lecithin-cholesterol monolayers[11]. Willmer[11], De Bernard[12] and Snart[13] all concluded that this type of behavior results from the formation of distinct surface structures. In this case of ganglioside/cholesterol monolayers there are 2 different surface structures: one due to excess cholesterol with the surface structure being composed of 1 molecule ganglioside:3 molecules cholesterol and the other surface structure due to excess ganglioside being composed of 1 molecule cholesterol:2 molecules ganglioside. The intermediate compositions being mixtures of these 2 basic structures.

In an attempt to study in more detail the miscibility of gangliosides in cholesterol mixed melting points were determined from which the phase equilibrium diagram shown in figure 3 has been obtained. In mixtures containing an excess of cholesterol 2 temperature dependent phase changes were noted: as the temperature was increased softening at the edges of the mixtures was noted. Rapid melting followed by irreversible decomposition of the mixture occurred a few degrees higher. At mole fractions of cholesterol less than 0.7 the softening at the edges of the mixture was not observed; final melting of the mixtures became less distinct with the mixture 'charring' and decomposing rather than melting. The phase behavior exhibited by mixtures containing an excess of cholesterol (mole fraction cholesterol greater than 0.7) is of the type associated with constituents that are completely miscible in both the solid and liquid states; although such behavior is not normally associated with complex formation the diagram (fig. 3) is interpreted in terms of the formation of a cholesterol/ganglioside complex in the molecular ratio of 3:1 with this complex and cholesterol being completely miscible. The phase equilibrium diagram however, provides no evidence for the formation/behavior of a 1:2 cholesterol: ganglioside complex.

The property of gangliosides to form complexes with other membrane constituents (cholesterol) raises the possibility of such complexes having a role in the structure of hormone receptors where gangliosides are known to form at least part of the receptor – a problem which is under investigation in this laboratory at the present time.

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Effects of N-ethyl maleimide on urea facilitated transport across toad gall bladder

C. Ardizzone and C. Lippe

Institute of General Physiology, University of Bari, 165/A, via Amendola, I-70126 Bari (Italy), June 28, 1982

Summary. An SH reactive agent, N-ethyl maleimide (10^{-3} M for 2 min in the luminal fluid) selectively inhibits the urea transepithelial flux across the toad gall bladder. Thiourea and antipyrine flux is not inhibited. The inhibitory effect on urea flux seems to be exerted on the carrier mechanism of urea transport.

It is known that the gall bladder is the site of an active transport mechanism for urea and presumably other amides, and that such transport exhibits saturation kinetics and is selectively inhibited by phloretin[1,2].

Little can be said about the chemical nature of the carrier molecules involved; however, the selective inhibition by cycloheximide[3] seems to indicate that they are of a protein nature. The activity of several carrier molecules possessing sulphydryl groups is strongly inhibited by SH-reactive agents such as N-ethyl maleimide (NEM) and mersalyl[4].

In order to define the urea carrier molecule more precisely, we have studied the effects of NEM on urea transport across toad gall bladder.

Methods. Gall bladders were isolated from female toads *(Bufo bufo)* and then opened, washed free of bile with Ringer solution and mounted between 2 lucite chambers containing 7 ml of incubation fluid, gassed with air at 22 ± 2 °C. The exposed area was 0.2 cm^2 and both sides were bathed with the same fluid containing (in mmole/l): NaCl 112, KCl 5, $CaCl_2$ 1, $NaHCO_3$ 2.5, test molecule 1, pH = 8.1.

Transepithelial fluxes from the serosa to the mucosa were measured using the following labeled molecules: ^{14}C-urea, ^{14}C-thiourea and N-methyl ^{14}C-antipyrine (obtained from N.E.N., Frankfurt, FRG).

The labeled molecule was added to the serosal compartment (final activity 1 μCi/ml) and after 2 h of equilibration, samples were withdrawn every hour from the opposite compartment. After a control period, NEM (10^{-3} M) was added to the mucosal side for 2 min; samples were withdrawn every hour thereafter for 2 h.

For counterflow experiments, the isolated tissue, perfused as previously reported, was loaded for 70 min with Ringer solution containing ^{14}C-urea (10^{-6} M); final activity on both sides was 5 μCi/ml. During this period the perfusion fluids were stirred continuously with magnetic bars. At the end of the loading period the perfusion fluids were recovered and the chambers were thoroughly washed twice

Effect of NEM (10^{-3}M for 2 min in the luminal fluid) on nonelectrolyte fluxes across toad gall bladder

Molecule	A	B	C	B-A	C-A
Urea (6)	155.8±30.5	129.9±25.9	126.4±22.3	−25.9±6.6*	−29.4±9.1**
Thiourea (5)	28.6±7.0	28.9±7.3	40.8±9.0	0.3±2.0	12.2±4.3
Antipyrine (7)	121.2±21.5	126.0±21.3	158.3±31.7	4.8±8.2	37.1±17.8

*$p < 0.02$; **$p < 0.05$. A, Serosa mucosa fluxes: control; B, serosa mucosa fluxes: 1st h after NEM treatment; C, serosa mucosa fluxes: 2nd h after NEM treatment. The fluxes are expressed as nmoles/cm^2 · h. The mean values ±SEM are reported. Number of experiments in brackets.

with nonradioactive Ringer solution. Both chambers were then filled with the same solution, which was replaced every 5 min, 3 times. Afterwards, Ringer solution containing 10 mM urea was added to both sides and removed every 5 min, 5-6 times.

In tissues treated with NEM, the drug (10^{-3} M) was added for 2 min to the mucosal fluid, before the loading period. The radioactivity was counted with a liquid scintillation spectrometer.

Results and discussion. Studies on frog skin have shown that long term incubations with NEM 10^{-3} drastically increase the permeability of this tissue for urea and phenylalanine[5].

One could argue that in our experimental conditions as well a long term incubation of the gall bladder epithelium with NEM 10^{-3} M would aspecifically increase its basic permeability, making unmeasurable other specific effects of the SH-group reagent. In order to exclude this possibility we exposed the tissue to NEM 10^{-3} M for only a short incubation period (2 min).

As shown in figure 1 even this short incubation time is able to slightly increase the permeability of the tissue to thiourea, a molecule which crosses the gall bladder epithelium by simple diffusion[1]. On the other hand, after incubation with NEM, the transepithelial fluxes of urea are significantly decreased. This observation could suggest a specific inhibitory effect on its carrier mediated transport system[2], but it could as well be explained by an aspecific mechanism. In fact, it is known that the antibiotic amphotericin B[1], which is able to increase the permeability of the luminal membrane of the gall bladder, causes, as a side effect, a cellular swelling which reduces the extracellular lateral spaces of the epithelium[7]. This effect has been well documented by Curci et al.[1], who demonstrated that, under amphotericin B treatment, the gall bladder transepithelial fluxes of urea and other amides are significantly reduced. They have observed that this effect is linked to the cellular swelling induced by the antibiotic and have made the hypothesis that the space between the lateral membranes is reduced in such a way that the diffusion of the molecules which normally use this pathway can be drastically reduced.

Further support of this hypothesis comes from the observation of Cremaschi et al.[9] who, using an electromicroscopic approach, have shown that the lateral membranes of toad gall bladder are highly folded.

To answer the question of which of the 2 mechanisms was responsible for the decreased transepithelial fluxes, we checked the effect of the NEM on the transepithelial fluxes of antipyrine, a lipid-soluble molecule which easily diffuses through the cellular plasma membrane and whose fluxes should be negatively influenced by a reduced accessibility of the lateral membranes.

As shown in the table, the incubation of the tissue with NEM does not affect the transepithelial fluxes of antipyrine; this result strongly supports the idea that the NEM specifically interacts with the urea carrier mediated transport system in the gall bladder and that its effect cannot be related (as a side effect) to the increase of permeability which NEM produces on the luminal side of the tissue.

It is known that countertransport is a characteristic property of mobile carrier systems[8]. In a previous work[6] we demonstrated a counter transport mechanism for urea on the luminal side of *Bufo bufo* gall bladder: on the basis of these results we demonstrated the existence of a mobile carrier system for urea on the gall bladder luminal membrane. To gain further insight into the problem of the reactive site of the NEM we carried out counter transport experiments for urea in the presence and in the absence of the SH-group inhibitor. It can be noted (fig. 1) that the discharge rate of 10^{-6} M urea undergoes a considerable increase when the tissue is perfused with Ringer solution containing 10 mM

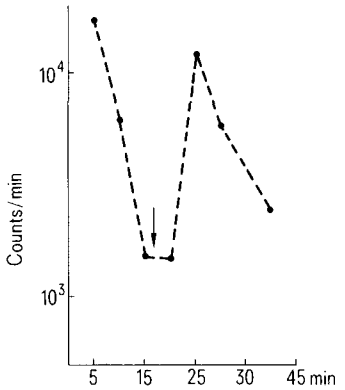

Figure 1. Mucosal efflux of urea in the toad gall bladder, the arrow indicates the addition of urea 10 mM on both sides of the preparation.

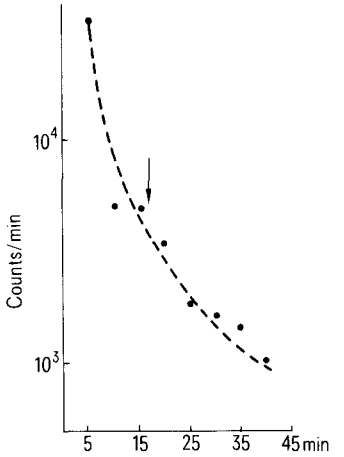

Figure 2. Mucosal efflux of urea in the toad gall bladder pretreated by NEM (10^{-3} M for 2 min), the arrow indicates the addition of urea 10 mM on both sides of the preparation.

urea. This result confirms the existence of countertransport for urea as previously reported[6].

Figure 2 shows that, after incubation with NEM, the countertransport phenomenon of urea is completely abolished. The results of figure 2 give further support to the hypothesis that the SH-group reagent inhibits the urea transport interacting directly with the carrier molecule.

On the basis of our experimental results, we have reached the conclusion that the urea carrier molecule has a proteic nature and that SH-residues are involved in the translocation mechanism of the amides.

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Increased concentration of plasma cholesterol in veal calves fed soyabean lecithin

A.C. Beynen and L.G.M. Van Gils

Department of Human Nutrition, Agricultural University, De Dreijen 12, NL-6703 BC Wageningen (The Netherlands), and Institute for Scientific Research in the Field of Animal Nutrition, Trouw & Co., N.V. International, NL-3881 LA Putten (The Netherlands), November 8, 1982

Summary. Veal calves (aged about 1 week) were fed a milk replacer without or with soyabean lecithin (2 and 4%; on dry matter basis) for 18 weeks. It was found that lecithin increased the level of plasma cholesterol in a dose-dependent manner.

Lecithins constitute a large family of related compounds differing in the kinds of fatty acids attached to positions 1 and 2 of the glycerol moiety. Soyabean lecithin contains relatively high amounts (up to 70%) of polyunsaturated fatty acids, mainly linoleic acid. There have been reports of beneficial effects of soyabean lecithin supplementation, either i.v. or orally, in human atherosclerosis.[1].

Literature reports as to the effect of dietary soyabean lecithin on the level of plasma cholesterol, a major risk indicator for atherosclerotic diseases, are not conclusive. A striking reduction of the concentration of plasma cholesterol was observed in hypercholesterolemic rhesus monkeys after feeding of soyabean lecithin[2]. In normocholesterolemic chimpanzees soyabean lecithin did not affect plasma cholesterol levels[3]. In hypercholesterolemic patients dietary soyabean lecithin has been found to be ineffective[4,5], or to produce a slight decrease in plasma cholesterol concentrations[6]. In healthy humans given capsules containing soyabean lecithin, a slight (3–6%) fall in plasma cholesterol was seen[7].

We are investigating cholesterol metabolism in the young bovine, which is an excellent model animal for the study of human atherosclerosis. The present study was carried out to see whether dietary soyabean lecithin affects the level of plasma cholesterol in veal calves.

For this investigation male Friesian-Holstein calves were used; they were purchased at a market at the age of about 1 week. The mean initial b. wt was 40.9 kg. The calves were housed individually in wooden boxes with slatted floors. On arrival in the calf house, the animals were randomly divided into 1 group consisting of 73 calves and 2 groups of 51 animals each. During the experiment the control animals were raised on a milk replacer containing 60% (weight percent; on dry matter basis) skim milk powder and 20% crude fat[8]. The other animals were fed either 2 or 4% native soyabean lecithin (Lucas Meyer, Hamburg, FRG). The lecithin was added to these diets at the expense of the fat. The milk replacers were reconstituted in hot water. On arrival the animals received 147 g air-dry feed per meal, this amount being gradually increased to 1575 g in the 18th week. The calves were pail-fed twice a day (at 09.00 h and 20.00 h). Blood samples were taken from the jugular vein between 10.00 and 12.00 h in the 18th week of the experiment. Plasma was collected by low speed centrifugation. Plasma total cholesterol was determined enzymatically according to Röschlau et al.[9], using the kit (CHOD-PAP) supplied by Boehringer-Mannheim GmbH, FRG. The reproducibility (coefficient of variation) was routinely less than 1.1%.

In the 20th week of the experiment the calves were slaughtered. The mean body weight of all the animals was 215 kg; the average daily body-weight gain was 1243 g. Thus, it can be concluded that the performance of the animals was excellent (cf. Beynen and Van Gils[8]).

The table shows the plasma cholesterol concentrations of the calves after 18 weeks of feeding soyabean lecithin. The inclusion of 2% soyabean lecithin in the milk replacer diet increased the level of plasma cholesterol by 6%, but this increase failed to reach a level of statistical significance. However, when the diet contained 4% lecithin, a significant ($p < 0.01$) increase in the level of plasma cholesterol was

Plasma cholesterol concentrations of veal calves fed liquid diets containing soyabean lecithin

Soyabean lecithin added to diet (weight %)	Number of animals	Plasma cholesterol (mmoles/l)	Relative value
None	72	3.70 ± 0.87	100
2.0	49	3.94 ± 0.82	106
4.0	49	4.20 ± 0.90*	114

Values are expressed as means ± SD; during the trial 5 calves died. *Vs control group (2-tailed Student's t-test): $p < 0.01$.