Metabolism of Insulin-I[131]

Studies in Isolated, Perfused Rat Liver and Hind-limb Preparations

Glenn E. Mortimore, M.D., Frank Tietze, Ph.D., and DeWitt Stetten, Jr., M.D., Ph.D.
Bethesda

The incubation of insulin with slices, homogenates or extracts of mammalian tissues has been shown to result in its biological inactivation and proteolytic degradation.[1-5] Inasmuch as crude extracts of liver have shown some specificity for insulin,[4,6] the view that a specific insulin degrading system, termed by Mirsky "insulinase," may exist has been proposed.[4] Although detectable levels of insulin protease are widely distributed, the greatest concentrations are found in homogenates of liver and to a somewhat lesser extent in kidney.[1] Muscle, a prime target of insulin, appears to contain very little of such activity in its extracts.[1] The rapid destruction of insulin in the intact animal could be attributed to hepatic and perhaps renal activity. However, such an assumption presupposes that circulating insulin has access to intracellular proteases of liver and kidney, and that "insulinase" activity is not an artifact of sliced and homogenized preparations. Since all endogenously produced insulin must pass through liver prior to reaching skeletal muscle and other target organs, the consequences of this passage are of considerable interest.

The purpose of this communication is to report the results of experiments designed to assess the relative magnitude of iodoinsulin degradation by perfused isolated, intact rat liver and hind-limb. Since at least the appearance of cellular integrity was well sustained, it is hoped that the present results more closely approximate reactions in normal tissues than do those obtained from slice and homogenate experiments.

EXPERIMENTAL

Perfusion technic. Fed, male Sprague-Dawley rats, weighing between 170 and 190 gm., were used as liver and hind-limb donors in the perfusion experiments to be described. The livers, which averaged 7.9 gm. in weight, were prepared for perfusion as follows: After etherization the rat's abdomen was widely opened and a small cannula, carrying oxygenated blood, was inserted and tied into the portal vein, care being taken not to disturb the bile duct. The chest was then opened and a cannula of larger diameter was placed in the inferior vena cava through an incision in the right atrium. This served to carry the effluent "venous" blood back into the oxygenating flask. To prevent loss of perfused blood by retrograde leakage, the inferior vena cava was ligated between the liver and right kidney. After an interruption of hepatic blood flow for a matter of one or two minutes, it was thus possible to establish a closed, isolated circulation.

Hind-limb preparations were perfused through a cannula tied into the abdominal aorta slightly above its bifurcation. Since it was not possible to collect the perfusate quantitatively from the inferior vena cava because of anastomotic connections with the vertebral veins, the operated animal was cleanly bisected just above the aortic cannulation, the hind-limb quarters were inverted, and the effluent blood was allowed to drip into a small funnel and thence to the oxygenator. All viscera except the urinary bladder, testes, prostate and seminal vesicles were removed. The hind-limbs ranged between 60.5 and 62.8 gm. in weight.

The perfusing blood volume of 50 ml. was collected immediately before the above procedure by exsanguinating five or six mature Sprague-Dawley male rats from the abdominal aorta, drawing the blood into heparinized syringes.

The effluent or "venous" blood entered the upper end of the perfusion oxygenator, a sloping glass cylinder 35 x 9 cm., which was rocked about its long axis through a 90° arc at eighty cycles per minute. The blood which collected at the lower end of the cylinder was filtered through nylon gauze and peristaltically pumped through Tygon tubing to the "arterial" cannula. The gas mixture, 95 per cent O_2—5 per cent CO_2, saturated with H_2O, flowed rapidly through the cylinder. In most experiments the blood flow was 7.0 ml./min.,

Presented at the Forty-ninth Annual Meeting of the American Society of Biological Chemists in Philadelphia on April 18, 1958.

From the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, United States Department of Health, Education and Welfare, Bethesda, Maryland.

creating a pressure gradient of 17—32 mm. Hg across the liver and 63—75 mm. across the hind-limbs. The entire perfusion procedure was conducted in a constant-temperature room at 37° C.

Treatment of blood samples. Individual 6 ml. samples were withdrawn from the perfusion vessel at 7.5, 15, 30 and 60 minutes after iodoinsulin was added. These samples, together with a zero-time control, were treated as follows: Aliquots of whole blood and plasma were directly assayed for radioactivity. One milliliter of plasma was diluted with 3.0 ml. of water and then 4.0 ml. of 10 per cent trichloroacetic acid (TCA) were added to precipitate the proteins. Five milliliters of the resultant clear supernatant fluid were removed for counting. All counting was performed in a well-type scintillation counter. From these data and the packed-cell volume it was possible to compute, for each milliliter of blood, the radioactivity contained in whole blood, in the cell fraction and in the TCA-soluble and precipitable fractions of plasma. For comparison all values were normalized, setting the whole blood radioactivity at zero time equal to 100 c.p.m./ml. blood.

Iodoinsulin was obtained from the Abbott Laboratories, Oak Ridge, and after dialysis contained not less than 97 per cent trichloroacetic acid (TCA)-precipitable radioactivity. The estimated degree of iodination was 1.0 atom of iodine per molecule of insulin (6,000 m.w.). Iodoinsulin preparations similar to those used in this study have been reported to retain full hypoglycemic activity.[7] The iodoinsulin was diluted with a solution containing 0.9 per cent saline and 0.5 per cent bovine serum albumin, and was then dialyzed at 4° C. against 0.9 per cent saline for twelve hours. Further dilution with the saline-bovine albumin solution was made just before use to achieve a final iodoinsulin concentration of 1 μg. per ml.

Paper chromatography. Unidimensional descending chromatography was carried out on 8 x 22½-inch strips of Munktell No. 20/150 filter paper[8] with the organic phase of a 3:1:4 mixture of n-butanol:acetic acid:water.[9] This system was chosen on the basis of its ability to resolve the components of a mixture consisting of insulin, 3-iodotyrosine, 3,5-diiodotyrosine, and inorganic iodide. No evidence has been found for the lability of organically bound iodine in this system. In more recent unpublished experiments we have obtained superior resolution of the above mixture with the butanol-ethanol-ammonia system of Tong and Chaikoff.[10]

After thorough air drying of the chromatogram, appropriate strips 1⅜ inches in width were cut lengthwise and scanned for radioactivity in a recording gas-flow paper strip counter.[11]

RESULTS

In all the experiments reported the perfused livers retained their normal moist, red-brown appearance and there was no evidence of swelling. Histologic examination* of a perfused liver following a typical experiment revealed generally normal-appearing cells except for occasional small, well-demarcated areas suggestive of localized ischemia. Oxygen extraction, denoted by marked darkening of the effluent blood, continued throughout the sixty-minute perfusion period and in one experiment, where the duodenum was cannulated, the continuous secretion of bile was observed.

In five experiments 1 μg. of iodoinsulin was added to the perfusion reservoir, and the rate of degradation followed by the changing distribution of radioactivity between the plasma TCA-soluble and precipitable fractions (figure 1). The initial concentration of iodoinsulin was 0.02 μg./ml. of blood, an amount which, it was hoped, might roughly approximate physiological levels in rat portal vein blood. There was a rapid decline in TCA-precipitable radioactivity, reaching a mean level of 29.4 per cent by sixty minutes (table 1). By contrast, the rate of appearance of plasma TCA-soluble activity was initially very slow and by 7.5 minutes there was only a negligible rise. The initial rapid capture of plasma TCA-precipitable activity by the liver with the delayed release of plasma TCA-soluble radioactivity was

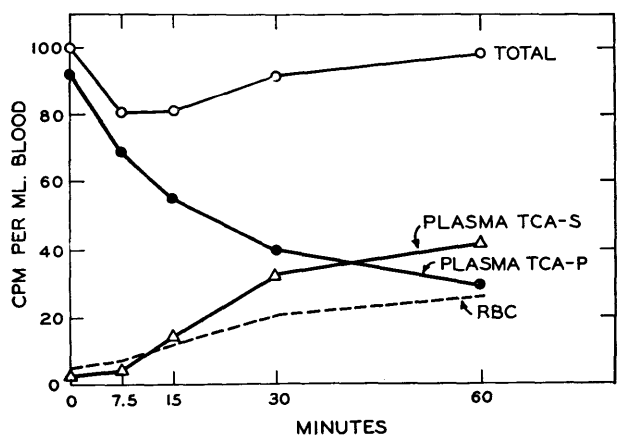

FIG. 1. Distribution of I^{131}-radioactivity among various blood fractions during sixty-minute rat liver perfusions. Each point represents the mean of five separate experiments in which 1.0 μg. of iodoinsulin was added at zero time. The plasma TCA-precipitable values are presented in table 1.

*This histological examination was kindly performed under the guidance of Dr. Samuel Spicer of the Laboratory of Pathology and Histochemistry, National Institute of Arthritis and Metabolic Diseases.

TABLE 1

Decrease of plasma TCA-precipitable radioactivity in c.p.m./ml. blood with time in five liver perfusions. One microgram of iodoinsulin was added at zero time

Experiment	16	17	19	20	77	Mean
Minutes						
0	89.0	93.8	89.0	93.8	94.4	92.2
7.5	69.3	76.9	65.9	70.0	61.6	68.7
15	58.8	56.3	53.4	53.5	50.7	54.5
30	39.6	38.4		41.6	38.0	39.4
60	26.9	27.2		32.5	30.8	29.4

undoubtedly responsible for the dip in the whole blood radioactivity concentration shown in figure 1. Later, as the rate of appearance of TCA-soluble material increased, total blood radioactivity approached its initial value. Although a possibility exists that this dip was related in part to a dilution by the fluid volume of the liver, we feel it is unlikely in view of our failure to detect a significant fall in total radioactivity in later experiments employing higher concentrations of insulin. Radioactivity associated with the cellular fraction of blood (labeled RBC in figure 1) in general followed the plasma TCA-soluble curve and in the latter half of the perfusion amounted to about 60 per cent of the plasma TCA-soluble activity, a distribution in substantial agreement with that of Berson.[12] It would appear that the RBC radioactivity is derived from the TCA-soluble material of plasma. With one exception all of these experiments were conducted with a uniform rate of blood flow of 7.0 ml./min., a rate which approximates reported estimates of splanchnic[13] and hepatic blood flow[14] in rats. When the flow rate was decreased to 3.8 ml./min. in one experiment no change occurred in rate of distribution of radioactivity among the various fractions and it was concluded the blood flow generally employed was great enough to provide adequate hepatic flow and prompt mixing of blood among the various components of the perfusion system.

The possibility that insulin degradation might occur in the plasma per se by virtue of an elution of intracellular proteolytic enzymes from the liver was dispelled by the following experiment. Plasma from a control sixty-minute rat liver perfusion was incubated with a trace amount of iodoinsulin for 1 hour at 37° and the degree of degradation compared with a similar incubation employing fresh rat plasma. No significant degradation occurred in either sample. Although the livers were isolated only in a "vascular" sense there was no extrahepatic loss of radioactivity via bile secretion, lymph drainage or diffusion since the radioactivity added could be quantitatively recovered at the end of the perfusion (table 2).

TABLE 2

Recovery of radioactivity in c.p.m. (not normalized) in four rat liver perfusions where the liver was assayed for radioactivity

Experiment	16	17	20	77
Remaining in blood	297,920	190,400	196,512	734,080
Removed by sampling	142,620	95,400	99,018	339,480
Liver	24,240	14,580	17,430	65,400
Total recovered	464,780	300,380	312,960	1,138,960
Total added	465,500	295,000	323,050	1,169,500
Per cent recovered	100	102	97	97.5

If it is assumed that: (a) Iodoinsulin is destroyed by the liver at a rate proportional to its instantaneous concentration in the blood presented to the liver, and (b) the recycling rate is sufficient to bring about essentially "instantaneous" mixing between the reservoir blood and the blood in the liver, the rate of decline in concentration of iodoinsulin in blood, —dC/dt, will be inversely related to the volume, V, of the cyclically perfusing blood.

$$-V\frac{dC}{dt} = k(C - C_\infty) \qquad (1)$$

Where V = total blood volume, in milliliters, C = concentration of iodoinsulin-I^{131} in c.p.m. per ml. of blood, C_∞ = asymptote approached by C after prolonged perfusion and k = reaction velocity constant, differing from the traditional first order constant in having dimensions of milliliters per minute. This constant k can be shown* to equal the product of the traditional first order constant and the volume of blood contained in the liver. Upon integration, equation (1) becomes:

$$k = \frac{2.3V}{\Delta t}\log\frac{C_a - C_\infty}{C_f - C_\infty} \qquad (2)$$

where C_a and C_f are initial and final concentrations over a time interval Δt.

An extrapolation from data in table 1 gives a mean value of 29.2 c.p.m. per ml. for C_∞. The physical meaning of this quantity is not entirely clear. It might signify that of the "iodoinsulin" initially added, some 29 per cent had been altered in preparation so as to be no longer susceptible of attack by liver. Alternatively, it might represent a stable fragment derived from iodoinsulin by hepatic proteolysis but of sufficient molecular

*The authors are indebted to Dr. J. Z. Hearon of the National Institute of Arthritis and Metabolic Diseases for his valuable assistance in the present mathematical treatment. A rigorous development of this and related kinetic problems will shortly be published by Dr. Hearon.

size to retain the property of TCA-precipitability.

The form of equation (2) permits adjustment to be made for the stepwise decrease in V which resulted from the sampling procedure. For each successive time interval the value for k has been computed (table 3) and a mean value of 3 ml./min. has been obtained. This quantity has a superficial similarity to "clearance" of the renal physiologists and indicates that of the 7 ml. of blood pumped through the liver each minute, iodoinsulin was extracted from 3 ml., or approximately 40 per cent.

The mean distribution of radioactivity in blood fractions from five rat hind-limb perfusions is shown in figure 2. The same experimental design used in the liver perfusions was employed in each instance. When compared with the liver perfusions (figure 1) one important difference was recognized, namely, the much slower rate of appearance of the TCA-soluble product in the hind-limb perfusate. This difference is magnified several-fold when the same comparison is made by weight of tissue. Although the rate of fall of TCA-insoluble activity appears to be comparable to that observed in the liver experiments, it is suspected that much of this decrease is related to dilution of the substrate by the large mass of perfused tissue. In a separate determination of the *inulin* space of a 60-gm. hind-limb preparation a 30 per cent reduction in the circulating concentration of *inulin* by dilution with tissue fluids was observed. Because neither the rate nor volume of dilution was measured directly in the above hind-limb experiments, it was not possible to compute the rate of disappearance of TCA-precipitable radioactivity as was done for the liver perfusions. A comparison of the time-concentration curves of the two groups of perfusions by inspection, however, leaves little doubt that the rate of uptake by hind-limb tissue is significantly smaller than by liver.

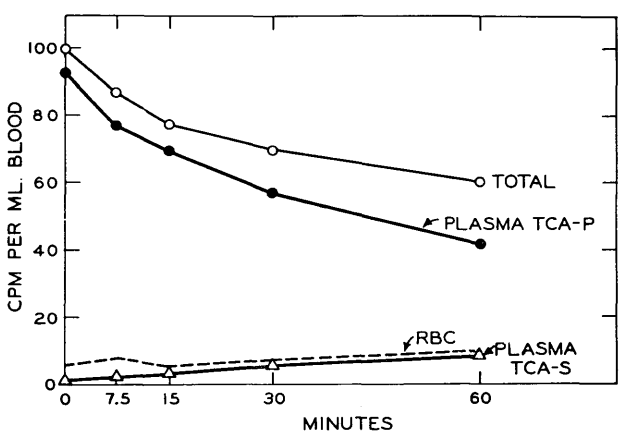

FIG. 2. Distribution of I^{131}-radioactivity among various blood fractions during sixty-minute rat hind-limb perfusions. Each point represents the mean of five separate experiments in which 1.0 μg. of iodoinsulin was added at zero time.

TABLE 3

Determination of k (ml./min.) for the hepatic removal of plasma TCA-precipitable radioactivity in five liver perfusions. Equation (2) in the text was applied to each time interval between samples. C∞ is the extrapolated asymptotic concentration of 29.2 c.p.m. per ml. blood

Time of sampling (min.)	C (mean plasma TCA-precipitable radioactivity in c.p.m./ml. blood. From table 1)	C — C∞ (c.p.m./ml. blood)	Δ t (min.)	V (ml.)	k (ml./min.)
0	92.2	63			
			7.5	50	3.11
7.5	68.7	39.5			
			7.5	44	2.63
15	54.5	25.3			
			15	38	2.30
30	39.4	10.2			
			30	32	4.16
60	29.4	0.2		mean	3.05

In view of the uncertainty regarding the mechanism of action of the sulfonylurea hypoglycemic agents and the claim by some that they will inhibit the degradation of insulin,[15] we thought it of interest to explore such an effect in isolated liver preparations. The sodium salt of tolbutamide,* dissolved in distilled water, was given intraperitoneally at a level of 225 mg. per kg. body weight both to the blood-donor and "liver"-donor rats three hours prior to the experiment. This amount of the drug was sufficient to cause a 40 per cent lowering of blood glucose in control animals. In figure 3 are shown the results of perfusing 1 μg. of iodoinsulin in a manner identical to the control liver perfusions. Since the time-concentration curves for the various fractions in figure 3 are essentially superimposable on those in figure 1, it may be concluded that tolbutamide did not (directly or indirectly) alter the fate of iodoinsulin.

Evidence that the degradation of iodoinsulin and native insulin may share a common reactive step is presented in figure 4. It may be seen that the perfusion of mixtures of 10, 100 and 1,000 μg. of crystalline insulin separately with 1 μg. of iodoinsulin was associated with decreasing rates in the cumulative hepatic removal of plasma TCA-precipitable radioactivity. This falling rate of removal of radioactivity in the presence of increasing amounts of

*A generous amount of sodium tolbutamide (Orinase) was kindly supplied by Dr. C. J. O'Donovan of The Upjohn Company, Kalamazoo, Michigan.

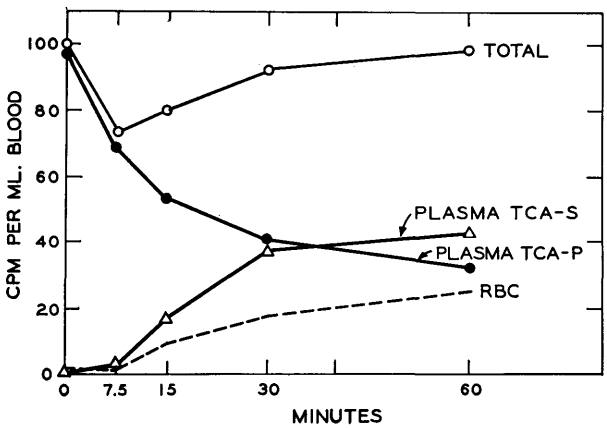

FIG. 3. Lack of effect of tolbutamide (Orinase) on the capture and degradation of iodoinsulin by perfused rat liver. Experimental conditions were otherwise identical to those whose results are depicted in figure 1. Each point represents three separate experiments.

native insulin is indicative of substrate competition for a rate-limiting step or steps. Such evidence, although inconclusive, is compatible with the thesis that the pathways of iodoinsulin and native insulin degradation are identical. Similar effects with intact mice,[16] eviscerated nephrectomized rabbits[71] and liver extracts,[4,18] have been previously reported.

An attempt was made to follow with paper chromatography the degradation of iodoinsulin during liver perfusion. Samples of plasma removed during the course of a perfusion in which 10 μg. of iodoinsulin had been introduced, were spotted on paper and chromatographed

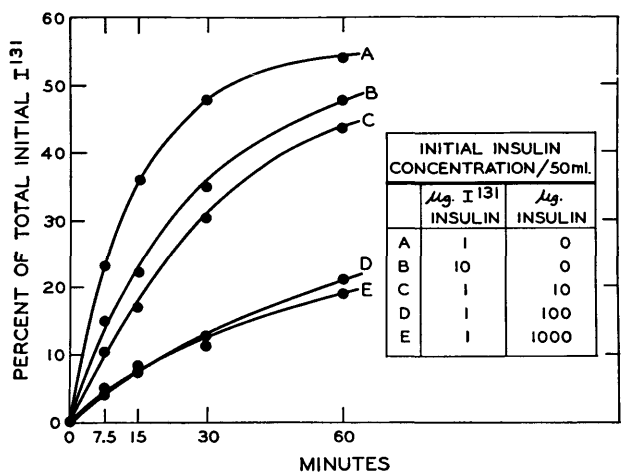

FIG. 4. Cumulative net removal of plasma TCA-precipitable I^{131} by perfused rat liver at various insulin concentrations. In calculating the values for each point a correction was made for the prior removal of radioactivity by sampling. The points of curves C, D and E represent means of two experiments each; B represents one experiment; A represents the mean of the five experiments shown in figure 1.

as described previously. A reciprocal change with time in the two major peaks on the radio chromatogram, bearing mobilities identical to insulin-I^{131} and iodide-I^{131} may be noted (figure 5). It will be seen in this figure that a relatively constant fraction of radioactivity of unknown nature remained fixed at the origin. This phenomenon, encountered in all similar chromatograms, is undoubtedly associated to some extent with the presence of a plasma protein of low mobility in this system. Since liver is known to contain a specific deiodinase for mono- and diiodotyrosine,[19] it seemed reasonable to assume that a substantial portion of this iodide-I^{131} was derived from iodotyrosine-I^{131} subsequent to the hydrolysis of iodoinsulin. Despite its unlikelihood, the possibility of deiodination occurring before the liberation of iodotyrosine—from peptides or iodoinsulin, itself—was investigated.

Ten micrograms of iodoinsulin were perfused in the presence of unlabeled mono- (MIT) and diiodotyrosine (DIT). Ten milligrams each of MIT and DIT were added just before the iodoinsulin, and the same amount adminis-

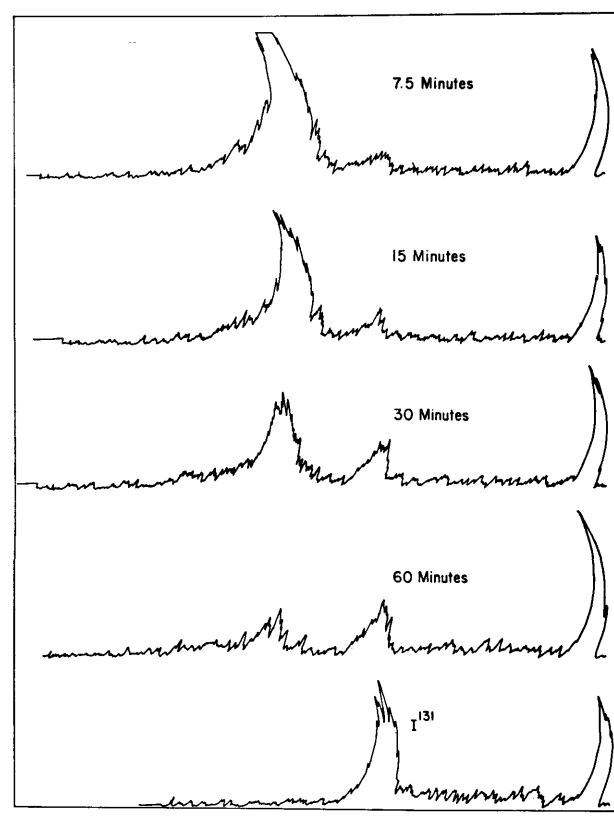

FIG. 5. Radiochromatograms of plasma sampled at the various times indicated during a rat liver perfusion in which 10 μg. of iodoinsulin was administered at zero time. The point of origin for each strip is the peak on the right. The peak which decreases with time on the left has the mobility of iodoinsulin.

tered by a motor-driven syringe continuously during the sixty-minute perfusion period. Plasma samples were then chromatographed in the same system previously described. As shown in figure 6 the iodoinsulin peak diminished at virtually the same rate as in figure 5, but the appearance of iodide-I^{131} was abolished. A small peak having the mobility of MIT was observed and is especially prominent in the thirty-minute sample (figure 7). These findings are compatible with the assumption that mono- and/or diiodotyrosine-I^{131}, generated by hepatic lysis of insulin-I^{131}, were being diluted by their nonradioactive analogs. Under these circumstances, the iodide generated by the action of hepatic deiodinase on iodotyrosine would be expected to be of low specific activity. These results argue against significant deiodination of intact iodoinsulin.

DISCUSSION

A postulated sequence of iodoinsulin degradation by the intact rat liver is presented in figure 8. The main purpose of this illustration is to underscore several questions concerning the interaction of insulin and intact cells. With regard to the perfusion experiments

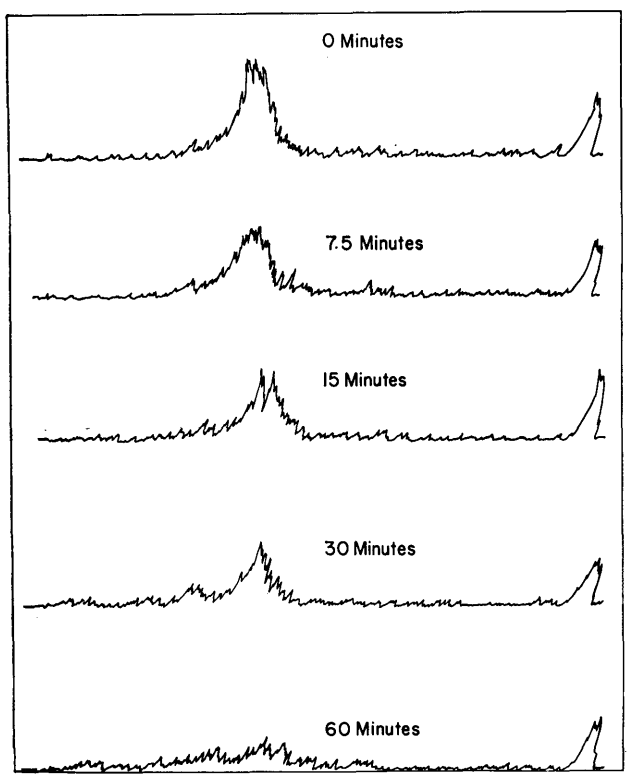

FIG. 6. Radiochromatograms of plasma sampled during a rat liver perfusion in which 10 μg. of iodoinsulin + 20 mg. each of mono- and diiodotyrosine were added. Points of origin are the peaks on extreme right; the large central peak has the mobility of iodoinsulin; no iodide-I^{131} is apparent.

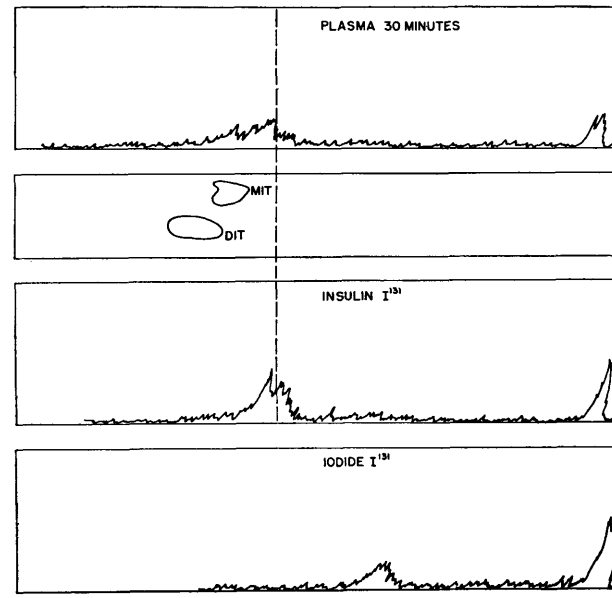

FIG. 7. Radiochromatogram of the thirty-minute plasma sample from the experiment in figure 6. Here the relative mobilities of MIT, DIT, iodoinsulin and iodide-I^{131} are shown for comparison. A peak to the left of iodoinsulin is apparently MIT. The origins are indicated by the peaks on the right.

one may ask whether or not insulin is captured and degraded by the intact cell in a manner different from the simple analogy of passive membrane diffusion and subsequent attack by soluble intracellular enzymes. Of interest in this connection is a report by Lee and Williams[20] in which the hepatic subcellular distribution of radioactivity was measured at different times following the injection of iodoinsulin into rats. Radioactivity was found to be rapidly incorporated within microsomal, mitochondrial and residual (largely soluble) fractions of the homogenized liver cell, giving a pattern of distribution which was significantly different from that obtained after the in vitro addition of either iodide-I^{131} or insulin-I^{131}. It was concluded that the liver cell may handle iodoinsulin in a way peculiar to its intact organization. The acceptance of such a view is tempting since it would prove useful in explaining the rapid uptake of insulin observed here as it has been in the interpretation of insulin effects on the isolated rat diaphragm[21] and on the enhancement of hexose penetration into eviscerated dog tissues.[22]

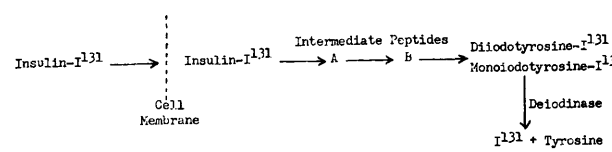

FIG. 8. Postulated pathway of insulin-I^{131} degradation by mammalian liver.

The nature of the intermediate steps before iodotyrosine release is equally obscure. Letters A and B (figure 8) indicate the possible occurrence of more than one reaction and thus more than one intermediate peptide product formed. Such reasoning is in keeping with the patterns of enzymatic protein hydrolysis observed in other situations. Although it seems certain from the experiments reported in this paper that the release of iodide requires the preliminary hydrolytic liberation of iodotyrosine, the full extent of insulin degradation is not known. Whether or not TCA-precipitable fragments are released from intact liver during insulin breakdown is a question currently under investigation.

The use of iodinated proteins as tracers in biological systems poses a problem of homogeneity, or lack thereof, within the substrate. It is known that the heavy iodination of insulin is associated with the nearly total loss of biological activity,[23,24] some of which may be restored by its partial deiodination.[23] In view of the reported greater ease in iodinating monoiodotyrosine than tyrosine,[25] it is conceivable that a substantial portion of the iodine may be added to a relatively few insulin molecules.* Under such conditions one might expect a significant fraction of the label to exist in biologically inactive insulin. This fraction, being small by weight, would not necessarily be detected in estimates of biological potency of the insulin sample as a whole. In addition to iodination per se the ionizing effects of gamma irradiation may give rise to further molecular alterations.[26,27]

As mentioned in the earlier kinetic discussion, a possible explanation for the apparently incomplete degradation of our iodoinsulin is the existence of an altered, nonreactive contaminant. Using a hydrodynamic flow paper electrophoresis technic Berson and associates[12] observed that from 2 to 25 per cent of the radioactivity from different preparations of lightly iodinated insulin migrated with serum proteins, leaving the larger fraction bound to paper at the origin. Although the paper-bound material disappeared from plasma logarithmically with time following the injection of iodoinsulin into rabbits, the fraction migrating with proteins remained relatively stable. These observations were confirmed by Scott and associates[28] who in addition were able to correlate the rate of disappearance of the paper-bound fraction with the disappearance of plasma insulin activity when iodoinsulin plus native insulin were administered to rabbits. Close quantitative agreement between the "apparent" inactive fraction of 29 per cent observed in our studies and those of Berson and Scott suggests that these fractions may be similar in constitution.

*The authors are presently investigating the chromatographic distribution of radioactivity in hydrolysates of iodoinsulin. The results to date, with four preparations similar to those reported herein, indicate that from 26 to 29 per cent of organically-bound radioactivity was incorporated in DIT; the remainder was in MIT.

SUMMARY AND CONCLUSIONS

1. The degradation of insulin-I^{131} by isolated, cyclically perfused rat livers was investigated by following with time the changing distribution of radioactivity among the plasma TCA-soluble, plasma TCA-precipitable and cellular fractions of the whole blood perfusates.

2. At relatively low concentrations of insulin-I^{131} the rate constant for the disappearance of plasma TCA-precipitable radioactivity was about 3 ml./min. Thus, at a hepatic flow rate of 7.0 ml./min., approximately 40 per cent of the insulin-I^{131} presented to the isolated, perfused liver was removed during any single passage.

3. The rate of degradation of insulin-I^{131} by perfused hind-limb preparations was far less than that of similarly perfused livers.

4. The administration of tolbutamide (Orinase) at a level of 225 mg. per kg. body weight to both blood- and liver-donor rats three hours prior to perfusion did not affect the rate of degradation of insulin-I^{131}.

5. A progressive decrease in the rate of degradation of insulin-I^{131} concomitant with increasing dilution by native insulin was observed and is indicative of substrate competition for one or more rate limiting reactive steps. Such evidence is compatible with the thesis that the degradative pathway for insulin-I^{131} may be shared in part or in its entirety by native insulin.

6. The addition of unlabeled mono- and diiodotyrosine to perfusing blood abolished the chromatographic appearance of iodide-I^{131} without affecting the hepatic uptake of insulin-I^{131}. Moreover, a small peak having the mobility of monoiodotyrosine-I^{131} was recognized. Such evidence strongly suggests that "deiodination" is not an initial event, but necessarily follows the hydrolytic cleavage of insulin-I^{131} to yield iodinated tyrosine.

SUMMARIO IN INTERLINGUA

Studios Del Metabolismo De Insulina A I^{131}

1. Le degradation de insulina a I^{131} per isolate, cyclicamente perfusionate hepates de ratto esseva investigate per sequer—como function del tempore—le alterationes in le distribution de radioactivitate in tres fractiones del perfusionato de sanguine integre, i.e. le fraction de plasma solubile in acido trichloroacetic, le fraction de plasma precipitabile per acido trichloroacetic, e le fraction cellular.

2. A relativemente basse concentrationes de insulina a I^{131}, le magnitude constante del disparition de radioactivitate in le fraction precipitabile per acido trichloro-

acetic esseva circa 3 ml per minuta. Assi, in le presentia de un fluxo hepatic de 7,0 ml per minuta, approximativemente 40 pro cento del insulina a I^{131} presentate al isolate e perfusionate hepate de conilio esseva eliminate durante un sol passage.

3. Le intensitate del degradation de insulin a I^{131} per perfusionate preparatos de gamba posterior esseva multo inferior a illo constatate in le caso de similemente perfusionate hepates.

4. Le administration de tolbutamido (Orinase) in un dosage de 225 mg per kilogramma de peso corporee al rattos contribuente le sanguine e etiam al rattos contribuente le hepates tres horas ante le perfusion non afficeva le intensitate del degradation de insulina a I^{131}.

5. Un progressive relentation del degradation de insulina a I^{131}, concomitante con un dilution progressive per insulina native, esseva notate e pare indicar le competition del substrato pro un o plure reactiones relentatori. Tal observationes es compatibile con le these que le degradation de insulina a I^{131} passa per un sequentia de passos que es partialmente o completemente identic con illo de insulina native.

6. Le addition de non-marcate monoiodotyrosina e diiodotyrosina al sanguine de perfusion aboliva le apparition chromatographic de iodo a I^{131} sin afficer le acceptation de insulina a I^{131} per le hepate. In plus, un micre picco con le mobilitate de monoiodotyrosina a I^{131} esseva recognoscite. Tal observationes es un forte suggestion que le "disiodation" non es un evento initial sed seque le fission hydrolytic de insulina a I^{131} que resulta in le formation de tyrosina iodate.

REFERENCES

[1] Mirsky, I. A., and Broh-Kahn, R. H.: The inactivation of insulin by tissue extracts. I. The distribution and properties of insulin inactivating extracts (insulinase). Arch. Biochem. *20*:1-9, 1949.

[2] Mirsky, I. A., Perisutti, G., and Dixon, F. J.: Destruction of I^{131} labeled insulin by liver slices. Proc. Soc. Exper. Biol. & Med. *86*:228-30, 1954.

[3] Tomizawa, H. H., Nutley, M. L., Narahara, H. T., and Williams, R. H.: The mode of inactivation of insulin by rat liver extracts. J. Biol. Chem. *211*:285-94, 1955.

[4] Mirsky, I. A., Perisutti, E., and Dixon, F. J.: The destruction of I^{131} labeled insulin by rat liver extracts. J. Biol. Chem. *214*:397-408, 1955.

[5] Vaughn, M.: The inactivation of insulin by an enzyme from rat liver. Biochim. et Biophys. Acta *15*:432-33, 1954.

[6] Tomizawa, H. H., and Williams, R. H.: Studies on the specificity of an insulin-inactivating system of the liver. J. Biol. Chem. *217*:685-94, 1955.

[7] Elgee, N. J., Williams, R. H., and Lee, N. D.: Distribution and degradation studies with insulin-I^{131}. J. Clin. Invest. *33*: 1252-60, 1954.

[8] Light, A., and Simpson, M. V.: Studies on the biosynthesis of insulin. I. The paper chromatographic isolation of ^{14}C-labeled insulin from calf pancreas slices. Biochim. et Biophys. Acta *20*:251-61, 1956.

[9] Bouman, J., and Homan, J. D. H.: A method for the determination of insulin by paper chromatography. Biochim. et Biophys. Acta *29*:417-22, 1958.

[10] Tong, W., and Chaikoff, I. L.: Hyrolysis of I^{131}-thyroprotein by pancreatic enzymes. J. Biol. Chem. *232*:939-50.

[11] Eisenberg, F., Jr., and Leder, I. G.: Improved scanner for radioactive paper strips. Anal. Chem. *31*:627-28, 1959.

[12] Berson, S. A., Yalow, R. S., Bauman, A., Rothschild, M. A., and Newerly, K.: Insulin-I^{131} metabolism in human subjects: Demonstration of insulin binding globulin in the circulation of insulin treated subjects. J. Clin. Invest. *35*:170-90, 1956.

[13] Spector, W. S.: Handbook of Biological Data. Philadelphia, W. O. Saunders Company, 1956, p. 283.

[14] Benacerraf, B., Biozzi, G., Halpern, B. N., Stiffel, C., and Mouton, D.: Phagocytosis of heat-denatured human serum albumin labeled with I^{131} and its use as a means of investigating liver blood flow. Brit. J. Exper. Pathol. *38*:35-48, 1957.

[15] Mirsky, I. A., Perisutti, G., and Diengott, D.: The inhibition of insulinase by hypoglycemic sulfonamides. Metabolism *5*:156-61, 1956.

[16] Mirsky, I. A., Perisutti, G., and Jinks, R.: Destruction of insulin by intact mice. Endocrinology *56*:484-88, 1955.

[17] Wick, A. N., and Drury, D.: Effects of superimposed native insulin on disposal of iodoinsulin in the body. Proc. Soc. Exper. Biol. & Med. *97*:514-16, 1958.

[18] Narahara, H. T., Tomizawa, H. H., Miller, R., and Williams, R. H.: Intracellular distribution of an insulin-inactivating system of liver. J. Biol. Chem. *217*:675-84, 1955.

[19] Roche, J., Michel, R., Michel, O., and Lissitzky, S.: Sur la déshalogénation enzymatique des iodotyrosines par le corps thyroide et sur rôle physiologique. Biochim. et biophys. Acta *9*:161-69, 1952.

[20] Lee, N. D., and Williams, R. H.: The intracellular localization of labeled insulin in mammalian liver. Endocrinology *54*:5-19, 1954.

[21] Stadie, W. C., Haugaard, N., and Vaughn, M.: The quantitative relation between insulin and its biological activity. J. Biol. Chem. *200*:745-51, 1953.

[22] Levine, R., Goldstein, M. S., Huddlestun, B., and Klein, S. P.: Action of insulin on the 'permeability' of cells to free hexoses, as studied by its effect on the distribution of galactose. Am. J. Physiol. *163*:70-76, 1950.

[23] Harrington, C. R., and Neuberger, A.: Electrometric titration of insulin. Preparation and properties of iodinated insulin. Biochem. J. *30*:809-20, 1936.

[24] Fraenkel-Conrat, J., and Fraenkel-Conrat, H.: The essential groups of insulin. Biochim. et Biophys. Acta *5*:89-97, 1950.

[25] Li, C. H.: Kinetics and mechanism of 2,6-di-iodotyrosine formation. J. Am. Chem. Soc. *64*:1147-52, 1942.

[26] Drake, M. P., Giffee, J. W., Johnson, D. A., and Koenig, V. L.: Chemical effects of ionizing radiation on proteins. I. Effect of γ-radiation on the amino acid content of insulin. J. Am. Chem. Soc. *79*:1395-401, 1957.

[27] Berson, S. A., and Yalow, R. S.: Radiochemical and radiobiological alterations of I^{131}-labeled proteins in solution. Ann. N.Y. Acad. Sci. *70*:56-68, 1957.

[28] Scott, G. W., Prout, T. E., Weaver, J. A., and Asper, S. P.: A comparison of the behavior of insulin and insulin labeled with I^{131} in serum. Diabetes *7*:38-45, 1958.