Insulin is ubiquitous in extrapancreatic tissues of rats and humans
(hormones/biosynthesis/radioimmunoassay/tissue extraction)

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ABSTRACT  Insulin has been detected, at levels higher than those in plasma, in a broad range of extrapancreatic tissues in both rats and humans. Rat liver insulin was shown to be indistinguishable from genuine insulin by radioimmunoassay, Sephadex chromatography, bioassay, and antibody neutralization. Liver insulin (like brain insulin) was unchanged in ob/ob mice, in rats treated with streptozotocin, or in fasted rats, despite marked alterations in pancreatic secretion of insulin and in liver content of insulin receptors. Insulin was found in cultured human IM-9 lymphocytes and cultured fibroblasts at concentrations greater than 100 times the levels in the media. IM-9 lymphocyte insulin also was shown to be indistinguishable from genuine insulin, by the same criteria used for liver insulin. The insulin concentration in cultured human cells was unaffected by depletion of insulin from the culture medium or by addition of beef insulin to the medium. The data suggest that a part, if not all, of the extrapancreatic tissue insulin is independent of plasma insulin and may be synthesized by the tissues themselves.

Recently, we demonstrated the presence of insulin in the brain in concentrations higher than in plasma (1). This brain insulin was indistinguishable from genuine insulin by multiple criteria, and its tissue concentration did not vary at all with extreme changes in plasma insulin (2). In the present study we show that insulin immunoreactivity is present in essentially all tissues of humans and rats as well as in cultured lymphocytes and fibroblasts at concentrations that are 2–100 times those present in the plasma or culture medium. The material is similar to (or identical with) genuine insulin by radioimmunoassay, gel filtration, bioassay, and antibody neutralization. The concentration of cellular insulin in vivo changes little or not at all in response to extremes of hyperinsulinemia and hypoinsulinemia.

MATERIALS AND METHODS

Tissue Preparations. Male Sprague–Dawley rats (250–300 g) fed ad lib were decapitated between 1400 and 1600 hr; "fasted rats" had been denied food for 50–72 hr before they were killed. Rats injected with streptozotocin (65 mg/kg intravenously) and control rats (injected with isotonic saline) were sacrificed 1 month after treatment. Obese (ob/ob) mice and their thin littermates (mixtures of ob/+ and +/+ of the C57BL/6J strain) were killed at 8–10 weeks of age. Organs and plasma were collected and stored as reported (1, 2).

Human tissue samples, obtained either during operations or at autopsy within 3 hr after death, were frozen on dry ice prior to extraction. Whole blood (500 ml) from a fasting (overnight) normal human volunteer was separated into mononuclear and granulocytic fractions by a modification (3) of the method of Boyum (4). The cells were washed in phosphate-buffered saline (pH 7.5), at 4°C and extracted with acid/ethanol. The granulocyte fraction contained 10% erythrocytes and 80% granulocytes, and the mononuclear cells consisted of 4% erythrocytes, 20% monocytes, and approximately 70% lymphocytes as determined by examination of stained smears or counts of cells after exposure to latex beads. Cultured IM-9 lymphocytes were grown in RPMI-1640 medium with 10% fetal calf serum for 4 days (to confluence), centrifuged at 500 X g for 5 min, resuspended in phosphate-buffered 0.85% saline (pH 7.5) with bovine serum albumin (1 mg/ml), and recentrifuged to yield a pellet for acid/ethanol extraction. The individual cell volumes for the fractions were determined in a cell-sizer (Coulter), and insulin concentration was expressed as ng/ml of cell volume.

Insulin Extraction. A standard type of extraction procedure was used (1, 2, 5). When known amounts of unlabeled insulin were added to rat tissues before extraction, the percentage of 125I-labeled insulin (125I-insulin) recovered after the extraction was similar to the percentage of the additional insulin recovered as measured by radioimmunoassay.

In all of our previous studies (1, 2) albumin was added at two steps, prior to lyophilization and during the final reconstitution. In the present study the first addition of albumin was omitted for all except the ob/ob and streptozotocin studies.

Gel Filtration. Extracts of liver and IM-9 lymphocytes were lyophilized, dissolved in 0.05 M (NH4)2CO3 containing bovine serum albumin (1 mg/ml), applied to a Sephadex G-50 (fine) column (1.5 X 90 cm), and eluted with the same solution. Fractions (1 ml) were collected, and the insulin concentration in each fraction was directly measured (final dilution, 1:10) in the radioimmunoassay.

Radioimmunoassay. Insulin radioimmunoassay was performed by a double-antibody method as reported (1, 2). To exclude the possibility that the apparent immunoreactivity in the tissue extract was due to some interference in the immunassay, IM-9 and rat tissue extracts, as well as three peak fractions from the rat liver extract gel filtration, were incubated for 3 days in the radioimmunoassay. Trichloroacetic acid was added to a final concentration of 5% to some tubes; guinea pig anti-porcine insulin antibody was added to a duplicate set of tubes to a final concentration of 1:1000 (antibody excess) and incubated for 4 hr at 4°C before addition of the second antibody (rabbit anti-guinea pig). Radioactivities estimated in the pellet and in the supernatant were identical to values in control tubes incubated with assay buffer alone, indicating that the extracts were not causing degradation of 125I-insulin or otherwise interfering in the radioimmunoassay.

1 Although the insulin immunoreactivity of the extrapancreatic tissues has not yet been purified and its sequence determined, the material in brain, liver, and IM-9 lymphocytes has been characterized (to almost the same extent as insulin in plasma) and, for convenience, we refer to these materials as "insulin."
Bioassay. Biological activity was measured as the conversion of 
[U-¹⁴C]glucose to ¹⁴CO₂ by isolated fat cells prepared from 
epididymal fat pads of young rats (6). The bioactivity of our rat 
insulin standard was 55% that of the pork insulin standard, 
which is similar to values reported for commercially prepared 
rat insulins (7).

RESULTS
The concentrations of insulin in rat tissues, measured by 
radioimmunoassay, were higher than the concentration of hor-
mone in plasma (Fig. 1 Upper, open bars). The insulin concen-
tration was highest in heart tissue, with a mean value that 
was 100 times the plasma level; it was lowest in fat, with a value 
5 times that in plasma (for fat versus plasma, P < 0.005). To 
monitor the losses of hormone during the extraction procedure, 
¹²⁵I-insulin was added during homogenization, and the total 
radioactivity in the final extract was used to calculate the re-
cover. The actual insulin content of the tissues may have been 
somewhat greater because some of the radioactivity that was 
recovered was no longer insulin (i.e., up to 10% of the recovered 
radioactivity was not precipitated by trichloroacetic acid and 
up to 30% was not recognized by anti-insulin antibody). From 
the open and solid bars in Fig. 1 Upper it can be seen that re-
cover of the ¹²⁵I-insulin varied widely among the different 
tissues—e.g., 7% in heart and 43% in fat. However, even the uncorrected insulin concentrations were significantly greater than 
the plasma insulin for all of the tissues (P < 0.05) except liver and spleen (P ≤ 0.1).

Tissue insulin was similar to genuine insulin in the radioim-
muassay. Pooled extracts of rat liver over a 20-fold range of 
dilution gave a curve that was similar to that of rat insulin (Fig. 
2A). When tissue extracts were filtered on Sephadex G-50, the 
immunoreactivity eluted as a single peak (Fig. 2B) in a position close to that of labeled insulin standard (and similar to that re-
ported for brain insulin) (1). The effluent fractions that corre-
sponded to the insulin peak were pooled and concentrated; the 
bioactivity of this extract (1.9 ng/ml), measured as glucose 
oxidation in isolated fat cells (Fig. 2C), was similar to the value 
predicted by radioimmunoassay (3.5 ng/ml). The bioactivity of 
the tissue insulin and that of the rat insulin standard were 
neutralized by anti-insulin antibody (Fig. 2C).

Human tissues and blood cells were also examined for insulin 
(Fig. 1 Lower); immunoreactive insulin was found in all those 
examined except granulocytes and erythrocytes. The insulin 
concentration in most of the human tissues was somewhat less than 
that in tissues of the rat. However, the concentrations of insulin in human plasma are typically less than those in rats, so 
that tissue levels were still higher than plasma. In comparison, 
the insulin concentration in mononuclear cells isolated from 
the blood of normal human donors averaged 49 ng/ml of cell 
volume, whereas plasma insulin concentration was 0.5 ng/ 
ml.

Human lymphoblastoid cells of the IM-9 line had insulin concentra-
tions averaging (± SEM) 21 ± 5 ng/ml of cell volume 
(corrected for insulin losses in the extraction). Similarly, the 
insulin concentration in cultured adult and fetal human fi-
broblasts averaged 10 ng/ml of cell volume whereas the insulin 
concentration in the culture media (that could have been con-
tributed by the fetal calf serum) was 15 pg/ml.

When an extract of the IM-9 lymphocytes was filtered on 
Sephadex G-50, the insulin immunoactivity was recovered as 
a single peak corresponding to that of the insulin standard (Fig. 
2E). When the effluent fractions that corresponded to the re-

dition of the insulin peak were pooled and concentrated, they 
were similar to genuine insulin on serial dilution in the radio-
imunoassay (Fig. 2D). The bioactivity of this material was 
6.0 ng/ml (Fig. 2F) compared to 12 ng/ml predicted by the 
imunoassay. The bioactivity of both standard pork insulin and 
of the lymphocyte insulin was neutralized by an excess of 
anti-insulin antibody.

To study the regulation of cellular insulin and the effect of 
plasma insulin on cellular insulin, several rodent models were 
examined. In the genetically obese (ob/ob) hyperglycemic 
mouse, concentrations of insulin in plasma and pancreas are 
markedly increased (and insulin receptors are substantially 
decreased in liver and other tissues except brain). The insulin 
concentrations in brain (previously reported (2)) and liver in the 
ob/ob mice were the same as in their thin littermates, al-
though the mean insulin levels in the plasma of the ob/ob mice 
were 50 times those of the thin animals (Fig. 3 Top).

Rats treated with streptozotocin (65 mg/kg intravenously) 
became diabetic within 36 hr as shown by urinary glucose 
(Clinitest, 3+ to 4+), ketonuria (Acetest, trace to large), and 
weight loss. Serum insulin had decreased to one-half the 
concentration in control rats (Fig. 3 Center). The insulin concen-

FIG. 1. Insulin concentrations in rat (Upper) and human (Lower) 
tissues. Tissue insulin contents are expressed as ng of insulin per g 
(wet weight) of tissue; plasma levels are ng/ml. Solid portions of the 
bars represent the concentrations of insulin (± SEM in Upper) 
measured directly in the radioimmunoassay; open portions represent 
the additional insulin representing correction for ¹²⁵I-insulin losses 
in the extraction. Rat tissue was measured against a rat insulin 
standard, and human tissue was measured against a pork insulin 
standard. The plasma levels shown were measured by direct addition 
of plasma to the radioimmunoassay; approximately 50% of the im-
munoactive insulin in plasma is lost during acid/ethanol extraction. 
(Upper) A, heart; B, kidney; C, skeletal muscle; D, spleen; E, lung; 
F, testis; G, brain; H, small intestine; I, liver; J, fat; K, plasma. (Lower) 
A, liver; B, spleen; C, lung; D, stomach; E, testis; F, kidney; G, colon; 
H, skeletal muscle; I, small intestine; J, fat; K, brain.
Fig. 2. Characterization of tissue insulin. (A) Serial dilutions of acid/ethanol extract of rat liver (Δ) compared with standards of rat and pig insulin (solid lines) in the radioimmunoassay. (B) Pooled rat liver extracts were filtered on Sephadex G-50, and the immunoreactive insulin was measured in each 1-ml fraction. Arrows (at bottom) indicate the void volume (150), labeled thyroglobulin) at the left, the 131I-insulin peak in the middle, and the salt peak (NaCl) on the right. (C) The effluent fractions from the column in B) were pooled and concentrated. Biological activity was measured as the conversion of [U-14C]glucose to 14CO2 by isolated rat adipocytes (6). The 14CO2 produced is plotted as a function of pork insulin standard (●) and the volume of extract of rat liver (Δ). The solid horizontal line represents the 14CO2 production in the absence of insulin. Symbols under the arrows represent the bioactivity in samples to which anti-insulin antibody (1:1000) had been added. (D) Serial dilution, in radioimmunoassay, of gel-filtered IM-9 extract (▼) compared to pork insulin (solid line). (E) Elution pattern of acid/ethanol extract of IM-9 lymphocytes filtered on Sephadex G-50 as in B. (E) Activity of IM-9 insulin (Δ) in glucose oxidation bioassay as in C.

...trations in the brain [previously reported (1)] and liver of the rats treated with streptozotocin were the same as in the normal rats. (As elaborated below, kidney insulin decreased to approximately one-third of the concentration in control animals.)

Prolonged fasting is another condition characterized by a marked decrease in insulin output by the pancreas. In rats fasted for 2-3 days, the insulin concentrations in brain and liver were indistinguishable from those in fed controls (Fig. 3 Bottom). In contrast, prolonged fasting was associated with a decrease in the insulin concentration in kidney. However, it should be noted that the decrease in insulin concentration seen with streptozotocin treatment and fasting was to levels normally seen in both brain and liver. Tentatively, we interpret the findings in kidney to indicate that the kidney insulin represents two compartments, one that is analogous to liver and brain insulin and is independent of plasma insulin concentrations, and one that is derived by reabsorption of the glomerular filtrate, which is responsive to changes in plasma insulin concentration (9-11).

The concentration of insulin in human cells in culture was independent of the insulin concentration in the medium in
Our detection of high concentrations of insulin by radioimmunoassay in numerous tissues of rats and humans is in accord with earlier observations, although little significance had been assigned to these measurements in the past (11, 12, 13). It has been recently suggested that extrapancreatic sources of insulin exist but are confined within the gastrointestinal tract (14, 15) or the nervous system (1, 2, 16). It has been demonstrated that circulating insulin persists after evisceration, suggesting that sources other than pancreas can supply insulin to the plasma (17). Other than that in the brain (1), extrapancreatic tissue insulin measured by radioimmunoassay has never been characterized previously.

We showed in the present study that insulin extracted from rat liver and from human IM-9 lymphocytes was similar to genuine insulin by radioimmunoassay (Fig. 2 A and D), gel filtration (Fig. 2 B and E), bioassay (Fig. 2 C and F), and neutralization of bioactivity by anti-insulin antibodies. On the basis of these data, the material cannot be proinsulin, split proinsulin, or one of the insulin-like growth factors or somatomedins; it is either insulin or something extremely similar to insulin.

When the tissue concentrations of insulin measured here are extrapolated to the total tissue space in vivo, in both rats and humans, the extrapancreatic tissues may contain as much as 5% of the total insulin of the organism in vivo, equivalent in amount to 40% of the total insulin released from the pancreas into the circulation within a 24-hr period. It must be emphasized, however, that the concentration of insulin in the pancreas itself is still as much as 10,000 times the concentration in the extrapancreatic tissues.

The finding of insulin at high concentrations in multiple extrapancreatic sites raises questions: How did the insulin get there? What is it doing there? Surely, under some circumstances, some of this insulin represents insulin of pancreatic origin that now is residing on receptors. Insulin receptors are

FIG. 3. Effects of hyperinsulinemia and hypoinsulinemia on tissue insulin levels. In each panel, values (mean ± SEM) for controls (open bars) are compared with values for experimental animals (hatched bars). The solid portions of the bars represent insulin concentrations measured directly in the radioimmunoassay and not corrected for insulin losses in the extraction. (Top) Weight and serum and tissue insulin values in obese (ob/ob) mice. Insulin values were measured with a pork insulin standard in the radioimmunoassay. * = 6, except samples were pooled for serum. (Center) Tissue insulin values in streptozotocin-treated rats (measured with a pork insulin standard). * = 6 except for kidney it was 8. (Bottom) Effect of fasting in rats (measured with a rat insulin standard).

which they had been grown. When IM-9 lymphocytes or human fibroblasts were grown in media in which the serum had been depleted of insulin or enriched with insulin, cellular insulin concentrations were unchanged from the concentration in cells grown in normal media (Fig. 4).

**DISCUSSION**

Our detection of high concentrations of insulin by radioimmunoassay in numerous tissues of rats and humans is in accord with earlier observations, although little significance had been assigned to these measurements in the past (11, 12, 13). It has been recently suggested that extrapancreatic sources of insulin exist but are confined within the gastrointestinal tract (14, 15) or the nervous system (1, 2, 16). It has been demonstrated that circulating insulin persists after evisceration, suggesting that sources other than pancreas can supply insulin to the plasma (17). Other than that in the brain (1), extrapancreatic tissue insulin measured by radioimmunoassay has never been characterized previously.

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The finding of insulin at high concentrations in multiple extrapancreatic sites raises questions: How did the insulin get there? What is it doing there? Surely, under some circumstances, some of this insulin represents insulin of pancreatic origin that now is residing on receptors. Insulin receptors are

**Diagrams**

**Fig. 4.** Insulin concentration in human cultured cells. Cellular insulin concentrations are shown for IM-9 lymphocytes (○) and human fibroblasts (□). Insulin in the medium (15 pg/ml) was depleted to ≤4 pg/ml by passage over an anti-insulin antibody affinity column. Beef insulin was also added to the medium in some cases to a final concentration of 104 pg/ml. Tissues insulin values are those measured directly in the radioimmunoassay (i.e., uncorrected for losses in the extraction procedure).

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for humans, calculations assume an extrapancreatic tissue insulin concentration of 6.8 ng/g (average of our values) distributed over 80% of the total body mass of a 70-kg man (intravascular fluids and bone are excluded). Data for total pancreatic insulin (8 mg) and for 2-hr pancreatic output (1 mg) are taken from well-established sources (18). In rats, an average insulin concentration of 45 ng/g of extrapancreatic tissue is assumed to be distributed over 80% of a total body mass of 280 g (average weight of fed rats). The total pancreatic insulin (250 μg) is the mean value determined by radioimmunoassay of tissue extracts from pancreas in four fed rats. Twenty-four-hour insulin output is assumed to be one-fifth of the total pancreatic insulin. In this discussion we have assumed that the extrapancreatic tissue insulin is distributed roughly uniformly but we have not totally excluded (except in brain) the small possibility that our findings could be explained by the presence, in every tissue, of one rare cell type that is extremely rich in insulin.
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present on nearly all cells (19), and the concentrations of receptors are about the same on nontarget cells as on classical targets such as liver, muscle, and fat cells. Theoretical predic-
tions and experimental results indicate that receptor concentra-
tions in vivo are sufficiently high (relative to receptor affinity and hormone concentration) that the concentration of hormone bound to cells may be equal to or possibly exceed the hormone concentration in plasma (20). However, that would be insufficient to account for the levels observed here. In experi-
ments with liver (21) and fat (22) cells in vitro, intracellular uptake of 125I-insulin was associated with rapid degradation. With insulin that has a fluorescent label, intracellular entry of hormone can be followed by noting an orderly arrangement of fluorescence within the cytoplasm of the cell (23). Although it gives exquisite localization, this method fails to allow an accurate estimate of what fraction of total hormone remains intracellularly or what fraction of the fluorescence represents intact insulin.

The data in the hyperinsulinemic mice, the hypoinsulinemic rats, and the cultured cells grown in insulin-depleted media are the most difficult to reconcile with a model of pancreatic synthesis and transport in plasma followed by receptor-mediated or receptor-independent uptake and storage. The obese mice have plasma insulin concentrations that are 50–100 times nor-
mal but receptors are decreased to 30–50% of normal (2, 24). The streptozotocin-treated rats have a modest decrease in basal insulin (and presumably a marked decrease in postprandial insulin) and a 2- to 3-fold increase in receptors (2). That both of these groups of animals have the same concentration of liver insulin as the thin animals suggests that (i) the concentrations of receptors and of hormone are exquisitely regulated over an extreme range to keep the concentration of intracellular hor-
mon constant, (ii) the cells have an extraordinary transport and storage system (i.e., very high affinity and relatively low capacity), or (iii) that the cells are synthesizing insulin themselves and the intracellular hormone is largely of local origin.

The hypothesis that insulin may be synthesized locally by all cells may seem somewhat unconventional at first, but sup-
portive data for it are available. Although it is generally assumed that hormones are produced by specialized cells, mostly con-
ained within a special gland, this concept is being challenged and extended by new data. Neoplastic tissues of diverse origin can produce different hormones (25) and can cause, in some instances, specific clinical syndromes. Human chorionic go-
nadotropin, which is found in the trophoblast and in tumors derived from it, has been identified in normal tissues as well, albeit as an asialo form (26). Releasing factors, originally thought to be present in the hypothalamus only, are now found in all brain areas and in the gastrointestinal tract (27). Hormones such as cholecystokinin and vasactive intestinal polypeptide, originally thought to be specific for the gastrointestinal tract, are now being found in the central nervous system (27). Pre-
liminary unpublished data from our laboratory suggest that human growth hormone is present in various human tissues. It may be that, during differentiation, genes are never completely repressed and all proteins encoded within the genome are synthesized regularly at a low rate during the cell’s life-span. This is supported, at least on a transcriptional level, by studies in sequence diversity of RNA transcripts in the nucleus (28), although it has not yet been shown whether any of the hetero-
genous nuclear RNA transcripts have any function in protein synthesis.

If insulin is synthesized locally for a purpose, numerous possibilities exist for its action. Insulin (as well as other peptides) may be involved in intracellular or intercellular (paracrine) signaling in organelle organization, protein storage, or immuno-
logical self-recognition. If insulin is produced by the pancreas only and its presence within the cell is explained by a transport mechanism, intracellular insulin may represent the pool that is destined for degradation. Different functions of transported insulin may be postulated but, at the present time, there are no data supporting an intracellular action of insulin. It is note-
worthy in that respect that streptozotocin-treated animals die from hyperglycemia due to insulin deficiency even though liver insulin content is normal, suggesting that it is unlikely that in-
tracellular insulin is involved in carbohydrate metabolism.

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