one animal that had minimal evidence of hepatitis. However, the other nuclear alterations (irregularity in shape and so forth) were pronounced and seen in almost every hepatocyte.

We also examined five other chimpanzees that had non-A, non-B hepatitis after inoculation with infectious material from a variety of sources not specifically related to strains F or H. These included commercial antihemophilic factor concentrates and plasma from patients with non-A, non-B hepatitis. In addition we studied three chimpanzees with acute hepatitis type A and two chimpanzees with acute and two with chronic hepatitis type B infections (Table 1). We found the cytoplasmic structures in four and the nuclear structures in one of the five chimpanzees with non-A, non-B hepatitis. Neither structure was detected in the livers of the chimpanzees with hepatitis types A or B. Cytoplasmic and nuclear structures were never seen in biopsies from the same animal.

Additional evidence that the cytoplasmic and nuclear changes were specific for non-A, non-B hepatitis was obtained by reexamining selected biopsies under code. Coded biopsies were correctly interpreted as having been taken from an animal inoculated with strain F (cytoplasmic structures detected), strain H (nuclear changes detected), or normal, in the case of preinoculation or convalescent phase biopsies.

In chimpanzees chronically infected with hepatitis B virus (but not those acutely infected), we found structures in the cisternae of ER and core particles in nuclei of hepatocytes characteristic of hepatitis B virus infection. These structures were morphologically distinct from the cytoplasmic and nuclear structures associated with the non-A, non-B hepatitis we describe here (*5*).

Recently, we reported the detection of 24- to 27-nm viruslike particles observed in the cytoplasm of liver cells from a marmoset infected with hepatitis A virus. These particles were shown to contain hepatitis A antigen by peroxidase immunoelectron microscopy (*3*). Similar hepatitis A antigen was detected by peroxidase immunoelectron microscopy in the cytoplasm of hepatocytes from a chimpanzee infected with hepatitis A virus. However, hepatitis A virus particles could not be detected by standard thin-section electron microscopy in the hepatocytes of the three acutely infected chimpanzees studied herein. Nuclear changes were not seen in such hepatocytes. In contrast, Schaffner *et al.* (*6*), demonstrated clusters of dense heterochromatin-like granules, 35 to 40 nm in diameter, in nuclei of hepatocytes of chimpanzees acutely infected with hepatitis A virus. The nuclear structures reported here are morphologically different and have a smaller diameter.

We believe that the cytoplasmic and nuclear structures described herein are specifically associated with non-A, non-B hepatitis and that they may be of significance as markers of infection, although we cannot exclude the possibility that they might represent hitherto unknown nonspecific responses of hepatocytes to injury. The relationship of the structures to the specific infectious agent or agents of non-A, non-B hepatitis remains uncertain. Specificity will have to be shown by some means such as immunoelectron microscopy. To date, attempts to identify an antigen-antibody system for non-A, non-B hepatitis have been unsuccessful.

The possibility that there may be more than one infectious agent as the cause of non-A, non-B hepatitis is suggested by a report of multiple bouts of non-A, non-B hepatitis in patients, whose biopsies showed evidence of acute infections for each bout (*1*). The present observations of different morphological changes at the electron microscopic level produced by different inocula add support to the earlier data suggesting the possibility of more than one non-A, non-B hepatitis agent. Furthermore the finding that every transmissible agent examined by us produced one or the other morphological change offers hope that the total number of non-A, non-B virus types will be small.

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Erythrocytes: A New Cell Type for the Evaluation of Insulin Receptor Defects in Diabetic Humans

Abstract. *Human erythrocytes have specific insulin receptors. When studied in an insulin radioreceptor assay, erythrocytes from adult-onset, nonobese diabetic subjects bound at least 42 percent less insulin than the normal subjects at insulin concentrations from 0.1 to 100 nanograms per milliliter. The diabetic subjects had 190 insulin receptor sites per cell as compared with the 380 insulin receptor sites per cell for the normal subjects. The deficit of insulin binding in the diabetic subject was thus associated with a fewer number of insulin binding sites per cell with little or no change in affinity. The erythrocyte is a readily available cell for the evaluation of cellular insulin receptor activity.*

Insulin action in humans has been studied at the cell receptor level in obesity (*1–6*), diabetes mellitus (*6–10*), pregnancy (*10*), uremia (*11*), acromegaly (*12*), ataxia telangiectasia (*13*), and the syndrome of severe insulin resistance with acanthosis nigricans (*14*). Insulin receptors have been defined in human mononuclear cells (*1–3, 5, 7, 8, 13–16*), granulocytes (*17*), adipocytes (*3, 4, 6, 18*), placental cells (*10*), and cultured fibroblasts (*19, 20*). Intracellular binding sites have been identified in nuclei (*21*), on the Golgi apparatus (*22*). These studies of insulin receptors have been done with cells that, although accessible, are obtained and isolated only after time-consuming processes. The mature human erythrocyte is easily obtained, easily isolated, and is the most abundant circulating cell.

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Recently, we determined that normal human erythrocytes had the same insulin binding characteristics as human monocytes, hepatocytes, and adipocytes (*23*). Erythrocytes, thus, have specific insulin receptors. However, their physiological significance in normal and insulin-resistant man remains unclear. Having defined the character of the human erythrocyte insulin receptor in normal subjects, we now present data on the insulin receptors of erythrocytes from diabetic subjects.

Twelve nonobese, adult-onset, diabetic volunteers, ranging in age from 25 to 82 years, were compared with 17 normal volunteers, 18 to 35 years of age, with neither a family history of diabetes mellitus nor evidence of glucose intolerance. The fasting glucose levels for the diabetic subjects ranged from 125 to 486 mg/100 ml (Table 1).

Erythrocytes from the diabetic subjects bound less ^{125}I-labeled insulin than erythrocytes from the normal subjects. The 12 diabetic subjects bound a mean of 5.9 specific percent [±0.96 percent, standard deviation (S.D.)] of insulin per 3.52×10^9 cells when exposed to insulin at 100 pg/ml as compared to a mean of 10.1 specific percent (±1.4 percent S.D.) per 3.52×10^9 cells for 17 normal adults. At every insulin concentration from 0.1 to 100 ng/ml, the diabetic subjects bound significantly less ($P < .001$) insulin than the normal subjects. In the normal physiological range of insulin concentrations, from 0 to 10 ng/ml, the diabetic subjects bound at least 42 percent less insulin than the normal subjects (Fig. 1).

When the data for the diabetics and normals were subjected to Scatchard analysis (*24*), curvilinear plots were obtained. The diabetic subjects had 190 receptor sites per cell, while the normal subjects had 380.

To investigate the possibility that the diabetic subjects had antibodies or a circulating inhibitor to the receptor site, we pooled the serum from the diabetic subjects and incubated it with normal erythrocytes. After 1 hour of incubation, the binding of ^{125}I-labeled insulin to the normal subjects' cells was unchanged.

Erythrocytes from diabetic subjects have specific insulin receptors with characteristics similar to the insulin receptors found in other human and animal cells. In the diabetic subjects' and in the normal subjects' erythrocytes, there was a progressive inhibition of ^{125}I-labeled insulin binding over the entire range of insulin concentrations studied. In this study, when the erythrocytes were incubated with insulin at 0.1 ng/ml, normal subjects bound 42 percent more insulin than the diabetic subjects. Further, at each insulin concentration, from 0.1 to 100 ng/ml, the normal subjects bound more insulin. The diabetic subjects studied thus have a defect of insulin binding to erythrocytes similar to the defects previously reported on monocytes for diabetic subjects. The chemical diabetic subjects studied by Olefsky *et al.* (*7*) bound 46 percent less insulin than the normal subjects when monocytes were incubated with 0.2 ng of insulin per milliliter. Goldstein *et al.* (*9*) found that monocytes from prediabetic subjects, incubated with insulin at 0.5 ng/ml, bound 35 percent less than the normal subjects. In our study, we found a similar defect in insulin binding for the diabetic subjects.

Because valid plasma insulin levels were available for only six of the 12 patients and were not markedly elevated, it is difficult to evaluate the effect of circulating insulin levels on the defect of insulin binding in the diabetic subjects. It should be noted, however, that a defect of insulin binding and receptor number was also present in those diabetic subjects with normal insulin levels. In these patients, this defect of insulin binding in the presence of normal levels of immunoassayable insulin suggest either a genetic regulatory influence on insulin binding or some membrane receptor regulating mechanism other than the circulating levels of insulin.

The curvilinear Scatchard plot of the diabetic subjects was similar in shape to

Table 1. Diabetic patient profile.

Patients	Age	Sex	Percent ideal body weight	Fasting serum glucose (mg/100 ml)	Radioimmunoassay plasma insulin (μU/ml)
R. M.	60	F	86	225	21
S. G.	25	M	93	125	12
M. M.	43	F	102	312	31
W. L.	82	M	104	203	27
C. B.	45	M	108	146	54
R. S.	58	M	113	167	83
M. M.*	60	M	99	486	
S. G.*	58	F	115	223	
L. M.*	59	M	112	208	
M. N.*	66	F	84	300	
P. J.*	51	F	104	309	
P. F.*	43	F	116	252	

*Patients previously treated with insulin.

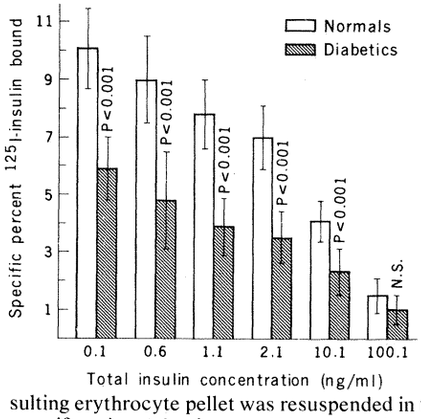

Fig. 1. ^{125}I-Labeled insulin binding to erythrocytes from adult-onset, nonobese, diabetic patients. Erythrocytes from 17 normal volunteers and 12 adult-onset, nonobese, diabetic subjects were studied. Blood (10 ml) obtained in a heparinized tube was centrifuged (15 minutes, 400*g*, 20°C). The plasma was aspirated, and the cell pellet was mixed with two parts of physiological saline and layered on a mixture of 3 ml of Hypaque (33.9 percent) and Ficoll (9 percent) (*25*). After centrifugation for 20 minutes, the saline phase, monocyte phase, Hypaque-Ficoll phase, granulocyte phase, and the upper layer of the erythrocyte phase were aspirated. The remaining cell pellet was then mixed with a double volume of saline, the above procedure was repeated, and the resulting erythrocyte pellet was resuspended in two parts of a Hepes-tris buffer (*26*). After another centrifugation (10 minutes, 400*g*, 4°C), the buffer was aspirated and the cell pellet was again suspended with a volume of buffer, resulting in a suspension containing 4.4×10^9 cells per milliliter. More than 95 percent of the erythrocytes were viable, as determined by the trypan blue dye-exclusion technique (*27*). The binding of ^{125}I-labeled insulin to human erythrocytes was determined by incubating 400 μl of cell suspension containing 1.76×10^9 erythrocytes, 50 pg of ^{125}I-labeled insulin, and various concentrations of unlabeled insulin (0 to 0.5×10^5 ng) resulting in a total volume of 0.5 ml. After incubation at 15°C for 3.5 hours, 200-μl portions of the incubated suspension were placed into chilled microcentrifuge tubes containing 200 μl of buffer G and 200 μl of dibutyl phthalate and centrifuged (Beckman Microfuge) at 4°C for 2.5 minutes. To determine the amount of ^{125}I-insulin bound, the cell pellets were cut with a heated scalpel and counted in a gamma counter (Searle, model 1185). Significant binding is indicated. Abbreviation: *N.S.*, not significant.

that of the normal subjects and to the Scatchard plots from other patients with insulin resistance (*1-10, 13, 14*). Further analysis of the data indicated that the decrease binding observed in the diabetic subjects was associated with a decreased number of receptor sites per cell as compared to the normal subjects. Erythrocytes from normal subjects had approximately 50 percent more insulin binding sites than those from patients with adult-onset diabetes mellitus, whereas the monocytes from normal subjects reported by Olefsky and Reaven (*7*) also had approximately 50 percent more sites than those from patients with chemical diabetes.

Although the physiological significance of the decrease in receptor number is unclear, it is possible that this decreased number of receptors in the diabetic subjects is in part responsible for the cellular metabolic deficits that enhance the morbidity associated with this insulin resistant state.

In summary, these studies demonstrate that insulin binding to erythrocytes is decreased in patients with adult-onset diabetes. This defect of insulin binding is associated with a decrease in the number of insulin receptor sites per cell. The erythrocyte is a readily available cell for the evaluation of cellular insulin receptor activity. Human erythrocytes may also be useful for studying the actions of other hormones at the cellular level.

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Angiotensin Convertase Activities in Human Alveolar Macrophages: Effects of Cigarette Smoking and Sarcoidosis

Abstract. *Angiotensin I convertase activity has been found in human alveolar macrophages from normal volunteers and patients with pulmonary sarcoidosis. This activity is higher in the alveolar macrophages from smokers than from nonsmokers, and is even more elevated in sarcoid patients. The activity can be detected with both angiotensin I and bradykinin analogs and appears to require protein synthesis, but the enzyme is not secreted by alveolar macrophages in culture.*

The activity of angiotensin I convertase (ACE) in the serum is increased in patients with the granulomatous disorder, sarcoidosis (*1-5*). The enzyme ACE is well recognized as a vascular endothelial cell ectoenzyme (*6*), and is also present in sarcoid granulomata (*7*). The cell types present in granulomata in general include the tissue macrophage and its close relative, the epithelioid cell (*8*). One type of tissue macrophage, the pulmonary alveolar macrophage (AM) of the rabbit does not normally exhibit ACE activity, but such activity may be induced when the cells are cultured in the presence of $10^{-7}M$ dexamethasone (*9*). We confirmed this observation and obtained similar results with the addition of beclamethasone dipropionate ($10^{-7}M$) to the culture medium (*2*). In the experiments described here, we examined the ACE activities of human AM's from both healthy subjects (cigarette smokers and nonsmokers) and patients with sarcoidosis to determine whether such cells contribute to the increased serum ACE activity in sarcoidosis.

The ACE activities in the cells from the three groups of subjects are shown in Fig. 1. In Fig. 1A, the ACE activity is expressed per milligram of cell protein. On this basis, when compared with the activity in nonsmokers, the ACE activity is increased in the cells from both smokers and sarcoidosis patients ($P < .05$). Although the lavage cells from all three groups contained from 1 to 2 percent polymorphonuclear leukocytes, the cell populations in these groups differed. The cells from smokers were 98 ± 2 percent AM [mean ± 1 standard deviation (S.D.)]. However, in nonsmokers and patients with sarcoidosis, the AM comprised 88 ± 8 percent and 77 ± 10 percent, respectively. The remaining cells were lymphocytes as judged by morphologic examination of cells treated with Wright's stain and the failure of these cells to stain with nonspecific esterase stains. The differential counts by these two methods were in close agreement. Since we could not detect ACE activity in a lymphocyte-rich- (75 percent) human peripheral blood mononuclear cell preparation (separated by Ficoll-Hypaque gradient), we have expressed the ACE activity on the basis of 10^7 AM (Fig. 1B). These data indicate that, compared with AM's from nonsmokers, the ACE activities in AM's from smokers and patients with sarcoidosis are, respectively, three and five times greater ($P < .01$).

To define further the characteristics of the ACE activity in AM's, we used SQ 20881 (H-<Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro-OH), a specific inhibitor of angiotensin convertase (*10*). With hippuryl-l-histidyl-l-leucine as substrate, SQ 20881 ($10^{-5}M$) totally inhibited the activity of the macrophage enzyme. A single enzyme acts as a dipeptidyl carboxypeptidase catalyzing both the conversion of angiotensin I to angiotensin II and the hydrolysis of bradykinin (*11, 12*). Since the hippuryl-l-histidyl-l-leucine substrate

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