cians that the increased metabolic processes of pregnancy place a burden on the kidneys. The term "low reserve kidney" is frequently mentioned in obstetrics and usually denotes a kidney nearly exhausted by pregnancy. This concept has led many obstetricians to advise against pregnancy in the presence of any renal abnormality. In view of the increase in renal circulation and in glomerular filtration rate in pregnancy, this concept seems to be untenable.

Summary. 1. Renal function studies were performed on 9 patients during 1st. 2nd and 3rd trimester of pregnancy and in the postpartum period. 2. A progressive increase in renal plasma flow and glomerular filtration occurred, reaching maximum in 3rd trimester and returning to non-pregnant levels after delivery. 3. These changes speak against an added burden imposed on the kidneys by pregnancy.

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Effects of Superimposed Native Insulin on Disposal of Iodoinsulin in the Body.*† (23791)

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Iodoinsulin is widely used as a tracer in work designed to study the physiology of insulin. It is assumed that the iodinated compound is treated like native insulin by the tissues of the body. The validity of this assumption is supported by the observation that the breakdown of iodoinsulin in the body can be slowed by simultaneous administration of large doses of native insulin(1). This suggests the degrading processes of the two compounds are related and that some at least of the degrading mechanisms do not distinguish between them. Additional support for this assumption was given by our finding that biological activity resulting from a dose of native insulin decayed at the same rate as labeled insulin given at the same time to eviscerated-nephrectomized rabbits(2). In the intact animal the decay rate of iodoinsulin is very rapid. Greeley found the decay of biological activity to be much slower(3). There are many possible causes for this discrepancy. The kidney and liver, which actively degrade iodoinsulin, may treat native insulin differently. Another possible cause of the discrepancy is that iodoinsulin is ordinarily used in tracer amounts whereas appreciable dosages of insulin must be given to follow biological activity. Large doses of native insulin definitely lower the decay rate of iodoinsulin when the two are given together(1). It is possible that the smaller doses used to study biological activity could also produce such an effect. The experiments reported in this paper were planned to study this factor. The decay of iodoinsulin in the intact animal is so rapid that it would be difficult to detect small changes that might be effected by moderate doses of native insulin. Very large doses of

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native insulin are needed to give enough effect to be detected. The decay rate is very much lower in eviscerated-nephrectomized rabbits and we made use of this preparation with the view that much smaller effects could be detected with it. Several animals were so prepared. Each was given a tracer dose of iodoinsulin. In addition some were given simultaneously a dose of native insulin which varied over a wide range.

Methods. Insulin-I^{131} Degradation Assay. The insulin-I^{131} was purchased from Abbott Laboratories. The degradation of insulin-I^{131} was measured by the rise in radioactivity in the 10% trichloracetic acid (TCA) soluble fraction. This is based on the fact that the radioactivity of insulin-I^{131} is at least 95% precipitable with TCA, and it is assumed that the non-precipitable radioactivity represents breakdown products of the insulin-I^{131} molecule. Accordingly, the I^{131} content of the plasma and of the TCA soluble plasma fraction were determined. All radioactivity measurements were made in a well-type scintillation counter. All glassware used in the transfer of the insulin-I^{131} was washed with 10% NaOH and assayed for its radioactivity content so that corrections for the amount of radioactivity injected into the animals could be made. In no experiment did the I^{131} absorption by the glassware exceed 2% of the anticipated injected radioactivity. The rabbits were eviscerated by the method previously described(4). Native and iodoinsulin were mixed in about 4 ml saline which was injected intravenously followed by 3 rinses of 2 ml saline each. The total volume injected amounted to about 5 ml per kilo body weight. Volume of distribution of iodoinsulin was calculated by dividing the radioactivity per ml plasma into the total activity given. The plasma radioactivity includes both the TCA-soluble and precipitable fractions. We also calculated the volume of distribution after correcting for the estimated TCA-soluble activity. Since the degraded iodine appears to be largely associated with quite small molecules (5) it might rapidly distribute in the extracellular compartment. We estimated the total activity of the "degraded" iodine by multiplying the TCA-soluble activity per ml plasma by the estimated volume of the extracellular compartment (25% of body weight). Such a correction did not change the results significantly.

TABLE I. Effect of Superimposed Doses of Native Insulin on Degradation of Iodoinsulin at Various Times after Injection in Eviscerated-Nephrectomized Rabbits. Figures represent % of total plasma activity that is TCA soluble.

	Time after inj. in min.			
Units insulin	15	30	60	120
Tracer*	11	19	34	56
1.4		10	18	28
1.42		15		40
1.65	6	13	24	39
1.7	9	14	21	37
10	10	13	21	27
100	6	9	15	26
200	3	5	11	26
500	2	4	8	19
1000	2	3	12	27

* Tracer alone; avg of 9 animals.

Results. Table I shows the effects of superimposing a dose of native insulin on the rate of decay of iodoinsulin in the eviscerated-nephrectomized rabbit. As to be expected, very large doses definitely delayed the breakdown of the labeled compound. Much smaller doses (10 down to 1.4 units per kg) also had an appreciable effect. These intermediate doses are in the range of those ordinarily used to follow biological activity.

While carrying out these experiments we made the incidental finding that superimposed native insulin delays the apparent distribution of iodoinsulin in the body. In the eviscerated-nephrectomized animal tracer doses of iodoinsulin appear to distribute in a volume somewhat more than that of the extracellular compartment(2). The results shown in Table II might suggest that the native insulin slowed up the passage of iodoinsulin from the intravascular, to the interstitial, compartment and reduced the final volume of distribution. However another explanation is more likely. Some special tissue may take up iodoinsulin and hold it by some process that could become saturated if the concentration of the hormone were high. This tissue could not distinguish between native and labeled insulin so then carrier insulin would retard the passage of the labeled compound from the blood stream. This sequestration cannot be very large since

TABLE II. Effect of Superimposed Doses of Native Insulin on Apparent Volume of Distribution of Iodoinsulin at Various Times after Injection in Eviscerated-Nephrectomized Rabbits. Figures represent calculated volume of distribution of iodoinsulin expressed as ml/100 g body wt.

	Time after inj. in min.			
Units insulin	15	30	60	120
Tracer*	14	21	29	32
10	14	17	21	24
100	9	12	17	20
200	11	16	21	22
500	6	11	16	20
1000	7	10	16	20

* Tracer alone; avg of 8 animals.

the apparent volumes of distribution of labeled insulin with and without carrier are 20 and 30% of body volume respectively at the end of 2 hours. This concept is supported by the fact that the volume with carrier is close to that of the extracellular space. A compound with the relatively low molecular weight of iodoinsulin must at least distribute in the extracellular compartment.

Discussion. Our results explain in part why insulin action as measured by its effect on sugar metabolism seems to indicate a decay rate of the hormone in the body much less than that of iodoinsulin as measured by conventional methods. To measure biological activity, one must use much larger doses of insulin than workers ordinarily use in studying the fate of iodoinsulin in the body. On the basis of our findings we believe that moderate doses of native insulin must depress the breakdown of the labeled compound in the intact animal although this would be difficult to demonstrate because of the rapid degradation and the high variability between animals. However this factor cannot entirely account for the discrepancy. Insulin binding by specific plasma globulin produced as a result of previous insulin injections(5) could have prolonged the biological activity of the hormone in the animals studied by Greeley. These dogs were pancreatectomized in order to eliminate the variability of endogenous insulin and had to be maintained on insulin. To what extent insulin binding could have affected the results would have to be determined by comparing the biological effects of doses of insulin given soon after pancreatectomy and some weeks later in the same animal.

The effect of large doses of native insulin in reducing the apparent volume of distribution of iodoinsulin is most likely the result of a mechanism similar to that acting on the degradation process. Our results suggest that when iodoinsulin is given in tracer doses there is sequestration of a portion of it by some process that can be saturated by a large dose of native insulin. Experiments designed to study the mixing of iodoinsulin in the body and its ultimate volume of distribution should be carried out with the addition of a large amount of native insulin. The values for these which we give in this paper should be more correct than those given in our earlier publication(2).

It would follow from our findings that when iodoinsulin is used as an indicator of the behavior of natural insulin in the body, the latter should be added in physiological amounts, or in larger dosages if the investigator is concerned with the disposal of such.

Summary. In eviscerated-nephrectomized rabbits the decay of iodoinsulin is retarded by addition of moderate and physiological doses of native insulin. The apparent volume of distribution of iodoinsulin is reduced by superimposed native insulin. This suggests a sequestering of part of the tracer compound by a process that can be saturated by added native insulin. The results indicate that in studying the physiology of insulin by use of iodoinsulin, investigators should add native insulin in appropriate dosage. Determinations carried out in this way indicate that iodoinsulin distributes in the eviscerated-nephrectomized rabbit in a volume equal to the extracellular compartment.

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