

## In vivo Effects of the Synalbumin Insulin Antagonist\*

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**Summary.** Albumin prepared from serum of diabetic patients inhibits the effect of insulin on rat diaphragm muscle but not on their epididymal fat pads when injected intravenously to make a foreign protein concentration of 3.8 g/100 ml in the plasma of the rat. At this concentration, albumin from normal subjects had no insulin inhibitory effect on either tissue. — These observations confirm, under *in vivo* conditions, that diabetic albumin is more antagonistic to insulin than albumin prepared from normal subjects.

**Effets in vivo de l'antagoniste de l'insuline, la synalbumine**

**Résumé.** L'albumine préparée à partir du sérum de sujets diabétiques inhibe l'effet de l'insuline sur le muscle du diaphragme du rat mais n'a pas d'effet sur le tissu adipeux épiddymaire, quand elle est injectée par voie intraveineuse pour produire une concentration de protéine étrangère de 3.8 g/100 ml dans le plasma du rat. A cette concentration, l'albumine de sujets normaux n'a pas

d'effet inhibiteur sur l'insuline pour les deux tissus. — Ces observations confirment, dans les conditions *in vivo*, que l'albumine diabétique est plus antagoniste de l'insuline que l'albumine obtenue à partir de sujets normaux.

**In vivo Effekte der Synalbumin Insulin Antagonisten**

**Zusammenfassung.** Aus diabetischen Seren präpariertes Albumin hemmt die Insulinwirkung am Zwerchfellmuskel, aber nicht am epididymalen Fettgewebe der Ratte, wenn bei i. v. Zufuhr eine Konzentration des Fremdeiweißes von 3.8 g% im Plasma der Ratten erreicht wird. Dagegen hemmt in der gleichen Konzentration verarbeitetes Albumin, das aus Normalseren gewonnen wurde, den Insulineffekt an keinem der beiden Gewebe. Diese Beobachtungen bestätigen, daß auch unter *in vivo* Bedingungen diabetisches Albumin die Insulinwirkung stärker hemmt als das Albumin von Normalpersonen.

**Key-words:** Antagonism, fat, glycogen, insulin, *in vivo*, muscle, synalbumin.

Human plasma albumin inhibits the effect of insulin on the rat diaphragm muscle *in vitro* [8, 13, 16, 18], and several workers have confirmed the original observations of VALLANCE-OWEN et al. [18], that there is more antagonism to insulin with albumin prepared from the plasma of diabetic patients [1, 2, 6, 11, 12, 16]. In each case the antagonistic effect is not due to the albumin itself but to some substance associated with it — hence the term, synalbumin insulin antagonist [17].

However, when human albumin (Cohn fraction V) previously shown to be antagonistic to insulin *in vitro*, was infused over three hours into the femoral vein of lightly anaesthetised non-fasting rats, no antagonism to insulin or diabetogenic effect could be demonstrated [4]. Initially the albumin was infused at a rate of 180 mg/hour for three hours achieving a foreign protein concentration of 3.4 g/100ml and later the rate was increased to 450 mg/hour for three hours being equivalent to 5.5 g/100ml foreign protein. In these studies there was no effect on the plasma insulin concentration or on the establishment of steady blood sugar levels and diaphragms from the animals were not less insulin sensitive *in vitro*.

Nevertheless JERVELL [9], and JERVELL and VALLANCE-OWEN [10], using the intraperitoneal technique of RAFABLSON [14, 15], showed that this synal-

bumin antagonist opposed insulin action on muscle *in vivo* although not modifying the effect of insulin on fat tissue.

*In vitro* assays and intraperitoneal injections of albumin can be criticised because of the unphysiological contact of the protein with the diaphragm and clearly intravenous administration of the albumin would be preferable. Accordingly we have investigated the effect of the synalbumin insulin antagonist on glycogen formation in muscle and fat tissue of rats when this material is injected intravenously.

### Material and Methods

Albumin was prepared from the fresh serum of fasting healthy volunteers and of fasting diabetic patients by the trichloroacetic acid/ethanol procedure of DEBRO [5, 18], and by the method of FERNANDEZ et al. [7] which does not involve trichloroacetic acid or dialysis. Ordinarily, single preparations of albumin were used, although in some experiments two albumin samples were pooled, in order to provide sufficient protein.

Male Wistar rats weighing 90–130 g were investigated, but on any one experimental day animals of the same weight were used. The rats, which were fed but unanaesthetised were injected intravenously, using the tail vein, with Gey & Gey buffer (2.5 ml per 100 g bodyweight) containing 1000  $\mu$ U insulin and 4  $\mu$ C <sup>14</sup>C-labelled glucose alone or together with either

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albumin from normal subjects or from diabetic patients in a concentration of 250 mg per 100 g rat. This achieves, at the time of injection, a *human* albumin concentration of 3.8 g/100 ml in the plasma of the rat. The concentration in the total blood volume would be 2.8 g/100 ml.

Nine animals were used in any one experiment, three being injected with insulin and  $^{14}\text{C}$ -labelled glucose alone, three with insulin,  $^{14}\text{C}$ -glucose and normal albumin and three with insulin,  $^{14}\text{C}$ -glucose and diabetic albumin. After 30 min the rats were killed and the radioactivity was determined in glycogen isolated from each half of the diaphragm muscle and each epididymal fat pad after the method of RAFAELSEN et al. [15]. Both the hemidiaphragms and fat pads were immediately weighed on a torsion balance. The epididymal fat tissue was extracted with a mixture of two parts of chloroform and one part of methanol for 24 h, after which the tissue was dried. The hemidiaphragms and fat pads were placed in 0.5 ml 30% potassium hydroxide and hydrolysed by boiling for 30 min in a water bath. Following this the glycogen was precipitated by the addition of 0.1 ml 2% Sodium sulphate and 1.5 ml absolute ethanol for each 0.5 ml hydrolysate, and allowed to stand at room temperature for 24 h. The samples were centrifuged and the supernatant discarded while the precipitate was washed once with 2 ml 70% ethanol and subjected to a second centrifugation. After drying, the precipitates were dissolved in 1.0 ml 0.1N  $\text{H}_2\text{SO}_4$  and boiled for three hours in a water bath. Following this 0.1 ml from the diaphragm preparation and 0.2 ml from the fat pad preparation, was pipetted into 5 ml scintillation fluid and the counts/minute were determined by a Packard Tricarb Counter. The results were calculated as counts per minute per mg wet tissue and expressed as a percentage of the incorporation with insulin and glucose alone, in order to facilitate comparison between separate experiments.

All albumin samples were tested at a concentration of 1.25% for insulin antagonism *in vitro* [18, 19] — those from diabetic patients being antagonistic, those from normal subjects non-antagonistic.

### Results and Discussion

The results of intravenous injection of albumin from normal subjects and diabetic patients into fed unanaesthetised rats are shown in Fig. 1. There is a clear antagonistic effect of albumin from diabetic patients on the rat diaphragm muscle with no such action on the fat pads.

At this concentration (3.8 g/100ml) and under these conditions, albumin from normal subjects has no antagonistic effect on the muscle tissue, however in one experiment the concentration was doubled when antagonism was achieved.

It is noteworthy that diabetic albumin antagonises insulin action on muscle to the same extent whether

it is prepared by the trichloroethanol method of DEBRO or by the FERNANDEZ method which involves no dialysis.

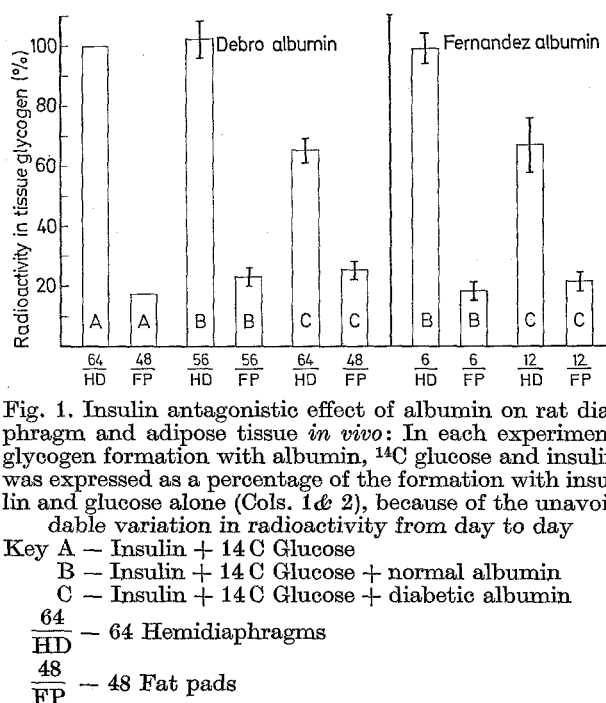


Fig. 1. Insulin antagonistic effect of albumin on rat diaphragm and adipose tissue *in vivo*: In each experiment glycogen formation with albumin,  $^{14}\text{C}$  glucose and insulin was expressed as a percentage of the formation with insulin and glucose alone (Cols. 1 & 2), because of the unavoidable variation in radioactivity from day to day

Key A — Insulin + 14 C Glucose

B — Insulin + 14 C Glucose + normal albumin

C — Insulin + 14 C Glucose + diabetic albumin

$\frac{64}{\text{HD}}$  — 64 Hemidiaphragms

$\frac{48}{\text{FP}}$

— 48 Fat pads

These results indicate, *in vivo* as *in vitro*, that albumin from diabetic patients is more antagonistic to insulin than albumin prepared from normal subjects.

Much *in vitro* work has also shown that the synalbumin insulin antagonist, although antagonistic on the rat diaphragm, does not reduce the effectiveness of insulin on the fat pad [2, 13]. We have also confirmed these observations under *in vivo* conditions.

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