Degraded and Undegraded Insulin in the Bile of Rabbits After Intravenous Administration of Tracer Amounts of Labeled Insulin (32788)

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The influence of the liver on the metabolism of insulin has been reported by several authors. Elgee *et al.* (1) have demonstrated that there is a rapid accumulation of radioactivity in the liver and kidney after the intravenous administration of insulin-^{131}I. Mortimore *et al.* (2) have shown that when insulin-^{131}I is incorporated in a liver perfusion medium, about 40% disappears even when the perfusion has passed through only once. It is generally admitted that the degradation of the labeled insulin is followed by urinary excretion.

In recent years several research workers have studied different aspects of the metabolism of insulin-^{131}I (3, 4). The iodinated hormone seems to have a longer average half-life than the endogenous hormone (5). There is, however, evidence, confirmed more than once, that the intact labeled insulin and the native insulin are degraded by the same mechanism (6).

Labeled insulin is virtually completely precipitated by TCA. When radioactive insulin was given to an animal and the protein fraction was precipitated with 10% trichloroacetic acid (TCA) the protein-bound radioactivity was assumed to represent undergraded labeled insulin, whereas the TCA-soluble radioactivity was assumed to be comprised of the degradation products of the labeled hormone (7).

We have recently demonstrated the presence of insulin in bile (8) and the biliary excretion of injected unlabeled heterologous insulin (9). These results were recently confirmed by Daniel and Henderson (10).

The aim of this study is to investigate the possible role of the liver in the elimination of degraded and undergraded insulin through the bile, after the intravenous administration of tracer concentrations of labeled insulin.

Material and Methods. Normal rabbits weighing from 1.5 to 2 kg were studied. They were kept without food for 18 hours before the beginning of the experiment. In order to obtain the bile, the animals were anesthetized with ether. A catheter was placed in the bile duct through a medial abdominal laparotomy, and after ligation of the cystic duct the bladder was removed and the catheter was exteriorized and the bile was collected in a test tube in 10-min samples. The amount of bile that could be collected in the course of 6 hours following cannulation of the choledochus turned out to be 8–10 ml/hour.

The labeled insulin was obtained from the Radiochemical Centre, Amersham, Buckinghamshire, England.

Four rabbits were injected with 0.1 μg (2500 μU) of labeled insulin, injected into the vena porta of two of the rabbits and into the abdominal vena cava in the other two. Four other rabbits were given the iodinated hormone in both ways, but at concentrations of 0.04 μg (1000 μU). Other animals were used to study the possible recovery of undergraded insulin in the bile, and for studying the TCA-soluble fraction.

The following experimental procedure was carried out in order to study the presence of immunologically active insulin (undergraded) in the bile: Aliquot parts of bile collected at the different times were submitted to incubation for 18–24 hours in the presence of anti-insulin serum (binding reagent). This binding reagent was obtained from Wellcome Laboratories. At the end of the incubation period, the material was filtered through an Oxoid membrane (11), and the radioactivity of the antigen–antibody complex was measured with a methane-flow counter.

The degradation of insulin-^{131}I in bile was measured by estimating the radioactivity in the 10% TCA soluble fraction, as measured with a scintillation counter. Other measurements of radioactivity in the blood and in suitable homogenates from various organs, such as the liver, thyroid, spleen, and kidney were made at the end of the experiment.

Results. Bile flow radioactivity after iv injection of insulin-^{131}I at two different concentrations in normal rabbits. The results

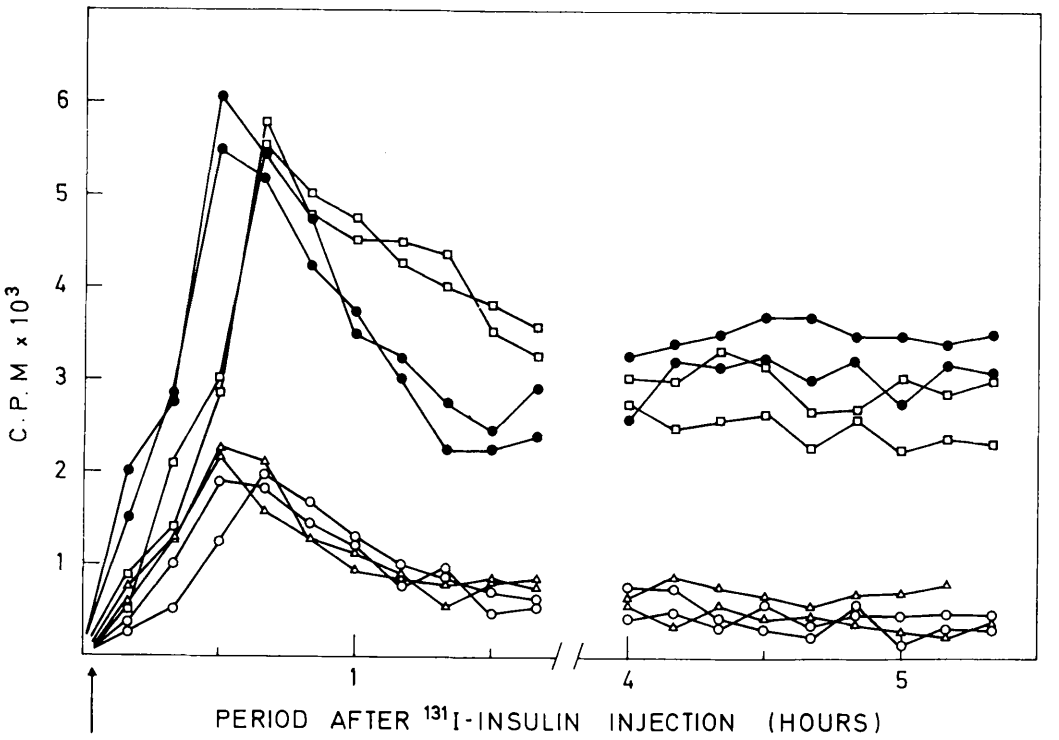

FIG. 1. Recovery of radioactivity in the bile of rabbits after the injection of different amounts of labeled insulin. Insulin was injected rapidly into either the vena porta (vp) of the abdominal vena cava (avc) at the time indicated by the arrow. Two animals were used in each experiment: bile radioactivity after insulin-^{131}I administration into the vp (●) = 0.1 μg; (Δ) = 0.04 μg and into the avc (□) = 0.1 μg; (○) = 0.04 μg. First samples were taken 10 min after insulin administration.

of measurements of radioactivity in the bile flow after iv administration of labeled bovine insulin in normal rabbits are summarized in Fig. 1. It can be seen clearly that after injecting 0.1 μg of insulin-^{131}I into the portal vein as well as into the abdominal vena cava, significant radioactivity could be recovered in the bile as long as 5.5 hours after the injection of the labeled insulin.

Other experiments shown in Fig. 1 indicate that even after the injection of as little as 0.04 μg of labeled insulin, significant amounts of radioactivity were recovered in the bile.

Antibody-bound insulin-^{131}I of the bile at different times after iv administration of labeled insulin. Figure 2 shows that during the first hour of the experiment, significant amounts of biliary insulin could be found in the antibody-bound fraction, even when only insulin-^{131}I were injected.

Percentage TCA-precipitable and non-TCA-precipitable insulin-^{131}I remaining in the bile at various times after iv administration of 0.1 μg of labeled insulin. Table I illustrates the percentage distribution of degraded and undegraded insulin present in the bile after the injection of 0.1 μg of labeled insulin into both the portal and the posterior vena cava. It can be shown that during the first hours the undegraded (TCA-precipitable) insulin represented up to 43% of the total radioactivity, whereas after the first hour precipitation did not exceed 20% and remained rather constant up to 5 hours.

Total radioactivity recovered in the bile during the experiment. A study was made of the distribution of the radioactivity left in certain organs and fluids at the end of the experiment in order to have some idea of the total radioactivity eliminated in bile over the

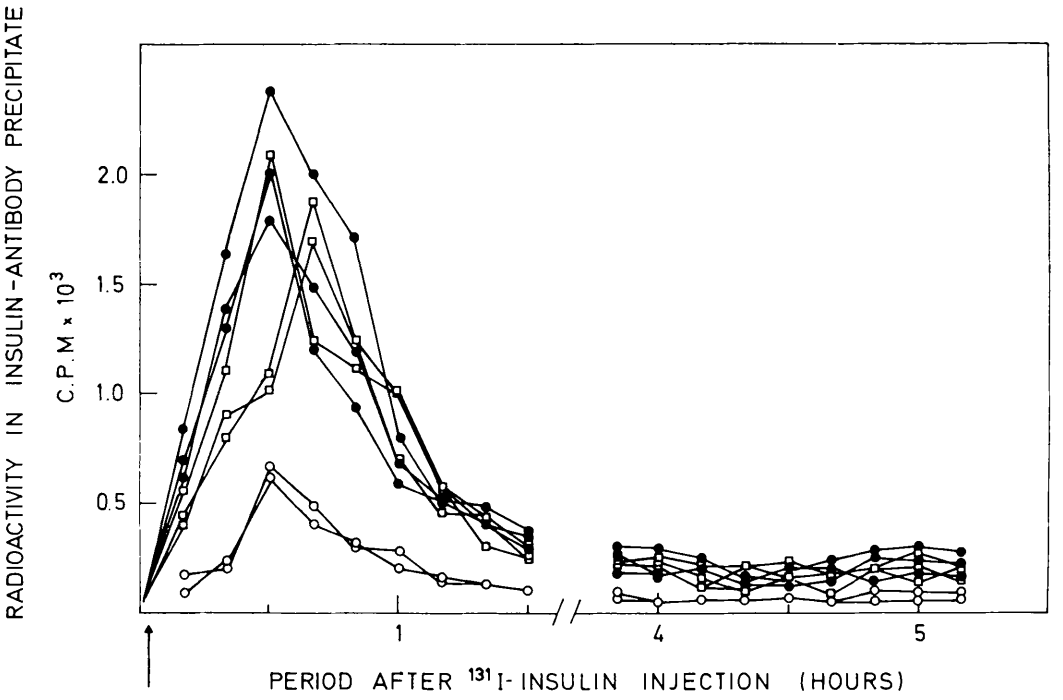

FIG. 2. Antibody-bound insulin-^{131}I in the bile at different times after iv administration of labeled insulin. Insulin recovered in bile at the indicated times after the injection of 0.1 μg of insulin-^{131}I into the vena porta (●) and the abdominal vena cava (□), and after the injection of 0.04 μg of labeled insulin into the vena porta (○). The labeled insulin was injected rapidly at the time indicated by the arrow. Experimental details are given in the text.

5.5 hours of the experiment. Table II shows that the percentage of radioactivity recovered in the bile during this period is larger than that remaining in the liver when the animal was sacrified. The highest percentage of radioactivity was recovered in the urinary system, and yet the TCA-precipitable fraction in the urine never exceeded 7–8% in further experiments carried out by us and not reported here. The spleen contained no significant part radioactivity. The small percentage of radioactivity recovered in the thyroid is the expression of a minimal degree of deiodization, especially as no protective agent (such as potassium iodide) was used in these experiments.

Discussion. Little information is available regarding the distribution of insulin in body fluids other than the blood. However reports of the renal clearance of insulin have appeared while this manuscript was in preparation (12, 13). It is generally accepted that insulin is destroyed in the liver (2, 14). The presence of degraded and undegraded insulin in the bile must be a consequence of insulin metabolism in liver cells. Although the ability of the liver to inactivate insulin does not appear to be firmly established, the degradation of insulin by the liver might very well indicate a definite influence on the amount of undegraded insulin that can be excreted into the bile.

During the first hour after the intravenous injection of insulin-^{131}I, perhaps due to the rapid accumulation of radioactivity in the liver, a significant amount of radioactivity appeared in the bile. About 43% of this radioactivity represents undegraded insulin. The recovered radioactivity later dropped to a lower, but constant level, for at least 5 hours, during which time the degradation of the hormone was intensified in such a way that at the end of the experiment no more than 15–18% of the recovered radioactivity represented immunologically active insulin. Beck *et al.* (15) have recently shown that intact insulin molecules can penetrate the liver cells

TABLE I. Percentage TCA-precipitable and Non-precipitable ^{131}I Compounds Remaining in the Bile Flow.

Time after injection[a] (min)	Insulin-^{131}I (0.1 μg) injected in: Vena porta TCA-pptable	Vena porta TCA-non-pptable	Abdominal vena cava TCA-pptable	Abdominal vena cava TCA-non-pptable
10	—	—	—	—
20	46	49	41	54
30	51	45	47	42
40	39	55	42	53
50	46	—	40	55
60	36	60	30	68
70	—	—	25	69
80	32	63	27	68
90	39	56	—	—
120	30	63	—	—
140	—	—	27	70
150	25	75	20	73
170	16	80	19	80
190	20	78	19	75
210	20	75	20	73
230	25	72	—	—
240	—	—	21	75
250	20	75	20	73
270	18	75	15	75
290	20	73	20	76
300	15	78	15	80
310	20	75	—	—
330	15	78	15	80

[a] Bile was taken at the indicated times after injection of labeled insulin.

of mammals. The degradation of insulin in the liver apparently is not total, and this suggests that the liver not only shows a capacity for "taking up" radioactivity from the blood, but can also retain undegraded insulin. The liver's ability to attract this circulating insulin coincides in time with the disappearance of insulin from the plasma as shown by Croughs *et al.* (16).

When the labeled insulin reaches the liver through the portal vein, the radioactivity appeared in the bile slighly earlier than when insulin was injected into the vena cava; otherwise, no significant differences were observed between the two routes of insulin administration. The excretion of significant amount of unlabeled insulin into the bile, requires the administration of 1.4 μg of the hormone (9). However working under the same experimental conditions with labeled insulin, significant amounts of the hormone can be detected in bile after injecting only 0.04 μg of insulin-^{131}I. These differences in sensitivity are due to a higher accuracy of the method and also probably to the fact that the average life of the iodized hormone is more securely guaranteed against degradation (16).

These results confirm that the liver determines the output of undegraded insulin into the intestine through its biliary function, even when at physiologic concentrations of the hormone, and they provide evidence that at least part of the insulin-^{131}I degraded in the liver is subsequently excreted in the bile.

Summary. During the first hour after the intravenous administration of tracer concentrations of labeled insulin into the portal vein as well as into the abdominal vena cava, significant amounts of radioactivity appear in the bile of rabbits. About 43% of this radioactivity represents undegraded insulin. The total recovered radioactivity drops after the

TABLE II. Distribution of Radioactivity 5.5 Hours after Injection of Labeled Insulin.[a]

	Insulin-^{131}I (0.1 μg) injected in: Vena porta	Abdominal vena cava
Bile flow	6.95	6.47
Thyroid	1.43	2.02
Total blood	8.08[b]	8.17[b]
Bladder	1.09	0.93
Urine	32.50	31.88
Kidney	5.53	4.52
Liver	5.50	4.77
Spleen	0.02	0.02
Recovery	61.10	58.78

[a] Data are expressed as percentages of total radioactivity injected. A 0.1 μg dose of labeled insulin was injected either into the vena porta or into the abdominal vena cava of rabbits. Bile was collected for 5.5 hours as indicated in the text and total radioactivity was estimated. At the end of the experimental period measurements of radioactivity in the blood and suitable homogenates of the required organs were carried out.

[b] Most of these values (89–90%) were found in the plasma obtained after separation by centrifugation of the blood cells.

first hour, remaining constant thereafter for at least 5 hours. During this time the degradation of the hormone is intensified and no more than 15–18% of the recovered radioactivity represents immunologically active insulin.

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Studies on the Site of Synthesis of Transcobalamin-II* (32789)

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Mammalian serum binds exogenous radioactive vitamin B_{12} (B_{12}) and in most species, two or more protein binders have been separated by cellulose chromatography (1, 2). Two binders in human serum, characterized by Hall and Finkler have been designated transcobalamin-I (TC-I) and transcobalamin-II (TC-II). Mouse serum unlike the serum of other mammalian species appears to have only one B_{12} binder, characterized by Coffey *et al.* (3) which elutes from cellulose ion exchange columns at the same pH and which has the same molecular size, i.e., equivalent to proteins with molecular weights of 35,000 (4) as the TC-II of human serum. Tan *et al.* have demonstrated that TC-II of mouse, rat, dog, and human, all have a molecular size similar to proteins that have molecular weights of 35,000 (4). This size has been confirmed recently for human TC-II by Grasbeck (5). A rapid assay for TC-II has recently been achieved by Hansen *et al.* (6,7), employing zirconyl phosphate gel (Z-gel). Zirconyl phosphate gel of pH 6–7 specifically binds TC-II, but does not bind TC-I which if present, is present only in small amounts in normal human serum, but elevated in serum of chronic myelogenous leukemia patients (6). TC-I has a molecular size similar to proteins with molecular weights of 117,000 (5). The charcoal method devised by Miller (8) differentiates between protein bound and free B_{12}, and thus, the difference between the two assays represents TC-I (6). Zirconyl phosphate gel of pH 5 binds intrinsic factor of human, rat, mouse, and hog, R of normal human gastric juice, but does not combine TC-I (3,6,9,10). These B_{12} binders are not adsorbed

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