THE EFFECT OF RENAL DISEASE ON THE DEGRADATION OF INSULIN

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Reduced requirements for insulin following development of the Kimmelstiel-Wilson syndrome have been observed in some patients.[1, 2, 3] Similarly, amelioration of glycosuria and hyperglycemia has been reported after induction of renal disease in alloxan diabetic rats.[4, 5] The mechanism by which diabetes is thus improved is obscure.

The demonstration by Williams and co-workers[6] that the normal rat kidney has a great avidity for injected I^{131} insulin led us to postulate that the diseased kidney might not sequester or inactivate insulin as well as the normal kidney, thus leaving more of the active hormone free to circulate.

The Influence of Induced Renal Disease on Diabetes

Before testing this hypothesis, we were able to confirm the observations of others that the production of nephrotoxic serum "nephrosis" in rats with experimental diabetes was followed by a striking reduction in levels of blood and urinary glucose (Table 1). The tube feeding of a constant diet[7] throughout the experiments precluded variations in food intake as an explanation for the phenomenon.

The Disposal and Distribution of Adminstered Insulin

In experiments dealing with the disposal and distribution of administered insulin, the half disappearance time (HDT) from serum and the localization in organs (kidney, liver and muscle) of intravenously injected I^{131} insulin were measured at suitable intervals in five groups of rats under light nembutal anesthesia: 1) normal; 2) diabetic (partial pancreatectomy or alloxan); 3) nephrotic (nephrotoxic serum);[8] 4) diabetic (partial pancreatectomy), later made nephrotic; and 5) acutely nephrectomized. All animals were tube fed identical diets[7] throughout, kept in metabolism cages and weighed daily. Determinations of blood[9] and urinary[10] glucose in diabetic animals and of urinary albumin[11] in nephrotic animals were made periodically. No insulin was given therapeutically.

Blood samples were taken at 15, 25 and 40 minutes after injection of the

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TABLE 1

Effect of Nephrosis on Severity of Diabetes in 12 Partially Pancreatectomized Rats

	Proteinuria mg/day	Glycosuria gm/day	Blood Sugars	
			NFBS	FBS
Before nephrosis.....	14.3*	2.2	290	94
During nephrosis.....	664.6	0.56	162	74

* Mean of 11 determinations in 5 normal rats.

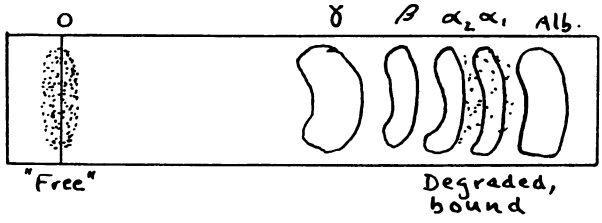

FIG. 1. The distribution of I^{131} insulin in serum after paper electrophoresis.

labeled insulin and the unaltered and unbound ("free") hormone in the serum was measured by paper electrophoresis. With this procedure the unaltered insulin remains at the site of application to the paper strip and its radioactivity can be counted there, whereas degraded insulin and that bound to serum proteins migrate outward with the latter[12] and can be disregarded (Fig. 1). The half disappearance time of free insulin from serum was calculated by the formula:

$$b\ (=\text{slope}) = \frac{1}{317}\,(15y_{15} + 25y_{25} + 40y_{40}) - \frac{80}{3}\,(y_{15} + y_{25} + y_{40}) \quad (1)$$

$$t_{\frac{1}{2}} = \frac{.3010}{b} \quad (2)$$

where

b = slope of a line describing the fall of undegraded radio-insulin in serum for the period 15 to 40 minutes.

y = log of counts at origin at the time designated in subscript.*

Immediately after the 40 minute blood sample had been drawn, the kidneys, liver and diaphragm were removed and homogenized separately in iced saline with a Waring blender. Trichloracetic acid was added and the

* Formula derived by Professor K. Alexander Brownlee, Department of Statistics, University of Chicago.

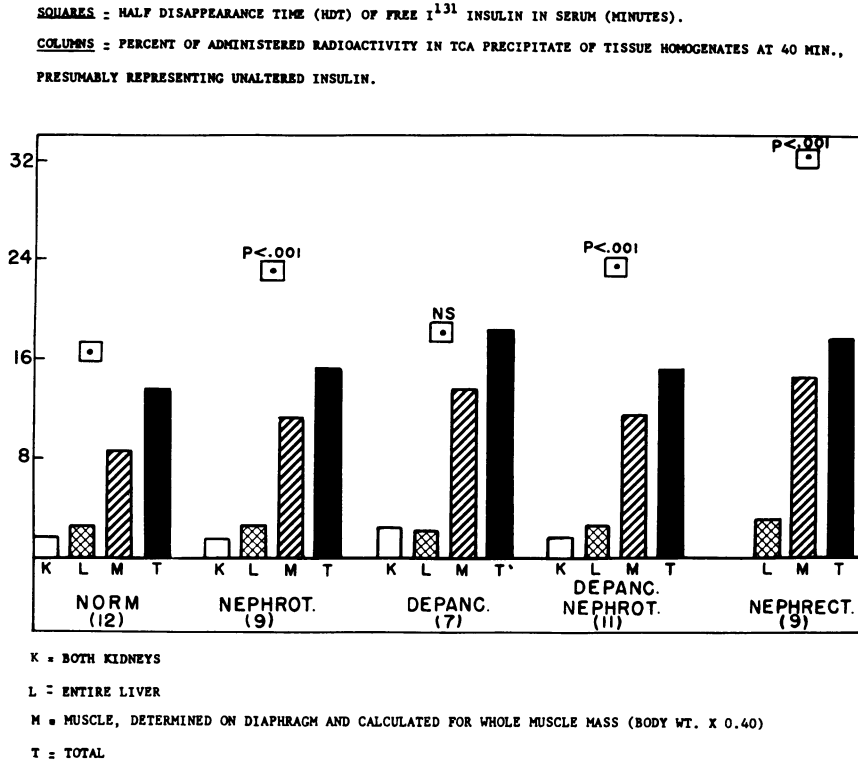

FIG. 2. The half disappearance time from serum and the localization in tissues at 40 minutes of intravenously injected I^{131} insulin.

radioactivity of the TCA precipitate or aliquots thereof, presumably representing undegraded insulin both free and bound to tissue protein, was determined in a well scintillation counter. Kidney, liver and muscle have been reported to account for about 50 per cent of total body radioactivity 15 minutes after intravenous injection of I^{131} insulin[6] and our data at 40 minutes are in substantial agreement.

Fig. 2 shows that in both the nondiabetic nephrotic and the depancreatized-nephrotic rats there was a significant ($p < .001$) increase in HDT as compared with values for normal rats. The increase in HDT in acutely nephrectomized rats was striking and was accompanied by appreciable increases in radioactivity of the remaining organs (liver and muscle). In both nephrotic groups, however, differences from normal with respect to levels of TCA precipitable radioactivity in kidney, liver and muscle, whether considered separately or combined, were insignificant and values did not change uniformly in a direction consonant with the HDT. This

TABLE 2

Hypoglycemic Effect in Normal Rats of Insulin Incubated with Tissue Extracts

	Insulin (10)	Insulin and Kidney Extract		Insulin and Liver Extract	
		Normal (20)	Nephrotic (13)	Normal (13)	Nephrotic (9)
Average of 30 and 40 minute blood sugars as per cent of initial value	51.5	70	61.5 ($p < 0.01$)	81.5	61 ($p < 0.002$)

Figures in parentheses indicate number of animals.

suggested that fixation of the hormone in these organs does not determine the rate of disappearance of insulin from the serum. The high levels of tissue radioactivity accompanying normal HDT in pancreatectomized rats remain unexplained.

If the slowing of disappearance rates in renal disease is not due to decreased *sequestration* of insulin in organs, one might postulate a diminished *degradation* of insulin as a result either of kidney damage itself or of the general metabolic effects of such damage.*

Evidence that the presence and integrity of renal tissue per se influence disappearance rates is provided by the finding that HDT was shortest in normal rats (16.7 min.), longer in those with nephrosis (23.1 min.), and longest in those nephrectomized immediately before injection (32.6 min.) (Fig. 2). In the latter, metabolic effects presumably had not had time to make their appearance. It seemed likely, then, that the partial inactivation of insulin that normally takes place in tissues[13] was being interfered with as a direct result of change within the kidneys or, of course, their surgical removal. Accordingly, the degradation of insulin by extracts (insulinase?)[13] of organs from normal and nephrotic rats was next investigated.

Inactivation of Insulin by Tissue Extracts

Saline extracts of homogenized normal rat kidney incubated with commercial insulin for one hour at 37° C. and injected into normal rats (0.2 u. insulin per rat) reduced the blood sugar of the latter to 70 per cent of the initial value. Extracts of nephrotic kidneys produced a reduction to 60.5 per cent ($p\Delta < 0.01$), while insulin alone gave a reduction to 51.5 per cent (Table 2). There was a similar difference in hypoglycemic effect between extracts of normal and of "nephrotic" liver incubated with

* No means were available to us for measuring renal excretion of insulin. In the nephrotic rats, however, increased rather than decreased loss of insulin by this route would be expected in view of the marked proteinuria—a situation hardly compatible with the prolonged HDT exhibited by these animals.

insulin. There was no difference, however, in the effect of extracts of normal as compared with "nephrotic" diaphragm.

In another approach, cold saline homogenates of rat tissues were centrifuged under refrigeration. The supernate was removed, incubated for one hour at 37° C. with 0.005 millicuries of I^{131} insulin per ml and diluted to 200 volumes with 25 per cent human serum. After the counting of aliquots to determine total radioactivity, an equal volume of 10 per cent TCA was added, the resulting suspension centrifuged and the supernate filtered and counted in a well-type counter. The radioactivity of the precipitate was calculated by difference. In this procedure, degraded I^{131} insulin is found in the filtrate while undegraded insulin, presumably active, is brought down with the other proteins. It was found that pooled extracts of kidney, liver and diaphragm from 8 normal rats contained 43, 39 and 48 per cent, respectively, of the total radioactivity in the precipitate, while such extracts from 10 nephrotic rats contained 51, 52 and 68 per cent of the total radioactivity in the precipitate. Extracts inactivated by heating at 80° C. for 10 minutes showed 97 per cent of the total radioactivity in the precipitate.

It would thus appear that extracts of kidney, liver and, by some tests, muscle, contain a heat labile substance which inactivates insulin, that "nephrotic" organs contain less of it than normal organs, and that the difference in HDT of I^{131} insulin between normal and nephrotic rats is owing to a difference in the rate of degradation of insulin and not to a difference in its sequestration in kidney, liver or muscle.

Summary

The abatement of experimental diabetes in rats following induction of nephrotoxic serum nephrosis has been confirmed.

Intravenously injected I^{131} insulin persists for a longer time in the blood of both nondiabetic and diabetic rats with experimental nephrosis than in the blood of normal rats, and for a still longer time in acutely nephrectomized rats. These differences in insulin disappearance time are not accompanied by decreased sequestration of insulin by the kidneys, liver or muscle of the nephrotic animals but appear to be correlated with diminished degradation of the hormone by such organs or their products.

The relation of these observations to the reduced insulin requirements of some patients with diabetic glomerulosclerosis must remain conjectural until similar experiments are carried out in human subjects.

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DISCUSSION

Dr. Joseph H. Holmes (Denver): I would like to ask if there was a wide spread in the results for your nephrotic animals and does this give us any area for speculation of the marked difference between renal patients in their response to insulin and to glucose loads.

Dr. George W. Thorn (Boston): I think that the approach given by Dr. Ricketts is a very interesting one to a problem which all of us have been interested in and I wonder in this postulation whether one might, as he originally stated, be interested in the disappearance of insulin as a function of the type of binding of insulin in the serum. I was also interested to note that the animals tested had lower blood sugar values than the controls. Therefore, if one assumes that the pancreatectomy was fairly complete or, let us say, that the insulin reserve was severely limited, then one also has to assume an improvement in blood sugar on a basis other than insulin action to begin with or as associated with the phenomenon.

Therefore, I was wondering if in your hypothesis you included how effectively your nephrotic serum might impair new glucose formation (gluconeogenesis), which would be an added factor, of course.

Another question I wanted to ask is whether you have any thoughts in connection with any of the patients on an alteration in renal glucose thresholds, whether that would be a phenomenon. It did not seem to be in the animals. If renal threshold for glucose were increased a bit then this would also prove to be a helpful mechanism for the organism and, by a complicated series of events, one could imagine some improve-

ment. After all, the kidney is a very potent glucose producer and whether it does this under normal circumstances as to what quantity, we do not know. However, most certainly, at least in the diabetic individual, insofar as glucose over-production is any part of the picture, the kidney might be expected, on this metabolic pathway, to augment diabetes. I wonder what your thoughts are on that.

DR. RICKETTS (Closing): In reply to the question raised by Dr. Holmes—I believe the "p" values that I indicated gave the answer.

With regard to Dr. Thorn's comments, I believe he covered a number of points that deserve attention.

If I understand him correctly, one of the questions was concerned with whether or not endogenous insulin is inactivated by the kidney as well as injected insulin. I think one has to assume that in our rats the diabetes was not very severe. They were probably secreting some insulin of their own and, therefore, if any analogy is to be made between our experimental observations in rats and those in man, one would have to assume that the kidney normally inactivates some endogenous insulin as well as some injected insulin. However, whether this is actually true is something that cannot be established at the present time.

As to the contribution of the kidney to the glucose pool, this is a well known fact, as Dr. Thorne has indicated. However, my impression as to the magnitude of the contribution is a little different from his. The phenomenon can be demonstrated, but in the reports I have seen it did not seem to me that it was as important as his remarks indicated. I am doubtful that it would have had an effect on our results, but this remains a possibility.

As to the question of whether the renal threshold has changed in the nephrotic animals, this almost surely has to be answered in the affirmative. Probably it was altered. However, it surely was not altered so much as to lower the blood sugar. If we can analogize from the renal losses of glucose in nephrotic patients, such losses in our rats are not likely to have been very large. Furthermore, it is difficult to see how an alteration in renal threshold for glucose would affect the half disappearance time of the insulin.