The absorption spectrum of the substance less polar than estrone when dissolved in sulfuric acid showed maxima at 400 and 420 mμ which are not shown by any estrogen available to us. The fluorescence of this compound in sulfuric acid was found to be maximal at 480 mμ with maximal excitation at 440 mμ which is characteristic of the estrogens.

Studies with the fraction corresponding to estradiol-17β have shown that the fraction may contain phenols other than estradiol-17β. The absorption spectrum of the fraction in sulfuric acid showed maxima at 420 and 490 mμ with an inflection at 455 mμ which estradiol-17β does not show. In sulfuric acid fluorescence properties were the same as those of estradiol-17β. However, this fraction when studied for estrogenic potency produced a positive vaginal smear in castrate female mice.

The amounts of estrone in 2 of 15 blood samples from pregnant women were 7.6 and 2.3 μg per 100 ml of plasma. The amounts of the fraction corresponding to estradiol-17β in the 15 samples averaged 6.5 μg/100 ml blood, calculated as estradiol-17β. It must be emphasized that this figure includes the increment that is not estradiol-17β.

These results agree with the preliminary report of Purdy, Engel and Oncley(9) that estrone is not found in free form in blood. They showed that estrone sulfate was the main metabolite found in blood after administration of estradiol-17β. The nature of the unknown materials is being investigated.

Summary. A detailed examination of the free estrone fraction of alumina column chromatograms of extracts of blood plasma has shown that when elution is performed with 0.25, 0.50 and then 1% ethanol in benzene instead of only using 1% ethanol in benzene, the free "estrone" fraction actually consisted of a substance slightly less polar than estrone. Free "estrone" is seldom found in plasma. Two of 15 samples contained 7.6 and 2.3 μg/100 ml plasma. The fraction which presumably contained estradiol-17β contained other material. The so-called estradiol-17β fraction averaged 6.5 μg/100 ml plasma.

1. Veldhuis, A. H., *J. Biol. Chem.,* 1953, v202, 107.
2. Nakao, T., Aizawa, Y., *Endocrinol. Japan,* 1956, v92, 2.
3. Aitken, E. H., Preedy, J. R. K., *Biochem. J.,* 1956, v62, 15.
4. Slaunwhite, W. R., Jr., Sandberg, A. A., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 544.
5. Oertel, G. W., West, C. D., Eik-Nes, B., *J. Clin. Endocrinol. and Metab.,* 1959, v19, 1619.
6. Brown, J. B., *Biochem. J.,* 1955, v60, 185.
7. Stimmel, B. F., *J. Biol. Chem.,* 1946, v162, 99.
8. Engel, L. L., *Rec. Progress in Hormone Res.,* 1950, v5, 335.
9. Purdy, R. H., Engel, L. L., Oncley, J. L., *Fed. Proc.,* 1959, v18, 305.

Received November 12, 1959. P.S.E.B.M., 1960, v103.

Factors Affecting Drug Metabolism by Liver Microsomes. IV. Starvation.* (25509)

ROBERT L. DIXON, ROBERT W. SHULTICE AND JAMES R. FOUTS
Dept. of Pharmacology, State University of Iowa, Iowa City

We have studied effects on drug metabolism of factors which affect liver structures, especially liver microsomes(1). One such factor is starvation, since electron micrographs of liver tissue showed that differences do exist between normal and fasted animals. Cytoplasmic basophilia decreases sharply after starvation of a few days, and most hepatic cells lose ergastoplasm(2) and undergo significant change in dispersion of this structure(3). We believed that these cellular changes in liver structure: namely, reduction in amount of ergastoplasm, and changes in microsome subunits, might have an effect on certain microsomal drug metabolisms. A variety of drugs are metabolized through the action of en-

* This investigation supported in part by U.S.P.H.S. Grant.

TABLE I. Effects of Starvation on Drug Metabolism by 9000 × g Supernate from Pooled Mouse Liver. (Micromoles drug metabolized†/g of liver.)

	Incubation time (hr)	Normal S.T.* 5-15 min.	Starved animals S.T.* 20-40 min.	S.T.* 41-60 min.	S.T.* 61-80 min.	S.T.* greater than 80 min.
Hexobarbital	1½	4.46 (4) [2.40–5.96]	2.79 (4) [1.86–4.00]	1.86 (4) [.84–2.34]	.88 (2) [.56–1.20]	.77 (4) [.32–1.11]
Chlorpromazine	"	2.80 (3) [2.49–3.33]	1.98 (3) [1.27–2.38]	1.70 (3) [1.26–2.22]	1.37 (3) [1.21–1.56]	1.19 (3) [.98–1.36]
Pyramidon	"	1.48 (4) [1.42–1.53]	1.11 (4) [.74–1.25]	.74 (4) [.60–.80]	.57 (4) [.35–.70]	.46 (4) [.36–.57]
Acetanilid	"	2.03 (4) [1.30–2.50]	2.22 (4) [1.27–3.42]	1.68 (4) [1.00–2.71]	1.21 (3) [.96–1.68]	.93 (3) [.86–1.03]
p-Nitrobenzoic acid	1	8.22 (3) [5.76–12.30]	17.03 (3) [15.72–18.78]	14.62 (3) [14.16–15.12]	11.88 (3) [11.49–12.60]	8.43 (3) [7.76–8.92]
Neoprontosil	1	15.55 (3) [12.06–19.38]	17.65 (4) [12.39–20.55]	16.44 (3) [10.50–20.91]	15.37 (2) [11.37–19.38]	12.59 (3) [9.90–14.85]

* Groups made on basis of hexobarbital sleeping times (S.T.). See *Methods*.

† Amount of drug disappeared in the case of hexobarbital or chlorpromazine, amount of product formed in case of pyramidon, acetanilid, p-nitrobenzoic acid, or neoprontosil. These values are avg of several experiments. Figures in parentheses are No. of experiments made, and figures in brackets are ranges obtained in the determinations.

zymes present in liver microsomes(4). A greater sensitivity to drugs of the fasted animal may be in part due to deficiency of these enzymes. To gain a picture of the effects of starvation, various drug pathways were studied. These included side chain oxidation of hexobarbital, N-dealkylation of pyramidon, hydroxylation of the aromatic ring of acetanilid, oxidation of the ring sulfur of chlorpromazine, reduction of aromatic nitro-group of p-nitrobenzoic acid, and reduction of aromatic azo-group of neoprontosil.

Methods. Male mice of initial weight of 25-30 g were used. After 36 hours starvation, hexobarbital (80 mg/kg administered intraperitoneally) sleeping times were determined, and mice grouped as to those which slept 20-40, 41-60, 61-80, and more than 80 minutes. Normally, control mice given the same dose of hexobarbital slept less than 10 minutes. Any starved mice sleeping less than 19 minutes were considered essentially unaffected by starvation; this group never amounted to more than 15-20% of total animals used. Twelve hours later (total starvation of 48 hours) metabolism *in vitro* of various drugs by pooled livers from groups of mice was determined. Supernate fraction (9000 g), containing microsomal and soluble enzymes, was prepared from the homogenate of pooled liver samples by high speed angle centrifuge. Methods used in measuring drug metabolism are the same as in the first paper of this series (1 a). Incubation times were the same for liver samples from normal and starved animals.

Results. Table I shows that *in vitro* metabolism correlated well with prolongation of hexobarbital sleeping times. Thus, animals which slept longest were also those whose livers were least able to metabolize most drugs studied. Exceptions are pathways involved in reduction of aromatic nitro group or azo linkage. *In vitro* metabolism of compounds like p-nitrobenzoic acid and neoprontosil is not depressed by starvation which causes a marked effect on most oxidative pathways. Whether or not starvation results in a true stimulation of reduction as compared with marked depression of oxidation is now being studied.

We have also shown that the metabolism of drugs in these fasted animals returns completely to normal within 24 hours after access to food. Under investigation is the effect of various diets on return to normal, of ability of animal to metabolize drugs.

We determined that the dose of hexobarbital used in grouping starved mice, *i.e.*, in assessing effects of starvation on metabolism *in vivo*, does not result in differences in drug enzyme activity, when animals so treated are

compared with uninjected controls. Thus, drug metabolisms in livers from animals injected 12 hours previously with a dose of 80 mg/kg of hexobarbital were compared with those in livers from uninjected animals and shown to differ by no more than 10%. Therefore, when we assayed metabolisms *in vitro*, previous drug administration had had a minimal effect. Such experiments were prompted by the work of Conney *et al.*(5) who showed drug administration to stimulate certain microsomal drug metabolisms measured 24 hours to several days after such drug administration.

Apparent lack of enzyme activity in livers from starved animals could be due to (I) actual absence of enzyme protein; (II) deficiency of cofactors, *i.e.*, reduced triphosphopyridine nucleotide (TPNH); or (III) presence of inhibitors of drug metabolizing enzymes.

We tried to eliminate the second possibility by adding TPNH-generating system of glucose-6-phosphate, glucose-6-phosphate dehydrogenase, triphosphopyridine nucleotide (TPN),† and nicotinamide.

Presence in starved liver of inhibitors of drug metabolism was not suggested in experiments where metabolism by starved and normal livers determined separately was compared with metabolism by mixtures of starved and normal livers.

We are left with the probability that the decrease in enzyme activity is due to an actual loss in enzyme protein.

Discussion. Electron micrographs of samples of liver from starved animals reveal changes in structure of the endoplasmic reticulum from which microsomes are derived. Such structural changes may be associated with marked depression of drug metabolism which we have shown to occur in starved animals as compared with normal animals. The nutritional status of the animal therefore becomes important as a possible reason for some of the so-called biological variation in drug response. Animals may vary in ability to metabolize drugs on the basis of whether or not they have had sufficient food prior to drug administration. A properly controlled experiment should include regulation of food intake before any drug administration.

Our findings may have possible therapeutic importance in administration of drugs to severely debilitated and/or malnourished patients. Our finding that depressed drug metabolisms recover fairly rapidly upon refeeding is encouraging in this connection. However, it should be pointed out that a state of starvation reached quickly may not be fully comparable with a similar state reached slowly. Animals maintained on insufficient diets for extended periods of time may not respond as quickly upon refeeding, in recovering normal levels of drug metabolism, as the experimental animals used.

Summary. Starvation depresses hepatic microsomal drug metabolism both as measured *in vitro* and *in vivo*. This depression is believed to be caused by an actual loss of enzyme protein in the microsome. Oxidative pathways are affected more than reductive pathways and indeed starvation may result in an activation of reduction of nitro and azo groups. The nutritional status of the animal prior to drug administration becomes important as a factor in the response obtained. Possible therapeutic implications are discussed.

† Chemicals obtained from Sigma Chemical Co., St. Louis, Mo.

1. (a) Fouts, J. R., Adamson, R. H., *Science,* 1959, v129, 897. (b) Adamson, R. H., Aleu, F., Fouts, J. R., *The Pharmacologist,* 1959, v1, 54. (c) McLuen, E., Aleu, F., Fouts, J. R., *ibid.*

2. Bernhard, W., Rouiller, C., *J. Biophys. Biochem. Cytol.,* 1956, v2 (Suppl.), 73.

3. Fawcett, D. W., *J. Nat. Cancer Inst.,* 1955, v15 (Suppl.), 1475.

4. Brodie, B. B., Gillette, J. R., La Du, B. N., *Ann. Rev. Biochem.,* 1958, v27, 427.

5. Conney, A. H., Davison, C., Glasgow, C., Burns, J. J., *The Pharmacologist,* 1959, v1, 64. Conney, A. H., Burns, J. J., *Nature,* 1959, v184, 363. Conney, A. H., Gillette, J. R., Inscoe, J. K., Trams, E. R., Posner, H. S., *Science,* 1959, v130, 1478.

Received November 12, 1959. P.S.E.B.M., 1960, v103.