The Renal Clearance of I^{131} During the Infusion of NaI^{131} and Insulin-I^{131}

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Although detectable levels of insulin proteases are widely distributed in mammalian tissues, the greatest concentrations have been found in homogenates of liver and kidney.[1] It seems to be well established that the liver degrades insulin and that its "insulinase" activity is not an artifact of sliced and homogenized preparations.[2] Recent work by Stadie, Williams, and others, showed that after the injection of I^{131} insulin into rats, the highest concentration of bound radioactivity was found in the kidney, which suggests that this organ may participate in insulin catabolism.[3,4]

The role of the kidney in insulin degradation is of additional interest in the light of the clinical observation that insulin requirements often decrease in diabetics with advanced intercapillary glomerulosclerosis.[5,6] Although decreased dietary intake may play a role,[7] the existence of decreased insulin catabolism in diabetics with advanced renal disease remains an attractive hypothesis. This study was designed to investigate the role of the human kidney in insulin degradation.

MATERIALS AND METHODS

The purpose of this study was to compare the renal clearance of I^{131} during the infusions of NaI^{131} and insulin-I^{131}. Five patients, two female and three male, whose ages ranged from fifteen to fifty-eight years, and who had no known renal or cardiac disease, were studied. One of the patients had diabetes and had been taking 40 units of NPH insulin daily for ten years. Each of the patients had his thyroid suppressed with two 30 mg. doses of Methimidazole given twelve and three hours before the experiments. Each patient received 1,500 cc. of water twelve and three hours before the experiments, and sufficient water (usually 250 cc. every half hour) to insure a urine flow of at least 2 cc. per minute. The iodide^{131} and insulin-I^{131} infusions were carried out on separate days in random order with at least a six-day interval between the two studies. In determining the renal clearance of I^{131}, a priming dose of 50 μc of NaI^{131}* was injected intravenously, followed by a continuous intravenous infusion of NaI^{131} in normal saline at 0.33 μc/min. The total amount of NaI^{131} administered was approximately 100 μc. This rate was found to be optimal for providing a slowly rising blood level of radioactivity. After a thirty-minute period of equilibration, urine was collected by means of an indwelling Foley catheter for four consecutive thirty-minute periods, and a blood sample was drawn at the midpoint of each period.

In the determination of the I^{131} clearance during the infusion of insulin-I^{131},† a priming dose of 4 μc of insulin-I^{131} was given, followed by a continuous infusion of 0.65 μc/min. of insulin-I^{131} in normal saline. Again, this rate of infusion was found to be optimal for obtaining slowly rising blood levels of radioactivity. Urine and blood were collected in the manner previously described after a thirty-minute equilibration period.

In both types of experiments the infusions were carried out at the rate of 4 ml./minute.

Determinations of radioactivity were made in duplicate on 2 ml. aliquots in a scintillation well counter. Sufficient counts were obtained to maintain a counting error of less than 1 per cent. Samples of urine, whole plasma, plasma TCA precipitates, and plasma TCA supernatants were counted in this manner. The plasma was precipitated with an equal volume of 40 per cent trichloracetic acid. The supernatant was aspirated and counted. The TCA precipitates were washed three times with distilled water and digested with concentrated KOH.

Following NaI^{131} and insulin-I^{131} infusions the radioactivity in the TCA soluble fraction of the plasma was

Winner of the 1959-60 Graduate and Medical Student-Intern Essay Contest of the American Diabetes Association for the best paper in the field of diabetes reporting original work, whether laboratory investigation or clinical observation. This is the paper for which he received his award.

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* Abbott Laboratories.

† Abbott Laboratories — Specific activity = 4.7 millicuries per mg. Concentration: 0.1 mg./ml. Insulin activity: 27 μ/mg. Stored at 4° C. for maximum one week before use.

found to be mainly inorganic I^{131} because it was 97 per cent extractable with chloroform after the addition of iodide carrier and excess iodate.[8] The radioactivity in urine was found to be less than 0.1 per cent precipitable with TCA. In addition, the urinary radioactivity was identical with that of an NaI^{131} standard on descending paper chromatography[2] with a butanol-acetic acid-water solvent (3:1:4) and subsequent autoradiography with Eastman no-screen X-ray film. In one patient after insulin-I^{131} infusion, the urinary chromatograms were divided into 1 to 2 cm.-wide strips and counted in a scintillation well counter. Again, the radioactivity corresponded to the iodide^{131} spot; neither the presence of insulin-I^{131}, labeled monoiodotyrosine, nor diiodotyrosine could be demonstrated.

In both the NaI^{131} and insulin-I^{131} infusions, the renal clearance of inorganic I^{131} was calculated by the following formula:

$$I^{131}\ \text{clearance (ml./min.)} = \frac{\text{Counts/ml. urine x urine flow (ml./min.)}}{\text{TCA soluble counts/ml. plasma}}$$

"Endogenous creatinine clearances"[9] were carried out simultaneously in some experiments. No significant differences were observed between values obtained during the NaI^{131} and insulin-I^{131} infusions.

It was assumed that the renal clearance of iodide proceeding from the extrarenal breakdown of insulin-I^{131} should equal the clearance of infused NaI^{131}. If the kidneys effectively bind insulin and participate in its proteolytic destruction, I^{131} may be added to the urine by the kidneys. In this case, the "apparent clearance of iodide" during the infusion of insulin labeled with I^{131} should exceed the clearance of iodide during a NaI^{131} infusion.

RESULTS

Figure 1 illustrates the changes in plasma radioactivity during infusions of NaI^{131} and insulin-I^{131} in the same patient (P.H., twenty-nine-year-old female nondiabetic) on different days.

It can be observed that during the infusion of NaI^{131} practically all the counts are contained in the TCA soluble fraction of the plasma, and the TCA precipitate is negligible. During the insulin-I^{131} infusion, the radioactivity is distributed between the TCA precipitate and the TCA supernatant. The TCA soluble counts rise on a steeper slope than the precipitable counts because the destruction of insulin exceeds the rate of infusion.

Table 1 summarizes the iodide clearances measured during the infusion of NaI^{131} and insulin-I^{131} in five patients.

It can be observed that in all patients the mean clearance of iodide during the insulin-I^{131} infusion was great-

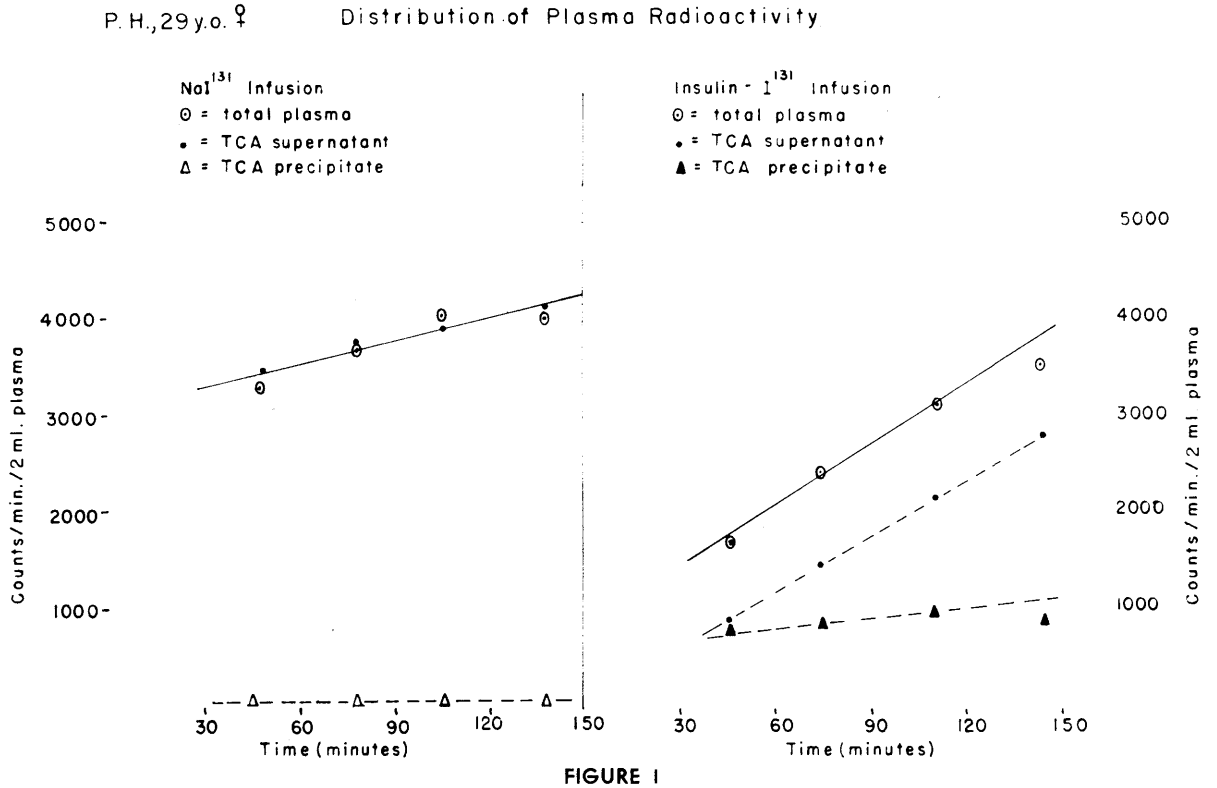

FIGURE 1

TABLE 1

Patient	Collection period	I^{131} clearance (ml./min.) NaI^{131} infusion	Insulin-I^{131} infusion	P
V.D.	1	35.3	50.9	
Age 50	2	34.8	47.6	
Male	3	30.7	40.7	
	4	22.1	44.1	
	Mean±S.E.*	30.7±3.8	45.8±2.2	<0.01
J.M.	1	—	60.2	
Age 36	2	40.1	65.7	
Male	3	44.1	64.6	
	4	44.7	58.1	
	Mean±S.E.	43.0±1.4	62.1±1.8	<0.05
F.W.	1	43.8	43.4	
Age 15	2	34.1	48.5	
Female	3	38.4	40.6	
	4	42.7	51.6	
	Mean±S.E.	39.8±2.2	46.0±2.5	<0.2
A.W.	1	36.8	42.8	
Age 58	2	32.9	44.3	
Male (diabetic)	3	28.2	40.2	
	4	27.0	37.9	
	Mean±S.E.	31.2±2.2	41.3±1.4	<0.01
P.H.	1	37.6	30.4	
Age 29	2	—	43.8	
Female	3	34.8	51.4	
	4	27.5	39.6	
	Mean±S.E.	33.3±2.4	41.3±4.4	<0.2
Average Mean±S.E.		35.6±2.5	47.3±3.8	<0.05

* S.E. = standard error of the mean.

er than the mean clearance of iodide during the infusion of NaI^{131}. In three patients, (J.M., V.D., A.W.) this difference was statistically significant. In two patients, (F.W. and P.H.) there was some overlay, but the trend was the same. The mean clearance of iodide in the five patients was 35.6 ml. of plasma/min., while the mean of the apparent clearance of iodide during the infusion of insulin-I^{131} was 47.3 ml. of plasma/min. The statistical probability of the differences between these means being due to chance is less than 5 per cent. When paired analysis of the five pairs of clearances is carried out, the probability of the differences being due to chance is less than 1 per cent.

Figure 2 compares the levels of TCA precipitable and soluble radioactivity during the infusion of insulin-I^{131} in a representative nondiabetic patient (F.W.) and a diabetic (A.W.). The latter patient had been taking 40 units of NPH insulin daily for the last ten years. In the nondiabetic, the rate of destruction of insulin-I^{131} exceeded the rate of infusion shown by the steeper slope of TCA soluble radioactivity as compared to the protein bound counts. When insulin-I^{131} was infused at the same rate to the diabetic patient the following differences were observed: (a) the levels of protein-bound radioactivity in plasma were higher than in any of the nondiabetic patients studied; (b) the rate of accumulation of protein bound radioactivity was greater, resulting in a steeper slope of the TCA precipitable counts; (c) the rate of destruction of insulin-I^{131} was decreased, as illustrated by the slower rise of TCA soluble radioactivity. These findings are best explained by the presence of insulin binding antibodies in the plasma of the diabetic patient treated with insulin.[10,11]

Although patient A.W. probably had circulating insulin binding antibodies, it is of interest to note that similarly to the nondiabetics, his "apparent clearance" of I^{131} during the infusion of insulin-I^{131} exceeded that observed during the infusion of NaI^{131}.

In three additional patients, an attempt was made to obtain a decrease in the apparent iodide clearance by diluting the infused insulin-I^{131} with nonlabeled insulin. Figure 3 illustrates the results of one of these experiments which were essentially the same as those obtained with the other two patients.

In these experiments, 100 microcuries of insulin-I^{131} were infused over a three-hour period at a rate of 0.0002 units of insulin per minute. After ninety minutes, the labeled insulin was diluted with a simultaneous infusion of 0.1 or 0.3 units of nonlabeled insulin* per minute (the nonlabeled insulin exceeding the labeled insulin by a ratio of 500-1,500:1). The rate of infusion was 4 ml./min. during the entire experiment. The solvent was 0.9 per cent saline during the first, and 5 per cent glucose during the second, experimental period.

The upper part of figure 3 shows that there was no break in the slope of inorganic iodide (TCA supernatant) which should be expected if the sites of insulin binding had been saturated and insulin breakdown inhibited by the infusion of nonlabeled insulin. The lower part of figure 3 demonstrates that there was no decrease in the apparent iodide clearance.

DISCUSSION

Our working hypothesis is that the kidney clears iodide derived from the extrarenal breakdown of insulin-I^{131} in the same manner as it clears infused sodium iodide. If this is correct, the apparent increase in iodide clearance during the infusion of insulin-I^{131} is a result of addition of iodide to the urine by the kidneys. This iodide could proceed either from the labeled insulin itself or from one of its circulating breakdown products from extrarenal sites. Iodinated insulin gives rise to monoiodotyrosine (MIT) and diiodotyrosine (DIT),[2] both

*Crystalline Zinc Insulin, Lilly.

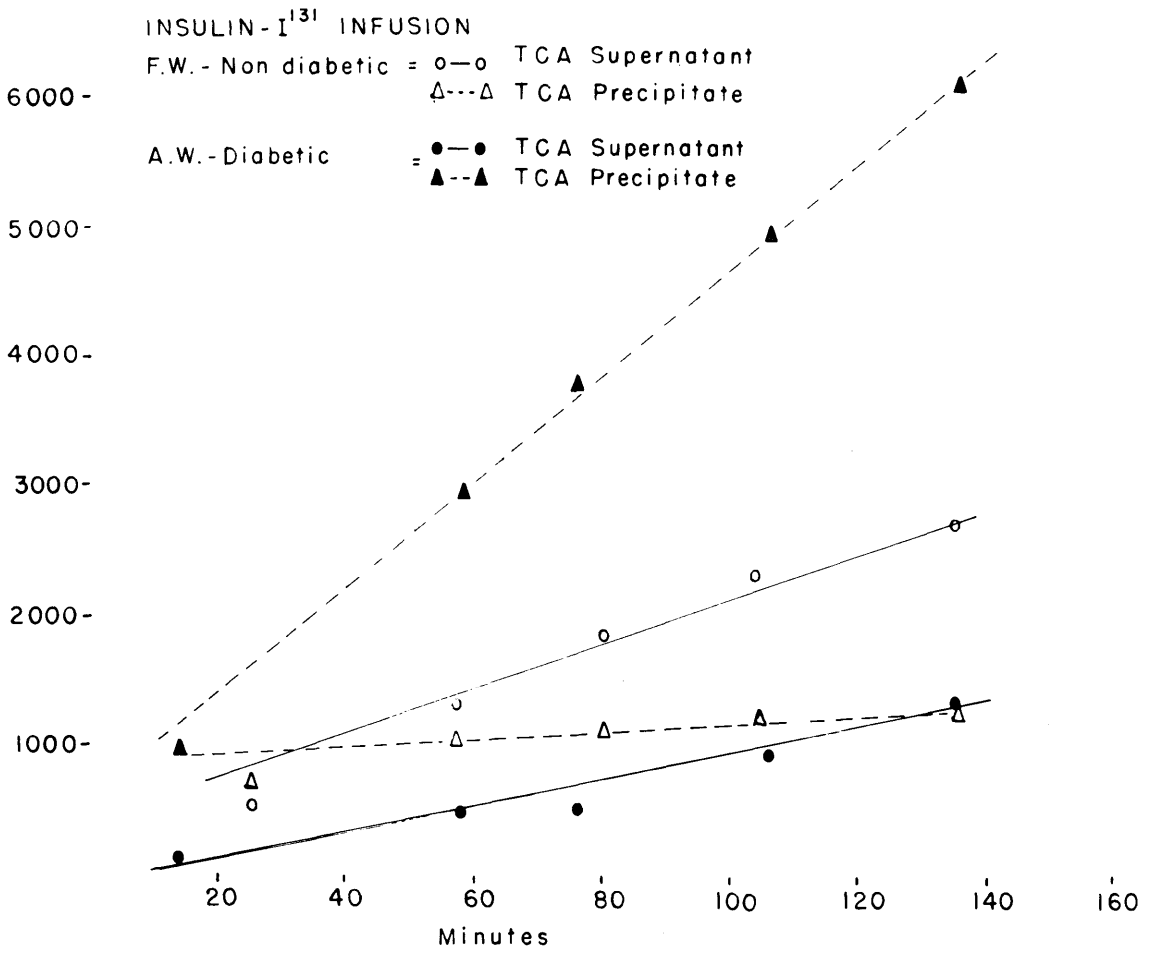

FIGURE 2

of which are deiodinated by renal tissue in vitro.[12] Lassiter and Stanbury[13] studied quantitatively the renal clearance and deiodination of infused DIT in humans. Their figures indicate that the amounts of DIT^{131} circulating in plasma at any given moment should be approximately half as much as that present in the form of $iodide^{131}$ to explain the observed increase in iodide clearance during the infusion of insulin-I^{131}. However, the circulating amounts of DIT and MIT during insulin-I^{131} infusions, if present at all, are extremely small mainly due to the rapid hepatic deiodination of the tyrosines.[2]

An attempt was made to block competitively the binding sites of insulin-I^{131} in the kidney by diluting the insulin-I^{131} infusion with nonlabeled insulin (figure 3). The failure to accomplish this was probably a result of not administering enough insulin. Prout and Evans[14] showed that in rats more than 100 units of insulin/kg. are necessary to achieve saturation of binding sites and decreased insulin catabolism.

The possibility of labeled and nonlabeled insulin having different degradation pathways has been shown to be unlikely by work with intact mice,[15] eviscerated nephrectomized rabbits,[16] liver extracts,[17] and perfused rat

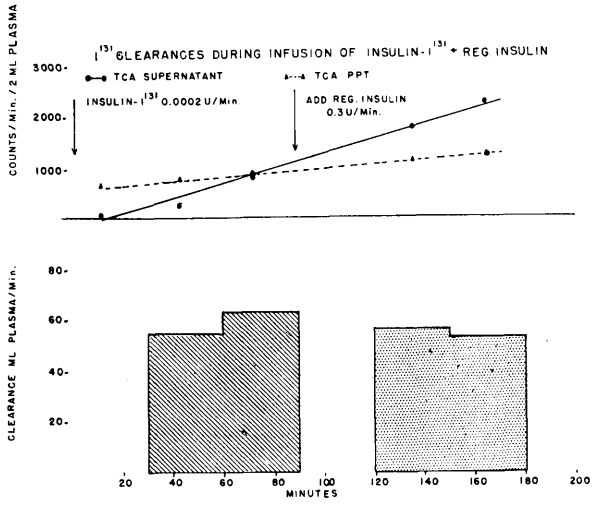

FIGURE 3

livers.[2] Further study of the I^{131} renal clearances of diabetics with intercapillary glomerulosclerosis will be necessary before the clinical implications of the renal breakdown of insulin can be evaluated.

SUMMARY

The renal clearance of iodide^{131} was studied during continuous infusions of NaI^{131} and insulin-I^{131}. It was assumed that the renal clearance of iodide proceeding from extrarenal breakdown of insulin-I^{131} would equal the clearance of infused NaI^{131}. In all experiments, the "apparent iodide clearance" during insulin-I^{131} infusion was greater than the clearance of infused NaI^{131}. The mean iodide clearance of five patients was 35.6 ml./min. $\pm$ 2.5, while the "apparent iodide clearance" during insulin-I^{131} infusion was 46.3 ml./min. $\pm$ 3.8 ($p<0.05$). It is probable that the excess I^{131} that appeared in the urine during the infusion of insulin-I^{131} proceeded from degradation and deiodination of hormone concentrated in the kidney.

Dilution of insulin-I^{131} by simultaneous infusion of 0.4 U./kg. unlabeled insulin did not change the "apparent I^{131} clearance."

SUMMARIO IN INTERLINGUA

Le Clearance Renal de I^{131} Durante le Infusion de *NaI^{131} e de Insulina-I^{131}*

Le clearance renal de ioduro^{131} esseva studiate durante le continue infusion de NaI^{131} e insulina-I^{131}. Esseva supponite que le clearance renal de ioduro resultante ab le decomposition extrarenal de insulina-I^{131} deberea esser equal al clearance del infundite NaI^{131}. In omne le experimentos, le "apparente clearance de ioduro" durante le infusion de insulina-I^{131} esseva plus grande que le clearance del infundite NaI^{131}. Le clearance medie de ioduro de cinque patientes esseva 35,6 $\pm$ 2,5 ml/min, durante que le "apparente clearance de ioduro" durante le infusion de insulina-I^{131} esseva 46,3 $\pm$ 3,8 ml/min ($p<0,05$). Il es probabile que le excesso de I^{131} que appareva in le urina durante le infusion de insulina-I^{131} resultava de degradation e disiodisation de hormon concentrate in le renes.

Le dilution de insulina-I^{131} per le infusion simultanee de 0,4 unitates per kg de peso corporee de non-marcate insulina non alterava le "apparente clearance de I^{131}."

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