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THE EFFECTS OF NOREPINEPHRINE TREATMENT ON DRUG METABOLISM BY LIVER MICROSOMES FROM RATS*

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Abstract—A single, large intraperitoneal injection of norepinephrine has little effect on the hepatic microsomal metabolism of hexobarbital, aminopyrine, or aniline, though hepatic glycogen levels are markedly depressed for at least 12 hr. Repeated injections of norepinephrine will depress both hepatic glycogen and the microsomal drug metabolisms studied. These effects are at least partially reversible when injections or norepinephrine are stopped. Phenoxybenzamine and dihydroergotamine, given i.p., can block the effects of norepinephrine on hepatic glycogen, but cannot be said to block effects of the catecholamine on microsomal drug metabolism because these adrenolytic drugs (and also dichloroisoproterenol) have a direct inhibitory effect on the hepatic drug metabolisms studied. The inhibition of the microsomal metabolism of hexobarbital and aminopyrine by phenoxybenzamine and dihydroergotamine is of the same order as that seen with SKF 525 (β-diethylaminoethyldiphenylpropyl acetate) and is the subject of further investigation.

PREVIOUS work[1–5] in this laboratory has shown that changes in smooth endoplasmic reticulum (SER) structure and microsomal drug-metabolizing activity are generally accompanied by changes in hepatic glycogen content. Hepatic glycogen content and drug-metabolizing enzyme activity may be related, since both drug-metabolizng enzymes and glycogen stores are found predominantly associated with areas of the smooth reticulum of the hepatic cell. To investigate a possible correlation between hepatic glycogen content, SER structure, and SER function (drug metabolism), a method was employed which is known to alter hepatic concentrations of glycogen: i.e. norepinephrine treatment.[6] This work was done in an attempt to correlate changes in hepatic glycogen concentration with changes in activity of drug-metabolizing enzymes of the smooth reticulum.

METHODS

Preparation of animals. Male Holtzman rats weighing 200–250 g were used. Norepinephrine bitartrate (Levophed, Winthrop) was injected intraperitoneally (i.p.) The animals were killed at various times after the administration of the catecholamine and their livers removed and prepared for glycogen, histological, and enzyme studies.

Preparation of tissue samples. Livers were homogenized in the cold with a Potter homogenizer having a plastic pestle. All homogenates were prepared such that each

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gram of liver was suspended in 2 ml of cold isotonic (1·15%) KCl. The supernatant fraction (9,000 *g*), containing microsomal and soluble enzymes, was prepared from the homogenate with a high-speed angle centrifuge (20 min) at 5°.

The supernatant was centrifuged for 60 min at 30,000 rev/min (78,000 *g*) to sediment microsomes. These microsomes were reconstituted with soluble fraction so that 1 ml contains the microsomes from 1 g of liver.

Determinations in vitro

One ml of liver fraction was incubated in a Dubnoff shaking incubator at 37° with oxygen as the gaseous phase for oxidative enzyme determinations. Final concentrations of the cofactors added were: triphosphopyridine nucleotide (NADP) (5·5 to 11 $\times$ 10^{-5} M), glucose-6-phosphate (5 $\times$ 10^{-3} M), nicotinamide (2 $\times$ 10^{-2} M), and $MgSO_4$ (5 $\times$ 10^{-3} M). All concentrations are superoptimal, and the final volume of all incubation mixtures was brought to 5 ml with 0·1 M phosphate buffer, pH 7·4.

The pathways studied, methods used in their assay, and substrate concentration in μmoles per 5 ml incubate were: the side chain oxidation of hexobarbital[7] (3 μmoles); the N-dealkylation of pyramidon (aminopyrine) to 4-aminoantipyrine[8] (40 μmoles); and the hydroxylation of aniline to *p*-aminophenol (0·5 μmole). In this last determination the metabolite, *p*-aminophenol, was measured by a method of J. R. Gillette (personal communication). Two g of NaCl was added to 4 ml of the incubate, and the mixture extracted by shaking with 30 ml ether for 20 min. Twenty ml of the ether phase was then extracted with 4 ml 1 N Na_2CO_3 containing 1% phenol (phenol added to the Na_2CO_3 just prior to use). One ml of a bromine–Na_2CO_3 solution (bromine water added to 1N Na_2CO_3 until a light yellow color appears) was added to 3 ml of the Na_2CO_3 phase, and the mixture read at 620 mμ on a Coleman spectrophotometer. Disappearance of substrate was measured after the metabolism of hexobarbital. The appearance of the metabolite listed (4-aminoantipyrine, *p*-aminophenol) was determined in the other pathways studied.

Determination of glucose-6-phosphate dehydrogenase was performed by the method of Kornberg and Horecker.[9] Hepatic levels of glycogen were determined as described by Montgomery.[10]

Statistical evaluation of data

The statistical methods used are described by Snedecor.[11] The Student's 't' distribution was used as a test of the null hypothesis. The level of significance used in all determinations was P equal to or less than 0·05. All values expressed in tables are means plus or minus standard deviation. Means which are significantly different from control are underlined. The number of animals represented by this mean is in parentheses.

RESULTS

In these experiments the observed decreases in drug-metabolizing enzyme activity were due to an alteration of the microsomal fraction, and not due to 'extra microsomal' factors. A NADPH-generating system contributes the only known cofactor which in the presence of oxygen completes the nonmicrosomal portion of this system.[12]

No deficiency of cofactor NADPH was suggested by any of the following methods

(a) All incubation mixtures contained optimal quantities of glucose-6-phosphate, NADP, nicontinamide, and magnesium ion. Glucose-6-phosphate, NADP, and the soluble-fraction enzyme glucose-6-phosphate dehydrogenase, make up the NADPH-generating system.

(b) Glucose-6-phosphate dehydrogenase activity was determined in all cases. When the activity of this enzyme was below normal, additional glucose-6-phosphate dehydrogenase (Sigma Chemical Co.) was added to the incubate. In no case did the addition of this enzyme alter results.

(c) In order further to rule out effects upon the soluble fraction portion of the *in-vitro* system, the following experiments were conducted. Soluble fraction from normal liver was added to hepatic microsomes from norepinephrine (NE)-treated animals, and hepatic microsomes from normal animals were combined with soluble fraction from livers of NE-treated animals (Table 1). In no case did the exchange of soluble fraction have a significant effect upon observed results.

TABLE 1. METABOLISM OF HEXOBARBITAL BY HEPATIC MICROSOMES

	Soluble fraction	
	NE-treated	Control
Microsomes Norepinephrine-treated	0·14*	0·17
Microsomes (Control)	0·79	0·87

* Metabolism of hexobarbital expressed as mean micromoles of drug metabolized by microsomes from $\frac{1}{3}$ g wet wt.liver/2 hr incubation. Mean is average of two determinations utilizing six animals per separation.

In these experiments liver microsomes from NE-treated animals were separated from the corresponding soluble fraction, and liver microsomes from control or normal animals were also separated from their soluble fraction by ultracentrifugation at 78,000 *g* for 1 hr. Combinations of microsomes with soluble fractions were made such that microsomes from NE-treated animals were incubated with soluble fraction from NE-treated or control animals (line 1, columns 1 and 2 respectively), and microsomes from control animals were incubated with soluble fraction from NE-treated or control animals (line 2, columns 1 and 2 respectively).

The experiments suggest that the explanation for an altered rate of drug metabolism was a defect in the microsomal fraction and not a deficiency of cofactors.

Rats were treated acutely and chronically with norepinephrine, which was chosen instead of epinephrine because of its more selective effect upon hepatic glycogen levels in the rat. Epinephrine is reported to alter both hepatic and skeletal muscle glycogen in the rat. Isoproterenol predominantly affects skeletal muscle glycogen in this animal. Norepinephrine causes an alteration in the amount of hepatic glycogen with little effect on skeletal muscle glycogen.[13]

Preliminary experiments established that isoproterenol had little effect on hepatic glycogen in doses as high as 40 mg/kg administered i.p. Similar results have been reported by Vrij *et al.*[13] The effects of epinephrine have been studied by our group, and these results have been reported in a recent review article by Fouts.[14] A dose of 1 mg norepinephrine/kg, calculated as free base, was injected i.p. as the bitartrate salt in a 0·01% solution. It was established in preliminary experiments that this dose and dilution would depress liver glycogen for a 12-hr period without being lethal to more than 5% of the animals treated.

Table 2 demonstrates that a single injection i.p. of norepinephrine, 1 mg/kg, has a rapid effect on hepatic glycogen concentrations. The effects were pronounced for at least 12 hr after injection, after which time recovery occurred. This dose of norepinephrine, which so markedly reduced hepatic glycogen concentrations after a single administration, was without significant effect upon the microsomal metabolisms of hexobarbital, aminopyrine, or aniline (Table 2). It is interesting to note what appears to be an initial stimulation of the metabolism of hexobarbital and aminopyrine 2 hr after injection of the catecholamine. A similar stimulation is noted in the data published by Fouts.[14]

TABLE 2. EFFECTS OF A SINGLE DOSE OF NOREPINEPHRINE ON HEPATIC LEVELS OF GLYCOGEN AND MICROSOMAL DRUG METABOLISM*

Hours after injection	No.	Glycogen†	Hexobarbital	Aminopyrine	Aniline
Control	3	5·88 ± 1·29	4·16 ± 2·02	0·43 ± 0·16	
2	3	*0·08 ± 0·07*	6·27 ± 0·50	0·70 ± 0·10	
4	3	*0·05 ± 0·03*	3·30 ± 0·07	0·41 ± 0·04	
8	3	*0·02 ± 0·02*	3·25 ± 0·35	0·39 ± 0·04	
Control	4	5·69 ± 0·91	3·77 ± 1.15	0·29 ± 0.09	0·26 ± 0·10
12	4	*0·81 ± 0·71*	4·30 ± 0·87	0·30 ± 0·11	0·37 ± 0·03
24	4	*2·11 ± 1·08*	2·87 ± 0·73	0·17 ± 0·04	0·26 ± 0·05

* Norepinephrine was injected i.p., 1 mg/kg, at various times prior to 8 a.m. (All animals were sacrificed at about 8 a.m.)

† Values for glycogen are expressed as mean gram % ± standard deviation. Metabolism of drugs expressed as mean micromoles metabolized per gram wet weight liver per 2 hr incubation ± standard deviation. All values in italics are significantly different from control.

Table 3 presents data concerning the effect of norepinephrine, 1 mg/kg injected i.p. every 12 hr starting at 8 a.m. In the upper half of Table 3 all assays (glycogen and drug metabolism) were made 12 hr after the last dose of NE. Thus a group of animals was killed for enzyme and glycogen determinations after two doses (24 hr after the start of treatment and 12 hr after the last dose of NE), four doses (48 hr after starting treatment and 12 hr after the last dose), and six doses (72 hr after starting treatment and 12 hr after last dose). After six doses of NE the injection of drug was stopped, and recovery of glycogen and enzyme activity was determined (lower half of Table 3). It can be seen that hepatic glycogen concentrations had returned to control levels 36 hr after injections of drug were stopped. However, the metabolism of hexobarbital and aminopyrine was significantly depressed at this time. Four and five days after treatment ceased (168 and 192 hr after first injection), metabolism of these two drugs was still below control values.

Table 4 demonstrates that hepatic glycogen concentrations can be maintained at control levels during most of the course of norepinephrine treatment with an adrenergic blocking agent, phenoxybenzamine (Dibenzyline; Smith, Kline and French). Of the blocking agents studied, phenoxybenzamine and dihydroergotamine were effective in blocking the glycogenolytic action of NE. Dichlorisoproterenol (DCI, Lilly) in doses as high as 20 mg/kg was without effect on the glycogenolysis after NE. These observations are in agreement with those of Claassen and Noach.[15] However, even with hepatic glycogen levels maintained at control levels, the metabolisms of hexobarbital, aminopyrine, and aniline are significantly depressed. It can

TABLE 3. EFFECTS OF CHRONIC NOREPINEPHRINE TREATMENT ON HEPATIC GLYCOGEN AND DRUG METABOLISM

Treatment*	No.	Glycogen	Hexobarbital	Aminopyrine	Aniline†
Effects of repeated NE Injection					
Control	3	7·18 ± 1·31	4·27 ± 0·83	0·54 ± 0·18	0·31 ± 0·09
NE, 2 doses (24 hr)	7	*1·27 ± 0·59*	*2·54 ± 0·49*	*0·20 ± 0·08*	0.30 ± 0.08
Control	5	6·51 ± 1·07	5·31 ± 0·91	0·48 ± 0·05	0·61 ± 0·19
NE, 4 doses (48 hr)	7	*1·01 ± 0·88*	*2·24 ± 0·74*	*0·10 ± 0·05*	*0·40 ± 0·13*
Control	7	6·20 ± 0·90	5·52 ± 1·00	0·47 ± 0·17	0·39 ± 0·11
NE, 6 doses (72 hr)	9	*1·78 ± 1·22*	*1·93 ± 0·87*	*0·19 ± 0·05*	*0·25 ± 0·07*
Recovery from NE Effects					
Control	3	6·69 ± 0·56	3·85 ± 0·45	0·34 ± 0·02	
NE, 6 doses (96 hr, 36 hr after last dose NE).	7	6·67 ± 1·55	*2·02 ± 1·32*	*0·06 ± 0·02*	
Control	2	5·27 ± 1·29	4·78 ± 0·16	0·34 ± 0·09	0·27 ± 0·10
NE, 6 doses (120 hr, 60 hr after last dose NE).	4	6·99 ± 0·65	*1·67 ± 0·40*	*0·10 ± 0·03*	*0·17 ± 0·04*
Control	2	6·70 ± 0·32	4·96 ± 1·55	0·39 ± 0·00	
NE, 6 doses (168 hr, 108 hr after last dose NE).	5	7·25 ± 0·41	3·86 ± 1·19	*0·23 ± 0·10*	
Control	2	7·29 ± 0·28	2·84 ± 0·10	0·30 ± 0·05	
NE, 6 doses (192 hr, 132 hr after last dose NE).	4	6·76 ± 1·20	*1·89 ± 0·57*	0.26 ± 0·05	

* Norepinephrine injected i.p., 1 mg/kg, twice daily for 3 days.

† Glycogen and drug-metabolizing pathways are expressed the same as in Table 2.

TABLE 4. EFFECT OF PHENOXYBENZAMINE ON NOREPINEPHRINE ALTERATION OF HEPATIC GLYCOGEN AND MICROSOMAL DRUG METABOLISM

Treatment	No.	Glycogen†	Hexobarbital	Aminopyrine	Aniline
(i) Control (saline)	3	7·18 ± 1·31	4·27 ± 0·83	0·54 ± 0·18	0·31 ± 0·09
(ii) NE, 2 doses	7	*1·27 ± 0·59*	*2·54 ± 0·49*	*0·20 ± 0·08*	0·30 ± 0·08
(iii) NE, 2 doses + phenoxy.	4	*3·07 ± 1·41*	3·65 ± 1·31	0·32 ± 0·06	0·37 ± 0·03
(iv) Saline, 2 doses + phenoxy.	4	*2·24 ± 1·16*	3·47 ± 0·85	0·32 ± 0·09	0·31 ± 0·12
Control (saline)	5	6·51 ± 1·07	5·31 ± 0·91	0·48 ± 0·05	0·61 ± 0·14
NE, 4 doses	7	*1·01 ± 0·88*	*2·24 ± 0·74*	*0·10 ± 0·05*	*0·40 ± 0·13*
NE, 4 doses + phenoxy.	4	6·64 ± 1·17	*2·84 ± 0·81*	*0·17 ± 0·05*	*0·23 ± 0·06*
Saline, 4 doses + phenoxy.	2	6·73 ± 0·14	*3·50 ± 0·01*	*0·23 ± 0·00*	*0·28 ± 0·14*
Control (saline)	7	6·20 ± 0·90	5·52 ± 1.00	0·47 ± 0·17	0·39 ± 0·11
NE, 6 doses	9	*1·78 ± 1·22*	*1·93 ± 0·87*	*0·19 ± 0·05*	*0·25 ± 0·07*
NE, 6 doses + phenoxy.	4	5·46 ± 0·71	*1·24 ± 0·65*	*0·25 ± 0·01*	*0·23 ± 0·02*
Saline, 6 doses + phenoxy.	4	5·99 ± 0·99	*2·30 ± 1·42*	*0·27 ± 0·08*	*0·24 ± 0·02*

* Metabolism and glycogen levels of four groups of animals were determined daily: (i) Control (saline injected); (ii) NE 1 mg/kg every 12 hr; (iii) phenoxybenzamine injected s.c. 5 mg/kg, 15 min before norepinephrine was injected i.p.; (iv) phenoxybenzamine injected s.c., 5 mg/kg, 15 min before saline treatment i.p.

† Glycogen expressed as mean gram % ± standard deviation. Values in italics are significantly different from saline control.

also be seen that phenoxybenzamine had an inhibitory action upon rates of microsomal drug metabolism. This is noted when one compares the metabolism by those animals treated with phenoxybenzamine plus saline to controls which received only saline (Table 4).

The adrenergic blocking agent dihydroergotamine was also investigated. Preliminary experiments indicated that this substance has a direct inhibitory effect upon microsomal drug metabolism similar to that seen with phenoxybenzamine.

Table 5 presents the concentrations of adrenergic blocking agents producing 50% inhibition (I_{50}), interpolated from plots of per cent inhibition against the logarithm of adrenergic blocking agent concentration. It can be seen from these *in-vitro* studies that all adrenergic blocking agents studied were effective inhibitors of microsomal drug metabolism. These effects are under further investigation.

TABLE 5. CONCENTRATION OF ADRENERGIC BLOCKING AGENTS* PRODUCING 50 PER CENT INHIBITION OF VARIOUS DRUG METABOLIC PATHWAYS *in vitro*.

Substrate	Product	Concentration required for 50% inhibition		
		Phenoxybenzamine	Dihydroergotamine	Dichloroisoproterenol
Hexobarbital	Ketohexobarbital	6 ± 6 (3)†	15 (9, 20)	60
Aminopyrine	4-Aminoantipyrine	19 ± 5 (4)	19 (17, 20)	42 (40, 44)
Aniline	*p*-Hydroxyphenol	300 (100, 500)	12 (11, 13)	120 (90, 150)

* Adrenergic blocking agents: phenoxybenzamine HCl (Dibenzyline, SKF), dihydroergotamine methanesulfonate (D.H.E. 45 Sandoz), and dichloroisoproterenol HCl (Lilly).

† Concentrations of inhibitors are M · 10^{-3}. Values are means ± standard deviation. The figures in parentheses represent either the number of separate experiments or actual values comprising average.

DISCUSSION

Norepinephrine was selected for these experiments instead of epinephrine even though its hyperglycemic effect is generally considered to be only about one eighth that of epinephrine. However, this is not the case in rats. Reports indicate that epinephrine causes an increased breakdown of both skeletal muscle and hepatic glycogen.[13] Isoproterenol has little effect on hepatic levels of glycogen but effectively reduces the skeletal muscle glycogen of the rat. Norepinephrine on the other hand, is relatively ineffective in altering the muscle stores of carbohydrate but is quite effective in reducing hepatic stores of glycogen.[13] Another consideration of the more specific action of norepinephrine on carbohydrate metabolism was the 'clinical impression' of the physical state of the rats. The animals treated with norepinephrine at these high doses appeared to be in much better physical condition than were the animals treated with epinephrine at the same dose.

In preliminary experiments our results agreed with those of Vrij *et al.*[13] Both epinephrine and norepinephrine were rapidly effective in lowering hepatic levels of glycogen, whereas isoproterenol in doses of 5, 10, and 20 mg/kg was without effect within 24 hr after i.p. administration. Preliminary experiments also indicated that in order to have a pronounced depression of glycogen level which would last at least 8–12 hr after administration of the norepinephrine, a dose of 1 mg/kg administered i.p. as a dilute solution (0·01%) was necessary.

Results with norepinephrine agree with the preliminary results reported by Fouts using epinephrine.[14] An acute injection of 1 mg of the norepinephrine/kg caused a rapid decrease in the concentration of liver glycogen. This effect lasted 8–12 hr. However, drug metabolism in the presence of these rapid changes of hepatic glycogen was not depressed. An initial stimulation of metabolism, 2 hr after drug, was seen. This effect seemed to be real although statistically not significant with the number of animals used. Fouts[14] has shown a similar increase in metabolism in experiments using epinephrine. Cooper and Rosenthal have reported that addition of norepinephrine or epinephrine to bovine adrenal homogenates accelerates hydroxylation of steroids at C-21 and C-17[16]. The hydroxylation of steroids by this system is also NADPH and oxygen dependent. In experiments *in vitro*, the addition of norepinephrine (10^{-3} to 10^{-6} M) to incubation mixtures increased the metabolism of hexobarbital and aminopyrine, although the increase was not statistically significant.

With repeated injections of norepinephrine a significant decrease in metabolism *in vitro* of hexobarbital, aminopyrine, and aniline was observed. After three days (six injections) of treatment with norepinephrine the treatment was stopped. Recovery of drug-metabolizing enzyme activity and hepatic glycogen concentrations were determined over a period of 132 hr (196 hr after first injection of catecholamine). It was noted that hepatic glycogen concentrations returned rapidly to control values. However, drug-metabolizing enzyme activity was still at less than control values four days after norepinephrine administration was stopped.

The next step in this investigation was to attempt to block the effect of norepinephrine on hepatic glycogen to determine its relationship to microsomal drug-metabolizing activity. Three adrenergic blocking agents were investigated: phenoxybenzamine hydrochloride (Dibenzyline, SKF), dihydroergotamine methanesulfonate (D.H.E. 45, Sandoz) and dichloroisoproterenol (Lilly). The blocking agent was administered subcutaneously 15 min before the i.p. injection of norepinephrine. In preliminary experiments it was established that phenoxybenzamine HCl (5 mg/kg), and dihydroergotamine (0·2 mg/kg) successfully blocked the glycogenolytic effect of norepinephrine. However, dichloroisoproterenol was without effect in doses as high as 20 mg/kg. These observations are in agreement with those published by Claassen and Noach.[15]

Phenoxybenzamine HCl was effective in chronic experiments in maintaining control levels of hepatic glycogen after administration of norepinephrine. However, this adrenergic blocking agent appears to have only a slightly protective effect in blocking the actions of norepinephrine upon drug metabolism. The metabolisms of hexobarbital, aminopyrine, and aniline were significantly lower than control even when hepatic glycogen concentrations were maintained at control levels. These results are complicated by the fact that phenoxybenzamine HCl itself has an inhibitory effect upon drug metabolism. The effect was seen when one compared the metabolism by rats treated with phenoxybenzamine and saline to animals receiving only saline.

Similar results were obtained with dihydroergotamine. That is, the drug maintained the level of hepatic glycogen after treatment with norepinephrine but apparently failed to prevent the decrease in rate of drug metabolism. Dihydroergotamine, like phenoxybenzamine, also is inhibitory to the microsomal drug-metabolizing enzymes.

In subsequent investigations it was determined that all the adrenergic blocking agents used are effective inhibitors of drug metabolism *in vitro* (Table 5). More studies are planned to investigate this effect and attempt to determine its mechanism.

It would be interesting to know whether inhibition of microsomal drug-metabolizing enzyme activity is a property common to all adrenergic blocking agents.

An explanation of the effect of norepinephrine upon microsomal drug metabolism is not possible at this time. Histological examination of livers from norepinephrine-treated animals did not suggest gross hepatic damage as a causative factor. Neither could the decreased drug metabolism be explained by deficient cofactors. It would seem that chronic treatment with this catecholamine results in an alteration of both microsomal drug metabolism and the amount of glycogen stored by the hepatic cell

These experiments and our previous investigations of the effect of starvation (in the mouse),[2] alloxan diabetes,[4] and SKF 525-A treatment[17] indicate that factors which affect glycogen storage by the hepatic SER often affect other functions of the SER (drug-metabolizing enzyme activity). And factors that influence the activity of the microsomal enzymes which metabolize drugs may also influence hepatic glycogen storage. However, the amount of hepatic glycogen does not always correlate with the activity of the drug-metabolizing enzymes. One cannot say that decreased glycogen levels are always associated with decreased microsomal drug-metabolizing enzyme activity, since the results given in Table 2 show this is not true. Conversely it is not true that decreased drug-metabolizing enzyme activity is always accompanied by decreased hepatic glycogen levels. The results in Table 4 as well as other experiments in which *p*-aminosalicylic acid was fed to rats[18] show that drug-metabolizing enzyme activity may be depressed while hepatic glycogen is normal or greater than normal. One point that deserves further study is whether the duration of abnormal glycogen levels may be important to an effect on microsomal enzyme activity. It has been our impression that glycogen must be maintained at abnormal levels for more than a few hours or drug metabolism will not be measurably affected. This may mean that glycogen levels are important only in so far as they affect cell ultrastructure. A rapid change in glycogen which is not sustained may not affect cell structure, whereas long-lasting changes in glycogen will affect structure. It is interesting to note that all conditions in which glycogen levels were abnormal (low in starvation, jaundice, diabetes; high in *p*-aminosalicylic acid treatment) and microsomal drug enzyme activity was also affected were conditions under which effects on glycogen were maintained for at least one and usually several days. We plan to study cell ultrastructure after acute vs. chronic norepinephrine administration to see whether ultrastructure migh change only after chronic treatment, thus giving a reason for the differences observed in this study in effects on drug-metabolizing enzyme activity.

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