Hepatic Necrosis Due to Bromobenzene and its Dependence upon Available Sulfur Amino Acids.* (19320)

DIETER KOCH-WESER,†‡ JESUS DE LA HUERGA,† AND HANS POPPER.

From the Hektoen Institute for Medical Research and the Department of Pathology, Cook County Hospital and Northwestern University Medical School, Chicago.

In many instances of hepatic injury, it is difficult to decide whether an excess of a toxic factor or a deficiency of an essential factor is the underlying cause. This problem, originally raised by the nutritional hepatic injury(1) and the demonstration of the importance of sulfur amino acids(2), has been recently stressed by the effect of ethionine upon the liver. This substance, being an analogue of methionine and thereby a possible inhibitor of protein synthesis(3), produces a fatty liver in female rats(4,5). In an attempt to find other examples of hepatic injury produced by depletion of amino acids, the acute effect of bromobenzene upon the liver was studied. The rationale for this experiment was the coupling of bromobenzene with cysteine to form bromobenzylcysteine, which is acetylated and excreted as mercapturic acid in the urine (6,7). Since methionine may serve as source for cysteine, bromobenzene could deplete the body of both sulfur amino acids. Therefore, the influence of preceding protein depletion and of sulfur amino acid supplements upon the hepatic injury was also investigated.

Material and methods. Wistar strain white rats were divided into 8 groups. All of them, except one control group, received by intraperitoneal injection 0.05 ml of bromobenzene per 100 g body weight, dissolved in 0.20 ml of corn oil. The fasting time before bromobenzene administration, the protein content of the diet fed for two weeks before the fasting period and the sex of the animals, varied (Table I). Supplements of methionine or cysteine were given in 4 doses 12 hours apart during the fasting and experimental period (*i.e.*, 16 hours before to 32 hours after the bromobenzene administration). All animals were killed 48 hours after the administration. The degree of hepatic necrosis in the histologic specimen was graded from 1+ to 4+ taking the estimated percentage of necrotic area of the lobule as criterion.

Results. In unprotected rats the centrolobular zone revealed necrosis 48 hours after the intraperitoneal injection of bromobenzene. This was characterized in some instances by diffuse coagulation and eosinophilia of the cytoplasm of the liver cells, the nuclei of which

TABLE I. Survival and Degree of Hepatic Damage 48 Hours After Intraperitoneal Injection of Bromobenzene to Rats.

No. of rats							
Surviving	Dead	Sex	Protein content of diet preceding fasting, %	Fasting preceding bromobenzene admin., hr	Bromobenzene inj. intraper., ml/100 g body wt	Supplement inj. intraper., 300 mg per 100 g body wt	Mean hepatic damage in surviving rats in +
24	2	♀	16	16	.05	—	3.2
13	1	♀	16	16	.05	Methionine	.9
9	0	♀	16	16	.05	Cysteine	1
7	0	♀	16	0	.05	—	1.9
10	4	♀	8	16	.05	—	3.7
7	8	♀	16	88	.05	—	3.9
11	1	♂	16	16	.05	—	3.3
5	0	♀	8	88	—	—	0

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† Solomon Foundation Fellow.

‡ Present address: Department of Medicine, University of Chicago, School of Medicine, Chicago.

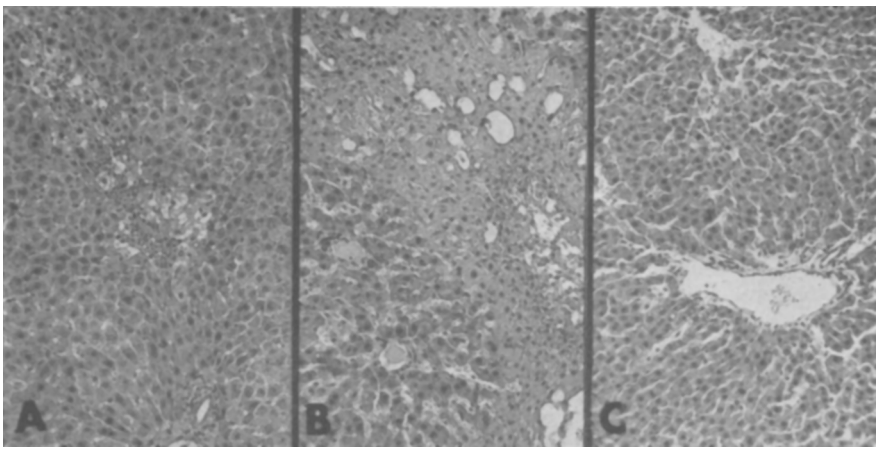

FIG. 1. Photomicrographs (220 ×) of livers of rats after administration of bromobenzene. A. Necrosis and disappearance of liver cells in the center of the lobule, which is infiltrated by inflammatory cells. The liver cells surrounding the necrosis reveal hydropic swelling. B. Disappearance and necrosis of the liver cells in the greater part of the lobule with only a small rim of liver cells remaining in the periphery. Extensive hemorrhage and dilatation of some sinusoids. C. With simultaneous administration of methionine. Intact structure.

were pyknotic. In other places the central zone showed complete disappearance of the liver cells and collapse of the framework which was surrounded by red cells intermingled with a few partly phagocytosing white cells. Some of the neighboring cells were hydropic. The Kupffer cells were proliferated and revealed phagocytosed material including non-iron-containing and non-fluorescent pigment. In the vicinity the architecture of the liver cell plates was distorted but little attempt at regeneration was noted (Fig. 1 A). In severe cases only a small rim of normal liver cells was preserved in the periphery of the lobule and the necrotic areas showed extensive hemorrhages (Fig. 1 B).

These changes were almost completely suppressed by supplementation of the diet with methionine (Fig. 1 C), and cysteine. Some protection was given by feeding the animals throughout the experiment. The severity of the liver damage was significantly enhanced and the mortality increased by either lowering the protein content of the preceding diet or increasing the fasting period. There was no difference in susceptibility to bromobenzene between males and females. The control group, kept on the low-protein diet and then fasted for 88 hours without the administration of bromobenzene, did not show significant liver damage or any mortality.

Comments. The extent of the acute centrolobular necrosis, which in severe instances is associated with hemorrhagic destruction of the lobular parenchyma, depends apparently upon the available protein stores, since the same dose produces far more extensive lesions if starvation or a low-protein intake precedes the administration of bromobenzene. The amount of cysteine available for coupling with the aromatic substance appears to be the determining factor. This is supported by almost complete prevention of the lesion by preceding administration of either methionine or cysteine in high doses. It remains to be proven that the mercapturic acid excretion is increased following the acute bromobenzene administration and that it rises even more if the hepatic lesion is prevented by the administration of either sulfur amino acid. In chronic experiments it has been proved that sulfur amino acid administration prevents on one side the growth inhibition produced by bromobenzene feeding(8,9) and increases, on the other side, urinary mercapturic acid excretion(10). The fact that the acute hepatic necrosis produced by bromobenzene injection can be prevented by the administration of sulfur amino acids, permits two interpretations: The lesion could be produced by acute deficiency of sulfur amino acids which are drained from the body during the formation

of mercapturic acid, detoxification taking preference over physiologic requirements. The massive hepatic necrosis produced by yeast diets and prevented by sulfur amino acids simulates morphologically the picture produced by bromobenzene. On the other hand, a primary hepatotoxic effect of bromobenzene cannot be excluded in view of the hepatic necroses produced by other halogenated hydrocarbons like carbon tetrachloride and chloroform, even though fatty changes present in those are missing in the bromobenzene lesion. In this case, the reported findings could be explained by a dependence of the extent of the necroses on the amount of bromobenzene not detoxified because of relative lack of sulfur amino acids. In both instances the bromobenzene lesion is a combination of a toxic and deficiency effect. How far similar mechanisms can be invoked to explain the toxicity and detoxification of other substances which produce acute liver damage, has to be investigated.

Summary. Intraperitoneal administration of bromobenzene produces acute centrolobular hepatic necrosis, its severity depending on the preceding protein depletion. Methionine and cysteine given prior to bromobenzene administration prevent the lesion. The lesion is an example of interlocked toxicity and deficiency effect, since bromobenzene drains sulfur amino acid from the body.

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