test after washing and resuspending in MEM+10% serum. Cells grown in spinner flasks as cell suspensions were washed from MEMJ+10% serum and resuspended in MEM+10% serum to reconstitute the normal concentration of Ca and Mg ions able to support adhesion.

Results. The rate of adhesion of a viable cell population treated for 10 min with trypsin is shown in Figure 1, compared with the rate of adhesion of untreated cells grown in suspension (spinner culture). The 2 functions follow an exponential pattern during the first hour followed by a plateau and a secondary exponential phase of growth. Trypsin-treated cells have a slower rate of adhesion than untreated ones showing a less pronounced plateau before the start of the cellular growth.

Cells treated for longer periods of time by trypsin (Figure 1) show proportionally slower rates of adhesion in which no plateau is present. This may be due to the fact that after cell growth is started, the adhesion of part of the population is still taking place and therefore the function obtained is the resultant of the simultaneous presence of these 2 phenomena.

The same experiment performed on EDTA-treated cells is shown in Figure 2. The rate of adhesion follows an irregular pattern during the first hour and later falls to zero, showing that these cells were not viable despite their capacity to adhere during the first few hours. This late toxicity of EDTA is also evident in the 20 min sample that reaches the value of the initial population only after 48 h. This sample, however, was released in suspension by scraping because its time of contact with EDTA was too short to release in suspension the cells.

Figure 3 reports the 3 functions obtained for nonadhering cells. It appears that 10 min trypsin-treated cells, EDTA-treated cells, and spinner cultured cells, all disappear from culture medium at the same rate while 30 min trypsin-treated cells disappear at a lower rate. This conclusion was reached by performing analysis of variance on the data obtained.

Discussion. It may first be noted that by the approach of measuring both the rate of adhesion of living cells and the rate of disappearance of non-adhering cells, it becomes much easier to separate the true process of cell adhesion from the cell growth and death, normally present in every cell population. In this way it can be seen that EDTA and trypsin have quite a different effect on adhesion and on the subsequent growth of KB cells.

EDTA does not affect adhesion but, in agreement with data on riaggregation[3], shows quite a toxic effect on living cells. EDTA being unable to modify the rate of disappearance of non-adhering cells, it appears that a non-viable population of cells may adhere to glass at the same rate as a living one.

Trypsin is able to decrease the rate of adhesion and apparently reduces the lag, period before growth resumption. Since any trypsin possibly adsorbed on the plasma membrane during the treatment[4] is readily inactivated by the serum added to the culture medium[7], the hypothesis that membrane adsorbed trypsin could interfere with the process of adhesion can be ruled out. At the contrary, the decrease of the rate of adhesion being proportional to the time of contact of cells with trypsin, the effect on adhesion may be interpreted as a removal of membrane proteins, as was suggested by other authors[8] investigating the process of cell aggregation.

In conclusion, the adhesion of KB cells to a glass surface is a process not necessarily related to the viability of the cell population. EDTA and trypsin, in the experimental conditions here utilized, may well serve the purpose to demonstrate this dissociation and to help to understand the mechanisms involved in cell adhesiveness[9].

Riassunto. La velocità di adesione al vetro di cellule pretrattate con Tripsina ed EDTA è stata comparata a quella di cellule cresciute in sospensione, utilizzando un metodo che permette di valutare la popolazine di cellule adese vitali. I risultati mostrano che il pretrattamento con Tripsina riduce la velocità di adesione senza ridurre la vitalità delle cellule, mentre il trattamento con EDTA pur essendo citotossico non altera la velocità di adesione.

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The Effect of Glibenclamide on Non-Esterified Fatty Acid Levels in the Plasma of the Severely Alloxan Diabetic Rat

In addition to their ability to stimulate insulin release from the pancreatic β-cell, it has also been postulated that the hypoglycaemic sulphonylureas exert an effect at certain extrapancreatic sites[1,2]. BEWSHER and ASHMORE[3] observed a direct inhibition of hepatic lipase activity by tolbutamide, and WEISS et al.[4] showed glycodiazin would inhibit triglyceride lipase bound to the lysosomal structure. These results explain the often observed inhibition of ketogenesis in vitro by the hypoglycaemic sulphonylureas.

SCHMIDT et al.[5] showed a reduction in the plasma concentration of non-esterified fatty acid (NEFA) and β-hydroxybutyrate in alloxan diabetic rats 1 h after the i.p. injection of glibenclamide 0.5 mg/kg. The effect on plasma β-hydroxybutyrate has been confirmed by other workers[6]. SCHMIDT[5] observed no change in plasma insulin levels at sacrifice both histologically and immunologically and suggested that an effect of glibenclamide on adipose tissue lipase was responsible for the fall in NEFA observed[7]. The animals used in the latter investigation were given alloxan i.p. (75 mg/kg), and left for 10 days to develop stable diabetes. Animals were then selected for the experimental group on the basis of blood sugars in excess of 300 mg/100 ml. In our laboratory rats given alloxan 75 mg/kg i.v. developed diabetes after 48 h. of a severity which closely paralleled that expected after pancreatectomy. Indeed, treatment of these rats with 100 mg/kg glibenclamide orally every 5 h for 15 h (4 doses in all), produced no detectable elevation of the plasma insulin level (FOY and STANDING, unpublished observation). The experiment of SCHMIDT was, therefore, repeated and then a similar investigation was carried out using our own animal model, comparing the response to glibenclamide with tolbutamide and chlorpropamide.

Materials and methods. Male C.S.E. (Bradford Strain) rats weighing 200–220 g, were used in all experiments. Diabetes was induced by slow i.v. injection of 75 mg/kg

alloxan monohydrate in 0.9% saline, and all animals were allowed food and water throughout the experimental period. The degree of diabetes induced was indicated by the condition and wasting of the animals, by positive urinary glucose and ketone (Labstix), and by high (> 300 mg/100 ml) blood sugar at death.

48 h after the alloxan injection, rats assessed as ketotic diabetic were divided into 2 groups. The first group was given 0.5 mg/kg glibenclamide i.p., the second group saline 1.0 ml/kg, and, after the appropriate time interval (15, 30, 60 min) the animals were stunned and 3 ml venous blood rapidly removed from the dorsal vena cava, just above the renal artery. Blood sugar was determined from this sample by the method of ASATOOR and KING[8]. The remaining blood was centrifuged at 2,500 rpm for 10 min at 3 °C, and the plasma assayed for NEFA content by the colorimetric method of ITAYA and UI[9], and for immunoreactive insulin content by the method of HALES and RANDLE[10]. This method has been shown to be sensitive to insulin levels well within those detected in this study and although standardized on beef insulin to measure effectively mouse and rat insulins[11,12]. The experiment was repeated using tolbutamide and chlorpropamide 50 mg/kg i.p.

The experimental procedure of SCHMIDT et al.[5] was also carried out. Rats were given alloxan 150 mg/kg i.p. and on the morning of the 10th day, a 0.1 ml blood sample was taken from the tail vein for blood sugar determination. Animals with blood sugar above 300 mg/100 ml, high urinary glucose + ketone (Labstix) and loss of weight, were selected and divided into 3 groups. 1 group served as control, the other 2 groups were given glibenclamide 0.5 mg/kg i.p. 1 group was sacrificed after 15 min, the other after 1 h, and blood glucose, insulin and NEFA in the plasma was determined in each case.

Results. Animals given 75 mg/kg alloxan 2 days previously showed the expected significant increase in the level of plasma NEFA and blood glucose (Figure 1). 1 h after glibenclamide, a marked suppression of plasma NEFA was observed without a corresponding fall in blood glucose. Tolbutamide and chlorpropamide (50 mg/kg), had no significant effect on either parameter. Figure 2 shows a small, but significant elevation of plasma insulin levels in the glibenclamide treated group after 1 h, whereas administration of tolbutamide and chlorpropamide had no significant effect. Using a separate group of animals, the plasma insulin levels were determined 15 and 30 min after the glibenclamide dose. Plasma insulin levels were markedly elevated at 15 min, whilst at 30 min and 1 h, the elevation was small but significant (Figure 3).

The experiment based on that of SCHMIDT[5] showed a significant fall in plasma NEFA, without a concomitant depression of blood glucose levels. However, plasma insulin levels were elevated significantly in the glibenclamide treated group after 15 min and 1 h (Figure 4).

Discussion. The observation that tolbutamide inhibits cyclic AMP phosphodiesterase in certain tissues[12] provides a paradox when considering tolbutamide as an antilipolytic agent. Such an inhibition would result in an increased cellular level of 3′5′-AMP and hence an expected lipolytic effect on the adipose tissue cell. However, tolbutamide's action has been shown to be unrelated to any change in cellular cyclic AMP levels[13,14]. CHAN and FAIN[15] have suggested that both tolbutamide and glibenclamide could inhibit lipolysis by uncoupling oxidative phosphorylation but tissue ATP levels did not appear to

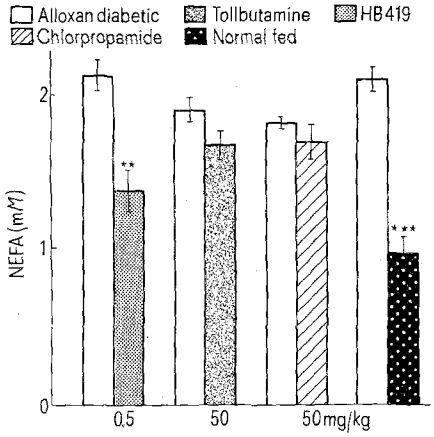

Fig. 1. The effect of induction of diabetes with alloxan 75 mg/kg i.v., on the plasma concentration of NEFA, and the influence of glibenclamide (HB 419), tolbutamide and chlorpropamide on the plasma level of NEFA, 1 h after i.p. administration ot the alloxan diabetic animal. n = 10–18 observations. Mean ± S.E. ** = $P < 0.01$.

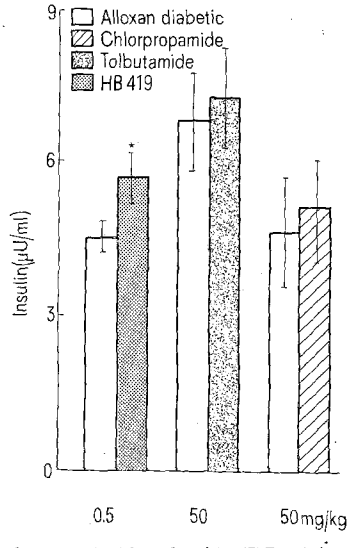

Fig. 2. The influence of glibenclamide (HB 419), tolbutamide and chlorpromapide on the plasma immunoreactive insulin concentration, 1 h after i.p. administration to the alloxan diabetic animal. n = 16–18 observations. Mean ± S.E. * = $P < 0.05$.

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be appreciably changed[14,17]. CORBIN and KREBS[18] and HUTTUNEN et al.[19] have demonstrated the existence of a 3′5′-AMP sensitive protein kinase which is responsible for the activation of adipose tissue triglyceride lipase. BROWN et al.[14] suggested that tolbutamide could inhibit lipolysis by inhibiting the binding of 3′5′-AMP to this protein kinase.

Many earlier clinical studies have shown that sulphonylurea drugs depress plasma NEFA levels[20–23], but none of these observations in maturity onset patients provide any evidence that the antilipolytic effect is mediated other than by improvement in the pancreatic insulin response. Clinical evidence from STONE and BROWN[24] showed that a single dose of acetohexamide or tolbutamide to ketoacidosis resistant diabetic patients caused an initial fall in plasma NEFA, although blood sugar showed no initial change. The blood sugar decrease was maximal at 6 h in these experiments, whereas a maximal fall in plasma NEFA occurred at 2 h. This early fall in plasma NEFA followed by a more latent blood sugar depression is a phenomena often observed when low doses of insulin are given to the pancreatectomised animal[25], the fat cell being uniquely sensitive to administered insulin[26]. KOVACEV and SCOW[27] using in vivo rat parametrial perfusion techniques in the pancreatectomized animal have shown that on the administration of 2 IU of insulin, a gradual reduction in blood glucose and ketone bodies was observed, reaching a maximal reduction in 100 min, whereas NEFA levels were completely suppressed at 30 min.

LOUBATIERES et al.[28] have stated that as little as $^1/_{12}$ of the normal amount of functional pancreatic tissue will still produce an insulinogenic response from the experimental animal. Although our animals were considered chronically diabetic, this, together with the high potency and intrinsic activity of glibenclamide did produce a pancreatic response 15 min after i.p. injection. Loubatieres also stated that glibenclamide may be 240 × more potent than its more classical sulphonylurea counterparts; tolbutamide and chlorpropamide which failed to elicit a detectable response.

The experiments of SCHMIDT were repeated in an attempt to confirm results from their laboratory. However, as can be seen in the diabetic animals so produced, some plasma insulin was always detectable after the 10-day period, and a plasma insulin reponse was seen in all animals receiving the i.p. glibenclamide dose. We were, therefore, unable to confirm their results, and unable to rule out at least a partial pancreatic locus of action for the observed glibenclamide suppression of NEFA in the alloxan diabetic animal.

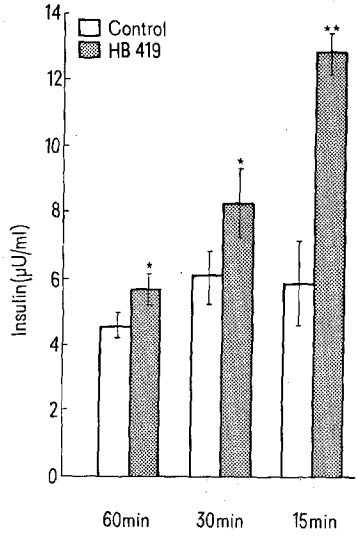

Fig. 3. The influence of glibenclamide (HB 419) on the plasma immunoreactive insulin concentration, 15, 30 and 60 min after i.p. administration to the alloxan diatebic animal. Mean ± S.E. $n = 16$–18 observations. * = $P < 0.05$. ** = $P < 0.01$.

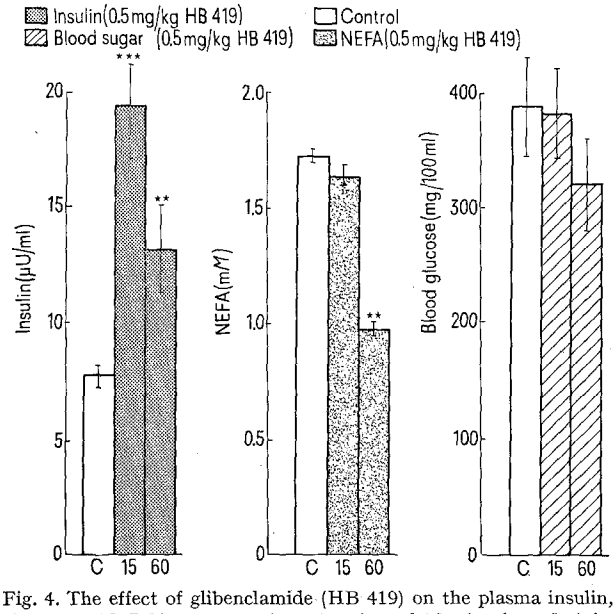

Fig. 4. The effect of glibenclamide (HB 419) on the plasma insulin, glucose and NEFA concentrations, 15 min and 1 h after i.p. administration to rats given 150 mg/kg alloxan i.p. 10 days previously and assessed as stable diabetic animals on the morning of the experiment. $n = 8$–10 observations. Mean ± S.E. ** = $P < 0.01$; *** = $P < 0.001$.

Zusammenfassung. Zugabe von Glibenclamid bei Alloxan induzierten, stark zuckerkranken Ratten vermindert den Gehalt freier Fettsäuren im Blutplasma. Kein entsprechend antilipolytischer Effekt wurde bei Wiederholung des Experimentes beobachtet, wenn Chlorpropamid und Tolbutamid hundertfach stärker als Glibenclamid verabreicht wurde.

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