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THE METABOLISM OF DRUGS BY REGENERATING LIVER*†

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Abstract—Liver during and immediately following the rapid regeneration caused by partial hepatectomy is deficient in ability to oxidize the side-chain of hexobarbital, oxidize the ring-sulfur of chlorpromazine, and reduce the nitro-group of *p*-nitrobenzoic acid. Full recovery of these enzyme activities occurs at about the same time as complete regeneration of hepatic mass (approx. 10 days after operation). The rate of O-dealkylation of codeine is affected only during the first (and most rapid) phase of regeneration; following this period of rapid regeneration (approx. 4 days after operation), this enzyme activity is the same as in unoperated control animals.

The lower enzyme activities are not caused by a lack of known cofactors or by the presence of inhibitors.

Sham-operation affects the rates of metabolism of hexobarbital and chlorpromazine, but not those of codeine or *p*-nitrobenzoic acid. The effects of sham-operation are significantly less than those of partial hepatectomy.

WORK in our laboratory has shown that hepatic tumors[1] and livers from new-born animals[2] are deficient in ability to metabolize a variety of drugs. It appeared that hepatic cells dividing rapidly may be absolutely or relatively deficient in such enzyme activities. Therefore, we studied the metabolism of several drugs by the microsomal fraction prepared from regenerating liver.

Von der Decken and Hultin[3] have reported that the microsomal enzyme systems which demethylate and cleave *p*-monomethylaminoazobenzene (MAB) were less active than "normal" in livers from animals after partial hepatectomy.

We have investigated (a) whether regenerating liver metabolized only one or several drugs more slowly than normal liver, (b) the extent to which enzyme activities were depressed, (c) whether the changes seen were fully reversible, and if so, how quickly, and (d) whether decreases in activity might be caused by deficiencies in cofactor (reduced triphosphopyridine nucleotide (TPNH)), or the presence of inhibitors.

MATERIALS AND METHODS

Male Holtzman rats weighing 200–250 g were used. Partial hepatectomies were performed according to the method of Higgins and Anderson.[4] Sham-operated animals were anesthetized with ether, the peritoneum was opened, the liver exteriorized, and

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the wound closed as in the hepatectomized animals. From seventy to eighty rats were operated on the same day. Every morning thereafter, control (unoperated), sham-operated, and hepatectomized rats were killed, the livers excised, and samples taken for histological examination by the Pathology Department*. Livers used in enzyme assays were chilled and homogenized with 2 vols of 1·15% KCl. The 9000 **g** supernatant fractions containing microsomal and soluble enzymes were prepared in the cold from homogenates using a high speed angle centrifuge.

The metabolisms studied were the side-chain oxidation of hexobarbital, the O-dealkylation of codeine, the ring-sulfur oxidation of chlorpromazine, and the reduction of the nitro-group of *p*-nitrobenzoic acid. Disappearance of substrate was measured in following the metabolisms of hexobarbital and chlorpromazine. The appearance of a metabolite (morphine and *p*-aminobenzoic acid) was measured to follow the metabolisms of codeine and *p*-nitrobenzoic acid, respectively. Hexobarbital was determined by the method of Brodie and Axelrod.[5] Chlorpromazine was assayed by the method of Salzman and Brodie.[6] The metabolism of *p*-nitrobenzoic acid was followed as reported previously.[7] Morphine was determined by using a phenol reagent[8] after extraction from tissue with chloroform. The incubation conditions used, cofactors added and their concentrations have been described.[9] Glucose-6-phosphate dehydrogenase was assayed by the method of Kornberg and Horecker.[10]

Duration of action of hexobarbital *in vivo* was assessed using "sleeping times" of rats injected intraperitoneally with hexobarbital sodium (100 mg/kg). The time during which the righting reflex was absent was called the sleeping time. No rat was used more than once in sleeping time determinations.

Statistical methods used are described by Snedecor.[11] We have chosen the value $P = 0{\cdot}05$ as the level of significance.

RESULTS

Tables 1, 2, 3 and 4 show that all drug metabolizing enzymes studied were present in livers regenerating after partial hepatectomy. From 48 to 192 hr after hepatectomy the metabolisms of hexobarbital, chlorpromazine, and *p*-nitrobenzoic acid were significantly slower than control.

The effect of sham-operation also is seen in Tables 1 and 2. Sham-operation affected the metabolisms of hexobarbital and chlorpromazine. At 72, 120, 168 and 192 hr after sham-operation the rates of metabolism of codeine and *p*-nitrobenzoic acid were not significantly different from control. At times of maximum depression, drug-metabolizing enzyme activities in hepatectomized animals were significantly less than those in sham-operated animals. Enzyme activity in regenerating liver leveled off at a minimum, for all metabolisms except codeine, between 72 and 192 hr after hepatectomy. The degree of depression of metabolism (expressed as per cent of control) was not the same for all pathways. Most affected was the metabolism of *p*-nitrobenzoic acid. Least depressed was the O-dealkylation of codeine. The rate of metabolism of codeine by livers from hepatectomized animals differed from the control rate only at 48 and 72 hr after operation. At all other times there was no significant difference in the rate of this metabolism between hepatectomized and control animals.

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TABLE 1. METABOLISM OF CHLORPROMAZINE BY 9000 g SUPERNATANT FRACTIONS OF RAT LIVER

Hours after operation	Metabolism by §						
	Unoperated (control) (μmoles*/g liver)	Hepatectomized (μmoles*/g liver)	P†	% of control activity	Sham-operated (μmoles*/g liver)	P‡	% of control activity
0	1·1 ± 0·28	—	—	—	—	—	—
48		0·63 ± 0·22	0·005	57	0·60 ± 0·19	>0·5	54
72		0·45 ± 0·28	<0·001	41	0·85 ± 0·27	0·025	77
96		0·35 ± 0·31	<0·001	32	1·3 ± 0·51	<0·001	118
120		0·42 ± 0·31	<0·001	38	1·2 ± 0·22	0·005	109
144		0·47 ± 0·24	<0·001	43	1·0 ± 0·43	0·010	91
168		0·32 ± 0·24	<0·001	29	0·64 ± 0·22	0·05	58
216		0·79 ± 0·17	0·025	72	1·3 ± 0·30	0·005	118
288		1·5 ± 0·10	<0·001	136	1·4 ± 0·26	0·2	127

* Values are average μmoles drug metabolized ± standard deviation per g liver (wet weight).

† Probability that there is no difference between the means of hepatectomized vs. control groups other than that caused by random error in measurement. Student *t*-test.

‡ Probability that there is no difference between the means of hepatectomized vs. sham-operated groups.

§ All groups had seven animals except control which had fourteen.

TABLE 2. METABOLISM OF HEXOBARBITAL BY 9000 g SUPERNATANT FRACTIONS OF RAT LIVER

Hours after operation	Unoperated (control) (μmoles*/g liver)	Hepatectomized (μmoles*/g liver)	n§	P†	% of control activity	Sham-operated (μmoles*/g liver)	n§	P‡	% of control activity
0	4·1 ± 1·2	—		—	—	—		—	—
48		1·7 ± 0·5	(15)	<0·001	41	2·5 ± 0·7	(7)	0·025	61
72		1·6 ± 0·5	(10)	<0·001	39	3·0 ± 0·8	(7)	<0·001	73
96		1·6 ± 0·6	(12)	<0·001	39	2·6 ± 0·4	(7)	<0·001	63
120		1·5 ± 0·6	(10)	<0·001	37	3·3 ± 0·8	(7)	<0·001	80
144		1·6 ± 1·1	(11)	<0·001	38	2·9 ± 0·6	(7)	0·005	71
168		1·5 ± 0·7	(11)	<0·001	37	3·0 ± 0·7	(7)	<0·001	73
192		1·4 ± 0·1	(8)	<0·001	34	—		—	—
216		2·3 ± 0·8	(15)	<0·001	57	2·7 ± 0·8	(7)	0·4	66
288		3·9 ± 0·6	(7)	>0·5	95	2·8 ± 0·3	(7)	0·001	68

* Values are average μmoles drug metabolized ± standard deviation per g liver (wet weight).

† Probability that there is no difference between the means of hepatectomized vs. control groups other than that caused by random error in measurement. Student *t*-test.

‡ Probability that there is no difference between the means of hepatectomized vs. sham-operated groups.

§ *n* = number of animals per group. Control groups had fifteen animals.

Full recovery of enzyme activities for the metabolism of hexobarbital, chlorpromazine, and *p*-nitrobenzoic acid occurred between 216 and 288 hr. This was at approximately the time when complete regeneration of hepatic mass has been reported to occur.[4] The rate of metabolism of codeine returned to control levels when the first phase of rapid regeneration had stopped—reported by Higgins and Anderson to occur 3 to 4 days after hepatectomy.[4]

Nicotinamide, TPN, $MgSO_4$, and glucose-6-phosphate were added in every assay. Glucose-6-phosphate dehydrogenase activity (generates TPNH) in livers from hepatectomized animals was at all times equal to or greater than control. As far as we could determine, the lower rates of metabolism of drugs *in vitro* were not caused by a deficiency of TPNH, the only known cofactor of these systems.

TABLE 3. METABOLISM OF CODEINE BY 9000 g SUPERNATANT FRACTIONS OF RAT LIVER

Hours after operation	Unoperated (control) (μmoles*/g liver)	Hepatectomized (μmoles*/g liver)	n‡	P†	% of control activity
0	1·6 ± 0·4	—		—	—
48		1·1 ± 0·3	(15)	0·005	69
72		1·2 ± 0·3	(15)	0·025	75
96		1·4 ± 0·4	(15)	0·2	88
120		1·3 ± 0·5	(11)	0·2	81
144		1·7 ± 0·4	(11)	0·4	106
168		1·5 ± 0·2	(11)	>0·5	94
192		1·4 ± 0·7	(4)	>0·5	88
216		1·8 ± 0·3	(11)	0·5	113

* Values are average μmoles drug metabolized ± standard deviation per g liver (wet weight).
† Proability that there is no difference between the means of hepatectomized vs. control groups other than that caused by random error in measurement. Student t-test.
‡ n = number of animals per group. Control group had fifteen animals.

TABLE 4. METABOLISM OF *p*-NITROBENZOIC ACID BY 9000 g SUPERNATANT FRACTIONS OF RAT LIVER

Hours after operation	Unoperated (control) (μmoles*/g liver)	Hepatectomized (μmoles*/g liver)	n‡	P†	% of control activity
0	1·7 ± 0·2	—		—	—
48		0·80 ± 0·09	(8)	<0·001	47
72		0·78 ± 0·17	(8)	<0·001	46
96		0·30 ± 0·10	(7)	<0·001	18
120		0·25 ± 0·01	(8)	<0·001	15
144		0·59 ± 0·12	(8)	<0·001	35
168		0·41 ± 0·01	(8)	<0·001	24
192		0·61 ± 0·12	(4)	<0·001	36
216		1·1 ± 0·17	(8)	<0·001	65
288		2·0 ± 0·22	(7)	0·025	116

* Values are average μmoles drug metabolized ± standard deviation per g liver (wet weight).
† Probability that there is no difference between the means of hepatectomized vs. control groups other than that caused by random error in measurement. Student t-test.
‡ n = number of animals per group. Control group had eight animals.

Mixtures of 9000 g supernatant fractions from control rats and from hepatectomized rats metabolized drugs at rates which were not less than those of the control supernatant fraction alone. It appeared that there were no enzyme inhibitors in livers from hepatectomized animals.

The duration of action of hexobarbital was used to estimate the rate of metabolism of this drug *in vivo*. Hepatectomized rats slept longer after a given dose of hexobarbital than did control or sham-operated animals. Table 5 shows that a prolonged sleeping

time was found at all times when the *in vitro*-assays showed the rate of hexobarbital metabolism to be less than in sham-operated animals. The depression *in vitro* of the metabolism of hexobarbital that was seen in sham-operated animals was not sufficient to affect the sleeping time *in vivo*. Thus, the duration of action of hexobarbital in sham-operated animals was not significantly different from that in control animals (Table 5).

TABLE 5. THE DURATION OF ACTION OF HEXOARBITAL IN CONTROL AND HEPATECTOMIZED RATS

Hours after operation	Sleeping times				
	Unoperated (controls) (min)	Sham-operation (min)	n*	Hepatectomized (min)	n*
0	22 ± 6	—	—	—	—
72		26 ± 4	4	283 ± 69	4
96		22 ± 2	5	204 ± 68	5
120		31 ± 6	5	88 ± 14	6
144		21 ± 5	6	80 ± 12	5
168		24 ± 4	5	85 ± 15	6
192		24 ± 6	5	54 ± 8	7
216		—	—	48 ± 8	6
240		—	—	50 ± 7	7
264		22 ± 5	5	42 ± 14	6
288		21 ± 3	5	27 ± 5	5

* n = number of animals in each group. Control group had thirty-two animals.

Histological examinations of sections of livers at all times after hepatectomy revealed no pathological changes which might be a cause of the lower enzyme activities.

DISCUSSION

Rapidly growing liver can be deficient in a number of microsomal enzymes which metabolize drugs. The pathways of metabolism affected and the extent of this effect depend upon the conditions causing rapid growth. In hepatic tumors[1] and in livers from newborn animals[2] the ability to metabolize hexobarbital, chlorpromazine, and *p*-nitrobenzoic acid is essentially absent. In regenerating liver after partial hepatectomy all of these metabolic activities are present, but their rates are less than control values. Between 48 and 192 hr after hepatectomy the rate of metabolism of *p*-nitrobenzoic acid is markedly depressed, while the rates of metabolism of hexobarbital and chlorpromazine also are depressed, but to a lesser extent.

Between 48 and 96 hr after partial hepatectomy, the rate of metabolism of codeine is less than in control animals This rate of metabolism is the same as in control animals from 96 to 216 hr after operation. Of all the drug metabolizing enzymes studied, only that for the metabolism of codeine returns to control levels of activity following the first phase of rapid regeneration (72–96 hr)

These results serve to emphasize again that not all drug-metabolizing enzymes are affected equally by the same conditions We have reported similar findings in studies on the metabolism of drugs in jaundiced rabbits,[9] starved mice[12] and diabetic rats.[13] von der Decken and Hultin[3] have shown that some microsomal enzyme activities were depressed after hepatectomy, while other activities were unchanged or elevated.

Their studies, showing a decrease in the rate of metabolism of MAB, are similar to our results, which show a decrease in the rate of metabolism of hexobarbital, chlorpromazine, and *p*-nitrobenzoic acid. However, one cannot generalize and say that all microsomal drug-metabolizing enzyme activities are depressed during the regeneration following hepatectomy.

The decrease in enzyme activities is unlikely to be caused by a deficiency of TPNH or the presence of enzyme inhibitors in regenerating liver At this time we cannot rule out a change in amount of active enzyme as the cause of the decreased activity.

The effects of sham-operation on the metabolism of drugs are not easily understood. It is apparent that all pathways are not affected by such operation. The rates of metabolism of hexobarbital and chlorpromazine are depressed, while those of codeine or *p*-nitrobenzoic acid are not affected. The effects of hepatectomy on the metabolism of hexobarbital and chlorpromazine are significantly greater than those of sham-operation at the times of the maximal effects of hepatectomy. The conclusions, though complicated by these effects of sham-operation, are still valid: the liver during and immediately after rapid regeneration is relatively deficient in its ability to metabolize certain drugs.

From such information as we have, one might speculate about the effects of certain hepatic injuries on the metabolism of drugs. In many cases, injury to the liver is followed by a period of regeneration. If such regeneration were extensive, the metabolism of drugs might be affected. Such effects on drug-metabolizing enzymes might not be predicted by presently used function tests or histological examinations, since these tests do not directly assess the status of the endoplasmic reticulum (or the microsomes which are derived from this reticulum).

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