inferred that VPA indeed acts via potentiation of GABAergic transmission. However, this interpretation cannot be sustained on the basis of the experiments dealing with the interaction of intraperitoneally given VPA with iontophoresed GABA and of VPA with GABA-mediated transsynaptic inhibition. Assuming that VPA acts through potentiation of GABAergic transmission one would expect a potentiation of GABAergic transsynaptic inhibition when the drug is applied in anticonvulsant doses. However, in our experiments we did not observe such an effect. Thus, in conclusion, our results indicate that the anticonvulsive mode of action of VPA is not mainly due to a potentiation of central inhibitory GABAergic transmission. Alternatively, a direct effect of VPA upon neuronal membrane properties has been suggested recently (Slater & Johnston 1977).

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Transport of liposome components in rat everted intestinal loops

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Many therapeutic agents that are poorly absorbed or biodegraded in the intestine have to be given parenterally with inconvenience and risk. A novel approach has recently been indicated through the use of phospholipid vesicles (liposomes) (Tyrrell et al 1976; Patel & Ryman 1976; Gregoriadis 1976; Dapergolas & Gregoriadis 1976; Dapergolas et al 1976). Oral administration of liposome-entrapped insulin caused a significant reduction of blood sugar concentrations in diabetic rats. However, in normal rats the insulin entrapped in egg yolk lecithin vesicles failed to produce such an effect (Patel & Ryman 1976; Dapergolas & Gregoriadis 1976). A possible explanation for the differences in the results between normal and diabetic rats could have been due to a difference in the rate of liposome transport through the gut wall. To test this possibility, we studied the transport of radioactively labelled constituents of liposomes through rat everted intestinal loops.

The efficiency of insulin entrapped in liposomes in lowering blood glucose concentration depended on the composition of the liposomes (Dapergolas & Gregoriadis 1976; Dapergolas et al 1976; Gregoriadis 1976). This also might be due to differences in the rate of uptake of various liposomes preparations through the gut wall. To examine this possibility, we tested the permeability of everted intestinal loops to labelled cholesterol and phosphatidylcholine incorporated in two types of liposomes, namely large multilamellar vesicles (LMV) and small unilamellar vesicles (SUV) and composed of various phospholipids.

Pure egg phosphatidylcholine (Egg PC, Makor Chemicales, Jerusalem), dipalmitoyl phosphatidylcholine (DPPC, Sigma), dicetylphosphate (DCP, Sigma) and cholesterol (CH, Merck), were obtained as commercial products and were used without further purification. Labelled ^{14}C-CH (CH, 57·7 mCi mol^{-1}) was purchased from Amersham and ^{14}C-PC (PC, 50–60 mCi mol^{-1}) was prepared according to Asher et al (1969).

Liposomes were prepared from PC and CH in the absence or presence of DCP (molar ratio—7 : 2 : 1). They were labelled either by ^{14}C-CH or by ^{14}C-PC. The components were dissolved together in chloroform, the solutions evaporated to dryness under vacuum and the residue dispersed in Krebs solutions to a final PC concentration of 5% (w/v), unless otherwise specified. Multilamellar liposomes (LMV) were obtained by mixing the aqueous suspensions. Small unilamellar vesicles (SUV) were prepared by sonication of the latter aqueous dispersions (by Heat System Model 350 sonicator) until they were clear (10–20 min).

Everted intestinal loops were prepared from normal and diabetic albino rats (150–180 g) of the Hebrew University (Sabra) strain. Experimental diabetes was achieved by subcutaneous injection of either alloxan (180 mg kg^{-1}) or by intravenous injection of streptozocin (50 mg kg^{-1}) and the rats were killed 2 or 9 days following the injection, respectively. The intestine was removed, rinsed with saline and everted. Loops of 5 cm length each were tied at the ends, after being filled with 0·7 ml Krebs solution and 0·3 ml of CO_2 (5%) in oxygen.

The everted loops were immersed in 10 ml of liposome dispersions in Krebs solution in 25 ml flasks which had been bubbled before with 5% CO_2 in oxygen. The dispersions were incubated for 1 h in a shaking bath at 37 °C. From each loop, 0·5 ml was then taken by syringe and transferred to a vial containing 3 ml Insta-Gel (Packard) and counted in a β-liquid scintillation counter (Packard, Model 2003). 0·05 ml of the

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incubation medium was also counted (after the addition of Krebs solution, up to 0·5 ml and 3 ml Insta-Gel). Blanks were prepared by adding 0·5 ml Krebs solution to 3 ml Insta-Gel. In estimating the rate of transport through the gut wall, we assumed that it was proportional to the ratio of inside/outside concentrations of the labelled compound. The amount transported is expressed as a per cent of radioactivity in the intestinal loops.

Transport of ^{14}C-PC, as well as that of ^{14}C-CH, contained in liposomes of various compositions, through gut loops of normal and diabetic rats is presented in Fig. 1. These results suggest the following conclusions:

1. The transport of ^{14}C-PC contained in liposomes was significantly greater than that of ^{14}C-CH. If the transport of ^{14}C-PC is due to transport of intact liposomes, then the lower transport of the ^{14}C-CH may be caused by exchange of CH between the liposomes and the cell membranes of the gut. However, if transport of the ^{14}C-PC was not due to transport of intact liposomes, but a result of transport of non-liposomal PC, then the rate of liposomal uptake is even lower. Such an uptake of non-liposomal PC could have been due to passage of monomeric or micellar PC or products of its degradation which had not been included in liposomes.

2. Transport of ^{14}C-CH from LMV was not different from that obtained with SUV when tested in loops of diabetic rats. In contrast, ^{14}C-CH contained in DPPC SUV passed through the gut of normal rats to a greater extent than ^{14}C-CH contained in egg PC multibilayers. Both ^{14}C-PC and ^{14}C-CH were transported similarly through intestinal loops of normal and diabetic rats, when contained in egg PC SUV and LMV. It is therefore unlikely that the excessive transport of ^{14}C-CH from DPPC SUV is an outcome of different behaviour of the gut of normal versus diabetic rats. It is more probable that the DPPC is responsible for the increased CH uptake, in accordance with the higher efficiency of DPPC vesicles in lowering blood sugar concentrations.

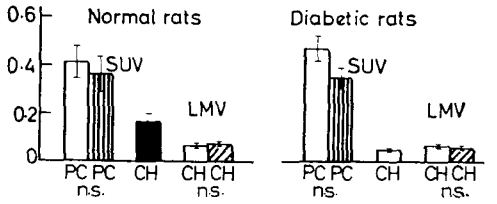

FIG. 1. The ratio of concentration of radioactivities between the content of rat everted intestinal loops (4 cm in length) and the incubation media. The ratio is expressed as per cent and denoted as "per cent transport". It was measured after 1 h of incubation under 5% CO_2 in oxygen at 37°C. Liposomes were either vesicular (SUV) or multilamellar (LMV), as indicated. Their composition was as follows:
□5% (w/v) PC + cholesterol; molar ratio = 7:2
▨2·5% (w/v) PC + cholesterol; molar ratio = 7:2
■5% (w/v) PC + cholesterol + diacetylphosphate (7:2:1)
■3% (w/v) DPPC + cholesterol (7:2)
The vesicles were labelled by labelled cholesterol ($\overset{*}{CH}$) or phosphatidylcholine ($\overset{*}{PC}$). Ordinate: % transport.

3. The fraction of ^{14}C-PC transported, did not change when the concentration of liposomes in the incubation was doubled, indicating a passive passage.

In conclusion, only a small fraction of the liposomes' components crossed the intestinal wall within 1 h. The fraction of intact liposomes that are transported might be even smaller. The very low permeability of the gut to liposomes is in accordance with the in vivo results of Ryman (1977). It might also account for the poor reproducibility of the experiments with oral insulin (Patel & Ryman 1977) since when very small amounts of the drug are transported through the gut wall, even minor individual changes could alter the results considerably. No differences were found between normal and diabetic rats. The difference in the hypoglycaemic effect of liposomal entrapped insulin noticed in vivo by Patel & Ryman (1976) must therefore be sought elsewhere. We were also unable to find a difference between SUV and LMV unless they have different ingredients.

Although uptake of intact liposomes seems clinically impractical, in the light of these results, their use by the oral route is not ruled out. They may still provide protection for drugs from enteric degradation along the gastrointestinal tract if they are released at the absorption site. The present experiments also do not rule out the possibility that a drug entrapped in liposomes may be transported through the intestinal wall when the liposomes fuse with the intestinal mucosa. Thus, in spite of the very low transport of intact liposomes, their content still may be transported.

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