly excreted in urine, it is proper to conclude that binding of liberated I^{131} to yield non-dialyzable radioactivity did not occur to any significant extent.

Results. 1. Bacteriological. A. Infusion of KI^{131} . Following administration of KI^{131} to normal rats, all cultures of blood, liver, lung, spleen and urine taken at intervals from nor-

mal rats were sterile, while 20% of cultures of blood and liver from cirrhotic rats were positive for *E. coli*.

B. Infusion of *E. coli* labelled with I^{131} . In normal rats *E. coli* were recovered from cultures of spleen to 2 hours after infusion; from blood cultures to 6 hours following infusion; and from cultures of liver and lungs to 12 hours following infusion. In cirrhotic rats, *E. coli* were recovered from blood, liver, and

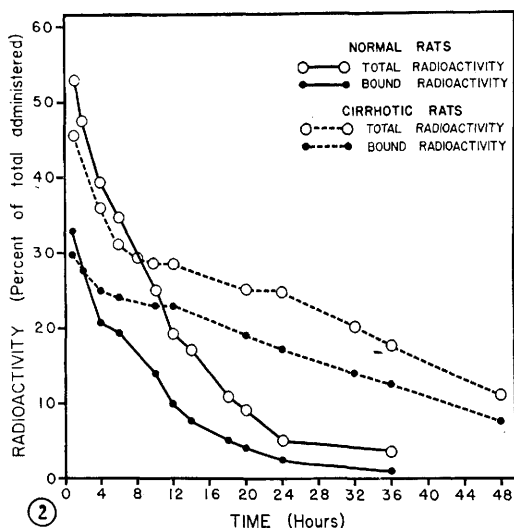


FIG. 2. Total and bound (non-dialyzable) radioactivity in liver of normal and cirrhotic rats at intervals after inj. of I^{131} labelled *E. coli* into splenic vein.

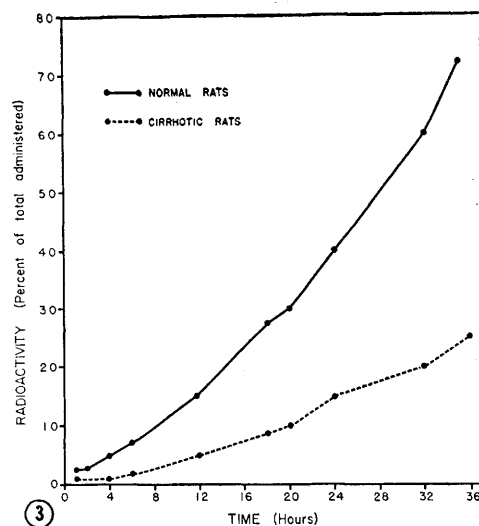


FIG. 3. Radioactivity (ionic) recovered from urine of normal and cirrhotic rats after administration of I^{131} labelled *E. coli* into splenic vein.

[‡] Blood volume was estimated to be 10% and skeletal muscle 40% of total body weight.

[§] Total radioactivity and iodine content of this solution was approximately equivalent to that of $\frac{1}{2}$ ml of suspension of labelled bacteria.

spleen to 48 hours, and from lung to 32 hours following infusion. In fixed sections of normal livers or lungs bacterial colonies were not seen. On the other hand, colonies of Gram negative bacteria were frequently seen in fixed sections of lungs and in portal areas of fixed sections of liver of cirrhotic rats. Occasionally bacteria formed micro-abscesses. Cultures were sometimes negative when colonies were observed in the sections. In both normal and cirrhotic rats all urine cultures were negative.

II. *Distribution of radioactivity in blood and tissues following injections of I^{131} labelled *E. coli* into normal and cirrhotic rats.* In normal rats, injected bacteria were promptly cleared from the circulation by the liver (Figs. 1 and 2) and lysed since the injected bound radioactivity was rapidly converted to ionic or dialyzable radioactivity; and the latter was promptly excreted in urine (Fig. 3). This rapid disappearance of bacteria from blood, and their uptake by liver, with lysis and release of I^{131} confirmed the bacterial culture data which indicated prompt sterilization of blood and liver.

In *cirrhotic* rats, on the other hand, though uptake of radioactivity by the liver was equal to that in normal rats, the bound or non-dialyzable radioactivity persisted at higher levels than in normal rats for 48 hours (Fig. 3). Only 25% of total radioactivity injected

was recovered from urine after 36 hours (Fig. 2). Furthermore, this impairment of bacterial lysis coincided with persistence of positive cultures in blood and liver.

Comment. These data show that the cirrhotic rat clears bacteria from the circulation as well as the normal rat. Persistence of viable bacteria in liver, lung, and spleen in cirrhotic rats suggests that the bactericidal defense in the R.E. system of lung and spleen as well as in liver is damaged. The persistence of viable bacteria in blood suggests that the blood is being reseeded from the persisting foci in the R.E. system.

Summary. Labelled (I^{131}) bacteria infused into portal vein of normal rats were promptly cleared and destroyed with release of ionic I^{131} and its prompt excretion into the urine. In cirrhotic rats the clearance process was normal, but capacity to destroy bacteria was impaired. This resulted in persistence of ingested bacteria and of "bacteria bound" radioactivity in liver, lung, and spleen, and continued reseeded of the blood stream from such foci.

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"Complete" and "Incomplete" Hemagglutinin Formation in the Mouse.* (24911)

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The presence of antibodies other than those active in a saline environment has been well documented with human serums following isoimmunization in pregnancy and transfusions. These varieties have usually been categorized as "univalent" or "incomplete" and are demonstrable in solutions of increased viscosity

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provided by adding serum, albumin, or polyvinylpyrrolidone. Other methods of detecting such antibodies include use of trypsinized erythrocytes and the antiglobulin or Coombs' test. "Incomplete" types of antibodies have also been observed in experimental animals (1, 2, 3, 4, 5, 6) but have not been studied in detail. The purpose of this paper is to report the appearance of high titered, "incomplete" hemag-