which are observed cannot be explained on the basis of the existence, in varying proportions, of a limited number of homogenous antibody subpopulations. Rather, purified antihapten antibody apparently consists of a highly heterogenous population of molecules. Similar conclusions have been reached by several previous workers (12,13).

Summary. Peptide maps of $F(ab')_2$ fragments of anti-DNP antibody fractions differing markedly in affinity for DNP-lysine were indistinguishable, suggesting that the structural differences corresponding to the differences in affinity were located in a highly variable portion of the antibody molecule.

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Hepatotoxicity of Phenothiazines *in Vitro* as Measured by Loss of Aminotransferases to Surrounding Media* (33066)

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The administration of chlorpromazine (CPZ), 10-(dimethylaminopropyl)-2-(chlorophenothiazine hydrochloride) leads to jaundice in 1–5% of the recipients (1). This phenomenon has been considered a manifestation of individual host idiosyncrasy or hypersensitivity to the drug. A closely related compound, promazine (PZ), 10-(dimethylaminopropyl)-(phenothiazine hydrochloride) however, has been attended by a very rare occurrence of jaundice, although extrahepatic manifestations of hypersensitivity occurs with both of these phenothiazines (1). Accordingly, the likelihood has been considered that the adverse effect CPZ may have upon the liver is aggravated by the coincidence of hypersensitivity. Some support for this view is provided by the demonstration that almost 50% of patients who receive CPZ develop some hepatic dysfunction (2–4). Previous studies utilizing a known hepatotoxin (carbon tetrachloride) demonstrated cytotoxicity in an *in vitro* system (5). The present study using an *in vitro* assay was undertaken to compare the relative adverse effects of CPZ and PZ. Leakage of intracellular enzymes which was presumed to result from injury to cellular membranes was utilized as an index of heptotoxicity.

Materials and Methods. Albino New Zealand strain rabbits were sacrificed by cervical fracture, their livers were removed and rinsed in cold saline. The tissue was blotted dry and from small chunks of tissue, slices were prepared using the Stadie-Riggs microtome. An intact tissue slice, weighing 50 mg

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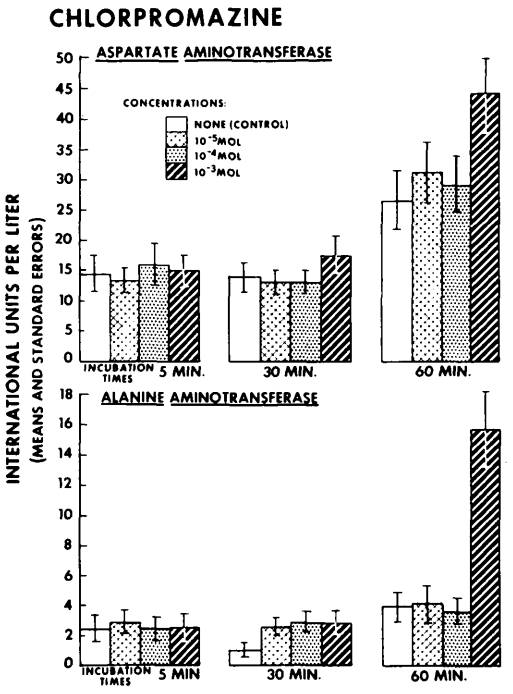

FIG. 1. Effect of chlorpromazine on GOT and GPT leakage to surrounding media; 10 experiments.

was placed into each vessel containing 10 ml of Krebs–Ringer bicarbonate buffer at a pH of 6.7, without added drug (control), or containing CPZ, or PZ[1] in concentration of 10^{-3}, 10^{-4}, and 10^{-5} *M*. The samples were incubated for periods ranging from 5 to 60 min in a Dubnoff metabolic shaker, maintained at 37°C under 95% O_2 + 5% CO_2. Each experiment was run in duplicate. After the incubation period was completed, the medium was decanted off and aliquots were assayed, in duplicate, by the method of Karmen for aspartic aminotransferase (GOT) (6) and Wroblekski and LaDue for alanine aminotransferase (GPT) (7).

Results. Exposure of rabbit liver slices for 60 min to a 10^{-3} *M* CPZ solution, led to an increase in the GOT activity of the surrounding medium significantly greater than that of the control preparation or that induced by exposure to 10^{-4}, and 10^{-5} *M* CPZ solutions. After 30 min of exposure, no significant increase in the GOT content of the medium could be demonstrated (Fig. 1).

The GPT activity in the medium was increased significantly after 60 min of exposure to CPZ concentration of 10^{-3} *M*, but not to concentrations of 10^{-4} and 10^{-5} *M*. At 30 min, no increase in the GPT content of the medium could be observed (Fig. 1). Exposure of the liver slices to PZ in the same concentrations led to no increase in the concentration of GOT or GPT in the medium at 60 min (Fig. 2).

Discussion. Exposure of rabbit liver slices to CPZ led to a significant increase in the

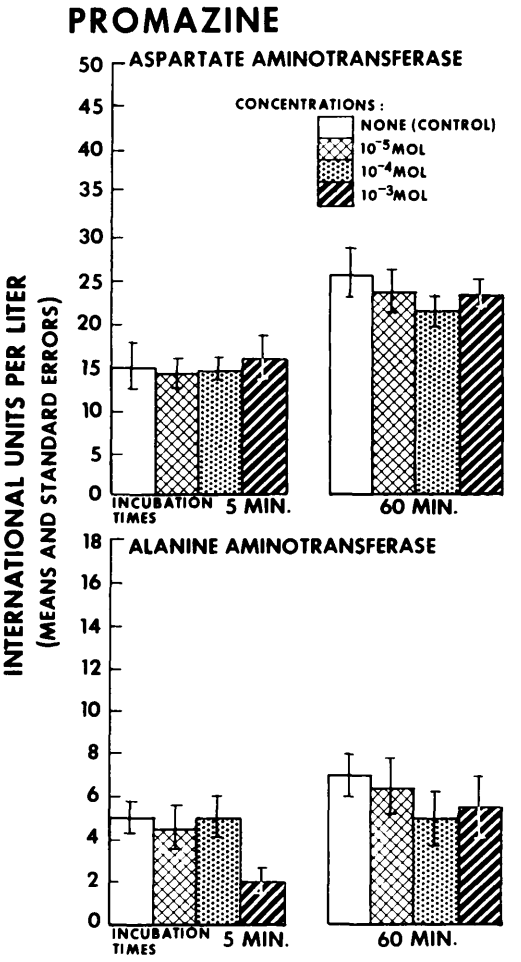

FIG. 2. Effect of promazine on GOT and GPT leakage to surrounding media; 6 experiments.

[1] Chlorpromazine hydrochloride (CPZ) and promazine hydrochloride (PZ), as the pure powders were each made available through the courtesy of the respective manufacturers: Smith, Kline, and French, Philadelphia; and Wyeth Laboratories, Radnor, Pennsylvania.

enzyme content of the surrounding medium attributable to leakage from the hepatocytes. Leakage of GOT and of GPT, however, occurred at concentrations approximately 100-fold greater than those usually found in serum, but less than 10-fold greater than those that can be found in the liver, both in humans as well as rabbits after therapeutic dosages (8,9). Leakage of enzyme from cells into medium may be assumed to represent membrane injury. Direct hepatotoxins, such as carbon tetrachloride, which are known to injur cellular membranes have been shown to produce effects *in vitro* analogous to those demonstrated with CPZ (5). The significance of the CPZ effect is supported by the observation that PZ in equal concentrations, induced no recognizable degree of leakage of enzymes from the cells. Patients who receive chlorpromazine develop a high incidence (50%) of mild hepatic dysfunction (2–4) and a significant incidence (1–5%) of jaundice (1). Promazine appears not to lead to hepatic dysfunction in patients who are serially studied (9) and has been associated with recognizable jaundice with extreme rarity (1). The difference in cytotoxicity for liver slices demonstrated *in vitro* appears to parallel the *in vivo* difference of occurrence of hepatic injury when either drug is administered to humans.

Summary. Exposure of rabbit liver slices to chlorpromazine in concentrations of 10^{-3} *M*, led to appreciable leakage of GOT and GPT into the surrounding medium. Promazine, in equal concentrations did not lead to an identifiable degree of leakage of these enzymes into the medium. The different effect of the two drugs on this system parallels what is known about their potential hepatotoxic effect in humans.

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^{137}Cesium Retention in Mice of Different Ages* (33067)

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The biological half-time for retention of ^{137}Cs in man has been of considerable interest. In a recent review, some investigators reported half-times as short as 10 days for newborn humans, 30–40 days for children, and 60–150 days for adults (1). We examined the retention of ^{137}Cs in mice as a function of age in order to have consistently acquired data for one mammalian species.

* Work performed under the auspices of the U. S. Atomic Energy Commission.

Methods. The ^{137}Cs chloride was injected intravenously into female mice of varying ages: 21 and 30 days; 3, 11, 22, and 32 months. Each injection of 0.1 ml contained about 0.2 μC carrier-free ^{137}Cs, which gave about 50,000 cpm in the mouse with our counting system. There were six mice in each age group except for the oldest group, which consisted of five mice. Each mouse was placed in a thin, perforated holder and counted under the face of an 8-inch diameter