their own. Unfortunately it is not possible to comment on their work at this time since this has not been published except for two experiments reported in four abstracts. What is clear from those abstracts is that the authors had also chosen to consider their animals as either diabetic or non-diabetic without reference to the asymptotically diabetic rats.

Yours sincerely,

J. M. Martin and R. B. Elliott

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Dr. Julio M. Martin
Research Institute
The Hospital for Sick Children
Toronto, Ontario
Canada

Insulin binding and internalization in human and chicken erythrocytes and the role of the cell nucleus

Dear Sir,

Nerurkar and Gambhir [1] have criticized a recent publication from this laboratory [2], based on their biochemical studies of insulin binding. Our studies reported our experiences with electron microscopy on the same topic. In these studies, we showed that insulin-gold complexes were internalized only by erythrocytes still displaying a nucleus (chicken, frog and trout) and also by human reticulocytes which had remnants of nuclear material. Mature erythrocytes did not internalize insulin-gold complexes.

The contradictory opinions about the internalization of insulin by mature human erythrocytes arise from two different methodological approaches. We must state, however, that in preparation for electron microscopy, we did carry out binding studies with ^{125}I -insulin, both at 15 °C and 37 °C with human and chicken erythrocytes. After an incubation time of 120 min, the cells were washed three times with an acidic incubation buffer (pH 6.0) to remove the non-covalent-bound hormone from the cell surface [3]. The remaining radioactivity in the cell pellets is shown in Table 1. Chicken erythrocytes revealed an almost ninefold greater percentage of covalently bound ^{125}I -insulin, which could be regarded as the internalized fraction. While the biochemical approach alone did not satisfactorily solve the question of whether insulin had or had not been internalized, the results of the complementary electron microscopy studies confirmed our hypothesis that insulin-gold complexes can be internalized only by nucleated erythrocytes and by human reticulocytes. On the other hand, in mature human erythrocytes, insulin-gold complexes were found only on the cell surface and not within the cell. Ackerman and Wolken [4] found a similar result using gold-labelled insulin albumin.

According to strict morphological criteria, reticulocytes are non-nucleated cells, although they can carry out synthesis of membrane

Table 1. The remaining radioactivity in the cell pellets of human and chicken erythrocytes before and after three washes with incubation buffer

Erythrocytes	% Specific ^{125}I -insulin bound to erythrocytes (4×10^9 cells/ml)	
	Initial binding	Binding after three washes with acidic buffer (pH 6.0)
Human ($n=6$)	7.6 ± 1.6^a	0.62
Chicken ($n=6$)	13.7 ± 2.9	5.22

Results expressed as mean $\pm$ SEM. ^a Reticulocyte count in the isolated fractions < 4.3 per 1000

proteins [5], a process which seems to be insulin-dependent and not seen in the mature erythrocyte [6]. Ginsberg and Brown [7] related the inability of mature erythrocytes to synthesize protein to the fact that these cells do not down-regulate their insulin receptors. Our referral to reticulocytes as nucleated cells was thus meant in a metabolic sense (= protein synthesis).

Ivarsson and Thorell [8] have documented that insulin binding to erythrocytes is a function of the percentage of reticulocytes in the isolated cell fraction. We have shown that reticulocytes can internalize insulin. The studies of Nerurkar and Gambhir [1, 9] showed that erythrocyte lysates can degrade ^{125}I -insulin. However, they have failed to determine the percentage of reticulocytes present in the isolated cell fraction. We assume that the degradation activity described by Nerurkar and Gambhir could well arise from a metabolically active part of the erythrocyte fraction, namely the reticulocytes.

Yours sincerely

Ch. Thun, X. Bihr, R. Moncayo and E. F. Pfeiffer

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Professor Dr. E. F. Pfeiffer
Zentrum für Innere Medizin
Universität Ulm
Steinhovelstraße 9
D-7900 Ulm (Donau)
FRG