Only the D29 phage–antiphage system was found to have exponential neutralization kinetics. The reason for this is not clear. Cross neutralization experiments have shown that these three phage–antiphage systems are specific and that no cross neutralization occurs between heterologous phage–antiphage systems.

Summary. The antigenicity of several known mycobacteriophages was determined in rabbits. The concentration of antibody production was low (K values 100 or less). The kinetics of neutralization showed that the D29, and not the Leo or R1, phage–antiphage system was exponential. The amounts, but not the rates, of antibody produced were dependent on the route used to inject the animals. No cross neutralization occurred between the heterologous phage–antiphage systems.

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Hepatic Drug Metabolism after Phenobarbital or 3-Methylcholanthrene Pretreatment of Rats Bearing a Pituitary Tumor (33034)

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A decrease in the microsomal metabolism of eight exogenous compounds by liver from rats bearing a somatotropin, corticotropin, and prolactin secreting pituitary tumor (MtT) (1) has been reported (2,3). The decreased metabolism of hexobarbital and aminopyrine by liver from MtT-bearing rats as compared with that of control rats was not mediated via the adrenals or testes, nor were any inhibitors of the *in vitro* hepatic metabolism of these two compounds demonstrated in the liver of MtT rats (4). In the present study, phenobarbital or 3-methylcholanthrene (3-MC) was administered to control and to MtT-bearing rats in order to (a) determine if liver microsomal drug-metabolizing enzyme activity (as measured by the metabolism of hexobarbital, aminopyrine, zoxazolamine, and benzpyrene) was increased after such treatment, and (b) investigate further the type of inhibition of this activity produced by growth of the MtT.

Materials and Methods. Male Fischer rats served as donors and recipients of the MtT [Furth strain MtT-F4 (5)]. After excision from donor animals, the tumor was homogenized in 0.9% aqueous saline with a glass homogenizer. The forty-first and forty-second generations of MtT were used, and at the time of autopsy, morphologic evidence for the secretion of corticotropin, prolactin, and somatotropin by the tumor was noted as previously described (3). Rats were given food and water *ad libitum.*

Rat livers were excised, and 1 gm of liver was homogenized with 2 ml of 1.15% KCl in a glass homogenizer which had a teflon pestle. The liver homogenate was centrifuged at 9000*g* at 4°C for 20 min. The liver 9000*g* supernatant fraction (0.25 ml equivalent to 1/12 gm of liver) was added to an incubation mixture which contained (as μmoles/2.5 ml of the mixture) glucose-6-phosphate, 12.5; $MgSO_4$, 12.5; and nicotinamide adeninedinucleotide phosphate (NADP), 2.08. The μmoles of substrate per 2.5 ml of reaction mixture

were: hexobarbital sodium, 2.25; aminopyrine, 20; zoxazolamine, 3; and benzpyrene, 0.3. The $MgSO_4$ was omitted from the reaction mixture when benzpyrene was used as the substrate, and semicarbazide HCl (25 μmoles /2.5 ml) was added when formaldehyde formed from aminopyrine was measured. The final volume of the incubation mixture was adjusted to 2.5 ml with 0.1 *M* potassium phosphate buffer, pH 7.35. Incubations were performed under oxygen at 37°C in a shaking water-bath incubator for 15 min (hexobarbital and aminopyrine) or for 30 min (benzpyrene and zoxazolamine).

The liver metabolism of hexobarbital (6), zoxazolamine (7), and of benzpyrene (7,3) was estimated by measuring the amount of substrate disappearance. Formaldehyde formed by the liver demethylation of aminopyrine was determined by the method of Nash (8) as modified by Cochin and Axelrod (9).

Results and Discussion. Although growth of the MtT in rats produced a marked decrease in the hepatic metabolism of hexobarbital and the production of formaldehyde from aminopyrine, phenobarbital pretreatment of MtT-bearing rats resulted in a significant increase in this metabolism as compared with that of saline-treated animals with the tumor (Fig. 1). The postphenobarbital increase in liver microsomal metabolism of these two compounds was not prevented by growth of the MtT, but the administration of phenobarbital did not bring this metabolism up to control levels.

A recent report (3) suggested that the percentage decrease in liver microsomal drug-metabolizing enzyme activity produced by growth of the MtT in rats was substrate dependent. That is, the liver metabolism of some drugs in MtT animals was decreased more than that of others. In the present study, there was a greater difference in the percentage increase of hexobarbital metabolized than there was of formaldehyde formed from aminopyrine following phenobarbital pretreatment of MtT-bearing rats (Fig. 1). Phenobarbital pretreatment of rats with the tumor increased the hepatic metabolism of hexobarbital 182%, whereas the production of formaldehyde from aminopyrine was increased only 36.6% as compared with that from MtT-bearing rats which did not receive phenobarbital.

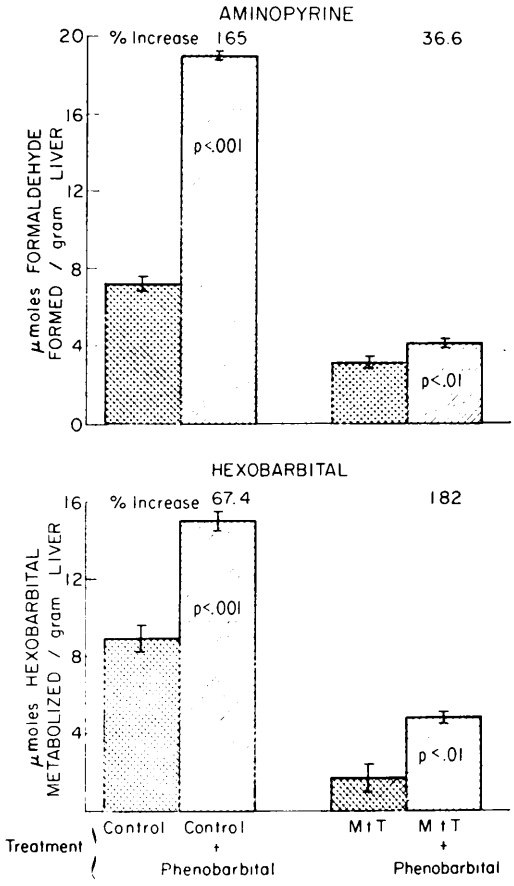

FIG. 1. The effect of phenobarbital pretreatment on the metabolism of hexobarbital and aminopyrine by liver from control and MtT-bearing male rats. Male Fischer rats (55 days old) were injected with MtT homogenate in each thigh (total dose equivalent to 1/3 gm of tumor). After 30 days of tumor growth, control and MtT-bearing animals were injected with phenobarbital sodium (75 mg/kg of body wt. i.p.) for 4 days. There were five rats per group. The μmoles of hexobarbital metabolized and of formaldehyde formed from aminopyrine per gram of liver are depicted as the mean ± SE of the mean. The liver metabolism of these two compounds in phenobarbital pretreated rats (control + phenobarbital and MtT + phenobarbital) was compared with that of nonphenobarbital-treated animals (control and MtT, respectively) in order to calculate the level of significance (p) and the percentage increase.

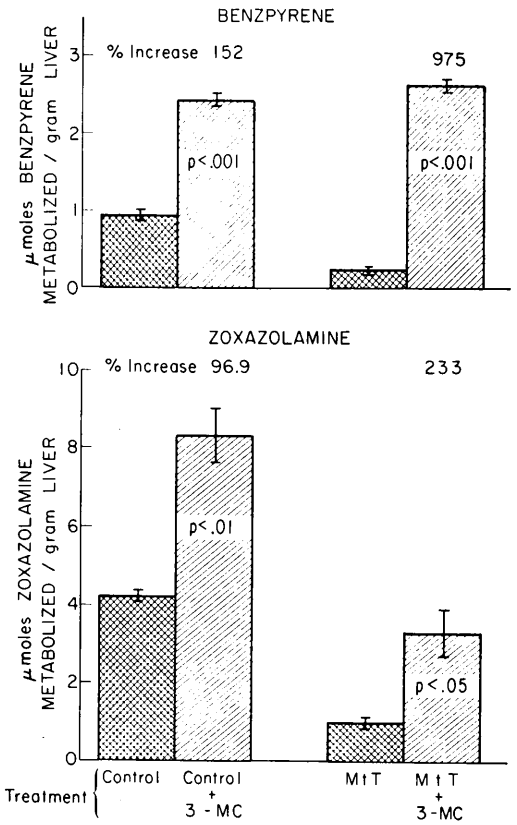

FIG. 2. The metabolism of zoxazolamine and benzpyrene by liver from control and MtT-bearing male rats after pretreatment with 3-methylcholanthrene (3-MC). Male rats (88 days old) were injected intramuscularly with 1 ml of MtT homogenate (equivalent to 500 mg of tumor). A solution which contained 3-MC in corn oil was injected (20 mg of 3-MC/kg of body wt.) intraperitoneally into control (control + 3-MC) and tumor-bearing (MtT + 3-MC) rats 48 hours before determinations were made. Normal control (control) and MtT control (MtT) rats received an injection of corn oil. The animals were used after 35 days of MtT growth, and there were 5 rats per group. The μmoles of zoxazolamine and benzpyrene metabolized/gram of liver are depicted as the mean ± SE of the mean. The level of significance (p) and the percentage increase in the liver/metabolism of each compound was calculated by comparing 3-MC pretreated rats with their respective controls (control + 3-MC vs control and MtT + 3-MC vs MtT).

The injection of 3-MC into MtT-bearing rats produced an increase in the liver metabolism of zoxazolamine and benzpyrene (Fig. 2). The increased liver biotransformation of these two compounds in MtT rats after 3-MC pretreatment also appeared to be substrate dependent, because this treatment increased the metabolism of benzpyrene by 975% and that of zoxazolamine by only 233% as compared with that of non-3-MC treated rats with the tumor. There was a difference in the percentage increase of zoxazolamine (96.9%) and benzpyrene (152%) metabolized by liver from 3-MC treated control rats, but this difference was less than that observed after 3-MC treatment of rats with the MtT.

The percentage increase in liver microsomal drug-metabolizing enzyme activity for four compounds in MtT rats after phenobarbital or 3MC pretreatment appeared to be substrate dependent. This percentage increase, however, may vary, because the MtT growth rate and possibly the decrease in hepatic drug metabolism may be different with each study. A description of hepatic hexobarbital and aminopyrine metabolism or of zoxazolamine and benzpyrene metabolism enhancement after pretreatment of MtT-bearing rats with phenobarbital or 3MC respectively was the primary purpose of this report.

The greater than expected effect of 3-MC, as well as phenobarbital on hepatic drug metabolism in MtT rats, may have resulted from a decreased biotransformation of these "inducing agents." In this regard, Shirasu *et al.* (10) noted that there was an increase in the excretion of *N*-hydroxy-*N*-2-fluorenylacetamide (N-OH-FAA) glucosiduranic acids in the urine of MtT rats pre-fed N-OH-FAA as compared with pre-fed but nontumor-bearing rats. Higher levels of N-OH-FAA which had not undergone dehydroxylation or deacetylation by rat liver were maintained in MtT-bearing rats.

The ability of drugs such as phenobarbital and 3-MC to stimulate the activity of several liver microsomal drug-metabolizing enzyme systems may be prevented by the prior or concurrent administration of ethionine (11, 12), actinomycin D (13), or puromycin (14). However, the inhibition of hepatic microsomal drug metabolism produced by growth of the pituitary MtT in rats appeared to be unlike that found with exogenous inhibitors, because (a) the normal level of this

metabolism was markedly decreased as compared with that of control rats, and (b) the response of this metabolism to phenobarbital and 3-MC was not prevented. If pituitary hormones (somatotropin, corticotropin, and prolactin) secreted by the MtT were responsible for the effects on liver drug metabolism described here and elsewhere (2,3,4), then the administration of these rat hormones (when available) to rats may provide information about the normal regulation of this liver drug-metabolizing enzyme activity.

Summary. The administration of phenobarbital to rats bearing a pituitary mammotropic tumor (MtT) produced an increase in the hepatic metabolism of hexobarbital which was greater than that of similarly treated control rats. The formation of formaldehyde from aminopyrine by liver from MtT-bearing rats, however, was not increased by an amount comparable to that of control rats following phenobarbital pretreatment. The metabolism of benzpyrene was increased more than that of zoxazolamine by liver from MtT rats after an injection of 3-MC, and the metabolism of both compounds was increased more than that found after control rats were injected with 3-MC. An increase in the liver metabolism of four drugs which followed phenobarbital or 3-MC pretreatment of control rats, therefore, was not prevented by growth in rats of a corticotropin, somatotropin, and prolactin producing pituitary tumor.

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The Excretion of the 3-Oxo-Conjugate of Aldosterone* by Normal Adolescent Boys† (33035)

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Considerable information is available in the literature concerning the excretion of the 3-oxo-conjugate of aldosterone by normal adults, but only a small amount of data has been published for normal children. Of the latter data, relatively little has to do with adolescent boys. Mattox *et al.* (1) have reported 24-hour excretion values of 0.7–6.5 $\mu g/m^2$ of body surface area for four boys

* Now also known as aldosterone 18-glucuronide.

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