In vitro RBC Uptake of Radio-Insulin: A Simple Method for Detecting Insulin Antibodies.* (26620)

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The *in vitro* uptake of I^{131}-labelled thyroxine or triiodothyronine by the red blood cell (RBC) is being employed for diagnostic and investigative purposes in relation to thyroid function. The concept underlying this technic presumes that *in vitro* distribution of thyroid hormones between RBCs and plasma is a function of the competitive binding affinities of the RBCs and certain of the plasma proteins. It may be possible to view the *in vitro* red blood cell uptake as reflective of hormone availability to the peripheral tissues. Substituting I^{131} insulin for I^{131} thyronines in a similar *in vitro* method, a study was undertaken of the insulin I^{131} partition between plasma and RBCs of normal and diabetic subjects, some of whom were, or had been, treated with insulin.

Method. Patients from Medical and Surgical services of the Outpatient Clinics, Michael Reese Hospital, served as subjects. Most determinations were made within 2 hours of collecting the blood specimens in heparinized tubes. Several specimens were examined 24 hours after storage in the cold with similar results.

The essential method and calculations are those previously described for the red blood cell uptake of I^{131} triiodothyronine(1) with the exception of using I^{131} insulin instead of I^{131} triiodothyronine. I^{131} insulin was added to 3 ml aliquots of whole blood in a stoppered, 10 ml Erlenmeyer flask and incubated, with shaking, at 37°C for 2 hrs. Radioactive insulin of a specific activity of 4.7-6.5 μc/mg was utilized, and 1×10^{-6} to 2.5×10^{-4} mg of insulin was added per flask. The radioactive content of two 1 ml aliquots of whole blood (at counting rates sufficient to permit 5% accuracy) was determined in a well type scintillation counter. The radioactivity of the RBC mass in each 1 ml aliquot of whole blood was determined after 5 washings in 10-fold dilutions of isotonic sodium chloride and centrifugation at 3,000 RPM for 10 minutes to separate cells from plasma or saline wash. The percent RBC uptake was calculated by the formula:

$$\%\ \text{RBC uptake} = \frac{\text{net counts in RBC} \times 100 \times 100}{\text{net counts/ml of whole blood} \times \%\ \text{hematocrit}}$$

This result yields an expression of RBC uptake for a hypothetical hematocrit of 100%, as previously described(1). "Criss-cross" experiments were performed by separating plasma and blood cells, washing the blood cells 3 times with isotonic saline, then resuspending normal cells in diabetic plasma and *vice versa.*

The influence of the white blood cell count was tested by centrifuging heparinized blood samples at 3,000 RPM for 10-15 minutes. The "buffy coat", adjacent cell layers and a proportionate amount of plasma were removed so as to preserve the original hematocrit. Each original sample accordingly yielded a white cell depleted and white cell enriched sample. White blood cells were counted in a conventional clinical chamber.

Results. Both single and multiple determinations were carired out in 113 adult patients. Of these, 37 subjects were non-diabetics who had never received insulin; 10 patients were diabetics managed with diet alone; 24 subjects were diabetics treated with oral sulfonylureas alone; 47 patients were diabetics having received insulin for variable periods of time in excess of 3 months and at variable dose schedules; and 8 patients had previously received chronic insulin therapy but had been without insulin from one month to 14 years.

Table I indicates that there is a distinct

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TABLE I. Uptake of Radioactive Insulin by RBC of Normal and Diabetic Subjects: Influence of Various Forms of Treatment.

Condition	Treatment	No.	% radio-insulin uptake by RBC*
Normal	—	47	6.2 ± .4
Diabetic	Diet alone	8	5.1 ± .7
"	Sulfonylureas alone	24	5.7 ± .4
"	Insulin for at least 3 mo	36	2.3 ± .3

* ± stand. dev.

reduction in uptake of radio-insulin by the RBC of the diabetic group receiving insulin currently as compared with both non-diabetics and diabetics treated by means other than exogenous insulin. No significant distinction can be made between non-diabetics and non-insulin treated diabetics. There was no correlation between degree of radio-insulin uptake by RBC with dosage of insulin administered or with duration of prior treatment in excess of 3 months.

After cessation of insulin therapy, the reduced radio-insulin uptake by RBC may persist for as long as 4 years or return to normal values within one year. Table II indicates the wide spread of findings, from 1.3% to 11.2% in 8 such patients whose insulin therapy was interrupted for variable periods of time.

The reduced uptake of radio-insulin by the RBC of insulin-treated diabetics is a function of the plasma and not the RBCs (Table III). So called "criss-cross" experiments in which plasma and cells were exchanged between a diabetic subject and a type-compatible normal indicate that the cause for the reduced RBC uptake is resident in the plasma of the insulin treated individual and is not influenced by any intrinsic property of the RBC.

TABLE II. Effect of Elapsed Time Since Interruption of Insulin Therapy on the Reduced Uptake of Radio-Insulin by RBC of Diabetic Subjects.

Patient	Elapsed time since insulin treatment	% radio-insulin uptake by RBC
1	1 mo	2.6
2	2 "	1.3
3	1 yr	11.2
4	3 "	2.2
5	3 "	6.5
6	3 "	4.7
7	4 "	2.6
8	14 "	3.8

Since a marked fixation of I^{131} insulin has been described for leucocytes in peritoneal exudates(2) of rabbits, the possible influence of white blood cells was examined. Artificially varying the white cell count of the same blood sample 4-5 fold does not reflect itself in any change in blood cell uptake (Table IV). It appears that circulating human leucocytes do not exhibit appreciable uptake of radioactive insulin in this technic.

TABLE III. Importance of Plasma in the Reduced Uptake of Radio-Insulin by RBC after Insulin Therapy As Seen in "Criss-Cross" of Normal and Diabetic RBC and Plasma.

Plasma + RBC	No.	% radio-insulin uptake by RBC*
Normal + Normal	6	7.4 ± 3.9
Diabetic + Diabetic	6	3.2 ± 1.4
Normal + Diabetic	6	6.5 ± 2.1
Diabetic + Normal	6	3.1 ± 1.4

* ± stand. dev.

Discussion. Berson and Yallow assayed the radioactivity of packed, unwashed erythrocytes following an intravenous injection of I^{131} labelled insulin. Erythrocyte radioactivity paralleled non-trichloracetic acid precipitable radioactivity. The erythrocyte radioactivity could represent insulin free of plasma binding protein or some *in vivo* breakdown product. In insulin-treated subjects, erythrocyte radioactivity appeared at a slower rate and to a lesser extent than in non-insulin treated subjects. This suggested increased binding of insulin in the plasma of the insulin-treated group and less available

TABLE IV. Lack of Influence of Leucocytes in Determining Radio-Insulin Uptake by Blood Cells: Artificial Enrichment and Depletion of Leucocytes in the Same Blood Sample.

Subject	Leucocytes/mm³	% radio-insulin uptake by RBC
1	Enriched 9,400	3.7
	Depleted 2,200	4.1
2	Enriched 6,700	2.8
	Depleted 1,200	2.4
3	Enriched 11,000	6.3
	Depleted 2,800	6.3

insulin for the erythrocytes. By a number of technics, this change in blood cell-plasma partition was demonstrated to be attributable to an insulin-binding globulin induced only in insulin-treated subjects, irrespective of their diabetic status. These technics included paper strip chromato-electrophoresis, starch block electrophoresis, ultra-centrifugation of insulin I^{131}-plasma mixtures, precipitation of insulin antibody complexes with rabbit anti-human globulin serum, etc.(3,4).

By use of a relatively simple technic, we believe that we have shown the presence of an insulin-binding plasma factor in subjects who have received insulin. We have not attempted qualitatively to correlate this with determinations done by other technics. However, because this plasma binding factor was insulin-induced and, as others have found (3,4), did not quantitatively correlate with either dosage or duration of insulin therapy, this procedure appears to demonstrate the presence of the reported insulin-binding globulin. In these studies the reduced RBC uptake appeared between the third week and third month of the institution of insulin treatment; it persisted for longer than 2 months and, in one patient, for 4 years after insulin treatment had been discontinued. This time relationship is consistent with the appearance and disappearance of the insulin antibody as determined by other technics(3, 4).

It must be noted that the present method cannot distinguish between red blood cells and leucocytes. However, we have demonstrated that the leucocyte contribution to the blood cell uptake may be regarded as unimportant in this technic.

Summary. The capacity of RBC to take up radioactive insulin (I^{131}) from whole blood has been studied in blood samples from normal and diabetic subjects. Prior insulin therapy induces a marked reduction in RBC uptake. Dietary management and/or sulfonylurea therapy does not induce a reduced RBC uptake of radio-insulin. These findings are interpreted to represent a simple technic for demonstrating the presence of insulin antibodies.

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Effect of Triparanol (MER/29) on Corticosterone Secretion by Rat Adrenals.* (26621)

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Triparanol (MER/29) is used as an agent to lower serum cholesterol. Studies by Avigan, Steinberg and coworkers suggest that triparanol inhibits conversion of desmosterol (24-dehydrocholesterol) to cholesterol, the final step of cholesterol synthesis(1). There is also evidence which shows that the total sterol pool is significantly decreased during triparanol therapy(2). Blohm, Kariya and Laughlin(3) have demonstrated that feeding triparanol to rats results in a marked reduction of adrenal cholesterol. Since cholesterol is an important precursor of adrenocortical hormones, it was thought that triparanol feeding might also result in decreased steroid hormone synthesis by this gland. For this reason the present study was initiated.

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Materials and methods. Young, male rats