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A New Sulfolipid: Application to Problems of Drug Transport

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Many mechanisms have been suggested to explain transport across biological membranes [PARK, 1961; BURGEN, 1962]. A constantly recurring suggestion has been that of 'carrier-facilitated diffusion' or 'carrier transport'. The general thesis of carrier transport as described by BURGEN [1962] is that a complex is formed between the substance to be transported and a membrane constituent at the membrane interface, which complex is 'miscible' with the lipid of the membrane. Such complexes are generally presumed to be based upon electrostatic or hydrogen bonding.

The association of the lipophilic complex with the membrane is energetically favorable. Where the overall process is 'uphill', however, carrier transport mechanisms include the use of ATP to account for the transfer of the substance being transported from the membrane into the cytoplasm.

Lipid soluble complexes have been used to explain only a few of the transport problems encountered. HOKIN and HOKIN [1963] used phosphatidic acid to explain the sodium 'pump' of the avian salt gland. STEIN [1961] has made the ingenious proposal that the transport of glycerol and glucose is effected by 'dimerizer' enzymes. He suggests that these enzymes are present in the membrane and that they combine two molecules of the polyhydric compound to form a lipophilic dimer which penetrates the membrane. Kinetic evidence is offered to support this proposal.

Lipid solubility has been a guiding principle in the design of antimicrobials for many years. Esters, for example, were believed to be more effective antimicrobials than the parent acids because of the greater lipid solubility of the esters [MOJE, MARTIN and

BAINES, 1957; GROVE, 1948]. There are a number of papers in the literature in which peculiarities of the actions of drugs may be interpreted profitably in light of the above. For example, TOWNSEND, BROWN, FELAUER and HAZLETT [1961] have found that certain fatty acids were effective against leukemic cells only below pH 5.6, whereas the esters of these acids were effective at neutrality. Presumably, the fatty acids form dimers at the low pH but are completely ionic above pH 5.6. As HARKINS [1952] has remarked, such an associated complex behaves more like a non-polar substance than do the single molecules.

We have isolated a sulfolipid from *Ochromonas danica*. The structure of this alkyl sulfate is 1,14-docosane disulfate. Several aspects of its biochemistry and physiology have been explored. It has been possible, for example, to isolate the S^{35}-labeled material from S^{35}-labeled cells. Upon feeding this labeled sulfatide to a culture of *O. danica* in the log phase of growth, it is found that the organism incorporates the sulfatide without cleavage of the esterified sulfate [HAINES and BLOCK, 1962].

Thus, the sulfatide is metabolically inert. This is specially interesting in view of the fact that the sulfatide is normally excreted in quantity into the medium [HAINES, 1965]. It would appear that this *inert* substance passes back and forth between the medium and the cells.

It was discovered by digestion of the cell extracts with proteolytic, as well as other enzymes, that the sulfolipid was attached to protein in the cell [HAINES, 1965].

A culture of cells was labeled with the purified S^{35}-sulfatide (50 μc). The cells were collected by centrifugation at 9000 *g* and washed with deionized water. The cells were suspended in isotonic pH 7.5 phosphate buffer and broken with a glass homogenizer. The resulting fragments were suspended on a sucrose density gradient (density = 1.255 to 1.129) and centrifuged at 35,000*g* in a Spinco Model L Ultracentrifuge for 16 h.

The white layer which remained at the top of the gradient was transfered to a Ficoll density gradient (density = 1.02 to 1.10) and centrifuged as before. The result was a white fluff at the top of the gradient and a thin white band further down the tube (density = 1.04). The tube was punctured at the base and fractions collected and counted. The major radioactive peak was the thin white band which was, judging from its density [KAMAT and WALLACH, 1965]

and its lack of color, the cell membrane. A detailed description of these experiments will be published elsewhere. The finding that the sulfolipid entered the membrane is consistent with the earlier finding that the sulfolipid is attached to protein in the cell.

AARONSON and BENSKY [1965] have suggested the use of *O. danica* for the screening of drugs affecting lipid metabolism. AARONSON [1964] had earlier used the organism to screen hypocholesteremic drugs. Conversely, the drugs' effect represents a means of ascertaining that it is transported.

SOKOLSKI, FERGUSON and GOFF [1962] have studied the uptake of neomycin, a lipophilic cation, by *O. danica*. They found that uptake was unaffected by cationic, non-ionic and amphoteric detergents. Alkyl sulfates, however, dramatically increased the uptake of the drug. As SOKOLSKI, FERGUSON and GOFF [1962] could not have known of the existence of a natural alkyl sulfate in the medium at that time, this effect was attributed to a special 'wetting of the membrane'. In view of the fact that the sulfatide exists in both the medium and the membrane, two alternative explanations of this data may be offered.

One possible explanation is that a complex is formed between the anionic detergent and the lipophilic cation, neomycin. This complex would behave as a non-polar compound. The complex, which has a greater affinity for the membrane than either of its component ions, is adsorbed and subsequently taken up by the organism.

The second possible explanation is that a carrier system exists in which the sulfatide carries the drug into the organism *actively*. One would not expect the organism to have a carrier system for lipophilic cations (if one exists at all) since *O. danica* is cultured in a simple medium which does not include lipophilic cations. Nevertheless, such a system may be in use for divalent cations. Such cations form the same kind of complex with lipophilic sulfate esters. Complexes of this sort have been observed for many years, including complexes of cerebroside sulfate [GREEN, ROBINSON and DAY, 1961] and steroid sulfates [MCKENNA and NORYMBERSKI, 1960]. This second explanation assumes that the anionic detergent is replacing the natural sulfatide as membrane constituent.

With each explanation, the synergistic action of the anionic detergent can be attributed to its forming an associated complex with the cationic neomycin.

If it is necessary for the anionic detergent to replace sulfatide in the membrane, then synergism would only apply in those organisms in which sulfatide is a constituent of the membrane. An extensive investigation has not been made for the distributation of the sulfatide in the microbial world. There is evidence, however, that the sulfate ester is in, or is excreted by, several protozoa, a sea water bacterium, an actinomycete, and algae. It was not present in several rat tissues [HAINES, 1965].

Anionic detergents might well be effective as synergists for cationic lipophilic drugs in tissues containing sulfatide or a similar constituent. If, on the other hand, a simple, non-polar, associated complex is the explanation for the data of SOKOLSKI, FERGUSON, and GOFF [1962], then cationic detergents will possibly have the same kind of action with anionic lipophilic drugs. It would seem that investigation into the use of such synergists would be profitable.

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