The increase in plasma UFA concentration during the first few hours after birth strongly suggests that UFA are rapidly mobilized from adipose tissue stores and serve to supply the energy needs of the organism. The associated hypoglycemia points to deficient carbohydrate oxidation as the probable major factor responsible for increased UFA concentration. An increase in sympathetic nervous activity could also account in part for the increase observed during the first few hours of life(10). The influence of various medications administered to mothers during labor cannot be defined fully, but it should be noted that the changes in UFA concentration were consistent in all infants studied.

The results presented raise the question of the effects of possible defective mobilization of UFA on survival of the full-term infant. The premature infant, furthermore, may lack sufficient stores of fat in adipose tissue to supply adequate quantities of UFA to vital organs. Further studies will be required to answer these and other questions raised by this investigation, such as source and fate of plasma UFA in the fetus, factors controlling the plasma level of UFA in the fetus and newborn, and the contribution of UFA to energy metabolism in fetal and neonatal periods.

Summary. Concentration of UFA in blood plasma of fetal sheep and newborn man was consistently much lower than in plasma obtained simultaneously from their mothers. The levels were comparable to those found in man after administration of glucose or insulin. In both species the plasma level of UFA rose rapidly within 2 hr of birth with an associated hypoglycemia. These findings support previous studies which suggested the occurrence of a rapid shift from carbohydrate to fat catabolism in the neonatal period.

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Isolation and Characterization of Glucuronic Acid Conjugates of Chlorpromazine in Human Urine. (25331)

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Salzman and Brodie(1) and independent investigation by this laboratory(2) showed chlorpromazine sulfoxide to be a metabolite of chlorpromazine in the dog and man. Ross, Young and Maass(3,4) reported demethylation of chlorpromazine in the rat, indicating the existence of demethylated products of chlorpromazine and possibly chlorpromazine sulfoxide as metabolic products of chlorpromazine. Walkenstein and Seifter(5) reported the presence of mono-demethylated products of promazine and promazine sulfoxide in urine of dogs treated with promazine. Hydrolysis of dog and human urine by acid, alkali or β-glucuronidase treatment increases the amount of phenothiazine-like extractable material(2, 6). The conjugated material represents a large fraction of chlorpromazine metabolites excreted in the urine. In this study, we have isolated such material from the urine of pa-

TABLE I. Paper Chromatographic Pattern of Urinary Chlorpromazine Metabolites.

Group	Phenothiazine metabolites	Endogenous constituents found also in control urine	Color developed by HNO_2 spray	Avg Rf
A. Rf <0.4	P_1		blue	.01
	P_2		purple	.03
	P_3		"	.05
		E_1*	blue	.23
	P_4		pink	.33
B. Rf >0.4	P_5		pink	.44
	P_6		blue	.53
	P mixture†		purple-pink	.57–.85
		E_2‡	pink	.95

* Constantly present. † Appears to be overlapping of at least 3 spots. ‡ Observed occasionally.

tients on chlorpromazine therapy and have characterized it as glucuronic acid conjugated chlorpromazine metabolites.

Materials and methods. Chlorpromazine urine. Pooled urine samples from hospital patients receiving 200-800 mg of chlorpromazine hydrochloride daily were preserved with toluene and refrigerated. Urine from normal individuals who had not received any phenothiazine served as control. *Paper chromatography of whole urine.* A descending paper chromatogram of 100 μl of urine was developed overnight on Whatman 3 MM paper with an "isoamyl" solvent system consisting of isoamyl alcohol, water, ethyl alcohol and formic acid in a volume ratio of 100:100:15:10. The paper was sprayed with a nitrous acid reagent consisting of concentrated hydrochloric acid containing a trace of $NaNO_2$. (All of a large number of phenothiazine compounds tested with this reagent developed colors—orange, pink, purple, violet and blue.) The development of colored spots thus indicated phenothiazine metabolites in the urine sample. *Separation of chlorpromazine metabolites by cation exchange resin. Preparation of column.* A slurry of Dowex 50 (200-400 mesh) in hydrogen form was poured into a 2 x 22 cm column to a height of 16 cm, and 100 ml of urine was applied followed by 100 ml of water. Gradient elution was carried out by coupling a flask containing 1000 ml of 1.4% ammonium hydroxide to another flask containing 1000 ml of water. *Examination of fractions.* A few drops from each collection tube were tested with a fresh HNO_2 reagent (12 ml of concentrated HCl + 1 ml of 0.01% $NaNO_2$ solution) for possible phenothiazine metabolites. Eluates that produced the same response to the HNO_2 reagent and appeared to contain the same phenothiazine(s) were pooled. Each of the pooled fractions was reduced *in vacuo* to a volume of approximately 25 ml, and a 100 μl aliquot of the concentrate was chromatogrammed with the "isoamyl" system described for whole urine. *β-glucuronidase hydrolysis.* A 5.0 ml aliquot was adjusted to pH 4.5 and incubated with 1.0 ml (5000 units) of mammalian β-glucuronidase at 37°C for 24 hours. A 100 μl aliquot was chromatogrammed. The pooled fractions, before and after hydrolysis, were analyzed for free and conjugated glucuronic acid by the method of Fishman(7). *Demonstration of released glucuronic acid.* The hydrolysates from the above fractions were treated with ion exchange resins to remove interfering material and chromatogrammed on 3 MM paper with a butyl alcohol, acetic acid and water (5:4:4) system. The paper was sprayed with an alkaline silver nitrate reagent and compared to the paper chromatograms of pure glucuronic acid and glucuronolactone.

Results. Paper chromatographic pattern of urinary chlorpromazine metabolites is shown in Table I. Group A phenothiazine metabolites (P_{1-4}) were not readily extractable from an aqueous medium by non-polar organic solvents.

The results of the separation of Group A from Group B chlorpromazine metabolites by Dowex 50 are shown in Table II. Group A metabolites were eluted from the cation exchange resin. Phenothiazine metabolites P_1,

TABLE II. Separation of Urinary Chlorpromazine Metabolites by Dowex 50.

Fractions from Dowex 50 column	HNO_2 test	Rf
Urine effluent	Negative (only non-phenothiazine urinary constituents)	
Water wash	Blue, endogenous constituent E_1	.23
Tube No. 2-5	Pink, " " E_2	.95
" " 35-50, *Fraction* P_{1-3}	Blue, metabolite P_1	.03*
	Purple, " P_2	.05*
	" " P_3	.10*
" " 51-79	Negative	
" " 80-110, *Fraction* P_4	Pink, metabolite P_4	.33
" " 111-200	Negative	

* These Rf's are higher than those found in whole urine (Table I), probably as a result of elimination of urinary constituents.

P_2 and P_3 were eluted as a mixture, Fraction P_{1-3}; P_4 was eluted separately. Group B metabolites were retained by the resin.

Both Fractions P_{1-3} and P_4 contained conjugated glucuronic acid but no free glucuronic acid or glucuronolactone. When similar fractions of control urine collected from Dowex 50 were tested, only the fraction corresponding to Fraction P_{1-3} contained conjugated glucuronic acid. Conceivably, Fraction P_{1-3} contained both phenothiazine glucuronides and other urinary glucuronides. Fraction P_4 contained only phenothiazine glucuronides.

β-glucuronidase hydrolysis of Fractions P_{1-3} and P_4 resulted in disappearance or reduction in quantity of metabolites P_{1-4}. Three new phenothiazines were observed on chromatographing Fraction P_{1-3} hydrolysate and 2 in Fraction P_4 hydrolysate. These compounds were different from chlorpromazine and chlorpromazine sulfoxide. The effects of β-glucuronidase hydrolysis are recorded in Table III. Some of these released phenothiazines were extractable by ether at pH 13. The extract from Fraction P_{1-3} hydrolysate showed a UV spectrum very similar to that of a mixture of chlorpromazine and chlorpromazine sulfoxide. The extract from Fraction P_4 showed a spectrum similar to that of chlorpromazine, with addition of a secondary absorption peak at 284 mμ. This is suggestive of a ring hydroxyl function. In contrast to chlorpromazine and chlorpromazine sulfoxide, these phenothiazines were also extractable from a weakly acidic medium at pH 5.

TABLE III. Effect of β-Glucuronidase Hydrolysis on Fractions P_{1-3} and P_4 (Paper Chromatographic Patterns).

Fraction		Before hydrolysis: Color with HNO_2	Rf	After hydrolysis: Color with HNO_2	Rf
P_{1-3}	P_1	Blue	.03	Absent	
	P_2	Purple	.05	Slightly affected	
	P_3	"	.10	Absent	
				New phenothiazine spots:	
				Purple	.47
				"	.75
				Blue*	.97
P_4		Pink	.33	Absent	
				New phenothiazine spots:	
				Purple	.57
				"	.85

* Trace, occasionally observed.

Discussion. Incubation under mild conditions with β-glucuronidase caused complete disappearance of 3 of the highly water-soluble metabolites and partial disappearance of the fourth. This observation accompanied by release of glucuronic acid and less polar, ether extractable phenothiazines indicated that these 4 metabolites excreted in urine were glucuronides of chlorpromazine. The extractability of the released phenothiazines from an acidic medium further indicated the presence of enolic or phenolic functions. These observations support the prevalent mode of detoxificaton of aromatic compounds through ring hydroxylation. The authors feel that the glucuronides reported here are conjugated products of glucuronic acid and phenolic metabolites resulting from hydroxylation of the phenothiazine nucleus and that hydroxylation constitutes an important aspect of chlorpro-

mazine metabolism. Other metabolic changes in the phenothiazine nucleus and dimethyl propylamine side chain are inferred by the presence of 4 glucuronide spots. The differences among these metabolites may be the result of hydroxylation at different positions of the nucleus accompanied by oxidation to sulfoxides and demethylation of the side chain.

Summary. Four polar, water-soluble metabolites of chlorpromazine were separated from urine by use of a cation exchange resin. These were shown to be glucuronides by release of free glucuronic acid and less polar, ether-extractable phenothiazines as a result of β-glucuronidase hydrolysis. Presence of enolic functions in these released phenothiazines was shown by their extractability from an acidic medium. Results strongly suggest hydroxylation of chlorpromazine and subsequent conjugation with glucuronic acid as an important route of chlorpromazine metabolism and detoxification in man.

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Metabolism of Propionate-1-C-14 in the Intact Mouse.* (25332)

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Previous *in vitro* studies indicated that adipose tissue may play an unique role in metabolism of propionic acid(1). It was noted, for instance, that incorporation of propionate-1-C-14 and propionate-2-C-14 into fatty acids of adipose tissue slices was 100 to 200 times the incorporation into fatty acids of liver slices. In contrast, total oxidation of propionic acid was comparable in both tissues. These results have led to a description of a new proposed metabolic pathway for fat synthesis in adipose tissue which includes propionate as a precursor(2). The present work was undertaken to determine whether this function of propionic acid in adipose tissue can be demonstrated in the intact animal, and whether this function is more pronounced in obesity.

Methods. Two groups of mice of the White Swiss strain were used: normal animals and animals made obese by injection of goldthioglucose(3). Both groups were fed *ad lib.* until time of experiment. Animals were injected intraperitoneally with 10 microcuries sodium propionate-1-C-14, in isotonic NaCl and were placed in all-glass, temperature-controlled metabolism cages with access to only water. CO_2 and urine were collected until time of sacrifice. Groups of animals (Table II) were killed by etherization at intervals up to 24 hours. The entire animal was immersed in alcoholic potassium hydroxide and heated overnight on a steam bath. Glycogen, fatty acids and non-saponifiable lipids were assayed

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