plus de 1 % de la radioactivité totale n'apparaît sur les autochromatogrammes, où les iodothyronines et les iodures sont seuls présents. Il n'a pas été possible de mettre en évidence des métabolites de T_3 ou de T_4 dans diverses conditions de température ou de milieu (α-cétoglutarate, phosphate de pyridoxal), conditions dans lesquelles l'effet des hormones sur le gonflement des mitochondries se manifeste. Enfin, il convient de signaler que les protéines mitochondriales migrent à 2 cm de l'origine des chromatogrammes, la migration de T_3 ou T_4 n'étant pas modifiée par leur présence, ce qui montre que les hormones fixées aux mitochondries ne le sont pas par liaison chimique. Les hormones semblent donc exercer leurs effets sur les mitochondries sans subir aucune transformation préalable, à la réserve d'une transformation réversible près.

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Some observations on insulin–receptor interaction

Previously, evidence was published to indicate the nature of the bonding of vasopressin at its receptor site in the kidney[1]. A mechanism of action for membrane permeability was proposed for the antidiuretic hormone[2] (vasopressin), based on a thiol – disulfide exchange reaction involving the vasopressin disulfide bond and a receptor thiol group. Studies on the action of vasopressin on the toad bladder[3,4] have also indicated a similar mechanism, giving further support to the hypothesis suggested. Since there are similarities in insulin, vasopressin and oxytocin, namely, each possessing a pentapeptide ring of 20 atoms with an intradisulfide bridge, we have been exploring, along with vasopressin studies, the possibility that the membrane-permeability action of insulin could also be explained by such an exchange reaction. We now have evidence to support this hypothesis. This study extends the early investigations of STADIE *et al.*[5,6] on the binding of iodoinsulin to muscle tissue and the membrane-permeability studies of LEVINE AND GOLDSTEIN[7].

In the experiments epididymal fat pads of Sprague–Dawley rats were removed as described by BALL *et al.*[8]. Diaphragms were carefully removed, washed in saline, blotted and cut in half. Both fat pads and hemidiaphragms were placed in flasks containing Krebs–Ringer bicarbonate buffer (pH 7.4), glucose and [^{131}I]insulin (biologically active), then gassed with a mixture of 95 % O_2 and 5 % CO_2 and incubated in a water bath at 37° for 20 min. Immediately after the incubation period the buffer solution was poured off and the tissue in a small amount of saline was heated for several minutes to inactivate enzymes. The tissues were then thoroughly washed with

0.01 *N* HCl followed by six washings with 8 *M* urea in 0.015 *N* HCl to a fairly constant radioactivity, as measured with a well scintillation counter. In experiments to release bound radioactive insulin from the tissues, thiol compounds in bicarbonate (pH about 8) were used. Results are as shown in Tables I and II.

TABLE I

RELEASE OF RADIOACTIVITY FROM RAT EPIDIDYMAL FAT PADS AND HEMIDIAPHRAGMS BY TREATMENT WITH VARIOUS THIOL COMPOUNDS IN 0.1 *M* HCO_3^- FOR ABOUT 16 h

For the release of radioactivity the tissues were treated with 10 ml of the thiol compound in bicarbonate and incubated in a water-bath shaker at 37°. The solution was decanted and the tissues rinsed several times with bicarbonate, then transferred to tubes and counted in a well scintillation counter.

Thiol compound	*Fat pads radioactivity released (%)*	*Hemidiaphragms radioactivity released (%)*
Control	12.2, 12,2	10.2, 12.0, 13.3
0.1 *M* cysteine	62.6, 64.8	60.6, 54.1
0.1 *M* thioglycolate	56.0, 43.6	42.8, 57.2
0.1 *M* β-mercaptoethylamine	61.3, 59.8	55.6, 66.0, 59.0

TABLE II

FURTHER RELEASE OF RADIOACTIVITY FROM RAT EPIDIDYMAL FAT PADS AND HEMIDIAPHRAGMS BY TREATMENT WITH VARIOUS THIOL COMPOUNDS IN 8 *M* UREA AND 0.1 *M* HCO_3^- FOR ABOUT 4 h

For the second release the procedure described above was used with the exception that the solution contained 8 *M* urea.

Thiol compound	*Fat pads radioactivity released (%)*	*Hemidiaphragms radioactivity released (%)*
Control	18.2, 17.3	14.8, 15.8, 17.3
0.1 *M* cysteine	80.3, 79.8	80.9, 74.9
0.1 *M* thioglycolate	71.4, 65.7	70.1, 68.6
0.1 *M* β-mercaptoethylamine	78.2, 77.4	79.3, 82.6, 80.9

The net release of radioactivity from the tissues, 31–54% and 48–66%, after the first and second treatment, respectively, by sulfhydryl compounds suggests that insulin interaction with tissue receptors is through a sulfur – sulfur bond or bonds formed between an insulin disulfide and a tissue-membrane – receptor thiol group or groups (thiol – disulfide exchange reaction).

Since insulin possesses three disulfide bonds, an intradisulfide bond and two interdisulfide bonds, we are cognizant of the fact that there can be a number of disulfide cleavages in the insulin – receptor complex. Of all the cleavages possible only the scission of the insulin – receptor sulfur – sulfur bond is a measure of the hormone – receptor interaction. Under the conditions of the experiments with the denatured insulin – protein complex it would be reasonable to expect a random cleavage of the disulfide bonds. In order to ascertain the hormone – receptor sulfur – sulfur bond cleavage successive treatments of the insulin – tissue complex with thiol compounds were resorted to and the ratio of ^{131}I in the A and B peptide chains of the iodoinsulin

was determined. The iodination of insulin by the triiodide method[9] was such that an average of 2 atoms of iodine per molecule of insulin was introduced. The ratio of the atoms of iodine in the two peptide chains was determined by adding carrier insulin to the iodoinsulin and oxidizing the mixture with performic acid. The oxidized peptide chains were separated by countercurrent distribution, following the method of CRAIG *et al.*[10]. Fractionation of the oxidized iodoinsulin showed nearly equal distribution of ^{131}I in the A and B peptide chains (45% in peptide A and 55% in peptide B).

Since disulfide cleavages of the denatured insulin–tissue complex are random on treatment with reductive agents and in view of our results showing nearly identical radioactivity in the A and B chains of the [^{131}I]insulin, the net release of up to 66% of the radioactivity from the tissue complex after successive treatments with reductive agents (Table II) would indicate a binding of insulin to the tissue via a disulfide bond. It is apparent that cleavages of disulfides other than the insulin–receptor disulfide cannot entirely account for the release of radioactivity.

The evidence presented indicates that the interaction of insulin with its receptor may possibly be described in a similar manner as that proposed for vasopressin[2].

The recent appearance of a preliminary communication by CADENAS, KAJI, PARK AND RASMUSSEN[11] on the inhibition of the insulin effect on sugar transport by a thiol-blocking agent, *N*-ethylmaleimide, lends further support to the hypothesis put forth.

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