Table 1. Excretion of urinary CGP.

Length of gestation (days)	Urine (μg/day)	Serum (μg/ml)
56	10.0	0.5
98	13.1	2.8
101	17.7	
135	56.9	
170	59.5	
178	31.1	
198	100.4	
203	69.2	13.9
222	76.4	
223	36.5	20.6
223	121.5	28.9
239	93.2	
265	105.0	

B/F ratio of CGP-^{131}I against the concentration of unlabeled CGP at a dilution of rabbit antiserum to CGP of 1:20,000 (Fig. 1) illustrates competitive inhibition of the binding of labeled hormone to antibody by unlabeled hormone. The upper dotted curve demonstrates the ineffectiveness of Wilhelmi HGH at concentrations of 0.05 to 10.0 mμg/ml to inhibit the reaction of CGP-^{131}I with antiserum to CGP. In addition, serum from normal adults and children and from patients with active acromegaly did not react in this assay system. The observation that CGP-^{131}I was not bound to rabbit antiserum to human serum at dilutions as low as 1:100 provided further evidence for the specificity of the assay.

The concentration of CGP in serums from pregnant women is shown in Fig. 2. At the earliest stage in pregnancy studied, 56 days after the last menstrual period, there was 0.53 μg of CGP per milliliter in the serum. A sharp rise in serum CGP was observed during pregnancy, a maximum being attained by the third trimester. In the few pregnant subjects studied on more than one occasion during gestation, there was a similar rise.

The amount of CGP excreted in 24-hour samples of urine was compared with its concentration in serum on the same day from the same pregnant woman (Table 1). The urinary CGP gradually rose during pregnancy, the pattern differing appreciably from that described for human chorionic gonadotropin. The data suggest a rough correlation of the amount of CGP excreted with the amount in serum. However, the exceptions observed may reflect an incomplete 24-hour collection of urine, variations in the metabolism of CGP, loss of activity with storage, or laboratory error.

At delivery, the amount of CGP in maternal serums was 50 to 200 times higher than that in the umbilical vein, the samples having been obtained simultaneously. In three samples of amniotic fluid, the concentration of CGP was 2.0, 3.3, and 11.1 μg/ml, respectively.

By 8 hours postpartum, CGP has been cleared from the circulation as indicated by assay of serum at dilutions as low as 1:5. The CGP was not detected in the serum of nursing mothers, in the postpartum period, or in individuals not harboring trophoblastic tissue. The serum of two nonpregnant women with galactorrhea did not contain CGP.

That the human placenta is the source of CGP is supported by these studies. The rise in serum CGP during pregnancy, its rapid disappearance postpartum, even in nursing mothers, supports this view and reflects the specificity of the assay. The localization of CGP within the cytoplasm of the syncytiotrophoblast layer of the human placenta (*6*) and the production of CGP by human chorionic tissue grown in vitro (*4*) indicate that, in addition to storing the hormone, the placenta synthesizes it.

The concentration of CGP in the serum of pregnant women is markedly elevated when compared to the concentration of HGH detected in acromegalic patients or in normal individuals after a hypoglycemic stimulus or a prolonged fast (*12, 13*). This substantiates earlier gel-diffusion studies in which precipitin bands were demonstrable when antiserums to HGH were reacted with pregnancy serum but not when reacted with acromegalic serums (*3*). These observations are consistent with data obtained by bioassay showing that the potency of CGP is considerably less than that of pituitary growth hormone, a finding supported by the failure to observe signs of acromegaly in normal pregnancy (*4*).

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Micropuncture Study of Inulin Absorption in the Rat Kidney

Abstract. *By means of a microinjection technique, inulin-carboxyl-C^{14} or inulin-methoxy-H^{3} was injected into single proximal tubules of the rat at various urine flow rates. Urine collected separately from the two kidneys showed negligible amounts of inulin activity on the noninjected side, thus demonstrating directly that there is no significant reabsorption of inulin by the renal tubular epithelium under these conditions.*

The use of inulin as a measure of glomerular filtration rate (*1, 2*) and renal tubular water reabsorption (*3*) is based on the assumption that it is freely filtered at the glomerulus and that no reabsorption or secretion takes place along the nephron. Although the proportional rise in inulin excretion with increasing plasma concentration supports this assumption (*2*), no conclusive evidence for the fate of inulin in the renal tubule has yet been presented. Micropuncture techniques (*4*) provide a possibility of testing this problem directly, and Shehadeh *et al.* have reported that inulin is extensively reabsorbed into the circulation after its infusion into rat proximal tubules blocked proximally by mineral oil (*5*). Their average recovery in the blood of 23 percent of injected inulin may represent an underestimate of inulin reabsorption, since urine was apparently not collected from either kidney during the first 20 minutes after infusion into the

tubule. There was similar reabsorption in *Necturus* (*6*). In view of the widespread use of inulin as a reference for water movement into and out of the renal tubule, it seemed important to clarify the question of inulin reabsorption.

We have carried out experiments different from those of Shehadeh *et al.* (*5*) and Scott *et al.* (*6*), using free-flow microinjection (*7*). The experiments were based on the assumption that inulin injected into a tubule in one kidney would be detected in the urine from the contralateral kidney if it were reabsorbed into the circulation. We used 18 male Wistar rats and anesthetized them by intraperitoneal injection of 50 mg of pentobarbital per kilogram of body weight. The left kidney was exposed for microinjection through an abdominal incision, and both ureters were catheterized with polyethylene tubing. Inulin-carboxyl-C^{14} or inulin-methoxy-H^3 (*8*) in isotonic saline colored with nigrosine was injected by micropuncture into proximal tubules over a period of 40 to 200 seconds. The injected tubule was not blocked with oil, and the rate of inulin infusion into the tubule was continuously adjusted to prevent backflow toward the glomerulus. Experiments were performed both during intravenous infusion of hypertonic sodium chloride solution (2.5 percent) and in nondiuretic rats in order to detect inulin reabsorption if it occurred only when the rate of urine flow was slow. Urine was collected over periods of 5 to 10 minutes for 40 to 75 minutes. To shorten the collecting period required in nondiuretic rats—the period was long due primarily to the dead space of the ureteral catheters—0.2 to 0.3 ml of 20 percent mannitol solution was generally administered intravenously 7 to 15 minutes after the injection of inulin into the tubule. This interval was sufficient for passage of the injected inulin through the nephron, so that the mannitol only flushed the urine from the renal pelvis and catheter into the collecting vial. Reabsorbed inulin was estimated as twice the amount in the urine from the contralateral kidney. At the end of the experiment puncture sites were localized by microdissection (*4*).

Table 1. Inulin reabsorption after microinjection into proximal tubules in the rat.

Site of injection (% of proximal tubule)	Inulin reabsorbed* (%)	Urine flow rate (μl/kg min)	Isotope
Intravenous infusion of 2.5 percent NaCl			
20	0	90	H^3
20	1.4	100	C^{14}
22	0	55	C^{14}
25	1.5	50	C^{14}
26	0	70	C^{14}
27	0	100	C^{14}
27	2.4	90	H^3
28	0.3	110	H^3
30	0	80	H^3
31	0	70	C^{14}
34	0	120	H^3
40	2.4	40	C^{14}
42	0	60	C^{14}
44	0.8	75	C^{14}
45	0	40	C^{14}
44	0.5	120	C^{14}
46	0.4	70	H^3
48	0.4	60	H^3
56	0	110	C^{14}
Nondiuretic rats			
23	0		H^3
23	0.7		H^3
26	0		H^3
28	1.4		H^3
30	0.6		H^3
33	0.6		H^3
34	1.8		C^{14}
38	0		H^3
39	0.8		H^3
46	0		H^3
46	2.0		H^3
47	1.6		H^3
48	0.4		H^3
50	1.2		H^3
51	0.6		C^{14}

* The reabsorbed inulin is calculated as follows: (2 × inulin activity in urine from right kidney/total inulin activity recovered) × 100. Where reabsorption is indicated as 0, radioactivity in the urine from the right kidney was less than 2 standard deviations above background level.

The results (Table 1) show that there was little or no reabsorption of inulin-C^{14} or inulin-H^3 (*8*) under these conditions. Further, the physiological significance of the small reabsorption in certain experiments seems highly questionable. There was no correlation evident between the percentage of reabsorption and the site of injection along the proximal tubule. Excretion by the contralateral kidney may well have resulted from unnoticed small droplets of inulin inadvertently left on the surface during micropuncture; inulin droplets deliberately deposited on the intact capsule of four kidneys were rapidly absorbed into the circulation, as evidenced by the prompt appearance of inulin in the urine from both kidneys at an equal rate. Conceivably, also, stretching of the tubule by too vigorous injection may lead to reabsorption by mechanical damage or direct injection into peritubular capillaries. Our results agree with those obtained on animals that had profuse osmotic diuresis (*7*), but are at variance with those reported by Shehadeh *et al.* (*5*). We conclude that inulin is a satisfactory marker for quantitative measurement of water reabsorption from the renal tubule of the rat under conditions of free flow.

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Isolation and Characterization of DNA from Kinetoplasts of Leishmania enriettii

Abstract. *The DNA of* Leishmania enriettii *can be separated by equilibrium sedimentation in cesium chloride into a major band of density 1.721 and a minor component of density 1.699. DNA from isolated kinetoplasts of this protozoan was identified as the less dense minor component.*

Within the protozoan family Trypanosomatidae an unusual organelle called the kinetoplast is situated in close proximity, if not actually connected, to the proximal end of the flagellum. The kinetoplast contains a substantial quantity of DNA and in part resembles a mitochondrion (*1*). Kinetoplast DNA has been isolated and found to differ in density from the nuclear DNA when sedimented to equilibrium in cesium chloride.

Leishmania enriettii was cultivated on Senekjie's medium (*2*) for 4 to 5 days and harvested by horizontal cen-