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Mobilization of Subcutaneously Injected Tritiated Insulin in Rats: Effects of Muscular Exercise*

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Summary. Previous studies in man and pancreatectomized dogs have indicated that alterations of the pharmacokinetics of subcutaneously injected insulin during physical activity may contribute to exercise-induced hypoglycaemia in insulin-treated diabetic patients. We have directly measured the appearance of subcutaneously injected insulin in the circulation and assessed its distribution to different tissues using a recently developed semisynthetic homogeneous [^{3}H]insulin as a tracer. Following subcutaneous injection in rats of [^{3}H]insulin in amounts insufficient to exert significant biological activity in intact animals, circulating levels of exogenous insulin were measured as plasma radioactivity co-migrating with insulin during gel filtration chromatography. Strenuous treadmill running accelerated the mobilization of subcutaneously injected [^{3}H] insulin and resulted in a significant elevation of circulating levels of exogenous insulin early during exercise, followed by decreased levels in the post-exercise period. In addition, exercise induced a redistribution of ^{3}H radioactivity in tissues, mainly increasing that found in skeletal muscle. This direct demonstration of altered pharmacokinetics of subcutaneously injected insulin during exercise provides, at least in part, a mechanism for the exercise-induced hypoglycemia seen following insulin injections in animals and during insulin treatment in man.

Key words: Exercise, tritiated insulin, subcutaneous insulin injections, exercise-induced hypoglycaemia.

Shortly after the introduction of insulin treatment it was observed that the hypoglycaemic effect of subcutaneously injected insulin was greatly increased by physical exercise [1, 2]. This results in frequent episodes of hypoglycaemia in insulin treated diabetics during or after physical activity. The precise mechanism of the exercise-induced hypoglycaemia in insulin-treated diabetics has remained poorly understood. Recent studies in pancreatectomized dogs [3] and in diabetic patients [4, 5, 6] suggest that increased absorption of subcutaneously injected insulin during exercise might contribute to the resulting hypoglycaemia.

In order to elucidate further the interaction between exercise and the efficacy of exogenous insulin, a systematic assessment of the pharmacokinetics of subcutaneously injected insulin appeared necessary. Previous attempts to perform such an investigation have been hampered by methodological problems, such as an inability to measure exogenous insulin in the presence of endogenous insulin production and the interference of insulin antibodies in insulin-treated juvenile diabetic patients with conventional insulin immunoassays. Furthermore, previously available preparations of radioactively labelled insulin were chemically heterogeneous [7] and their metabolic clearance rates were different from that of native insulin [8]. To overcome these difficulties we have used a recently developed semisynthetic tritiated insulin preparation which is both chemically

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homogeneous and indistinguishable from native insulin in its biological activity and its metabolism [9, 10].

This paper describes the use of this [^{3}H]insulin preparation in a study of the pharmacokinetics of exogenous insulin in rats at rest and during exercise.

Materials and Methods

Animals: All experiments were carried out using locally bred male Wistar rats of 200–250 g body weight. Except for the studies on biological effects of unlabelled regular insulin, in which 24 h fasted rats were used, animals were fed standard purina chow ad libitum and had free acess to water prior to the experiments, which were always started between 11.00 and 12.00 a. m. No anaesthesia was used.

Insulin Injections: NOVO-Actrapid® (monocomponent, pork) diluted prior to use in 0.9 g/100 ml NaCl containing 5 g/l bovine serum albumin (Pentex gradeV, Miles Lab. Ltd., Slough, U. K.) was used as unlabelled insulin. 4U/kg body weight was injected subcutaneously in a volume of 0.2 ml into the right gluteal region.

[^{3}H]insulin was prepared as described earlier [9, 10]: specific radioactivity was approximately 10 Ci/mmol. On the day of the experiment, the [^{3}H]insulin, which had been previously stored at $-20°$C, was repurified as described previously [10]. The pH of the repurified [^{3}H]insulin solution (in 0.2 mol/l glycine, 2.5 g/l bovine serum albumin) was adjusted to 7.2–7.4; 0.2 to 0.25 ml was injected either subcutaneously into the right gluteal region or into a tail vein. A total amount of 1 to 2.5 $\times$ 10^6 cpm/kg body weight (0.2 to 0.625 $\times$ 10^6 cpm per rat, mean 0.45 $\pm$ 0.03 $\times$ 10^6 cpm per rat, n = 73), was administered, equivalent to 0.017 to 0.04 U insulin/kg body weight. In some selected experiments up to 25 $\times$ 10^6 cpm/kg body weight, equivalent to 0.4 U/kg, was injected in order to study the distribution of ^{3}H radioactivity in skeletal muscle. Finally, in a few experiments, 4 $\times$ 10^7 cpm/kg was injected at the lower specific radioactivity of 3 Ci/mmol (equivalent to a dose of 2.4 U/kg), in order to study the effect of carrier insulin on the process under investigation. Within these limits, there was a linear correlation between the injected [^{3}H]insulin dose and resulting plasma concentrations of ^{3}H radioactivity (for example at 40 min after the adimistration of the tracer: $y = 315 + 0.0016x$; n = 11, r = 0.96, $p < 0.0001$; where y is the plasma ^{3}H concentration in cpm/ml and x is the injected dose of [^{3}H]insulin in cpm/200 g body weight).

Exercise: Immediately following the subcutaneous insulin injection, rats were subjected to exercise in a motor driven circular treadmill at a speed of 10 to 12 m/min for up to 30 minutes. Thereafter the animals were allowed to rest for up to 30 minutes. Only in the rats which were exercised in the fasting state after a subcutaneous injection of 4 U/kg body weight of regular unlabelled insulin were there obvious signs of fatigue during the last 10 minutes of the exercise period. Therefore, in these experiments the speed of the treadmill was slowed to 5–7 m/min. Control rats were left in their habitual cages following the insulin injection.

Sampling of Plasma, Tissue Extracts and Excretions: At indicated time points, rats were decapitated and neck blood was collected in chilled, heparinized tubes, centrifuged and plasma samples were processed for subsequent analyses. Immediately following decapitation tissue samples from several organs were excised, weighed, minced, and transferred into 2 g/100 ml sodium dodecyl sulfate (SDS) solution. The tissue pieces were subsequently homogenized, the SDS tissue homogenates heated for 2 minutes in boiling water and afterwards centrifuged at room temperature. The supernatant was kept at room temperature for subsequent analysis.

For radioactivity recovery studies, animals were kept separately in metabolic cages following the injection of [^{3}H]insulin. Faeces and urine were collected quantitatively over 24 hour intervals. Faeces were hydrolyzed in 4 mol/l HCl at 90°C for 6 to 8 hours; 20 mg/ml SDS were added to the urine before heating the solution in boiling water for 2 minutes. Seventeen days after the injection of [^{3}H] insulin the animals were sacrificed, plasma ^{3}H radioactivity was determined and the following organs were assayed for ^{3}H radioactivity as described above: heart, skeletal muscles, lung, liver, kidney, brain, spleen, perirenal adipose tissue and samples (subcutaneous tissue, skeletal muscle) of the injection site and its respective contralateral control. The radioactivity per organ was calculated on the basis of direct weight measurements of the entire organs (lung, liver, kidney, heart, spleen, brain) and based upon the assumptions that 45% of the total body mass consists of skeletal muscle [11] and 20% corresponds to extracellular space.

Analytical Methods: In aliquots of plasma samples, SDS-tissue homogenates and the respective excretion-solutions, ^{3}H radioactivity was determined in an automated liquid scintillation counter (Beckman model LS-230) using Instagel (Packard Instruments International SA., Zurich, Switzerland) as scintillant. Quenching was assessed for each plasma sample separately and for representative tissue homogenates by recounting the samples following addition of an internal ^{3}H standard. In addition, plasma samples (0.5 or 1.0 ml) were subjected to gel filtration on Sephadex G-50 Fine (Pharmacia, supplied by Instrumenten Gesellschaft, Geneva, Switzerland) columns (bed 0.8 $\times$ 60 cm) using 0.2 mol/l-Glycine/2.5 g/l bovine serum albumin, adjusted to pH 8.8 with 5 mol/l NaOH, as the elution buffer. In all fractions ^{3}H radioactivity was measured as described above. The yield of the columns was greater than 90%. Columns were calibrated with Dextran-blue (SIGMA), insulin and [^{3}H] phenylalanine (Radiochemical Center, Amersham, U. K.). Plasma ^{3}H radioactivity eluted from the column in several distinct peaks. The elution profiles were drawn on graph paper and the area of each peak was measured planimetrically (HAFF planimeter no. 317, Gebrüder Haff, GmbH, 8962 Pfronten, West Germany). Peak size was expressed as percent of the total ^{3}H radioactivity eluted from the column.

Glucose, lactate and 3-hydroxybutyrate were measured in plasma after deproteinization with perchloric acid 2 mol/l, using enzymatic methods. Insulin immunoassays [12] and affinitycolumn chromatography for identification of insulin immunoreactive material [10, 13] were performed using antiserum to pig insulin provided by Dr. Peter Wright (Indiana University School of Medicine, Indianapolis, Ill., U.S.A.). The limit of sensitivity of the insulin assay used is 0.125 ng/ml at the 95% confidence level and the interassay variance (at a concentration of 1 ng/ml) is 11.5%.

Presentations of Results, Calculations and Statistical Analyses: In order to allow for variation in rat weight and insulin dosage between different experiments, all data on plasma or tissue levels of ^{3}H radioactivity or [^{3}H]insulin were expressed as for an injection dose of 1 $\times$ 10^5 cpm [^{3}H]insulin/100 g body weight.

In the text, Tables and Figures, the results are given as means $\pm$ SEM and the statistical significance of differences were evaluated by Student's t-test for unpaired group comparisons.

Results

Metabolic Effects of Subcutaneously Injected Unlabelled Insulin and Exercise

In order to test whether rats might be used to study the exercise induced hypoglycemia following sub-

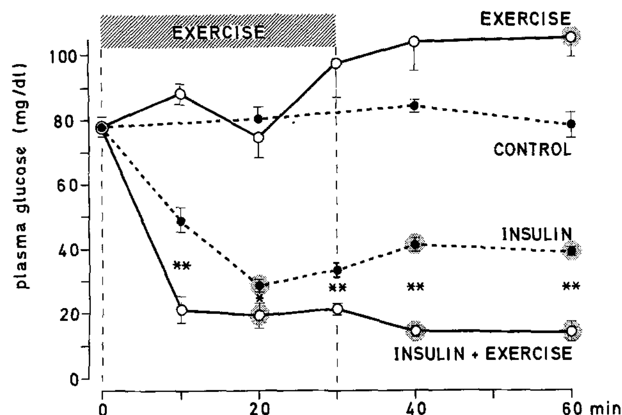


Fig. 1. Effects of subcutaneous injections of regular insulin 4 U/kg body weight and treadmill exercise on plasma glucose in 24 hours-fasted rats. Insulin was injected at 0 min. A total of 63 rats was used; each point represents the mean of 3–4 observations in individual rats. Points encircled by shaded areas are significantly different from controls ($p < 0.05$). Statistically significant differences between the insulin-injected group ("INSULIN") and the insulin-injected rats which were subjected to treadmill exercise ("INSULIN + EXERCISE") are indicated by * ($p < 0.05$) and ** ($p < 0.01$)

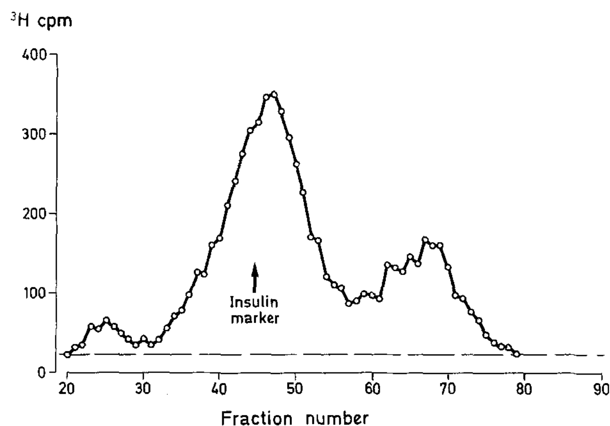
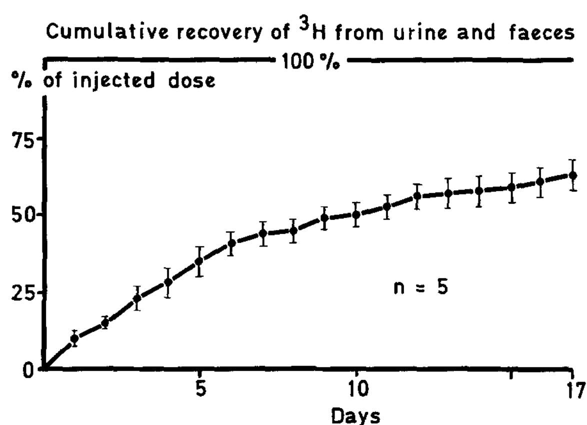


Fig. 3. Elution profile of G 50 Sephadex fine column chromatography of a representative plasma sample of a fed rat at 10 minutes after subcutaneous injection of [^{3}H]insulin. The fraction numbers are given on the abscissa; the total radioactivity per fraction is given on the ordinate. Each circle represents one fraction (the volume of which was 1 ml). The broken line indicates the background of the counting procedure. The first peak of radioactivity (between fraction numbers 21 and 29) eluted with the void volume of the column (as determined by dextran-blue). The column volume (as determined by [^{3}H] phenylalanine) corresponded to fraction numbers 65 to 76



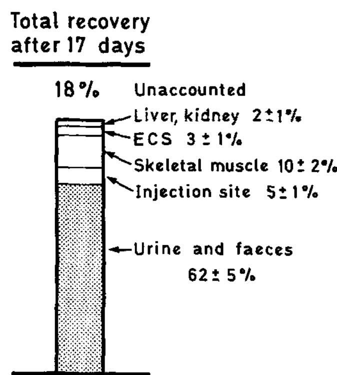


Fig. 2. Recovery of ^{3}H radioactivity after subcutaneous injection of [^{3}H]insulin in normal rats. ECS = extracellular space; the total radioactivity in plasma was assumed to be evenly distributed in the extracellular space

cutaneous administration of insulin, the effects of pharmacological doses of regular insulin and treadmill running on plasma glucose levels were studied in rats fasted for 24 hours. During exercise, plasma glucose levels were not different from controls; only at 60 minutes (post-exercise) was a slight, statistically significant, rise of glucose levels observed (Figure 1). Subcutaneous injections of 4 U insulin/kg body weight resulted in a prompt fall of plasma glucose, reaching a minimum value at 20 min. If the rats were subjected to treadmill exercise immediately following the insulin injection, the fall of blood glucose was both accelerated and more pronounced: plasma glucose concentrations were significantly lower than in the insulin injected group at rest at all measured time-points from 10 to 60 minutes. Furthermore, the insulin injection prevented the physiological rise of blood ketone body concentrations during the post-exercise period: plasma 3-hydroxybutyrate concentrations rose from 1.01 ± 0.07 mmol/l at 0 min to 1.63 ± 0.10 mmol/l 30 min after cessation of exercise in controls, as opposed to a fall to 0.40 ± 0.04 mmol/l 30 min after cessation of exercise in insulin injected rats.

Table 1. Plasma ^3H radioactivity and [^3H]insulin levels following injections of [^3H]insulin (10^5 cpm/100 g body weight) in normal rats

Time after injection min	0.5	1	5	10	20	30	40	60
Subcutaneous injection								
Total ^3H cpm/ml	–	132±129 (3)	332±77 (6)	589±59 (10)	764±60 (8)	705±60 (4)	722±41 (11)	871±119 (9)
[^3H]Insulin cpm/ml	–	–	286±66 (4)	386±43 (4)	471±43 (6)	430±33 (4)	336±32 (6)	311± 38 (8)
Intravenous injection								
[^3H]Insulin cpm/ml	10286±1584 (3)	4055±263 (3)	1482±214 (3)	529 (2)	494 (2)	–	266 (2)	98 (2)

Means ± SEM, number of observations in parentheses

Pharmacokinetics of Subcutaneously Injected [^3H]Insulin at Rest

Measurements of ^3H Radioactivity and [^3H]Insulin Levels in Plasma: To study the pharmacokinetics of subcutaneously injected insulin without affecting fuel and hormone homeostasis we have used tracer doses of [^3H]insulin, which had no effects on circulating levels of glucose or alanine (data not shown). Radioactivity in significant amounts was demonstrable in plasma even 5 minutes after subcutaneous injection of [^3H]insulin, with the levels rising to a plateau at 20 min (Table 1).

A quantitative recovery study of subcutaneously injected [^3H]insulin was performed in five rats (Figure 2). Over a period of 17 days the animals excreted 62 ± 5% of the initially injected radioactivity. Only in the first 24 hours following the [^3H]insulin injection was a significant amount of ^3H excreted in feaces (6 ± 1% of the injected dose). Thereafter, radioactivity was only excreted in urine, although the ^3H content of expired air was not measured. Even 17 days after the injection significant amounts of ^3H radioactivity could still be recovered in plasma and in several organs, most notably in the tissues of the injection site and in skeletal muscle (Figure 2). No radioactivity was detected in tissue extracts of brain, lung, spleen, heart, and adipose tissue. The ^3H radioactivity of the injection site (1923 ± 516) was 17 times higher than the respective value of the contralateral control specimen (114 ± 31 cpm/g wet weight, $p < 0.005$).

In order to investigate to what extent the total ^3H radioactivity in plasma at a given time point represented intact, tritiated insulin, plasma samples were analyzed using column chromatography. Figure 3 shows the result for a plasma sample taken 10 min after subcutaneous injection of [^3H]insulin. The radioactive material was found in three distinct peaks: the middle peak eluted with the insulin marker; on immuno-affinity column chromatography the material pooled from this peak was fully immunoreactive with an insulin antibody [13]. This material was thus assumed to be intact [^3H]insulin. In contrast, neither the labelled high molecular weight fraction nor the low molecular weight material reacted with insulin antibody. Treatment of the high molecular weight fraction with acetic acid or SDS did not result in the appearance of tritiated material, which, on repeated chromatography, would elute with the insulin marker.

The portion of total plasma radioactivity representing [^3H]insulin decreased continuously after injection from 80 ± 5% at 5 min to 38 ± 3% at 60 min (Figure 4). Two hours after the subcutaneous [^3H]insulin injection, 13% of the total plasma radioactivity still appeared to be intact [^3H]insulin (Figure 4), whereas by 3 hours [^3H]insulin was no longer detectable in the circulation (data not shown). One hour after subcutaneous injection of [^3H]insulin only about 1/3 of the total plasma radioactivity represented intact [^3H]insulin. Based on the assumption that the middle peak of the elution profiles (Figure 4) represents intact [^3H]insulin, we have calculated the plasma concentration of [^3H]insulin. Thus, the kinetics of appearance and disappearance of exogenous, injected insulin in the circulation were calculated following subcutaneous and, by contrast, intravenous injection of the labelled hormone (Table 1). The two modes of administration of an identical tracer dose resulted in characteristically different time courses of circulating [^3H]insulin levels: in accordance with the time course of the hypoglycaemic effect of subcutaneously injected unlabelled insulin in rats

(Figure 1) plasma [^{3}H]insulin levels reached a peak 20 min after its subcutaneous administration; the decay of circulating [^{3}H]insulin levels following intravenous administration of the tracer could be described by a double exponential decay curve with an initial rapid phase (half-life 2 min) followed by a slower phase (half-life 20 min).

Distribution of ^{3}H Radioactivity in Liver, Kidney and Skeletal Muscle: For further analysis of the fate of subcutaneously injected [^{3}H]insulin the distribution of ^{3}H radioactivity was measured in several organs: liver and kidney homogenates were analyzed, since 80% of the degradation of exogenous insulin appears to take place in these two organs [14]. ^{3}H levels per g wet weight liver paralleled roughly the respective plasma concentrations (Table 2). In accordance with studies using iodinated insulin preparations [15, 16] these findings indicate a free distribution of [^{3}H]insulin and/or its degradation products within the liver tissue without any significant hindrance by vascular or cell membrane barriers. By contrast, in the kidney ^{3}H radioactivity was concentrated 4.4-fold at 5 min and 8.6-fold at 30 minutes per g tissue wet weight over the respective plasma levels. Significant amounts of ^{3}H radioactivity were recovered from skeletal muscle homogenates only after 20 min. Thereafter, the ratio between the ^{3}H concentration per g muscle wet weight and the respective plasma radioactivity varied between 0.16 (at 20 min) and 0.24 (at 60 min) indicating an increasing distribution space of the tritiated material.

Effect of Exercise on the Pharmacokinetics of Subcutaneously Injected [^{3}H]Insulin

Measurements of [^{3}H]Insulin Levels in Plasma: The effect of treadmill running up to 30 minutes immediately following the subcutaneous injection of insulin was examined in fed rats. The strenuous character of the exercise procedure involved is demonstrated by a prompt rise of plasma lactate levels from 2.3 ± 0.1 mmol/l up to 13.6 ± 2.8 mmol/l. Except for a small, but statistically significant, rise of blood glucose at 5 minutes after the onset of exercise (from 7.32 ± 0.33 to 8.86 ± 0.61 mmol/l, $p < 0.05$), glycaemia remained unchanged; in particular, at no time was there any fall of blood glucose during the experimental procedure. Exercise induced marked changes in the pharmacokinetics of subcutaneously injected [^{3}H] insulin: early during teadmill running, at 5 and 10 min, plasma ^{3}H radioactivity was significantly elevated when compared with resting controls (Table 2). Thereafter, no significant differences were observed between the two experimental groups. In addition,

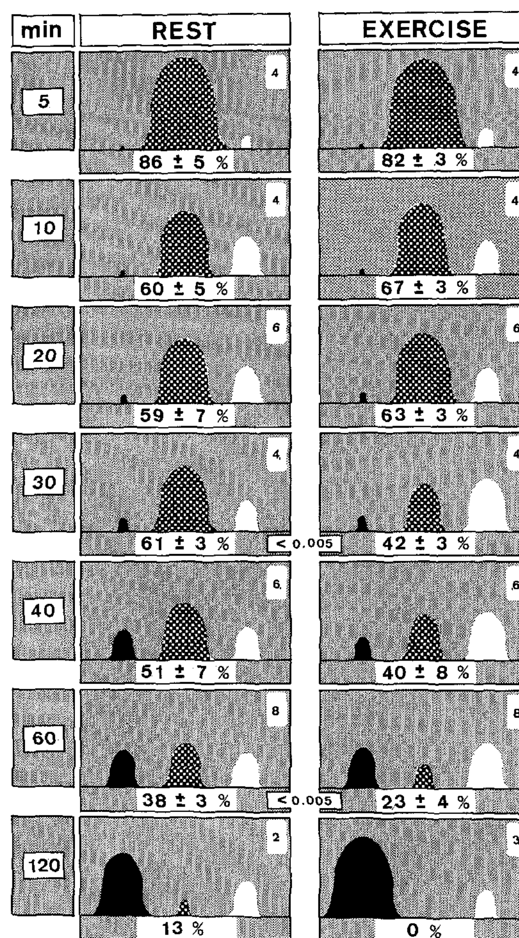


Fig. 4. Schematized distribution of total plasma ^{3}H radioactivity on column chromatography in three distinct peaks. The high molecular weight fraction (coloured black), [^{3}H]insulin (shaded) and small molecular weight degradation products of [^{3}H]insulin (coloured white) at indicated time intervals following the subcutaneous injection of [^{3}H]insulin in normal rats. The areas of the peaks indicate the respective percentages of the total plasma ^{3}H radioactivity. The size of the [^{3}H]insulin peak is given in % of the total ^{3}H radioactivity ± SEM at the bottom of each panel; the number of observations is indicated in the right upper part of each panel. The left part of the graph gives the results obtained from a total of 34 animals at rest. The right part depicts the effects of exercise (treadmill running from 0 to 30 min) and the subsequent post-exercise period. Significant differences between the relative [^{3}H]insulin portions of the total plasma radioactivity are indicated by p-values

exercise was associated with an acceleration of the decay of intact [^{3}H]insulin: towards the end of the treadmill running and during the post-exercise period, the relative portion of the total radioactivity eluting with the insulin marker was considerably lower in exercising animals; this difference was statistically significant at 30 and 60 min (Figure 4). Calculation of the plasma levels of [^{3}H]insulin in plasma revealed that exercise induced an accelerated appearance of [^{3}H]insulin in plasma, resulting in significantly elevated circulating levels early during the

Table 2. ^{3}H radioactivity in plasma and organ extracts following subcutaneous injection of [^{3}H]insulin (10^5 cpm/100 g body weight) in normal rats: effect of exercise

	Rest						Exercise (Treadmill running 0 to 30 min)					
Minutes	5	10	20	30	40	60	5	10	20	30	40	60
Plasma	332	644	764	705	659	818	816[c]	880[a]	880	882	803	771
cpm/ml	±77	±71	±60	±54	±62	±100	±95	±95	±104	±157	±89	±90
	(6)	(6)	(8)	(4)	(7)	(6)	(6)	(6)	(6)	(6)	(8)	(8)
Liver	207	493	677	624	642	771	375[a]	678	861	969	976	833
cpm/g	±43	±117	±141	±73	±133	±52	±59	±76	±181	±127	±152	±109
	(6)	(6)	(5)	(4)	(5)	(6)	(6)	(6)	(4)	(6)	(6)	(8)
Kidney	1478	4148	6725	6106	5594	6754	1867	4375	7146	7699	7286	7993
cpm/g	±369	±977	±1023	±1196	±1138	±904	±494	±600	±1755	±941	±472	±786
	(6)	(6)	(4)	(4)	(5)	(6)	(6)	(6)	(4)	(6)	(6)	(8)
Skeletal	16	56	119		150	198	149[c]	185[c]	286[a]	410	421[b]	289
muscle	±9	±41	±31	–	±47	±22	±27	±24	±68	±59	±77	±81
cpm/g	(4)	(4)	(7)		(5)	(5)	(4)	(4)	(6)	(4)	(6)	(5)

All values have been corrected for quenching by the addition of an internal standard.
Means ± SEM, number of observations in parenthesis.
Significantly different from resp. control values at:
[a] $p < 0.05$
[b] $p < 0.01$
[c] $p < 0.005$

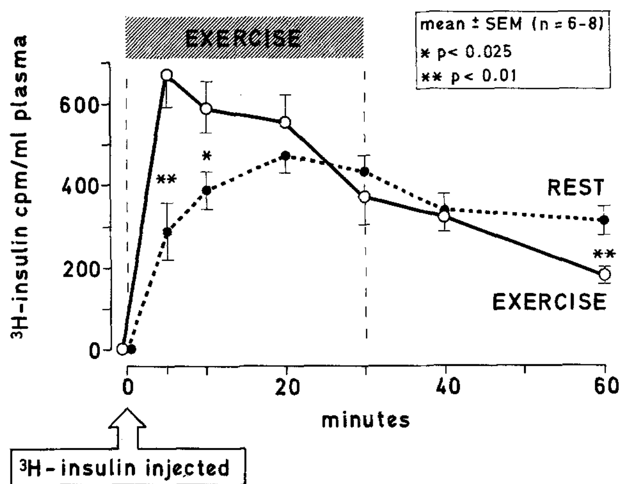


Fig. 5. Effect of treadmill running ("EXERCISE") on plasma levels of [^{3}H]insulin following subcutaneous injections of 10^5 cpm [^{3}H]insulin/100 g body weight in normal fed rats. [^{3}H]insulin concentrations were calculated based on measurements of total ^{3}H counts in plasma and on chromatographic evaluation of the samples as described in the methods

exercise period (Figure 5). Furthermore, in the post-exercise phase, plasma [^{3}H]insulin levels fell below the corresponding concentrations of exogenous insulin in control animals.

Distribution of ^{3}H Radioactivity in Liver, Kidney and Skeletal Muscle: Except for a small, but significant, elevation of ^{3}H radioactivity in the liver at 5 minutes, exercise had no effect on the distribution of tritiated material in liver and kidneys. Similarly, there were no alterations of the ratios between the concentrations of ^{3}H radioactivity per g tissue and per ml plasma; indicating that the distribution space of [^{3}H]insulin and its labelled degradation products in these organs remained unchanged during exercise. In contrast, ^{3}H levels in skeletal muscle were significantly elevated during exercise and still 10 minutes after cessation of exercise (Table 2). Already 5 minutes after the onset of exercise, significant amounts of tritiated material were extractable from skeletal muscle, and the ratio between tissue and plasma levels of ^{3}H rose to a maximum of 0.52, which was reached at 40 minutes (i. e. 10 minutes after the cessation of treadmill running). Since the extracellular space in rat skeletal muscle is approximately 20% [17] this finding seems to indicate a significant intracellular distribution of ^{3}H. Thirty minutes after the end of exercise ^{3}H levels in skeletal muscle were markedly decreased, suggesting a gradual washout of tritiated material which had accumulated in skeletal muscle during exercise.

Discussion

Although the replacement of insulin by subcutaneous injections has been a routine treatment of diabetes mellitus for more than fifty years, the pharmacokinetics of insulin delivered via administration of sub-

cutaneous depots have not been adequately studied. Such an investigation is important since the hypoglycaemic potency of subcutaneously injected insulin shows considerable, hitherto unexplained, variations, both from one patient to another and within the life course of one patient. It was suggested that such variations might be due to alterations of the pharmacokinetics of subcutaneously injected insulin, i. e. the rates of its absorption from the subcutaneous depot, its appearance in the circulation, its subsequent distribution, metabolism and excretion. In particular, attention has been focussed on the kinetics of the *absorption* of insulin from the injection site into the blood stream. Previous investigations are based on the external measurement of disappearance rates of radio-iodinated insulin preparations from the subcutaneous injection site [18, 6]. Among the several presumptions that these investigations are based upon, one has to assume that the injected insulin is not decomposed during the absorption process [18]. In contrast, this study demonstrates that 17 days after the injection of labelled insulin a considerable depot of radioactivity ($5 \pm 1\%$ of the initially injected dose) is still detectable in the tissue specimen from the injection site (Figure 2).

This may merely be a reflection on the initial impurities in the [^{3}H]insulin preparation, but a more likely suggestion is local degradation of [^{3}H]insulin and subsequent deposition of the metabolites in the injected tissue. We observe significant degradation at the site of injection even in the presence of cold, carrier-insulin. More recent results from our laboratory following subcutaneous injections of [^{3}H]insulin in rats have, in fact, revealed a significant local degradation of [^{3}H]insulin even during the first hour after the administration of the hormone. Therefore, to evaluate the absorption of insulin into the circulation, it appears essential to measure directly plasma levels of exogenous insulin following its subcutaneous injection. Such an investigation depends on the availability of an insulin marker, the metabolism of which is identical to that of native insulin. We have used the [^{3}H]insulin prepared according to Halban and Offord [9] as a marker for such studies.

This paper describes the application of [^{3}H]insulin to study whether physical exercise influences the pharmacokinetics of subcutaneously injected insulin. Such a possibility has been proposed since physical activity increases the hypoglycaemic effect of subcutaneously injected insulin in humans with and without diabetes mellitus [1, 2], a phenomenon which was entirely reproducible in normal rats during and after treadmill running, following the administration of a pharmacological dose of regular unlabelled insulin (Figure 1). Based on experiments with pancreatectomized dogs [3] and diabetic patients [4, 5, 6], it was suggested that exercise accelerates the mobilization of subcutaneously injected insulin. But studies in humans using external counting to measure the effect of physical activity on the absorption of iodinated insulin, led to conflicting results [19, 20, 6].

Using subcutaneous injections of tracer amounts of [^{3}H]insulin in normal rats, this study demonstrates clearly an accelerated appearance of exogenous insulin in the circulation; in the first ten minutes of the exercise period, plasma levels of the insulin tracer were markedly elevated. Previous studies using insulin infusions in pancreatectomized dogs have shown that the clearance of insulin from the circulation remained unchanged during exercise [21]. Hence, we assume that the elevated plasma concentrations of [^{3}H]insulin early during exercise are due to an increased rate of mobilization from its subcutaneous depot. Several possible mechanisms for this phenomenon may be considered: the exercise-induced acceleration of the absorption rate might be due to the increased circulation during physical activity – although an increased blood perfusion of subcutaneous tissues occurs only in certain types of physical activity [22]. On the other hand, the mechanical effect of contractions of the underlying musculature might induce changes in the interstitial pressure of subcutaneous tissues possibly leading to an accelerated absorption of insulin molecules into the capillaries and/or the lymphatic system. Finally, it might be speculated that any local degradation of insulin molecules could be inhibited due to primary (mechanical) or secondary (circulatory) consequences of physical activity. Further studies to clarify the mechanism of the increased mobilization of insulin from its subcutaneous depot might reveal a means of influencing and ultimately utilizing this phenomenon for therapeutic purposes.

The accelerated fall of [^{3}H]insulin plasma levels during the post-exercise period (Figure 5) could merely be a consequence of the initial acceleration of its absorption; if this hypothesis were true, the rise of plasma concentrations of exogenous insulin early during exercise would be due to a time shift in the absorption of the injected insulin at the cost of the availability of [^{3}H]insulin in the post-exercise period. On the other hand, the accumulation of ^{3}H radioactivity in skeletal muscle could be indicative of increased insulin degradation in muscular tissues in the post-exercise period. In fact, preliminary data show that the ^{3}H radioactivity extracted from skeletal muscle 40 and 60 min after the injection of [^{3}H]insulin represents almost entirely insulin degradation products.

The results of this study demonstrate significantly

increased circulating levels of exogenous insulin during the first 10 min of strenuous treadmill running in rats; we suggest that this observation can, at least in part, explain the exercise-induced hypoglycaemia seen following subcutaneous insulin administration (Figure 1). The rising needs of peripheral glucose utilization during strenuous exercise are most pronounced during the earlier phase of physical activity [23]. Under physiological circumstances this initial increase of peripheral glucose disposal is balanced by an adequate stimulation of hepatic glucose production, associated with a fall of circulating insulin levels [23, 24]. In contrast, a marked, even short-lived, rise of plasma insulin levels in the initial phase of exercise, as observed in this study (Figure 5), will inhibit the necessary increase in hepatic glucose production [3, 5] and hence, lead to hypoglycaemia.

We conclude that the amplification of the hypoglycaemic effect of subcutaneously injected insulin in rats by exercise is due to profound alterations of insulin pharmacokinetics; namely an acceleration of the appearance of exogenous insulin in the circulation early during exercise. Most probably due to anatomical differences, the time courses of both absorption and biological effect of subcutaneously injected insulin are considerably faster in rats than in humans; nevertheless, an analogous exercise-induced elevation of plasma [^{3}H]insulin up to 90 min after its subcutaneous injection was recently observed by us in juvenile type diabetics [25]. It is, therefore, conceivable that these findings, which are in accordance with recent studies in dogs and man [3, 4, 5, 6], represent a mechanism of exercise-induced hypoglycaemia in insulin treated diabetic patients.

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