TABLE II. Latex Agglutination* Inhibition Test by Immunologic Reaction of Human Growth Hormone with Rabbit Serum.†

Tube No.	Human growth hormone (μg)	Row I, antiserum	Row II, control serum
1	3.10	0	0
2	2.66	0	0
3	2.28	0	0
4	1.90	0	0
5	1.52	0	0
6	1.14	+	0
7	.76	+	0
8	.38	+	0
9	.19	++	0
10	.15	++	0
11	.11	++	0
12	.08	++	0
13	.04	+++	0
14	.02	++++	0
15	.01	++++	0
16	.00	0	0

* Degree of agglutination is expressed by No. of plus signs. No reaction is expressed by 0 sign.

† .005 ml of rabbit serum was used in each tube except for tube 16 in each row.

that there is one antigen-antibody reaction in the reaction between the Raben growth hormone product and its antiserum. Read(3) using rabbit antisera to the same product was able to show by a hemagglutination-inhibition technic the presence of an antigen in increased amounts in sera of acromegalic patients and decreased amounts in patients with hypopituitarism when compared to normal individuals. Assuming, then, on the basis of this evidence, that human growth hormone is antigenic, we conclude that latex agglutination as demonstrated by the results of this experiment is a manifestation of the reaction of human growth hormone with its antiserum.

The results in Table I show a zone of optimal concentration for antigen and antibody as manifested by a zone of no agglutination in the region of antigen excess and a zone of no agglutination where antigen concentration is too little. This follows well-known laws of other antigen-antibody systems using rabbit antisera(7).

It is hoped that latex agglutination by the reaction of human growth hormone with its antiserum may be useful for detection of growth hormone in human biological fluids. Experiments are now in progress to test this possibility.

Summary. A method has been described which demonstrated that the reaction between human growth hormone and its antiserum caused agglutination of a suspension of latex.

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Ammonia Detoxication in Liver from Humans.* (26274)

ALFRED L. KENNAN† AND PHILIP P. COHEN
Departments of Gynecology and Obstetrics and Physiological Chemistry, University of Wisconsin School of Medicine, Madison

The recent development of methods for assaying the individual enzymes of the urea cycle in a single homogenate(1) have made possible accumulation of data on ammonia detoxication by this enzymatic mechanism in human liver. With similar data for glutamic acid dehydrogenase and glutamine synthetase, it is possible to calculate the amount of ammonia which *theoretically* could be fixed by these separate mechanisms, and to obtain an over-all estimate for *in vitro* fixation of

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† Fellow of U.S.P.H.S.

ammonia by this tissue. Development of the enzymes for urea synthesis in fetal liver has also been studied. Previous studies have shown that there is an increase in human fetal liver arginase after the fourth month(2). Urea formation has also been demonstrated in human fetal liver at this stage with tissue slices and the reaction mixture described by Krebs and Henseleit(3). Glutamine synthetase and glutamic acid dehydrogenase activities have, to our knowledge, not been studied in human liver.

Materials and methods. A single specimen of fresh liver was obtained at 12, 20 and 40 weeks of gestation and from 2 adults during surgery.‡ Activities of the urea cycle enzymes were determined as previously described(1). Glutamic acid dehydrogenase was determined spectrophotometrically by following the decrease in optical density as a result of oxidation of DPNH during the course of reductive amination of α-ketoglutarate to glutamate. The reaction mixture was essentially that described by Solomon(4) and contained: 1 μmole of DPNH; 250 μmoles of phosphate buffer (pH 7.4); 300 μmoles of NH_4Cl; and 0.1 ml of appropriately diluted enzyme (1 part liver plus 99 parts of double distilled water) from a distilled water homogenate in each cuvette. Final total volume was 3 ml. After setting the spectrophotometer for the blank (all of the reagents except the DPNH), 20 μmoles of α-ketoglutarate were added to the other cuvette at 0 time and mixed rapidly with a stirrer. The decrease in optical density at room temperature was then recorded in a Beckman DUR spectrophotometer. One unit of glutamic acid dehydrogenase activity is defined as that amount which will oxidize 1 μmole of DPNH in one minute of the initial reaction. The value of the molecular extinction coefficient of DPNH employed was that determined by Horecker and Kornberg(5). Glutamine synthetase was determined by measurement of glutamyl hydroxamate formation according to the method of Lipmann and Tuttle(6). The reaction mixture contained: 200 μmoles of hydroxylamine hydrochloride (neutralized with KOH); 10 μmoles of ATP (di-K salt); 50 μmoles of K-glutamate; 260 μmoles of tris (trihydroxymethylaminomethane) buffer (pH 7.5); 20 μmoles $MgCl_2$; and 0.5 ml of homogenate (1 vol. of liver in 4 vol. of 0.15M KCl) in a final volume of 2.3 ml. The reaction was carried out at 38° for 15 minutes and deproteinized with 0.4 ml of 40% trichloroacetic acid. The ferric chloride reagent was then added and after centrifugation an aliquot of 1 ml was read at 500 mμ in the Beckman DU spectrophotometer. A standard curve was prepared using succinyl hydroxamate from which the value for the glutamyl derivative was approximated from the data of Meister *et al.*(7).

‡ Age, 46; gastric ulcer; age 18; asymptomatic gall stones. No clinical evidence of liver disease.

TABLE I. Urea Cycle Enzymes in Human Liver.

	Prenatal (wk gestation)			Adult (yr)	
	12	20	40	18	46
	Carbamyl phosphate synthetase				
U	91	95	121	256	222
SA	1.4	1.3	3.8	3.1	3.1
	Ornithine transcarbamylase				
U	599	1461	4234	9000	7910
SA	9.1	20	132	112	110
	Arginine synthetase*				
U	13	18	21	55	48
SA	0.29	0.23	0.52	0.78	0.77
	Cleavage enzyme				
U				168	180
SA				2.4	2.9
	Arginase				
U	2842	5054	5107		7750
SA	43	70	160		108
	Estimated urea synthesis, g (see text)				
	.021	.321	3.19		101 (avg)

The data of Jackson(8) were used for liver weights at 12 and 20 wk. Liver of 40 wk fetus weighed 105 g; 1400 g was used as wt for liver of adult female. Definition of U (units) and SA (specific activity) is given in the text under *Results.*

* Arginine synthetase represents activities of both condensing and cleavage enzymes(1).

Results. The values obtained for the urea cycle enzymes in units and specific activity are given in Table I. The units represent μmoles of product formed per gram of wet tissue per hour and specific activity represents μmoles of product produced per mg of protein per hour. If a calculation is made

TABLE II. Enzyme Systems Fixing Ammonia.*

	Glutamic acid dehydrogenase†				Glutamine synthetase†		
	μmoles DPNH oxidized/min.				μmoles glutamyl hydroxamate		
Liver tissue	Per ml	In cuvette	Units/g tissue	Units/mg prot.	React. mix	Per g tissue	μmoles glutamine, g prot./15 min.
Human	5.64×10^{-3}	16.9×10^{-3}	169	0.39	0.8	1.6	15.7
Rat	7.8×10^{-3}	23.4×10^{-3}	234	1.33	1.75	3.5	156.

* Urea cycle enzymes are given in Table I. † Assay conditions as described in text.

for amount of urea which could be formed *in vitro* in a 24 hour period from the rate limiting step (arginine synthetase system), one obtains the values appearing in the bottom line of the table. If calculations are made for NH_3-N which could be incorporated directly into glutamic acid and glutamine in adult liver, the following values are obtained for a 1 hour period: 40 g of NH_3-N for glutamic acid and 627 mg for glutamine (Table II). These are to be compared with values of 22.1 mg of NH_3-N as glutamic acid and zero as glutamine in fetal liver (20 wks). Since only half the nitrogen of urea comes from free ammonia, it is calculated that 23.6 g of NH_3-N could be fixed in a 24 hour period by this mechanism. Glutamic acid dehydrogenase would thus seem to have the greatest *potential ability* for ammonia detoxication.

Discussion. Duda and Handler(9) have shown by injection of $N^{15}H_3$ into rats that free ammonia is removed primarily by formation of glutamine and urea. In their studies, 65% of the administered isotope was present in glutamine and urea at the 15 minute interval and 90% in 30 minutes (72% as glutamine and 18% as urea). They have also made a calculation for glutamine turnover in the rat which suggests that 80% of NH_3 freed by the transamination and deamination reactions of glutamine is resynthesized into glutamine. The NH_3 lost during this turnover is of the same order of magnitude as the direct incorporation of NH_3 into urea during this period. Maximal rate of urea synthesis in the rat given an LD 99.9 dose of ammonia, also calculated by Duda and Handler(9), was about 1140 mg of urea for a 300 g rat. This can be compared with the figure given for urinary excretion of urea (720 mg) in the albino rat for the same period(10). Calculations of data from enzyme studies on urea formation in homogenates of rat liver indicate that the adult rat with a liver of 12 g(11) can synthesize of the order of 710 mg of urea in a day. These figures are all very close and suggest that the rat has very little *reserve synthetic ability* in the liver for urea synthesis.

In human beings, the enzymatic data suggest that only one-fifth of *potential synthetic ability* of the liver is used for daily synthesis of urea. In comparing data for adult human liver with that for the rat, one finds that, on the assumption that 20% of NH_3-N fixed as a result of glutamine synthesis is eventually converted to urea, the human could synthesize about 12.9 g of urea per day. Since this value is lower than the average daily excretion of the adult human and would be smaller by about 20% because of degradation in the intestinal tract(12), it is obvious that free ammonia, other than that arising from glutamine, is a major source for urea synthesis. In the case of fetal liver, glutamine cannot be a source of ammonia for urea synthesis since there is no demonstrable glutamine synthetase activity. This observation is consistent with the enzymatic findings that glutamine is not directly involved in urea biosynthesis.

The quantitative importance of glutamic acid dehydrogenase in ammonia detoxication in the liver is far from clear. Its role may be more important in the brain and in fetal tissues(4).

The greater capacity to synthesize arginine (by way of the ornithine-urea cycle) in the case of the human as compared with the rat could explain the need for arginine for maximum growth of the rat.

During human pregnancy, the urea formed

by the fetus is allegedly excreted by the mother. Balance studies show a positive nitrogen balance during pregnancy(13). From the present data, it is seen that the human fetus could produce as much as 3 g of urea daily. This amount of nitrogen would be within the normal daily variation of nitrogen excretion of the mother and thus nitrogen balance studies would not reflect the contribution of the fetus to maternal nitrogen excretion.

Summary. The detoxication of ammonia in homogenates of liver from fetal and adult human beings has been studied. Amount of urea synthesized in a day for each case has been calculated from the rate-limiting step of the urea cycle. Similar data are presented for glutamine synthetase and glutamic acid dehydrogenase. Values for human beings have been compared to similar values for the rat, and differences have been discussed.

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Experimental Steatorrhea Induced in Man by Bile Acid Sequestrant.* (26275)

SAMI A. HASHIM,† STANLEY S. BERGEN, JR. AND THEODORE B. VAN ITALLIE
Department of Medicine, St. Luke's Hospital and Inst. of Nutrition Sciences, Columbia University, N. Y. City

Bile acids are believed necessary for efficient digestion and absorption of dietary fat. An agent capable of binding bile acids in the gut might be expected to cause steatorrhea. In the present study, steatorrhea was induced experimentally in a series of otherwise healthy human subjects by means of a bile acid sequestrant (MK-135). This agent is the chloride salt of a basic anion exchange resin which binds bile acids *in vitro* and which, when administered orally, forms an insoluble, nonabsorbable complex with bile acids in the intestinal lumen(1,2).

Methods. Sixteen healthy adults aged 24-32 served as subjects. Effect of MK-135‡ administration on fecal fat excretion was determined in 4 subjects, and influence of this agent upon absorption and fecal excretion of I^{131}-labeled triolein or I^{131}-labeled oleic acid was measured in 12 subjects. *Fat balance studies.* To insure constant intake, 4 subjects were maintained on formula-type diets providing 40 calories/kg body weight/day for 5-6 weeks. Formula derived 40% of calories from fat (corn oil in two subjects, butter in one, and coconut oil in one), 43% from carbohydrate (dextrose) and 17% from

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† Postdoctoral fellow of Nat. Heart Inst., U.S.P.H.S.

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