Comparison of Disappearance from Plasma of Insulin I^{131} and of Insulin-Like Activity.* (29381)

ROBERT E. BOLINGER AND RONALD R. STEPHENS
Clinical Research Center, Kansas University Medical Center, Kansas City, Kan.

Insulin labeled with I^{131} has been used to study the parameters of plasma insulin disappearance in both man(1,2) and experimental animals(3,4,5). The use of insulin I^{131} can be justified only if it can be shown that the labeled molecules behave in a physiological system in a manner identical with or at least predictably different from native insulin(6). The present experiments are designed to study simultaneously the plasma disappearance characteristics of insulin labeled with I^{131} and of commercial crystalline insulin in normal and diabetic subjects, as biologically assayed on the rat.

Methods. Blood samples were drawn in the fasting state and the subjects injected intravenously with a mixture of insulin I^{131} (30-50 μc)† and crystalline insulin (0.2 unit/kg). Blood samples were drawn at 10, 20, 30 and 60 minutes. Radioactivity in TCA precipitates of plasma was assayed in the gamma well counter. Insulin-like activity (ILA) was assayed using the rat epididymal fat pad according to the method of Renold *et al*(7).

Results. The plasma disappearance curve for insulin I^{131} consisted of a rapid (10-20 minutes) initial component (Fig. 1) with a half life of 15 $\pm$ 2‡ minutes in the controls and 41 $\pm$ 9 minutes in the diabetics; and a slower (30-60 minutes) component with a half life of 45 $\pm$ 7 minutes in the controls and 88 $\pm$ 10 minutes in the diabetics.

The mean plasma disappearance curve for insulin as measured by insulin-like activity (ILA) consisted also of an initial rapid component with a half life of 15 $\pm$ 2 minutes in the controls and 42 $\pm$ 9 minutes in the diabetics; and a slower component with a half life of 30 $\pm$ 10 minutes in the controls and 78 $\pm$ 5 minutes in the diabetics.

Mean plasma ILA (767 $\pm$ 24 microunits/ml) for the controls after injection was significantly smaller ($P<.01$) than that for the diabetics (2470 $\pm$ 335 microunits/ml). Parameters of individual curves for ILA were difficult to estimate in the diabetic group because of the experimental error inherent within the bioassay.

Discussion. The composite curves of insulin disappearance as determined by insulin I^{131} generally showed longer half lives than those determined by ILA. The overestimation of the half life by the insulin I^{131} method was more marked in the diabetics although deviations were consistently in the same direction. Scott *et al*(3) have noted this overestimation by the insulin I^{131} method in rabbits and suggested that this is due to measurement of insulin I^{131} which has been altered, and not possessing the properties of true insulin.

Berson *et al*(8) stated that the slower phase of the disappearance of insulin I^{131} represents the disappearance of labeled non-insulin impurities.

It is apparent from these studies that the disappearance of insulin as measured by bioassay also shows a curve with an initial rapid and then a slower late component. It is necessary to conclude that this slow component is actually insulin-like activity and thus, reflects a parameter of true insulin disappearance. It is reasonable to suggest that this reflects the disappearance of bound insulin while the rapid phase represents free insulin. Prout and Katim(9) have presented evidence for a physiologic binding of insulin in the alpha globulin fraction, providing a possibility for binding of some of the insulin in normal controls.

Thus, although the ILA and insulin I^{131} methods for estimation of plasma insulin disappearance give different values, the deviations are relatively predictable. The

* Supported by grant from USPHS.
† Abbott Laboratories, 7 MC/mg.
‡ S.E. $= \sqrt{\frac{\Sigma\chi^2}{N(N-1)}}$.

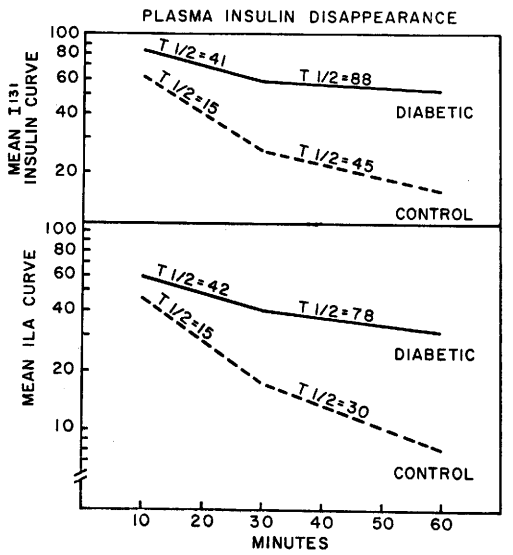

FIG. 1. Composite plasma disappearance curves for insulin measured simultaneously by assay of TCA precipitable radioactivity and by assay of ILA on the epididymal fat pad.

greater reproducibility of the insulin I^{131} curve renders this a more reliable measure of insulin dynamics in individual cases.

Summary. Plasma decay curves were determined in diabetics and normals after the injection of a mixture of insulin I^{131} and crystalline insulin. The general forms of the curves as determined by bioassay and assay of radioactivity were similar, consisting of an initial rapid component and a late slower component. Insulin I^{131} tended to overestimate the half life of disappearance determined by bioassay of insulin-like activity.

1. Welsh, G. W., Henley, E. D., Williams, R. H., Cox, R. W., *Am. J. Med.,* 1956, v21, 324.
2. Bolinger, R. E., Grady, H. J., *Ann. Int. Med.,* 1958, v48, 753.
3. Scott, G. W., Prout, T. E., Weaver, J. A., Asper, S. P., *Diabetes,* 1958, v7, 38.
4. Elgee, N. J., Williams, R. H., Lee, N. D., *J. Clin. Invest.,* 1954, v33, 1252.
5. Bolinger, R. E., Slinker, B. J., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 574.
6. Sheppard, C. W., *Ann. N. Y. Acad. Sci.,* 1963, v108, 29.
7. Renold, A. E., Martin, D. R., Dagenais, Y. M., Steinke, J., Nickerson, R. J., Sheps, M. C., *J. Clin. Invest.,* 1960, v39, 1487.
8. Berson, S. A., Yalow, R. S., Bauman, A., Rothschild, M. A., Newerly, K., *ibid.,* 1956, v35, 170.
9. Prout, T. E., Katim, R. B., *Diabetes,* 1959 v8, 425.

Received April 15, 1964. P.S.E.B.M., 1964, v116.

Effects of Pilocarpine on Exchange of K^{42} in Slices of Submaxillary Gland.* (29382)

L. H. SCHNEYER AND C. A. SCHNEYER
Department of Physiology, University of Alabama Medical Center, Birmingham

Secretion of electrolytes by salivary gland appears to involve a complex series of separate processes which have generally proved difficult to localize and identify(1,2). In the case of potassium, however, evidence suggests that transfer from cells to secretory fluid involves the participation of a great proportion of the cells of the gland and that the content of potassium in the cells is the primary source of the secreted ion(1-4). On this basis it appeared of value to determine whether an *in vitro* preparation of salivary gland could prove useful for investigation of secretion of potassium. Recent work with slices of submaxillary gland as the *in vitro* preparation showed that during incubation when net accumulation of potassium is occurring, addition of a parasympathomimetic stimulating agent results in reduction in the net accumulation of this ion(5). Since net loss of potassium from the tissue marks the initiation of secretion of this ion *in vivo*(6-8), a resemblance between the effect of the parasympathomimetic agent *in vitro* and tissue events in secretion of potassium after parasympathomimetic stimulation *in vivo* was suggested(5).

* Supported by grant from Nat. Inst. Health.