tibodies react not only to human growth hormone preparations which had been used as antigen, but also with an antigen located in eosinophil cells of human anterior pituitaries and eosinophilic adenomata from patients with acromegaly. The specificity of the reaction is demonstrated by the staining of anterior pituitary cells exclusively with fluorescent anti-H.G.H. and by the following controls. This staining can be inhibited with unlabelled human growth hormone; it can be absorbed out with human growth hormone; it does not localize in tissues other than human anterior pituitary glands and eosinophilic adenomata, and fluorescent antisera of heterologous nature do not localize in the parenchymal cells of the human pituitary gland.

These experiments strongly suggest that human growth hormone is either produced or stored in most, but not all, the eosinophil cells of the human anterior pituitary. The very few exceptions to the exclusive localization of the fluorescent anti-human growth hormone in the eosinophil cells in the adjacent section studied is probably within the experimental error, but another explanation may be required. It is unlikely that the localization of fluorescence in the few basophil cells is due to a contaminating antibody or we would have expected more basophil cells to be stained.

Conclusions. Using the fluorescent antibody technic it has been shown that labelled rabbit antibodies to Raben human growth hormone localized specifically in cells of the human anterior pituitary gland. This antibody probably localizes exclusively in eosinophil cells of normal human pituitaries and in eosinophilic adenomata from patients with acromegaly.

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Distribution and Excretion of Radiofluoride in the Human.* (25791)

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During recent years there has been increased interest in fluoride metabolism particularly in regard to adoption of water fluoridation for partial prevention of dental caries(1). Because the fluoride ion is a trace element and occurs biologically in very small quantities‡ use of a radiotracer for human metabolism studies has many advantages. The only useful

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‡ i.e., human plasma fluoride values are of the order of 0.1-0.3 ppm(2).

radioisotope of fluorine is F^{18} which is a positron emitter with a half-life of 109.7 minutes (3). The present study, utilizing radiofluoride to label 1 mg of fluoride, was undertaken to evaluate distribution and urinary excretion of fluoride ingested as a single dose by the human. One mg of fluoride was chosen because it is the quantity present in a liter of water fluoridated at 1 ppm.

Methods. Five microcuries§ of radiofluoride and 1 mg of stable fluoride as NaF contained in 250 ml of water were ingested by 2 volunteers. Each subject drank 250 ml of water at one hour and at 30 minutes before and 30 minutes after ingesting the fluoride solution (Table I). Additional water was taken over the remainder of observation period, lasting up to 5 hours, to permit collection of urine specimens at 10-15 minute intervals. Blood samples were obtained periodically by venapuncture and heparin was used as an anticoagulant (Table I). Salivary flow was stimulated by chewing paraffin and parotid gland saliva was collected by use of Lashley cups(4) placed over the orifice of each of Stenson's ducts. The saliva from the 2 glands was pooled in each sample. Radiofluoride concentrations of the fluids were determined by counting 4 ml volumes of whole blood, plasma, saliva and 10 ml volumes of urine in a well-type crystal scintillation counter. A probe type scintillation counter was used to evaluate, at frequent intervals, radiofluoride content of several body areas whose positions could be relocated. These measurements were made to indicate tissue retention of 1 mg of fluoride, labeled with F^{18}, ingested as a single dose. Owing to differences in instrumentation the activities measured over body areas cannot be directly compared with those of body fluids. All radioactivity measurements, after correction for background counts, and volume were adjusted for radioactive decay to a single point in time. Fluoride(2) in plasma and creatinine(5) and chloride(6) in plasma and urine were determined by standard methods. Renal clearances were calculated by standard procedures.‖ At the plasma fluoride levels in our studies 96-97% of plasma fluoride is freely diffusible through collodion membranes which would not pass albumin or larger molecules. Concentrations of fluoride in protein free ultrafiltrate and plasma water are equal(7) as a result of the Donnan membrane phenomenon. Plasma F^{18} concentrations were obtained from a curve delineated by the several physical measurements.

TABLE I. Determination of Renal Clearance of Radiofluoride.

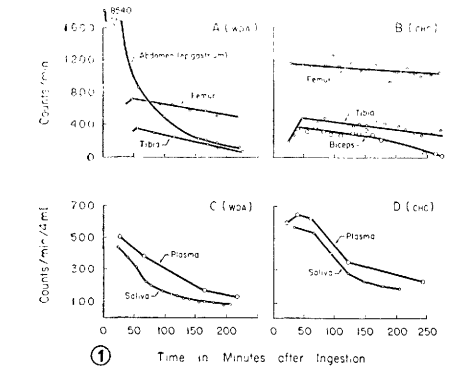
Time of procedure (mid-point in min.)	Urine vol	Blood vol	Water ingestion vol
		(ml)	
−60			250
−30			250
0			250
12	105		
22		20	
29.5	360		
30			250
40		20	
40.5	135		
46	116		
60		20	
62	104		
72.5	129		
84.5	102		
97	102		
108	38		
118	34		
120		20	
129	33		
140.5	46		
151	62		
161.5	44		
173	38		
180			180
185	32		
197	18		
208	14		
225	8		
240		20	
245			180
252.5	13		
282	87		
303.5	104		
321	163		

§ Radiofluoride (F^{18}) was assayed by gamma ray spectrometer comparison of annihilation gamma ray activity with that of standard Na^{22} samples obtained from Bureau of Standards, Washington, D.C.

Results. Fluoride contents of the plasma were 0.17 ppm ± 0.00 (3 samples, WDA) and 0.13 ppm ± 0.02 (5 samples, CHC). From Fig. 1 (C & D) it is seen that maximum plasma radiofluoride concentration was reached within 60 minutes and that parotid saliva radiofluoride concentrations were al-

‖ Renal clearance (ml/min) =

$$\frac{\text{Urine conc. (cts/min/4 ml)} \times \text{Urine flow (ml/min)}}{\text{Plasma conc. (cts/min/4 ml)}}$$



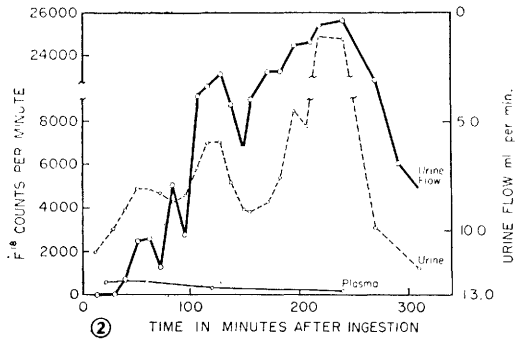


FIG. 1. A & B. Radiofluoride activity determined by portable crystal scintillation counter. C & D. Radiofluoride activity of 4 ml samples of blood and saliva.

FIG. 2. Radiofluoride content of each sample of plasma and urine corrected to a 4 ml volume is given on the left ordinate. Urine flow rate is shown on inverted right ordinate.

ways below corresponding plasma concentrations. Less than one percent of ingested activity was recovered in parotid saliva samples. Mean salivary flow rate for WDA was 0.78 ± 0.06 ml/min and for CHC was 0.37 ± 0.02 ml/min. Calculations from hematocrit values(8) of 9 samples of blood and radiofluoride concentrations of blood and corresponding plasma samples showed that 72 ± 5% of the isotope in the blood was present in the plasma. By using body weight to estimate total plasma volume it can be calculated that not more than 10% of ingested radioisotope was present in the entire plasma volume at any one time.

Fig. 1 (A &B) shows portable crystal scintillation counter observations over the indicated body areas.¶ The counts recorded over the abdominal (epigastric) area probably included the radioisotope present in liver and spine as well as that in the stomach. The initial rapid decline in these counts was undoubtedly due in large part to gastric emptying and to absorption. Rate of decline of abdominal area counts decreased with time, and after 150 minutes, was nearly equal to rate of decline of the counts over the greater trochanter of femur and tibia. This similarity in rate of change in isotope content suggests that the counts over the abdominal area recorded after 150 minutes were due mainly to radioisotope in the spine. The counts per minute measured at a right angle to the contracted biceps muscle declined at a rate similar to that measured over the femur and the tibia until after 150 minutes (Fig. 1 B). After that time biceps counts declined more rapidly than counts over the femur and tibia and at 250 minutes muscle counts were nearly zero whereas counts over the femur had decreased by only 15% from maximum value. This relative difference in retention of the isotope is due to association of fluoride with the bone mineral whereas in muscle fluoride is present only in tissue water. Furthermore, bone mineral, in contrast to muscle, contains a large amount of stable fluoride which acts as an exchange reservoir. These measurements indicate that movement of fluoride from epigastrium and uptake in the tissues occurs rapidly and that hard tissues retain their fluoride even though plasma activity during period of measurements has decreased markedly.

Renal concentration ratios (column 2 of Table II) ranged from a low of 7.8 to a high of 118. This degree of concentration is remarkable for a halogen. Radiofluoride clearance never exceeded creatinine clearance. Column 4 shows that clearance of fluoride in the human fluctuates, and increases with an increase in urine flow rate (columns 3 and 4). In particular it is to be noted (column 5) that fluoride clearances were always many-fold greater than chloride clearances. One-third of ingested radioactivity was recovered from the urine within 4 hours.

Fig. 2 summarizes in detail the measured

¶ The crystal detected the radiation through the depth of the body and within a solid angle of 120° from the aperture.

TABLE II. Renal Clearance Values.*

Subject and wt	F^{18} urine / F^{18} plasma	Urine flow, ml/min.	F^{18} clr, ml/min.	F^{18} clr / Cl clr	F^{18} clr / Creat clr	% tubular reabsorption of F^{18}
W.D.A. 80 kg	43.8	1.2	51	104	.40	60
	46.2	1.4	65	112	.39	61
	25.8	1.8	46	65	.52	48
	35.8	1.9	68	106	.40	60
	31.2	2.4	76	109	.49	51
	37.5	3.3	125	93	.47	53
	23.0	3.4	77	56	.41	59
	14.7	4.9	71	45	.41	59
	16.5	6.0	99	51	.50	50
	19.4	6.9	133	61	.40	60
	12.0	8.8	106	57	.70	30
	11.8	11.0	129	69	.68	32
	12.8	11.0	140	45	.59	41
C.H.C. 74 kg	118.3	.42	50	276	.43	57
	111.0	.57	63	176	.43	57
	28.5	1.4	40	111	.21	79
	29.4	1.5	44	102	.31	69
	18.6	2.7	50	98	.35	65
	14.4	2.7	38	98	.33	67
	20.2	2.8	56	88	.39	61
	15.8	3.1	49	126	.29	71
	18.7	3.4	64	85	.42	58
	15.0	3.8	57	58	.42	58
	13.0	4.0	52	108	.29	71
	16.0	4.2	67	97	.36	64
	13.3	6.2	82	82	.41	59
	11.1	6.9	77	171	.28	72
	8.6	7.9	67	749	.35	65
	7.3	8.2	60	142	.35	65
	10.2	10.2	104	868	.43	57
	8.2	10.4	85	947	.53	47
	7.8	10.5	82	748	.44	56
	8.6	11.7	101	1,119	.45	55

* Clearance periods are arranged in order of increasing urine flow rates.

and subsequently corrected individual radiofluoride contents of each 4 ml sample of plasma and urine from subject CHC and presents them in their proper sequence. Particularly, it should be noted that plasma content of radiofluoride is relatively constant when compared to urine content of radiofluoride and that the latter appears to be correlated with urine flow rates. There is a notable concentration of urine radiofluoride when urine flow decreases below 1 ml/min. This circumstance tends to maintain a large urinary clearance of fluoride even when urine volume decreases.

Summary. One mg fluoride labeled with radiofluoride was ingested by each of 2 adult humans. Observations of renal clearance of fluoride, chloride and creatinine were made. Fluoride clearance always exceeded chloride clearance by many fold and increased with urine flow, but fluoride clearance was always less than creatinine clearance. Renal tubules in 2 individuals, reabsorbed respectively 51 and 63% of fluoride in the glomerular filtrate which indicates a net process of glomerular filtration with a variable amount of tubular reabsorption. Plasma contained 72% of whole blood radiofluoride and radiofluoride concentration of plasma exceeded that of parotid gland saliva. Measurement of uptake and release of the radioisotope by soft tissues and by the skeleton showed characteristic differences of radiofluoride retention in these tissues. Skeletal tissues retained the isotope but soft tissues lost nearly all their activity within 4 hours after ingestion.

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Antibacterial Substances in Placentas and Serums of Mothers and Newborn Infants. (25792)

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The protection of the human fetus against infection has been an intriguing subject for many years. Since the placenta interposes a continuous though narrow wall of tissue between the blood of the fetus and that of the mother, and since the chorionic cells of the placenta may be phagocytic(1,2), the simplest hypothesis would assign the role of a barrier to the placenta. The final destruction of invading organisms would be relegated to the antimicrobial substances of maternal and fetal bloods(3,4,5,6), and perhaps to some inadequacy of fetal tissues as a culture medium(7) for certain organisms. However, some reports (8,9,10,11) suggest that the placenta may contain antimicrobial substances distinct from those of the blood. The present report records concentrations of certain antibacterial substances in the blood of mothers and newborn infants and attempts to determine whether there are additional antibacterial factors in the placenta itself.

Materials and methods. Venous blood was obtained from mothers, and blood from newborn infants was collected from the placental portion of the severed umbilical cord immediately after birth. After the blood had clotted at room temperature serum was separated by centrifugation in the cold and stored at –20°C. Placenta were frozen at –20°C, allowed to thaw, comminuted in the cold for 3 minutes using a Waring blendor, and centrifuged at 2000 rpm for 1½ hours at 5°C. The sterile, dark red supernatant was stored at –20°C. Some placental preparations were treated with bentonite to remove lysozyme (12) or mixed with washed, heat killed bacteria to absorb specific antibodies. Bactericidal activity of the serums and placental materials were tested against *Escherichia coli, Shigella flexneri, Salmonella choleraesuis, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus viridans* and a beta hemolytic *Streptococcus pyogenes*. Before testing, each organism was cultured for 24 hours in brain heart infusion broth (Difco). One-tenth ml of a 10^{-7} dilution of organisms suspended in 0.85% sterile NaCl solution was added to duplicate tubes containing either 1.0 ml cold serum, placental material or saline control. Mixtures were incubated for 30 minutes in a water bath at 37°C. Two ml molten nutrient agar (Baltimore Biological Labs.) was added to each tube, the contents mixed and allowed to solidify. *Pseudomonas* and *Salmonella* grew poorly below the surface of the agar. Mixtures containing either of these organisms were poured directly, therefore, onto the surface of nutrient agar plates. Colonies were counted after incubating tubes or plates for 24 hours at 37°C. By comparing colony counts of saline controls with counts of tubes containing placenta or serum, percentage of bacteria killed could be calculated. Hemolytic complement titers were expressed as the final dilution of serum or placental material required to lyse 50% of 1.5 x 10^8 optimally sensitized sheep erythrocytes in a reaction volume of 1.5 ml after 30 minutes of incubation at 37°C. Triethanolamine buf-