populations of 3 species of *Hemophilus* when diluted 10^4- or 10^5-fold and incubated in the defined medium is comparable to the high degree found when exposed to DNA after incubation of a 1:10 dilution in buffered saline, the function of the individual components of the medium requires further investigation. By analogy with the work of Fox and Hotchkiss(8) on the requirements for the "creation" or "recreation" of competence in pneumococcal populations, the amino acids and bivalent cations of the medium may function in formation of DNA binding sites and in the DNA-fixation process. Albumin, essential for competence in the pneumococcus is not required by *Hemophilus* under the conditions investigated.

Whether the components of the medium are required for synthesis of specific receptor sites, for a pinocytotic-like means of incorporation of the DNA molecule, or for active transport of DNA across the cell membrane cannot now be answered. However, demonstration of the emergence or expression of competence in the medium provides a tool for further study of both the emergence and decay of the competent state under chemically defined conditions.

Summary. Three species of *Hemophilus*, grown under the method of Goodgal and Herriott, were shown to emerge to a state of competence for transformation to SM-resistance in a chemically defined medium: L-aspartic, L-glutamic acids in buffered saline containing calcium and magnesium ions. The degree of competence of a "standard" culture was enhanced many-fold in 30 to 60 minutes even when diluted 10^5-fold in the defined medium. The emergence of competence in the synthetic environment was temperature dependent and inhibited by chloramphenicol; multiplication was not necessary. Competent populations washed in the defined medium retain competence; in populations which have lost transformability as a result of washing in buffered saline, competence may be restored. Data presented suggest that the competence which emerges reflects the final attainment of the competent state rather than its "restoration" in cells which were competent at an earlier period during growth of the population.

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CCl_4 Poisoning II: Serum Enzymes, Free Fatty Acids and Liver Pathology; Effects of Phenoxybenzamine and Phenergan®.* (27905)

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Carbon tetrachloride liver damage, as demonstrated by alterations in mitochondrial function, increased total liver lipid and necrosis, has been decreased by adrenalectomy, cord transection and administration of blocking agents(1,2). The changes involved in tissue necrosis and lipid deposition may be caused by separate mechanisms, since Phenergan® pretreatment can reduce tissue Ca^{++} imbibition and the efflux of liver enzymes

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into the blood without reducing total liver lipid(3). This investigation was undertaken to compare the effects of phenoxybenzamine and of Phenergan upon the CCl_4 mediated serum enzyme response, and to provide further information on the effects of CCl_4 and phenoxybenzamine upon free fatty acid (FFA) transport.

Materials and methods. Serum FFA were determined according to Dole(4). Male New Zealand white rabbits, 10 weeks old and 1.8-2.2 kg body weight were maintained on Purina nonmedicated rabbit chow and fed *ad libitum* prior to exposure. Two groups were exposed to 500 ppm CCl_4 vapors for 2 hours as previously described(5); one of these was pretreated 2 hours prior to exposure with 2 mg/kg phenoxybenzamine HCl (generously supplied by Smith, Kline and French Laboratories) administered subcutaneously. A third group (control) was air exposed for 2 hours, as was a fourth which was pretreated with phenoxybenzamine. Blood was drawn by left ventricular puncture immediately after exposure, and sera were extracted within one hour of sampling.

The serum activities of glutamic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), triphosphopyridine nucleotide specific isocitric dehydrogenase (ICD), glutamic acid dehydrogenase (GD) lactic acid dehydrogenase (LDH), malic acid dehydrogenase (MD), aldolase (ALD), and phosphohexose isomerase (PHI) were determined as previously described(5). Three groups of 6 rabbits, fed *ad libitum,* were exposed to 500 ppm CCl_4 for 6 hours following: 1) one hour pretreatment with Phenergan‡ 12.5 mg/kg intraperitoneally; 2) 2-hour pretreatment with phenoxybenzamine 2 mg/kg; and 3) no pretreatment. Two groups of animals were pretreated as above prior to a 6-hour air exposure. Twenty air exposed animals served as controls. Blood was sampled for enzyme analyses 24 hours after end of exposure period.

Liver and kidneys of treated and control animals were quickly removed; slices were fixed in cold methylal-calcium, sectioned and stained with H & E. Tissue for lipid examination was fixed, frozen, sectioned and stained with Oil Red O.

‡ N-(2′-dimethylamino-2′-methyl) ethyl phenothiazine · HCl, Wyeth Laboratories.

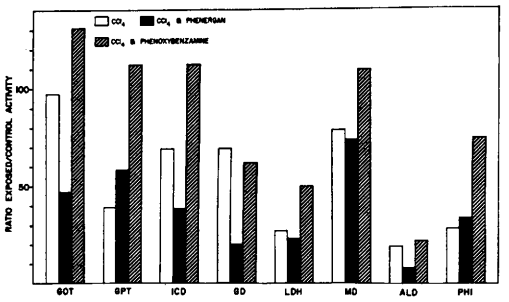

FIG. 1. Serum enzyme activities are expressed as the ratio of exposed to air-exposed control activities. Conditions of exposure and pretreatment are as stated in *Materials and methods.* Enzymes are abbreviated GOT, glutamic-oxalacetic transaminase; GPT, glutamic-pyruvic transaminase; ICD, triphosphopyridine nucleotide specific isocitric dehydrogenase; GD, glutamic acid dehydrogenase; LDH, lactic acid dehydrogenase; MD, malic acid dehydrogenase; ALD, aldolase; PHI, phosphohexose isomerase.

Results. Serum FFA were decreased to 50% of control levels immediately following a 2-hour CCl_4 vapor exposure. Phenoxybenzamine increased serum FFA by 50%; whereas, the simultaneous effects of the 2 agents appeared to be additive.

Vapor exposure to CCl_4 produced increases in serum enzyme activity (Fig. 1). Enzyme activity in sera of phenoxybenzamine of Phenergan pretreated, air exposed animals did not differ significantly from that of the air exposed controls. As compared with CCl_4 alone, phenoxybenzamine + CCl_4 resulted in no significant alterations of serum glutamic dehydrogenase but in marked increases in activity of the remaining serum enzymes. Phenergan pretreatment produced a dramatic decrease in the serum glutamic dehydrogenase activity of CCl_4 exposed animals. Significant decreases (P 0.05) were also observed for ALD, ICD and GOT; but the activities of the remaining serum enzymes were not altered significantly.

The pathological alterations of hepatic architecture were qualitatively similar in all 3 CCl_4 exposed groups. Quantitatively, the Phenergan pretreated + CCl_4 exposed animals demonstrated less centralobular ne-

TABLE I. Serum Free Fatty Acid (FFA) Response to CCl_4 and Phenoxybenzamine.

Treatment	Serum FFA (μeq/liter $\pm$ SE)	No. of animals	P*
1. Control	1180 $\pm$ 69	6	1 vs. 2, $P < .005$
2. CCl_4	525 $\pm$ 21	6	1 vs. 3, $P < .005$
3. Phenoxybenzamine	1820 $\pm$ 147	8	2 vs. 4, $P < .005$
4. " and CCl_4	1310 $\pm$ 129	8	3 vs. 4, $P < .025$

Conditions as stated in *Materials and methods.*
* P was determined by "Student's t-test."

crosis. Though hepatocytes showed granular alterations and lipid accumulations, there were more intact, normal-staining nuclei and less over-all inflammatory response than in the other 2 groups (Fig. 2, 3). Histologically, lipid appeared to be quantitatively greater in the Phenergan pretreated exposed animals. It was deposited more heavily and extensively throughout the liver lobules encroaching upon the portal area. There was no definitive consistent pathologic difference between the phenoxybenzamine pretreated CCl_4 exposed and the CCl_4 exposed animals. Histological evidence of kidney damage was not observed under any of the above conditions.

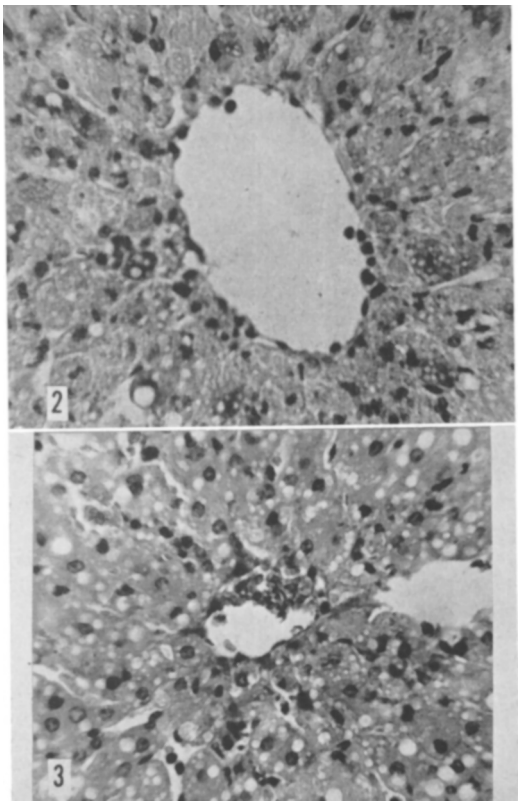

FIG. 2. (500 ppm CCl_4, 24 hr postexposure). Necrosis and inflammatory reaction with fatty phanerosis of cells about central vein. Note relative absence of cell nuclei though cellular margins are still discernible (H & E, x 1128).

FIG. 3. (Pretreatment, 12.5 mg/kg Phenergan®, exposed 500 ppm CCl_4, section 24 hr postexposure). Relatively more normal staining hepatocytic nuclei present, cellular outlines more discernible. Fatty phanerosis apparent (H & E, x 1098).

Discussion. The early CCl_4 induced decrease of serum FFA confirms the observations of Spitzer and Miller(6) but is in direct contradistinction to the more recent observations of Calvert and Brody(7). Neither is it consistent with the unaltered efflux of FFA from fat pads of CCl_4 treated animals(8). Phenoxybenzamine, when administered together *in vivo* with epinephrine, completely blocks the epinephrine induced FFA response of adipose tissue incubated *in vitro* (9). However, serum FFA were elevated by phenoxybenzamine (Table I). It is apparent that serum FFA may not reflect efflux from adipose tissue, since the serum level could be subject to alterations in the threshold of tissue uptake, circulatory flow and the availability of plasma protein binding sites. Therefore, a study of plasma FFA turnover is necessary to demonstrate the mechanisms by which CCl_4 and phenoxybenzamine influence plasma FFA levels.

The only measured change in CCl_4 response produced by phenoxybenzamine was an increase in serum activity of those enzymes§ present in the 105,000 $\times$ *g* supernatant of liver homogenates (Fig. 1). Al-

§ In rabbit liver, glutamic dehydrogenase in 100% mitochondrial. Malic dehydrogenase activity is equally divided between mitochondrial and supernatant fractions. The other 6 enzymes for which data are reported here exhibited less than 5% of the total activity in the mitochondrial fraction(5).

though phenoxybenzamine has proved effective in reducing hepatic necrosis and lipid accumulation(1,2), the means of CCl_4 treatment were different from that used in our experiments. When isolated livers were perfused with halogenated hydrocarbons, an abnormal resistance to perfusate flow was observed after a few hours(10). This correlates well with our pathologic data since the hepatocytes appeared hydropic and grossly swollen immediately following our 6-hour vapor exposure(5). Thus, in our experiments, phenoxybenzamine, by reducing either permissive or hypersecretory effects of catechol amines, could expose the liver to a greater quantity of blood-borne CCl_4. This could offset any blocking agent induced reduction of damage due to a possible hypoxic condition.

Phenergan, at the dose employed, was quite effective in decreasing the extent of necrosis (Fig. 2, 3). It also appeared that mitochondrial damage was reduced as evidenced by the pronounced decrease of serum glutamic dehydrogenase activity (Fig. 1). In contrast, however, Phenergan does not act by protecting the liver against the primary lesion since serum activity of many liver supernatant enzymes was relatively unchanged (Fig. 1), and lipophillic staining was actually increased. The increase in lipophillic staining could have been caused by a greater availability of high energy intermediates for lipid synthesis. This raises a question as to the effectiveness of Phenergan in preventing the early alterations in steady state concentrations of ATP and ADP in mitochondrial and supernatant fractions(11).

Since Phenergan has been shown to affect mitochondrial swelling and contraction(12, 13), our data suggest a mitochondrial locus of action but do not eliminate the possibilities of loci in the cell membrane, and in the endoplasmic reticulum if these structures are continuous.

Summary. Serum FFA were decreased by CCl_4 and increased by phenoxybenzamine. When phenoxybenzamine and CCl_4 were administered together, the effects appeared to be additive. Phenoxybenzamine was ineffective in protecting the liver from damage caused by vapor exposure to CCl_4. Phenergan reduced hepatic necrosis and serum activity of a liver mitochondrial enzyme, glutamic dehydrogenase. Lipophillic staining was increased while serum activities of liver supernatant enzymes were not decreased consistently. These data suggest a possible mitochondrial locus of action for Phenergan.

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