ditions, the soluble enzyme may be purified, firstly by chromatography on Sephadex G-200, then by electrophoresis on a polyacrylamide gel. At the end of this last operation, we observe a proteinic band which corresponds, after elution, with the maximum enzymatic galactose ^{14}C transfer activity on an exogenous glycoproteinic acceptor.

The solubilization process employed permits the conservation of an enzymatic activity in the soluble proteinic fractions. However, its yield is low, and the purification obtained does not allow one to conclude a pure enzymatic protein. Moreover, the technic cannot be used for a large preparative purpose. Thus other technics, such as electrofocusing, are being studied now.

Effects of Long-Term Feeding of Glibenclamide on Normal Rats

A. V. GEORGE and K. T. AUGUSTI

Department of Biochemistry, University of Kerala, Trivandrum (Kerala, India), 10 November 1975.

Summary. Prolonged administration of glibenclamide decreased blood sugar, liver glycogen and protein and increased liver and serum lipids and organic phosphates of liver in normal rats. A significant weight increase observed in glibenclamide group of rats is attributed to lipid accumulation.

The hypoglycemic action of sulphonylureas has been attributed mainly to their pancreatic stimulation of insulin secretion[1] or release of bound insulin[2,3]. In addition to their ability to stimulate insulin release from the β-cells of pancreas, it has also been suggested that these drugs exert an effect at certain extrapancreatic sites[4,5]. According to some reports, sulphonylureas exert activity independent of insulin, viz. the increased uptake of glucose by rat diaphragm[6,7] and fat pad[8], hypoglycemia without raising plasma insulin levels[9], ameliorating diabetic symptoms in cases where insulin alone proved inadequate[10]. BEWSHER and ASHMORE[11] observed a direct inhibition of hepatic lipase activity by tolbutamide, and WEISS et al.[12] showed that glycodiazin would inhibit triglyceride lipase bound to the lysosomal structure. Some of these results would explain the hyperlipidaemia observed in patients[13] and experimental animals[14] after long-term use of sulphonylureas. FOY and STANDING[15] found that the effect of glibenclamide on the insulin-secreting mechanism and its inhibitory effect on plasma non-esterified fatty acid release in alloxan diabetic rats were higher than that of other sulphonylureas. In 2 previous studies[14,16], long-term administration of tolbutamide and phenformin produced lipid accumulation in normal rats. In order to study the long-term effect of glibenclamide, which is 240 × more potent than other sulphonylureas[15,17], the present study was made.

Materials and methods. Young growing male Wistar rats (average weight 65 g) were divided into 2 groups of 12 animals each. They were fed ad lib. with normal laboratory diet. Group I rats were kept as control. Group II rats were orally given glibenclamide (dose 50 µg/kg/day). A fine suspension of glibenclamide in water (0.5 mg/ml) was prepared daily, diluted 100 times and from a dropper measured quantities of the same (1 ml/100 g body wt.) were administered into the mouth of each rat. Group I rats were given equal volums of water in a similar manner. During other times, all the rats were drinking water ad lib. The increase in weights of both the groups of rats were recorded monthly. After a period of 2 months, 6 animals from each group were starved for 6 h and then autopsied. Their liver glycogen was estimated by the method of CARROL et al.[18]. The remaining rats were starved for 18 h and their blood was collected from tail for blood sugar estimation. Later they were sacrified by decapitation. Following a previous procedure[19], blood was collected from the jugular vein and the sera and livers were separated for various estimations. Blood sugar was estimated by the method of ASATOOR and KING[20] using alkaline copper reagent[21]. Liver weights of each group were recorded and protein from the liver was separated as reported previously[14] and was estimated by LOWRY's method[22], using FOLIN CIOCALTEU reagent[23]. The protein values were calculated using a standard checked by Kjeldhal nitrogen determination. The acid-soluble, free amino acids of liver were neutralized and estimated by theninhydrin method of MOORE and STEIN[24]. Leucine was used as the standard. Lipid from liver was extracted by the method of ENTENMAN[25]. Total lipids in

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Liver (weight/100 g wet tissue)	Normal group	Glibenclamide group
Glycogen (g)	3.8 ± 0.20	2.4 ± 0.10[d]
Protein (g)	13.2 ± 0.20	11.6 ± 0.50[a]
Total lipids (g)	3.0 ± 0.1	4.4 ± 0.02[d]
Phospholipid (g)	2.0 ± 0.04	3.0 ± 0.08[d]
Triglyceride glycerol (mg)	332.5 ± 3.0	409.0 ± 1.2[d]
Total cholestrol (mg)	211.0 ± 3.0	264.2 ± 4.0[d]
Free cholestrol (mg)	126.5 ± 2.5	160.6 ± 5.0[d]
Aminoacids (mmole)	2.76 ± 0.20	1.81 ± 0.20[b]
Extra labile phosphate (mmole)	2.7 ± 0.01	3.0 ± 0.01[d]
Labile phosphate (mmole)	0.55 ± 0.1	0.80 ± 0.03[d]
Total phosphate (mmole)	4.6 ± 0.07	6.2 ± 0.1[d]
Liver weight (g)	6.0 ± 0.05	4.8 ± 0.10[d]
Serum (mg/100 ml)		
Blood sugar	61.2 ± 1.0	48.0 ± 0.30[c]
Total lipids	214.0 ± 3.0	284.0 ± 8.0[d]
Phospholipid	118.6 ± 1.5	139.3 ± 5.0[c]
Triglyceride glycerol	22.1 ± 0.4	29.7 ± 1.0[d]
Total cholesterol	59.6 ± 0.6	73.6 ± 0.8[d]
Free cholestrol	20.8 ± 0.2	25.8 ± 0.3[d]
Increase in body weight (g)		
After 1st month	74.1 ± 3.48	97.6 ± 5.28[d]
After 2nd month	131.0 ± 2.6	150.6 ± 2.41[a]

Mean values of 6 rats in each group ± SE. Student's t-test. The values of the glibenclamide group are significantly different from those of the normal group. [a] $p < 0.05$; [b] $p < 0.02$; [c] $p < 0.01$; [d] $p < 0.001$.

serum and liver were determined gravimetrically, as described by SPERRY and BRAND[26]. Total cholestrol was estimated by the method of CARR and DREKTER[27], free cholestrol by the method of SCHOENHEIMER and SPERRY[28], phospholipid by the method of ACKERMANN and TORO[29], and triglyceride by the method of VAN HANDEL and ZILVERSMIT[30]. The extra labile, labile and total phosphates of liver were determined by the method of FISKE and SUBBA ROW[31], following the modifications suggested by LELOIR and CARDINI[32].

Results. The results are recorded in the Table. Prolonged administration of glibenclamide produced significant effects as compared with the control values. Blood sugar ($p < 0.01$), liver glycogen ($p < 0.001$), protein ($p < 0.05$) and amino acids ($p < 0.02$) decreased significantly. Extra labile, labile and total phosphates of liver, triglycerides, phospholipids, free and total cholestrol and total lipids of liver and serum, all increased significantly ($p < 0.001$, except for serum phospholipid where $p < 0.01$). The lipid-raising effect of glibenclamide is more pronounced on the liver (47%) than on the serum (33%), and it is largely due to the increase in phospholipid and cholestrol. However the triglyceride was more increased in the serum (35%) than in the liver (23%). There was significant increase in body weight of the glibenclamide group as compared with the control. The increase in weight was highly significant after the 1st month ($p < 0.001$) and just significant after the 2nd month ($p < 0.05$). However the liver weigths of the glibenclamide group were significantly lower than those of the control ($p < 0.001$).

Discussion. As liver is the site of synthesis of proteins, the prolonged use of glibenclamide through its insulin-dependent and other actions on the liver might have affected the general metabolism. Previous experiments[14, 33] showed that tolbutamide and biguanides impair the liver with prolonged use to synthesize protein or glycogen. The results with glibenclamide also agree with those of tolbutamide. Just as with tolbutamide[14] glibenclamide decreased blood sugar, liver glycogen and liver protein and increased liver and serum lipids. The accumulation of lipids in the livers of the drug-treated group might have impaired the liver and retarded protein and glycogen synthesis. The lowered amino acid content of the liver may also be an adverse effect of the drug.

It has been found by several workers[34–36] that sulphonylureas inhibit lipolysis. Such effect of the drugs may contribute to the accumulation of lipids on prolonged use[13, 14]. In our findings, glibenclamide feeding produced certain effects similar to those of insulin, i.e. decreasing blood sugar, and increasing cholestrol and acid labile phosphate, but unlike insulin it raised the lipids. Its lipid-raising effects might not be insulin dependent as the hormone corrects lipid abnormalities. Hence the mechanism of action of glibenclamide cannot be definitely stated.

The prolonged use of glibenclamide has induced a derangement in the metabolism of protein and lipids, since even though there was a significant increase in body weight of the glibenclamide group of rats, that increase did not reflect in their liver weights. Actually this group had significantly lower liver weights as compared with the control group ($p < 0.001$). Lowered liver protein levels of the glibenclamide group indicate that the weight increase may not be due to protein retention but rather due to lipid accumulation. As chlorinated aromatic compounds have an adverse effect on liver (such as the production of fatty livers[37]) the effect of glibenclamide, a compound with a chlorinated aromatic ring, on liver is also a matter of concern. A detailed study of its effect on various enzymes such as lipogenic, lipolytic, glycolytic and glycogen and protein synthetic enzymes is warranted.

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