Extreme values of the 3 analytical values are represented in Figures 1 and 2. Protein was determined by the method of LOWRY et al.[10]. For the study of longevity, adult flies were maintaind at 25 °C. The flies were kept in small plastic cages with approximately 200 flies of each sex per cage and food was removed every other day. Flies were removed every 24 h and immediately processed.

Results and discussion. Figure 1 shows the results obtained during development of the insect. As it can be seen, the concentration of the nucleotide reaches a peak practically just at apolysis with a sharp decline in the pharate adult stage. As has been reported previously[4], the amount of adenyl cyclase increases during this stage; therefore it is quite possible to imagine that the much lower cyclic AMP level could indicate the presence of potent cyclic AMP phosphodiesterase activity in splitting the cyclic AMP. With this regard, we must underline that it is generally considered that phosphodiesterase governs the tissue level of cyclic AMP[11]. The possibility that this enzyme may be the rate limiting step for maintaining steady state levels of cyclc AMP in the insect must be determined. Levels of cyclic AMP are close to those described by KUO et al.[12] in silkmoth fat body. We must point out also that the fact that *Ceratitis capitata* produces cyclic AMP is not surprizing, since it has been shown to exhibit cyclic AMP dependent protein kinase[13].

Due to the ubiquity of cyclic AMP in nature and the tissues of different organisms, and because of the different nature of processes influenced by this nucleotide, it would play a very important role in the ageing phenomena. We have determined the levels of cyclic AMP during longevity, as is shown in Figure 2. A gradual increase takes place through the longevity reaching a maximum plateau at the end of life. Although it is very hard to explain such a pattern during ageing, one could think in a gradual maturation[14] of biochemical systems. Such phenomena in some enzymes in insects has been demonstrated and the increase of activity after several days of emergence has been related to the initiation of the flying activity[15,16].

The significance of the presence of cyclic AMP in *Ceratitis capitata* can only be speculated, but it can be imagined that the insect contains a primitive control system with several features in common with the intercellular metabolic system found in vertebrates.

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Glycoprotein Biosynthesis in Splenic Cells. Purification of a Microsomal Galactosyl-Transferase[1]

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Summary. One part of the microsomal galactosyl-transferase activity of splenic cells of rats can by solubilized by the action of Triton X-100 and Tween 20. Its purification on a Sephadex G-200 column and by electrophoresis on a polyacrylamide gel leads to a solution of high specific enzymic activity.

Previous work[2,3] has shown that diverse glycosyl-transferases activities exist in splenic cells. From this, we have been able to locate a mannosyl-transferase, a N-acetyl-glucosaminyl-transferase, a sialyl-transferase, a fucosyl-transferase and a galactosyl-transferase.

A more refined subcellular fractionation shows that these enzymes are distributed in the microsomal fractions sedimenting in the different zones of a sucrose gradient[2] or in the mitochondria[3]. Amongst the diverse galactosyl-transferases prepared from different tissues, some have microsomal localization[4,5], others are in a soluble state in the cell sap[6], and yet others are integrated in the membranal structures, but can be dissolved by the careful action of some detergents[7]. The microsomal galactosyl-transferase of splenic cells belongs to this last group. Conditions for its purification are the subject of this paper.

Material and methods. The preparation of microsomal fractions and the control of their quality are extensively

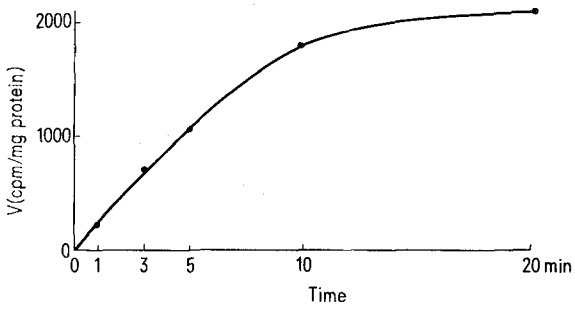

Fig. 1. Kinetic study of galactosyl-transferase (*Tris*-HCl buffer, 0.05 *M*, pH 6.8).

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described elsewhere[3]. The determination of galactosyl-transferase activity is made according to[8], using fetuine (Sigma), chemically treated to remove sialic acid and galactose, as exogenous acceptor.

Results. 1. Parameters of the galactosyl-transferase activity in the splenic cells. Figure 1 shows the kinetics of fixation of ^{14}C galactose on the endogenous acceptor. For the microsomal enzyme, there is no difference between the activities on endogenous or exogenous acceptor. Figure 2 shows that the optimal pH is equal to 6.8. Figure 3 shows the optimal temperature to be near 38 °C for the microsomal enzyme and 22 °C for the solubilized enzyme.

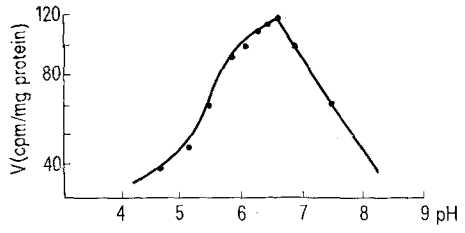

Fig. 2. Influence of pH on galactosyl-transferase activity (*Tris*-citrate buffer, 0.045 *M*).

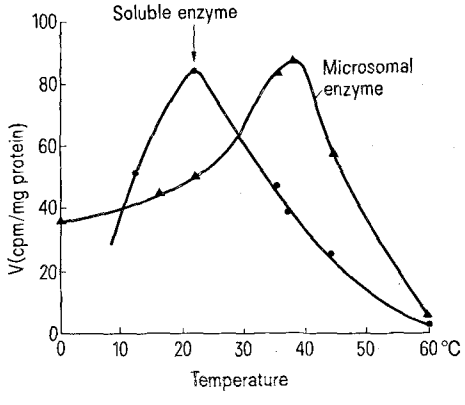

Fig. 3. Influence of temperature on galactosyl-transferase activity (*Tris*-HCl buffer 0.05 *M*, pH 6.8).

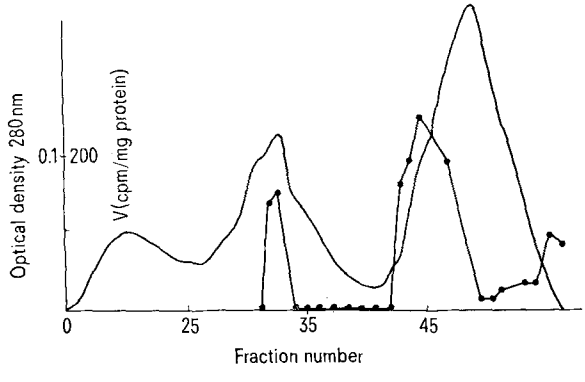

Fig. 4. Chromatography on Sephadex G 200 of galactosyl-transferase after solubilisation by Tween 20 (column 2.5 × 100, 15–20 ml/h, 1 fraction = 30 min). —, O.D. 280 nm; ▲—▲, specific activity of galactosyl-transferase (cpm/mg protein), incubation at 22 °C, exogenous acceptor.

2. Solubilization of the microsomal galactosyl-transferase by tensioactive agents. a) Effect of Triton X-100. The microsomes are incubated in a *Tris*-HCl 0.05 *M* pH 7.2 buffer at +4 °C under rapid agitation, in the presence of Triton X-100 (Triton/protein ratio equal to 1/1,000) for 30 or 60 min. After sedimentation of the suspension over 60 min at 145.000 *g*, the galactosyl-transferase activity on the endogenous and exogenous acceptor in the pellet and the supernatant is evaluated. This activity cannot be detected in the absence of exogenous acceptor in the supernatant. With the exogenous acceptor, 7% and 16% of this activity can be found in the supernatant for an incubation of 30 and 60 min respectively.

b) Effect of the Tweens. The microsomes are incubated in a *Tris*-acetate 0.12 *M* pH 7.2 buffer at +4 °C under rapid agitation for 3 min, in the presence of 2 different concentrations of Tween 20, 40, 60 and 80. After sedimentation under the same conditions as above, the galactosyl-transferase activity is distributed between the pellet and the supernatant as follows: without Tween, 0% is found in the supernatant, but 10%, 6%, 6% and 4% when Tween 20, 40, 60 or 80 is used respectively.

Thus it appears that the galactosyl-transferase can be solubilized by the Triton X-100 or by the Tween only in low proportions, but sufficiently to make a purification possible.

3. Purification of the galactosyl-transferase solubilized by the Tween 20. After solubilization by the Tween 20 under the above conditions, the galactosyl-transferase is chromatographied on a Sephadex G-200 column. 2 main peaks appear (Figure 4). The central fractions of peak 2 are chosen for purification by electrophoresis on a polyacrylamide gel. They are concentrated 10 times and after electrophoresis the gel is cut into sections: each section is eluted in a *Tris*-HCl 0.05 pH 7.2 buffer for 30 min at +4 °C (0.5 ml of buffer per section). Each eluate is studied for its galactosyl-transferase activity on exogenous acceptor. Parallel gels are coloured with Coomassie blue (proteins) and periodic acid-Schiff (glycoproteins). The results obtained are schematized in Figure 5.

Discussion. By the use of tensio-active agents, it is possible to detach a fraction of the enzyme from the membranal structures (between 10 and 16% of the total activity in the best cases). The best solubilization is obtained by Triton X-100 and Tween 20. Under these con-

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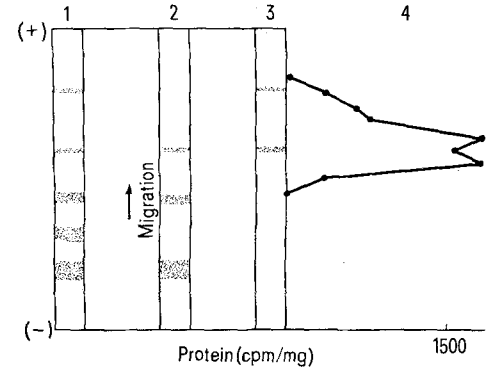

Fig. 5. Electrophoresis on polyacrylamide gel of peak 2 (from Sephadex column). 1. Protein (Coomassie blue) from Tween 20 supernatant. 2. Glycoproteins (periodic acid Schiff) from Tween 20 supernatant. 3. Protein (Coomassie blue) from central fractions of peak 2 (from Sephadex column). 4. Galactosyl-transferase activity, on exogenous acceptor, after elution from polyacrylamide gel (0.5 ml *Tris*-HCl buffer 0.05 *M*, pH 7.2, 30 min, 4 °C, per section).

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

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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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