in contrast to the 18 litters in which all pups died.

Summary. Potato sprouts are shown to be toxic to pregnant rats when fed at a level of 10% of the diet. When the potato alkaloid solanine was incorporated into the diet at a level approximating the concentration in the sprout diet, similar effects were observed. All of the pups in 18 of 33 litters born of rats eating the test diet died before reaching weaning age. Only one of 11 control litters was lost.

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Effect of Nephrectomy and Splanchnicectomy on Plasma Disappearance of Labeled Insulin in the Rabbit.* (26763)

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Studies have revealed that the disappearance rate of insulin-I^{131} from the plasma is influenced by a number of factors: plasma binding(1), state of hepatic and renal function(2,3), route and rate of administration (4), size of the endogenous insulin pool(5), and previous administration of heterologous insulin(6). Determinations based on accumulation of isotope in the liver and kidney after injection of insulin-I^{131} have implicated these organs in the process of insulin degradation(2). Such evidence is open to question since the concentration of radioactivity in these organs does not necessarily have any direct bearing on concentration of insulin. Berson *et al.*(1) and Scott *et al.*(7) have noted that the labeled material disappears from the plasma according to a curve with at least 2 different time components. On the basis of electrophoretic studies these authors have reasoned that the initial fast component of the disappearance is most representative of true insulin while the second slower component may represent the disappearance of some labeled product other than insulin. Bolinger and Slinker(3) studied the parameters of the slower component of the disappearance curve in rabbits and showed that the status of hepatic and renal function affected this component. The following study is designed to study the characteristics of the rapid component of the disappearance curve of labeled insulin in the rabbit and if possible arrive at a quantitative measure of the importance of the liver and kidney in this process. An attempt is made to define the system more specifically by including values obtained for biologic assay of insulin activity in the plasma, in order to arrive at a better estimate of the endogenous insulin pool.

Methods. Adult white rabbits were fasted for 48 hours. After light anesthetization with pentabarbital blood samples were drawn and I^{131} labeled insulin (.06 Unit) was injected intravenously. Blood samples were drawn by cardiac puncture at 10, 20, 30, 45, and 60 minutes. Plasma glucose was determined by the glucose oxidase method(8). Radioactivity was determined on both trichloracetic acid precipitates and supernatants of the plasmas, using a well counter and expressed as fraction of dose injected. Electrophoresis

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was carried out on paper strips using the Spinco apparatus. Insulin-like activity of the plasma was assayed using the rat diaphragm according to the method of Willebrands and Groen(9). Since the method does not discriminate between the small changes in plasma insulin activity occurring after the injected test dose and at the different sampling times, activity was determined on a pool of the timed plasma samples taken from each animal and the level of insulin activity assumed to be a constant during the test procedure. This assumption appeared valid in view of the insignificant changes in blood glucose after injection of 0.06 Unit of labeled insulin.

The animals were divided into 3 groups: Group I were normal animals; Group II, animals in which the kidneys were removed acutely just before the test procedure; Group III, animals in which splanchnic circulation was completely occluded acutely just before the test procedure. A group of sham operated controls showed no striking deviation from the normal.

Results. Assuming the steady state, rate of consumption of insulin can be theoretically defined:

$$R = k_1 S C$$

where R is consumption of insulin (milli-units/min/kg)

k_1 is a first order rate constant for disappearance of labeled insulin from the plasma (min^{-1})

S is the space of distribution of the labeled insulin (ml/kg)

C is concentration of insulin in the plasma as determined by biological assay (milli-units/ml). This is assumed to be constant for each animal.

The product k S expresses the volume of body fluid (per kg) which is cleared of labeled material per minute.

The curve of disappearance of the labeled material from the plasma can be resolved into at least 2 first order components (Fig. 1). The following determinations are made on the rapid component of the curve during the first 20 minutes.

Space of distribution. In the normal rabbits the labeled material distributed itself over a volume of about 18% of total body volume, approximately that of extracellular fluid (Table I). Nephrectomy produced an insignificant decrease in the space while splanchnicectomy produced a marked decrease to 4.7%.

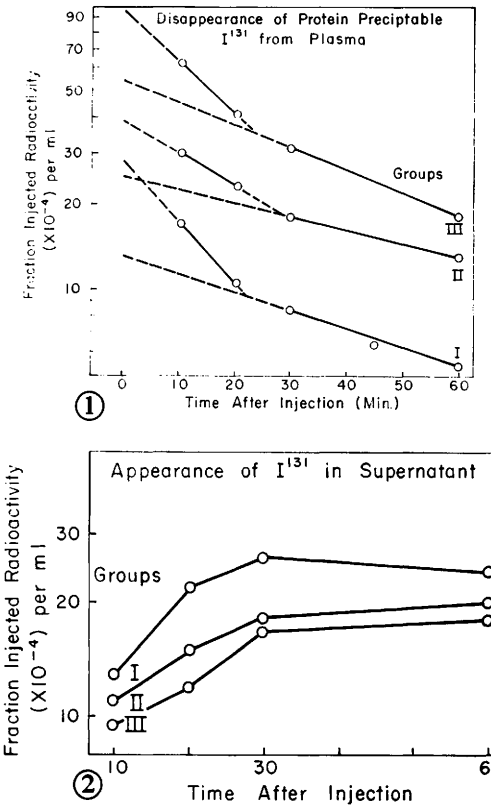

FIG. 1. Mean curves for disappearance of TCA precipitable radioactivity for each group. Actual curves are shown with solid lines and extrapolated curves with dotted lines.

FIG. 2. Mean curves for disappearance of TCA non-precipitable radioactivity for each group.

Disappearance constant in the normal animals was 0.0471 min^{-1} corresponding to a half time of 15 minutes. Nephrectomy produced a significant decrease in the disappearance constant while the rate in the splanchnicectomized animals was not different from normals.

The disappearance constant can not, however, be considered as a true measure of rate of disappearance of insulin since the space of distribution in each case is different. Thus a better measure of the true rate of disap-

TABLE I. Parameters of Plasma Disappearance of Labeled Insulin.

Group	No.	Insulin space, ml/kg	Rate constant, k_1 (min.$^{-1}$)	Half life, min.	Insulin conc., 10^{-3} U/ml	Insulin* consumption, 10^{-3} U/min./kg	Clearance* k S, ml/min.
I	9	186 ± 55	.0471 ± .0139	15 ± 5.1	.78 ± .32	7.04 ± 4.22	8.5 ± 3.7
II	4	145 ± 19	.0289 ± .0045	25 ± 6.2	.82 ± .13	3.64 ± 2.06	4.2 ± .7
III	7	47 ± 16	.0470 ± .0163	17 ± 5.4	5.61 ± 2.55	10.57 ± 2.14	2.1 ± .7

$$\text{S.D.} = \sqrt{\frac{\Sigma x^2}{N}}.$$

* Means of groups are significantly different from each other by analysis of variance.

pearance of insulin is the clearance factor (kS). In normals this was 8.5 ml/min/kg and nephrectomy was followed by a decrease. Splanchnicectomy produced an even greater decrease.

Consumption of insulin (R) in the normal animals is 7.04 milli-units/min/kg. Consumption of insulin is decreased after nephrectomy but not after splanchnicectomy. Actual increased insulin consumption after splanchnicectomy may be only apparent since it is based on the striking increase in assayable insulin activity from the plasma. The increased values for biological activity may be a result of exclusion of the liver from the circulation which could alter the assay by affecting the plasma binding of insulin(10) or by permitting accumulation of amino acids giving a false high value for insulin like activity(11).

Rate of appearance of radioactivity in the supernatant is a reflection of rate of breakdown of labeled insulin. This is decreased in both Groups II and III (Fig. 2).

The serum from each blood sample was electrophoresed and radioactivity from each protein fraction assayed. During the first 20 minutes, used for determination of the disappearance curves, 95% of radioactivity was in the beta globulin fraction.

Discussion. Evidence for the assumption that serum precipitable radioactivity is representative of true insulin after administration of labeled insulin, is largely indirect, since no direct experiment has yet been designed to demonstrate this. It is based, first, on the finding that after addition of labeled insulin to the serum, both biological activity and radioactivity are recovered primarily from the beta globulin fraction(12). Of the protein bound radioactivity in this experiment, 95% is in the beta globulin fraction. Furthermore Scott *et al.*(7) showed that the disappearance of biological activity from the plasma paralleled the same rapid component curve as that of the radioactivity from the globulin fraction, having the same mobility as that of insulin. That there are 2 different disappearance curves for the radioactivity from the serum does not exclude the possibility that both are representative of true insulin, since insulin may exist in the plasma in various states of binding with the plasma protein fractions.

Splanchnicectomy causes the greatest decrease in quantity of plasma cleared of insulin per unit of time. The capacity of the organism to consume insulin, however, is not compromised because of the ability of the remaining renal circulation to remove insulin at a higher plasma concentration of insulin. Conversely the greatest impairment in insulin consumption is produced by nephrectomy. These data are consistent with an hypothesis that both renal and splanchnic circulations can function in the degradation of insulin, but that at a higher level of plasma insulin concentration, the renal circulation can assume all of this function, in spite of a decreased insulin clearance. A true evaluation of insulin degradation must take into account not only the disappearance rate of the labeled material but also insulin concentration and space of distribution.

Summary. The plasma clearance and consumption of I^{131} labeled insulin was determined in normal, nephrectomized and splanchnicectomized rabbits. The rate constant for plasma disappearance of labeled material was decreased by nephrectomy but

not by splanchnicectomy. The plasma clearance of insulin was decreased by splanchnicectomy but the remaining renal circulation was able to maintain the consumption rate of insulin at a normal level.

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Electron Microscope Studies of Intact Epithelial and Fibroblast Cell Surfaces.* (26764)

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The earliest electron microscopic studies of tissue cell morphology utilized whole cells grown in tissue culture on formvar-coated copper wire grids. This technic, as described by Porter *et al.*(1), allowed visualization of some cell structures not seen by light microscopy, but did not permit the extensive studies of cellular morphology afforded by ultrathin sectioning. Consequently, the above method has had limited use and only a few additional studies(2,3,4) have been reported. Almost all of these appeared prior to the present widespread use of tissue culture as an experimental approach to various problems.

Although whole cell preparations are too thick to allow resolution of most structures in the nucleus and cytoplasm, the cell surface is clearly seen. Microvilli and other structures which project from the cell surface at various angles usually appear in thin sections as disconnected parts while in the whole cell mounts their structural integrity is retained. For certain studies in progress in this laboratory it was desirable to determine: (a) Whether the surface features of different tissue culture cell types are similar or vary significantly from one cell line to another, and (b) whether the surface morphology of one cell line studied over a prolonged period of time remains constant or varies. The present paper compares the surface morphology of 5 cell types commonly used in tissue culture and describes certain modifications of technic affording reproducible whole cell preparations in which microvilli and other surface structures are well preserved and remain free of most extracellular debris.

Materials and methods. Tissue culture. Cell types used included chicken fibroblasts, mouse fibroblast ("L"), HeLa, Chang's liver and chicken monocytes. These cells were grown and maintained in stock flasks of various sizes and after suitable sheets had been obtained were trypsinized into suspension. The cell suspensions were then adjusted to contain the following cell concentrations: 1-2 million chicken fibroblasts or 600,000 "L",

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