Comparative Changes in Serum Enzyme Levels in Beryllium- or Carbon Tetrachloride-Induced Liver Necrosis (37502)

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(Introduced by H. E. Stokinger)
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Several serum enzymes such as lactic dehydrogenase, gluatamic-pyruvic transaminase and glutamic-oxaloacetic transaminase have been used extensively in the detection of hepatic disease in man, as well as in evaluating chemically induced liver necrosis (1). Beryllium (Be) disease, although primarily respiratory in nature, has also produced liver damage in man (2). Be-induced liver damage has been reported in several animal species (3–7). Be first appears in the liver in the Kupffer cells and then diffuses into the liver parenchyma to produce midzonal necrosis (8) and reticulendothelial system blockage (9) within the cell. Be concentrates in the lysosome and nuclear fraction (10).

This comparative study was undertaken to evaluate the biochemical response of the liver, as measured by one lysosomal and several serum enzyme levels, to Be (midzonal necrosis) and carbon tetrachloride (CCl_4) (centrolobular necrosis) (11).

Methods. Male rats (150–200 g) were injected ip with Be as Be^7SO_4 (0.8 mg/kg body wt) or CCl_4 (2.5 g/kg body wt) or saline (controls). Since the level of the Be and CCl_4 compound injected was approximately an LD_{50} dose, enough extra animals were injected so that 10 animals from each chemically treated group, along with 4 controls, could be sacrificed by decapitation at 4, 8, 24 and 48 hr after injection. At each sacrifice time liver samples were taken from each group for histological examination and for "free" acid phosphatase assay, an indicator of lysosomal stability (12). The level of Be^7 in the liver was also determined in all Be-injected animals by counting the tissue in a Nuclear Chicago gamma counter.[1] The level of the serum enzyme isocitric dehydrogenase (ICD) (13), lactic dehydrogenase (LDH) (14), glutamic-pyruvic transaminase (GPT) (15), and glutamic-oxaloacetic transaminase (GOT) (16) were determined after each sacrifice. Enzyme levels were expressed as the number of times they were elevated over control values determined at the same time.

Results. The changes induced in serum enzymes (ICD, GPT, GOT, LDH) level by Be and CCl_4 are observed in Table I. In the Be-injected animals no elevation of any of the serum enzymes was observed until the 48-hr sacrifice when ICD, GOT, and GPT were elevated. In the CCl_4-injected animals, ICD, GOT, and GPT were progressively elevated with time start at the 4-hr sacrifice and continuing to the 48-hr sacrifice where LDH was also found to be elevated.

The important difference in response between the Be and the CCl_4 animals is the time of initial serum enzyme response following injection (48 vs 4 hr).

When the serum enzyme levels are compared at the time the initial response is observed (Be at 48 hr, CCl_4 at 4 hr), the degree of elevation of ICD, GOT, and LDH is similar in both Be and CCl_4 animals with only GPT showing a difference.

The cellular alterations occurring with $BeSO_4$ were quite different from those produced with CCl_4. At the 4- and 8-hr sacrifice some of the midzonal cells had clear cytoplasm which increased in extent by 24 hr.

[1] Mention of a commercial product or concern does not constitute endorsement by the National Institute for Occupational Safety and Health.

TABLE I. Beryllium- and Carbon Tetrachloride-Induced Changes in Serum Enzyme Levels.[a]

Time after injection (hr)	Isocitric dehydrogenase		Glutamic-pyruvic transaminase		Glutamic-oxaloacetic transaminase		Lactic dehydrogenase	
	Be	CCl_4	Be	CCl_4	Be	CCl_4	Be	CCl_4
4	N	9.5 ± 2.2	N	9.4 ± 2.0	N	3.5 ± 1.5	N	1.5 ± 0.7
8	N	14.5 ± 4.0	N	11.4 ± 2.0	N	5.1 ± 2.0	N	1.5 ± 0.5
24	N	9.8 ± 1.5	N	16.7 ± 1.4	N	6.3 ± 0.5	N	1.3 ± 0.2
48	8.3 ± 2.4	35.9 ± 5.4	6.3 ± 2.8	20.5 ± 2.1	3.4 ± 0.7	14.0 ± 2.4	1.8 ± 1.1	9.0 ± 1.4

[a] Expressed as number of times elevated above normal ± SE. N = normal (control animal sacrificed at the same time).

In addition, at 24 hr there was a generalized homogeneous staining of the liver cell cytoplasm in one rat. This had increased in extent at the 48-hr sacrifice, and in addition, there was focal midzonal and periportal coagulative necrosis.

Cellular alterations occurred in the livers of the animals injected with CCl_4 at all sacrifices. These changes began with small vacuoles in the centrolobular cells at 4 hr, progressed to widespread vacuolization and midzonal balloon cells (large, round cells with little stainable cytoplasm) at 8 hr, to focal coagulative necrosis at 24 hr and at 48 hr all the above changes were seen in addition to changes in all the centrolobular cells consistent with single cell necrosis, *i.e.*, the cytoplasm stained homogenously orange and contained large vacuoles.

By 8 hr after injection, the level of "free" acid phosphatase in the liver, an indicator of lysosomal stability, (80% increase above control) was at a peak in the Be-injected animals (Fig. 1). No elevation of "free" acid phosphatase was observed in the CCl_4-treated animals. The sequence of events following Be injection is as follows: (a) maximum elevation of "free" acid phosphatase (8 hr); (b) maximum Be^7 level in the liver (24 hr) (Fig. 2); (c) increase in the serum enzymes (48 hr).

Discussion. Be-induced liver necrosis appears in many respects to be completely different from necrosis induced by CCl_4. The much longer time (48 hr vs 4 hr) required in the Be-injected animal to exhibit a

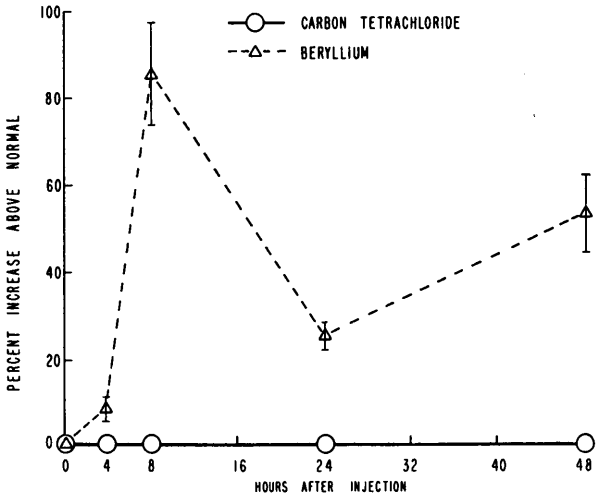

FIG. 1. Changes in "free" acid phosphatase levels in liver homogenates from rats injected intraperitoneally with different liver toxins.

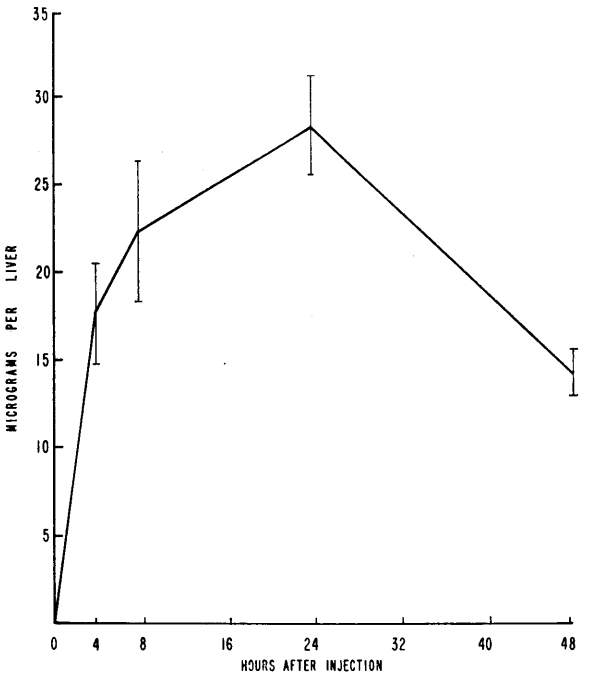

FIG. 2. Beryllium levels in rat liver at different time following intraperitoneal injection.

dramatic serum enzyme elevation is the major difference in the effect of these two liver toxins. The magnitude of the first observed response (Be 48 hr vs CCl_4 4 hr) however, is similar in each case.

The serum enzyme response varies according to the disease or injury (17, 19) as well as the subcellular location of these enzymes within the liver cell (20, 21).

One can conclude from this experiment that GOT, GPT, and ICD levels are excellent serum indicators of early subcellular changes, such as vacuolization, but these serum enzymes are reported to be poorly correlated at later stages of necrosis (1, 22, 23). The response of LDH, however, was not useful in the detection of early changes, although an increase was noted after coagulative necrosis.

Another major difference between the liver toxic agents appears to be in the role of the lysosome. Be-injected animals showed lysosomal rupture while no significant rupture was observed in the CCl_4-injected animals. Lysosome rupture has not been reported by others in the early stage of CCl_4 (24) necrosis indicating a secondary role for the lysosome. Other differences in the responses of the liver toxic agents have been reported in the area of body temperature, liver temperature and liver glycogen (4, 25, 26).

The role of the lysosome, especially as it relates to the delay in the appearance of Be liver necrosis, warrants further investigation.

Summary. Four serum enzymes (ICD, GOT, GPT, LDH) and one liver enzyme (acid phosphatase) were used to evaluate the difference in the rat response to Be-induced midzonal liver necrosis and CCl_4-induced centrolobular necrosis. Be produced a maximum elevation of liver free acid phosphatase 8 hr after injection, but the elevation of the serum enzymes ICD, GOT, or GPT were not observed until 48 hr after Be injection. LDH was not elevated at any time period. At equivalent doses in the CCl_4-injected animals, serum ICD, GOT, and GPT were all elevated by 48 hr, but no elevation of liver acid phosphatase was observed at any time period. These differences in response indicate a biochemical as well as an anatomical difference between these two types of

chemically induced liver necrosis.

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