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THE EFFECT OF A PITUITARY MAMMOTROPIC TUMOR ON HEPATIC MICROSOMAL DRUG METABOLISM IN THE RAT*

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Abstract—The effect of a transplantable hormone-producing pituitary mammotropic tumor (MtT) on the hepatic microsomal metabolism of eight compounds was studied in male and female Fischer rats. Growth of this tumor for about 31 days decreased the hepatic metabolism of most compounds studied as well as liver microsomal NADPH oxidase activity and the amount of hepatic cytochrome CO-binding pigment (P-450). The activity of glucose 6-phosphate dehydrogenase in the liver 104,000 *g* supernatant fraction of tumor-bearing rats, however, was increased as compared with that of control animals. Another tumor, adrenocarcinoma R-3230AC, did not decrease the hepatic metabolism of hexobarbital, aminopyrine or *p*-nitrobenzoic acid. High blood levels of rat somatotropin, prolactin and corticotropin produced by the MtT, individually or in combination, may have been responsible for the decrease in the metabolism of some compounds which was found in liver from MtT-bearing rats.

THE GROWTH of a pituitary mammotropic tumor in rats was found to produce high blood levels of rat corticotropin, prolactin and somatotropin[1] which resulted in splanchnomegaly and an increase in body weight as compared with non-tumor-bearing rats.[2] Bates *et al.*[3] were able to simulate some of the effects of this tumor on rat body and organ weights by the administration of large amounts of these three pituitary hormones. In order to observe the effects of these hormones on the hepatic microsomal metabolism of some compounds, this pituitary mammotropic tumor was implanted in rats to serve as a source of corticotropin, somatotropin and prolactin.

METHODS

Animals and preparation of tumor. Male and female Fischer rats were obtained from the National Institutes of Health or from a local distributor. Rats were housed in suspended metal cages approximately $3\frac{1}{2}$ in. above a refuse pan which contained wood shavings. A mammotropic tumor, MtT-F4 (hereafter denoted as MtT), was originally produced by Furth *et al.*[4] by treating rats with high doses of diethylstilbesterol. This tumor was obtained from male or female MtT-bearing Fischer rats which served as donor animals, and the thirty-ninth, fortieth and forty-first generations of tumor were used. At about 60 days of age, rats were injected intramuscularly with 1 ml of a mammotropic tumor homogenate prepared by homogenizing 1 g

* Part of this study was presented at the fall 1967 meeting of the American Society for Pharmacology and Experimental Therapeutics.

tumor plus 1 ml of sterile 0·9% aqueous saline in a ground-glass homogenizer. The tumor was allowed to grow for about 30–35 days, and tumor-bearing rats were used before any loss of body weight was noted. When the livers were removed, the body, liver, tumor and adrenal weights were recorded. Enlargement of mammary tissue and milk production were also noted. MtT-bearing animals which did not show mammary gland enlargement or an increase in liver weight in addition to adrenal weight as compared with that of control rats were not included in the study.

Preparation of liver. Livers were excised and homogenized (1 g liver plus 2 ml of 1·15% KCl) in a glass homogenizer with a teflon pestle. The liver 9000 *g* supernatant fraction, obtained by centrifuging the liver homogenate at 9000 *g* for 20 min, was used in most studies.

Preparation of incubation mixture. One ml of the liver 9000 *g* supernatant fraction (equivalent to $\frac{1}{3}$ g liver) was added to an incubation mixture which contained (as μmole/5 ml reaction mixture); glucose 6-phosphate (G-6-P), 25; nicotinamide, 100; $MgSO_4$, 25; nicotinamide adenine dinucleotide phosphate (NADP) 0·52*. Substrates and their final concentration in μmole/5 ml were: hexobarbital sodium, 3; aminopyrine, 40 (when formaldehyde was determined) or 10 (when 4-aminoantipyrine was measured); zoxazolamine, 3; *p*-nitrobenzoic acid, 12; neoprontosil, 7·8; codeine, 20; *N*-methyl-aniline, 20; benzpyrene, 0·6. Formaldehyde produced from aminopyrine or *N*-methyl-aniline was trapped by adding 50 μmole semicarbazide to the reaction mixture. The volume of the reaction mixture was brought to 5 ml with 0·1 M potassium phosphate buffer, pH 7·35. Samples were incubated in a Dubnoff shaking incubator at 37° for 30 min under oxygen, or under nitrogen for samples which contained *p*-nitrobenzoic acid or neoprontosil as the substrate.

Chemical assays. The rat liver metabolism of hexobarbital by side chain oxidation was determined by measuring the amount of substrate disappearance.[6] The hepatic formation of formaldehyde from aminopyrine or from *N*-methyl-aniline was estimated by the method of Nash[7] as modified by Cochin and Axelrod.[8] 4-Aminoantipyrine (4AAP) formed by the hepatic *N*-dealkylation of aminopyrine was measured after diazotizing and coupling of the compound with alpha-naphthol.[9] Liver hydroxylation of zoxazolamine and benzpyrene (estimated by substrate disappearance) was determined as described by Juchau *et al.*[10] The fluorescence of benzpyrene in hexane was measured with an excitation wavelength of 386 mμ and an emission wavelength of 406 mμ in a Baird-Atomic spectrophotofluorometer. The reduction by rat liver of *p*-nitrobenzoic acid (PNBA) to form *p*-aminobenzoic acid (PABA) and of neoprontosil to form sulfanilamide was estimated by a procedure described by Fouts and Brodie.[11] Morphine formed by the hepatic *O*-dealkylation of codeine was determined with the phenol reagent of Snell and Snell.[12] Liver microsomal NADPH oxidase activity was estimated by the method described by Hart and Fouts[13] as adapted from Gillette *et al.*[14] Hepatic cytochrome CO-binding (P-450) was determined with a Beckman DU spectrophotometer according to the method of Klingenberg.[15] Glucose 6-phosphate dehydro-

* The purpose of this study was to measure the metabolism of eight compounds by the 9000 *g* supernatant fraction of liver from control and MtT-bearing rats under the same reaction condition *in vitro*. Although nicotinamide may inhibit the demethylation *in vitro* of aminopyrine by rat liver to form formaldehyde,[5] it has not been shown to inhibit the metabolism of the seven other compounds which were studied. Furthermore, under the conditions for incubation of the control liver 9000 *g* supernatant fraction described in Methods, no decrease in the formation of formaldehyde from 5, 10, 20 and 40 μmole aminopyrine/5 ml reaction mixture was detected when 100 μmole nicotinamide was added and compared with a reaction mixture which did not contain nicotinamide.

genase (G-6-PD) activity in the liver 104,000 *g* supernatant fraction was measured by a method described by Kornberg and Horecker.[16]

The statistical methods outlined in Snedecor[17] were used, and the level of significance chosen was $P < 0{\cdot}05$.

RESULTS

Pathways studied. The metabolism of hexobarbital,* aminopyrine,* zoxazolamine, *N*-methyl-aniline, codeine, benzpyrene, PNBA* and neoprontosil by the liver 9000 *g* supernatant fraction from control and MtT-bearing male rats is depicted in Table 1. The metabolism of all compounds is expressed as micromoles of substrate utilized or product formed per gram of liver or per whole liver. Results were calculated on a "metabolism per whole liver" basis so that some estimate of the total hepatic metabolism (or total liver metabolism per rat) of these compounds could be obtained. In male rats with the MtT, the liver biotransformation of all compounds was decreased as compared with that of control rats when the results were calculated on a per gram liver basis. Expression of the results as "metabolism per whole liver" however, showed that the hepatic metabolism of hexobarbital, the formation of formaldehyde from aminopyrine and *N*-methyl-aniline, and the production of 4AAP from aminopyrine in MtT male rats remained lower than that of the control. Whereas the hepatic metabolism of zoxazolamine or benzpyrene, the production of morphine from codeine, and the formation of PABA from PNBA were similar (not statistically different) to that of control rats on a "metabolism per whole liver" basis, the μmoles of sulfanilamide produced from neoprontosil per whole liver was increased in MtT male rats as compared with that of control rats.

In female rats with the MtT (Table 2), the hepatic metabolism of all other compounds, except for that of benzpyrene and neoprontosil, was decreased when the results in these rats were expressed on a per gram liver basis and compared with the results from control female rats. The formation of 4AAP from aminopyrine by liver from MtT-bearing female rats appeared to be decreased more than the metabolism of the other eight compounds when these metabolisms were compared with those of non-tumor-bearing female rats on a per gram liver (— 93·3 per cent) or per whole liver (— 73·3 per cent) basis. In contrast to the male (Table 1) and with the exception of the formation of 4AAP from aminopyrine by liver from control and MtT rats, the hepatic metabolism of the remaining seven compounds seen in Table 2 was significantly increased in female MtT rats as compared with non-MtT-bearing controls when the metabolism was expressed as micromoles of substrate utilized or product formed per whole liver. This finding may be partly explained by noting that growth of the MtT in male and female rats seemed to decrease the hepatic metabolism of most compounds depicted in Tables 1 and 2 to a level that was similar for both males and females. For example, formaldehyde formed from aminopyrine by liver from males with MtT ($2{\cdot}20 \pm 0{\cdot}13$ μmole/g liver) was similar to that formed by liver from females with MtT ($2{\cdot}47 \pm 0{\cdot}22$ μmole/g liver). Thus, the greater percentage decrease in the liver metabolism of some compounds (expressed as metabolism per gram liver) by male rats with the MtT as compared with that of female MtT-bearing rats may have resulted from

* A study which describes the decrease in the metabolism of hexobarbital, the formation of formaldehyde from aminopyrine, and the production of PABA from PNBA by liver from MtT-bearing rats as compared with that of control animals has recently appeared.[18,19]

TABLE 1. METABOLISM OF SOME COMPOUNDS BY THE 9000 *g* SUPERNATANT FRACTION OF LIVER FROM CONTROL AND MAMMOTROPIC TUMOR-BEARING MALE RATS*

Compound studied	Compound measured	Metabolism/g liver†			Metabolism/whole liver†		
		Control	MtT§	%‡	Control	MtT	%‡
Hexobarbital	Hexobarbital disappearance	6·99 ± 0·42	0·38 ± 0·16	− 94·6	47·3 ± 2·4	8·2 ± 3·8§	− 82·7
Aminopyrine	Formaldehyde	6·42 ± 0·30	2·20 ± 0·13	− 65·7	44·4 ± 1·9	28·8 ± 1·4§	− 35·1
Aminopyrine	4-aminoantipyrine	0·51 ± 0·03	0·04 ± 0·01	− 92·7	3·4 ± 0·2	0·8 ± 0·3§	− 75·6
Zoxazolamine	Zoxazolamine disappearance	2·66 ± 0·09	1·02 ± 0·09	− 61·7	20·4 ± 1·1	20·8 ± 1·3	+ 2·2
N-methyl-aniline	Formaldehyde	2·63 ± 0·2	0·14 ± 0·02	− 94·7	17·8 ± 1·2	2·8 ± 0·3§	− 84·4
Codeine	Morphine	0·79 ± 0·02	0·45 ± 0·03	− 43·4	6·1 ± 0·3	9·4 ± 1·1	+ 55·9
Benzpyrene	Benzpyrene disappearance	0·44 ± 0·012	0·13 ± 0·02	− 69·8	3·4 ± 0·2	2·7 ± 0·4	− 19·0
p-Nitrobenzoic acid	*p*-aminobenzoic acid	0·80 ± 0·04	0·30 ± 0·03	− 62·7	6·2 ± 0·5	6·1 ± 0·3	− 1·1
Neoprontosil	Sulfanilamide	3·60 ± 0·02	3·17 ± 0·07	− 11·9	27·6 ± 1·2	66·0 ± 4·9§	+ 139·1

* In the mammotropic tumor (MtT) group, male Fischer rats (60–62 days old) were injected i.m. in each thigh with 0·5 ml MtT homogenate equivalent to 250 mg tumor. Livers were excised after the tumor had grown for 31–35 days. Control animals of a similar age were used, and there were 5 rats per group.

† Results are expressed as micromoles of substrate metabolized or of product formed per g (wet wt.) liver or per whole liver ± S.E. (S.E.M.).

‡ Per cent decrease (−) or increase (+) as compared with the control.

§ Significance, $P < 0·05$ as compared with the control.

TABLE 2. METABOLISM OF SOME COMPOUNDS BY THE 9000 *g* SUPERNATANT FRACTION OF LIVER FROM CONTROL AND MAMMOTROPIC TUMOR-BEARING FEMALE RATS*

Compound studied	Compound measured	Metabolism/g liver†			Metabolism/whole liver†		
		Control	MtT	%‡	Control	MtT§	%‡
Hexobarbital	Hexobarbital disappearance	1·40 ± 0·12	0·90 ± 0·04§	− 35·7	6·70 ± 0·1	10·7 ± 0·5	+ 60·3
Aminopyrine	Formaldehyde	4·15 ± 0·27	2·47 ± 0·22§	− 40·5	21·0 ± 1·1	44·9 ± 0·5	+ 113·8
Aminopyrine	4-Aminoantipyrine	0·104 ± 0·004	0·007 ± 0·004§	− 93·3	0·54 ± 0·05	0·15 ± 0·08	− 73·3
Zoxazolamine	Zoxazolamine disappearance	1·27 ± 0·13	0·62 ± 0·09§	− 51·2	6·5 ± 0·6	12·0 ± 1·4	+ 85·5
N-methyl-aniline	Formaldehyde	1·33 ± 0·10	0·67 ± 0·06§	− 49·6	7·0 ± 0·8	14·3 ± 1·2	+ 104·6
Codeine	Morphine	0·70 ± 0·01	0·43 ± 0·02§	− 39·1	3·6 ± 0·1	8·5 ± 0·5	+ 135·6
Benzpyrene	Benzpyrene disappearance	0·38 ± 0·04	0·26 ± 0·02	− 30·9	1·94 ± 0·17	5·22 ± 0·49	+ 169·1
p-Nitrobenzoic acid	*p*-Aminobenzoic acid	0·62 ± 0·05	0·31 ± 0·04§	− 49·8	3·2 ± 0·3	6·1 ± 0·6	+ 91·5
Neoprontosil	Sulfanilamide	3·77 ± 0·10	3·24 ± 0·12	− 14·1	19·4 ± 0·5	64·2 ± 1·6	+ 230·9

* Footnotes are the same as for Table 1 except that female Fischer rats were used.

a higher "normal" level of metabolism in male control rats, which growth of the tumor decreased to a level similar for both males and females. In addition, a lower "control" level of metabolism for these compounds by control female liver plus the observation that implantation of the MtT increased liver size 2- to 3- fold in both sexes may partly explain the increase in hepatic metabolism of most compounds per whole liver in female rats with MtT (Table 2) as compared with only one compound (neoprontosil) in males with MtT (Table 1). This explanation of the greater percentage decrease in the microsomal metabolism of some compounds by liver from MtT males as compared with that from MtT females seemed preferable to one which suggested that growth of the MtT more effectively decreased the metabolism of most compounds (expressed per gram liver or per whole liver) in male rats than it did in female rats.

The percentage decrease in hepatic microsomal drug-metabolizing enzyme activity produced by growth of the MtT for 31–35 days appeared to be substrate-dependent in both male (Table 1) and female rats (Table 2). That is, the liver metabolism of each substrate was not decreased by a similar percentage amount in tumor-bearing rats as compared with that of non-tumor-bearing animals. Although the metabolism of all compounds depicted in Tables 1 and 2 was not determined in liver from one group of tumor-bearing and one group of control rats, each compound showed a similar percentage decrease when its metabolism was studied in two separate experiments with liver from MtT and control rats. It should also be noted that the decrease in the amount of hexobarbital metabolized or formaldehyde formed from aminopyrine by liver from tumor bearing rats did not appear to be directly related to the total weight of the tumor when animals bearing the tumor for 31–35 days were used.

Effect on liver NADPH oxidase, G-6-PD and P-450. The activity of NADPH oxidase and the amount of CO-binding pigment (P-450) in hepatic microsomes as well as the activity of G-6-PD in the liver 104,000 *g* supernatant fraction were determined in male control and MtT-bearing rats (Table 3). The oxidation of NADPH by liver microsomes from rats implanted with the MtT was 46·7 per cent less than that of liver

TABLE 3. NADPH OXIDASE, G-6-PD AND P-450 IN LIVER FROM CONTROL AND MAMMOTROPIC TUMOR-BEARING MALE RATS*

Parameter studied	Control	MtT	%†
NADPH oxidase‡	0·375 ± 0·019	0·200 ± 0·014 §	− 46·7
P-450‖	164·3 ± 5·6	77·0 ± 5·3§	− 53·1
G-6-PD‡	2·6 ± 0·1	9·2 ± 0·4§	+ 249

* Male Fischer rats, 60–65 days old, were injected i.m. with 1 ml MtT homogenate equivalent to 500 mg tumor. Hepatic microsomes or the 104,000 *g* supernatant fraction was obtained after 30–34 days of tumor growth. Control rats were the same age as those bearing the tumor, and there were 5 animals per group.

† Per cent increase (+) or decrease (−) as compared with the control.

‡ Expressed as the change in o.d. per min per g (wet wt.) liver.

§ Significance ($P < 0·05$) as compared with the control.

‖P-450 was estimated in 3 ml liver microsomes (equivalent to 300 mg liver) at 450 mμ with a 1 cm light path. Results are expressed as optical density units × 10^{-3}.

microsomes from control rats. Similarly, the amount of P-450 formed by liver microsomes from tumor-bearing rats was reduced to 53·1 per cent of that found in control liver. However, liver G-6-PD activity in rats with the tumor was increased 249 per cent above that found in control animals. The decreased metabolism of some compounds by the liver 9000 *g* supernatant fraction from tumor-bearing rats, therefore, did not result from a deficiency of G-6-PD which, with G-6-P and NADP, was used as an NADPH-generating system.

Effect of another tumor. Adenocarcinoma R-3230AC, a rapidly growing mammary tumor,[20] was implanted in 68-day-old male Fischer rats in order to estimate the effects on hepatic microsomal drug metabolism of a tumor which was not known to produce pituitary hormones. After 13 days of growth, the adenocarcinoma R-3230AC tumor mass (8 g) was similar to that seen with the MtT (4–15 g) after approximately 31 days of growth. The amount of hexobarbital metabolized, formaldehyde formed from aminopyrine, or PABA produced from PNBA by the 9000 *g* supernatant fraction of liver from rats with the adenocarcinoma R-3230AC (after 13 days of growth) was similar to that of non-tumor-bearing rats (Table 4). Thus, growth of a nonpituitary tumor (adenocarcinoma R-3230AC) in male rats did not produce a decrease in the liver microsomal metabolism of three compounds which was observed with growth of the MtT in male rats (Table1).

TABLE 4. METABOLISM OF SOME COMPOUNDS BY THE 9000 *g* SUPERNATANT FRACTION OF LIVERS FROM CONTROL AND ADENOCARCINOMA-BEARING MALE RATS*

Compound studied	Compound measured	Control†	Tumor†
Hexobarbital	Hexobarbital disappearance	4·09 ± 0·22	4·00 ± 0·32
Aminopyrine	Formaldehyde	5·91 ± 0·13	5·19 ± 0·23
p-Nitrobenzoic acid	*p*-Aminobenzoic acid	0·90 ± 0·03	1·03 ± 0·05

* Male Fischer rats (68 days old) were implanted s.c. with small pieces of adenocarcinoma R-3230AC, and the tumor was allowed to grow for 13 days. Control rats were the same age, and there were 7 animals per group.

† Expressed as μmoles hexobarbital metabolized, formaldehyde formed from aminopyrine, or *p*-aminobenzoic acid produced from *p*-nitrobenzoic acid per g (wet weight) liver. No values were significant ($P < 0·05$) as compared with the control.

DISCUSSION

The hepatic metabolism of eight compounds in the male and of six compounds in the female rat was decreased by the growth of the MtT. This decrease occurred in rat liver which was exposed to high levels of rat pituitary tumor somatotropin, prolactin and corticotropin at the time microsomal drug-metabolizing enzyme activity was measured. In male rats with the tumor, liver microsomal NADPH oxidase activity was decreased, and hepatic 104,000 *g* supernatant fraction G-6-PD activity was increased as compared with that from control rat liver. The amount of microsomal P-450 in liver from animals with the MtT was also less than that of non-tumor-bearing rats but, since particle size may affect the absorbance of microsomes at 450 mμ when a Beckman DU spectrophotometer is used for the determination of P-450, the interpretation of this decrease must be postponed until the amount of P-450 in liver microsomes can be measured by other techniques.

In addition to the decreased liver metabolism of the drugs described here, the hepatic biotransformation of a carcinogen was found to be less in MtT-bearing animals as compared with non-tumor-bearing controls. Shirasu *et al.*[21] noted that the dehydroxylation and deacetylation of *N*-hydroxy-2-acetylaminofluorene were decreased in liver from rats with the MtT in contrast to that of control rats. Weisburger *et al.*[22] found an enhanced development of liver cell carcinoma in rats which were implanted with MtT and fed this carcinogen. When high doses of either of two hormones (somatotropin and corticotropin) produced by the MtT were administered to rats, the hepatic dehydroxylation and deacetylation of *N*-hydroxy-2-acetylaminofluorene may have been decreased.[23] Irving [24,25] showed that this carcinogen was metabolized by hepatic microsomes with NADPH as a necessary cofactor. Thus, the metabolism of *N*-hydroxy-2-acetylaminofluorene seemed to require hepatic microsomes, and this metabolism was probably decreased in rats which received two of the three pituitary hormones produced by the MtT.

Although Kato *et al.*[26] found that the hepatic metabolism of hexobarbital, strychnine and meprobamate was decreased in rats bearing the Walker carcinosarcoma 256, Rogers *et al.*[27] did not find a decrease in the metabolism of hexobarbital in liver from rats bearing the Morris hepatoma 7800 until the rats with large tumors became cachetic. A decrease in the liver *N*-demethylation of aminopyrine was not observed in hepatoma-bearing rats regardless of the time allowed for tumor growth. In the study by Kato *et al.*[26] the average tumor weight was 25·2 g per rat, whereas rats with the MtT had approximately 8 g of tumor per rat when hepatic microsomal drug-metabolizing enzyme activity was estimated. In the present study, growth of a tumor of non-pituitary origin (adenocarcinoma R-3230AC) did not decrease the liver metabolism of three compounds even though the weight of this tumor in rats was comparable with that of the MtT. If there were a factor common to most tumors which decreased the microsomal metabolism of some compounds in liver tumor-bearing rats, such a factor was not detected with the adenocarcinoma R-3230AC. The hepatic microsomal metabolism of some compounds in the rat, therefore, may have been decreased by some or all of the anterior pituitary hormones secreted by the mammotropic tumor MtT-F4.

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