BIOMEDICAL APPLICATIONS OF COMPLEXATION AGENTS/SOLUBILIZERS

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ABSTRACT. Non-toxic complexation agents/solubilizers have numerous applications in the biomedical field. Results obtained in the author's laboratory are reviewed here. This work resulted in: (a) the development of a system enabling more efficient therapeutic use of retinoids, (b) the development of an agent eliminating mycoplasma infections in cell cultures, (c) the development of a detoxification process for hypervitaminosis A, and (d) the development of a system for effective sublingual administration of sex hormones.

Alchemists coined the phrase "Corpora non agunt nisi soluta" to summarize their findings that compounds are not active unless they are dissolved. To explain his ideas about drugs and their receptors, Paul Ehrlich found it necessary to introduce a complementary phrase "Corpora non agunt nisi fixata" to express his opinion that a compound cannot act as a drug unless it binds to the proper receptor. Some progress in pharmacology has been made since the times of alchemists and Ehrlich; nevertheless, these two phrases still summarize the essence of our knowledge. The drug must be soluble enough in water and lipids to get to the proper receptors, and then bind to those receptors to elicit a pharmacological response. The study of solubilities and the process of dissolution of drugs and potential drugs is thus an integral part of pharmaceutical sciences. This fact has been only slowly and belatedly recognized and it is Higuchi and his school to whom the main credit should be given for this realization.

The solubility of organic lipophilic compounds can be manipulated to make them better suited for use as drugs; synthetic chemists can introduce polar groups into the non-critical part of the drug. This is not difficult to do technically, but the process requires knowledge of which structural parts of drugs are non-critical, and there is never absolute certainty to that point. Another disadvantage of such an approach is the extensive biosafety testing which must be made after any chemical modifications of a drug. The solubility of a potential drug can alternatively be modified by non-toxic solubilizers. There are disadvantages to this approach as well; instead of one compound (i.e., drug), two compounds (i.e., drug and solubilizer) have to be

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investigated. Furthermore, solubilizers are not specific--they can also move other lipophiles around in an organism and complicate the situation further.

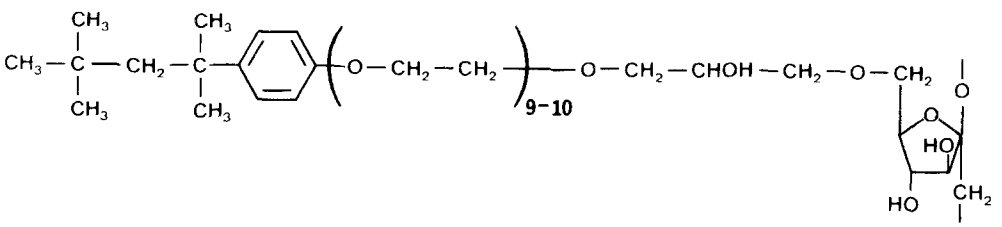
Before going into the specifics of the problems with solubilizers, it may be of help to describe the salient features of the systems involved. Drug receptors are present in target organs in concentrations of 1 p.p.m. or lower. A simple calculation shows that all these receptors in a man require only 0.1 mg or less of drug if binding would be infinitely strong. The binding of drugs to receptors is usually quite strong; dissociation constants of many drug-receptor interactions have been measured and many of them are in the 10^{-9}-10^{-8}M area. If such a drug would bind only to its receptor or remain in free form, then about 1 mg or less of drug would be satisfactory to achieve a full pharmacological response.

Drugs are administered at much higher doses; 5-200 mg are more realistic amounts. The excess of drug is obviously bound to other components of the body and some of these additional binding sites are common for many drugs. The majority of useful drugs have partition coefficient values between lipid and aqueous medium in the range of 0.1 and 10. Since lipids may form about 15% of body weight, quite a large portion of drug is segregated into the lipid spaces of the body. Furthermore, there are other hydrophobic spaces in an organism (e.g., interior portion of proteins, especially those involved in lipid transport and metabolism); drugs may enter and be segregated in those as well. Drugs segregated into those components of the organism do not perform any useful function. Nevertheless; there is an advantage to that process; such spaces form reservoirs or depos of drug and since some drugs are deactivated in the process of triggering their pharmacological response and/or cleared away, the drugs must be constantly resupplied to receptors in order for the treatment to be effective. The kinetics of this resupply of receptors by a drug from various depos is of critical importance and this resupply process forms the physical basis for the above mentioned requirement that the drug must have some solubility both in lipids and in water. Without some lipid solubility, depos of the compound in the body are too small and diffusion of the compound is too slow since it cannot cross cell membranes. Without some water solubility a drug is transported from depos in lipids to the receptors too slowly for a treatment to be really effective.

The above description makes apparent that difficulties encountered in the use of solubilizers may be quite numerous; nevertheless, some of these can be avoided by balancing the systems properly. In our studies we developed non-toxic solubilizers which could come into contact with cells without inflicting extensive damage and thus could be used on cells in culture or be injected into circulating blood of animals. Injection of such solubilizers into animals is equivalent to the introduction of additional carriers of lipophilic compounds and leads to a speedier distribution of a lipophilic drug through the organism. Furthermore, we used solubilizers to obtain very fast dissolution rates for drugs and used those systems to improve absorption of drugs when these are applied orally. In our experiments we were eclectic in the choice of bioassays or solubilizers; only the final results mattered.

Bio-evaluations were run on systems as different in complexity as isolated cells in culture and human beings. Solubilizers tested were also selected in an eclectic manner; they belonged to the class of polyethylene glycol derivatives, to the class of polymeric analogs of organic solvents, to saponins, and to cyclodextrins.

Detergents based on polyethylene glycols found extensive use as household and industrial detergents and eventually they found some limited use even in the biomedical field. The principal disadvantage of these detergents in their biomedical applications is rather their high potency. Consequently, we tried to diminish this potency; for this purpose we synthesized macromolecules in which these detergents were covalently attached to a polysaccharide (1). One of the main features of synthetic macromolecules is the difficulty with their purification. Therefore, several methods for preparation of these macromolecular detergents were tested with the goal to make them pure enough for direct use. The optimum method for synthesis of macromolecular detergents was found in the conversion of the terminal hydroxy group of polyethylene glycol detergent (e.g., Triton X-100) into the glycidyl ether group. Thereafter, these glycidyl ethers were reacted with polysaccharides; the most suitable of those was inulin, a polysaccharide which possesses reactive primary hydroxy groups. A structure of one unit of such a polymer is illustrated below:

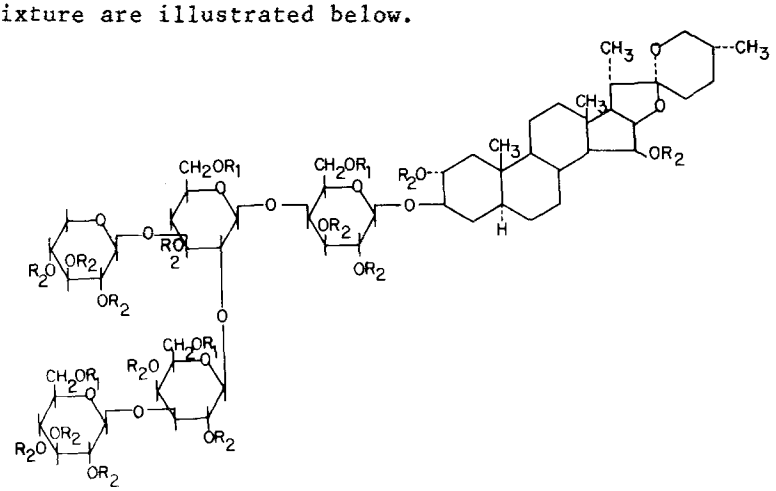


The resulting macromolecular detergents were evaluated for their physico-chemical and biological properties. The main lesson learned from these studies was from the comparison of the effects of parent detergents and of macromolecular detergents on the growth of mouse cells in culture. As expected, the macromolecular detergents were considerably less toxic than the parent compounds, but these differences were not great when evaluated after long exposures of cells to the compounds. On the other hand, there was a dramatic difference in toxicity when short exposures were used: cells could be treated for several hours with macromolecular detergents without any harm, whereas the toxic effects of parent detergents onset within a few minutes. Macromolecules diffuse and penetrate slowly and this obviously led to the observed decrease in cell damage.

Another class of solubilizers investigated was based on saponins. Digitonin, a rather mild saponin detergent, is very useful for the

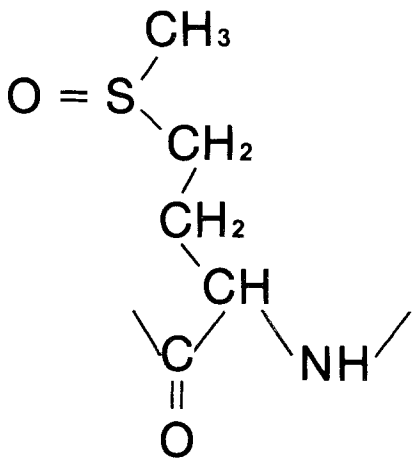
dissolution of drug receptors and other membrane components; these proteins, while solubilized by digitonin, still retain many of their biochemical functions. Digitonin also forms specific complexes with cholesterol; possibly this causes digitonin to be very toxic when in direct contact with unprotected cells (e.g., to cells in culture or when digitonin is parenterally injected). Our intent was to decrease this specific complexation with the hope to decrease the toxicity of digitonin as well. Eventually it was found that modification of the pentasaccharide part of digitonin with epoxides produced compounds of the desired low toxicity (2). These derivatives were still potent solubilizers of lipophilic compounds, but their intravenous toxicity was, compared to digitonin, decreased about five hundred times. The onset of toxic effects to cells in culture, similarly to the above macromolecular detergents, was also rather slow. With digitonin derivatives we managed to find practical use for this slow onset of toxicity.

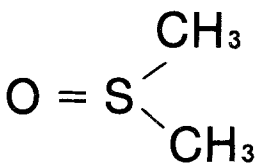
Cells in culture are not a laboratory curiosity: they have been used to manufacture viral vaccines or monoclonal antibodies; in these applications large quantities of valuable materials are involved. Infections are serious problems in such processes. From the different infectious agents, the most difficult to control are mycoplasmas. Mycoplasmas are microorganisms which have cell membranes similar to mammalian cells but are much smaller than mammalian cells. The slow diffusion of macromolecular detergents and differences in the size of cells and mycoplasmas was fortunately translated into a selective toxicity of these compounds to mycoplasmas. Low levels of these detergents were without effects on large mammalian cells, which obviously can repair some slow erosion on their periphery, whereas small mycoplasmas were eliminated (A. Nordin, private communication). The structures of components of the most effective mixture are illustrated below.



R_1=—CH_2–CHOH–CH_2–O–CH_2–CH_2–CH_2–CH_2–O–CH_2–CHOH–CH_2OH or H; R_2=H

Another solubilizer investigated was polymethionine sulfoxide. Dimethyl sulfoxide is a powerful organic solvent with strong and strange bioeffects: this simple solvent induces some cells in culture to differentiate, while other kinds of cells are inhibited from differentiation. Dimethyl sulfoxide also has distinct toxic effects, both on cells in culture and in animals. Our intent was to prepare and study a polymeric homolog of this interesting compound. Eventually, we chose to oxidize the commercially available polymethionine, a poly-amino acid which is insoluble in water, and found that the corresponding sulfoxide was well water soluble (3). The structure of one unit of that polymer is illustrated below on the left; on the right is the structure of dimethyl sulfoxide. Polymethionine sulfoxide is a powerful solubilizer

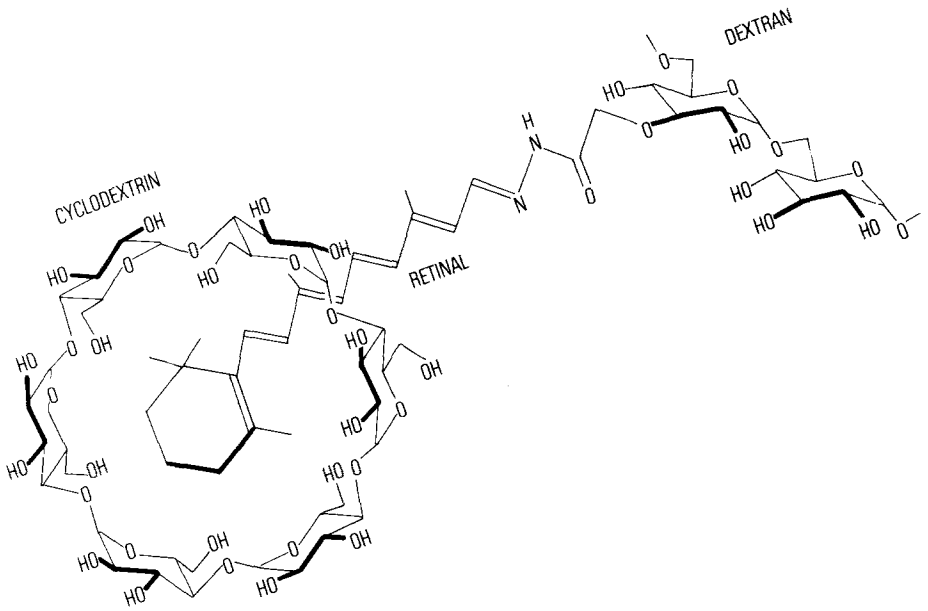




and is effective even in solubilization of vitamin A-related compounds which are rather resistant to other solubilizers. When biological effects of polymethionine sulfoxide were compared to those of dimethyl sulfoxide, the former was found non-toxic and inert in affecting the above mentioned differentiation changes in cells in culture. Polymethionine sulfoxide thus seems to be a suitable non-toxic solubilizer of drugs.

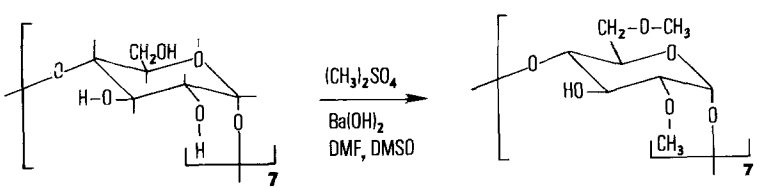
Cyclodextrins were important components of the remaining systems evaluated, in which mainly retinoids and sex hormones were studied. Retinoids, compounds related to vitamin A, are effective in the treatment of several skin disorders (e.g., acne) and are possibly chemopreventive agents of some forms of cancers. The therapeutic usefulness of retinoids is impeded by their high toxicity and thus, their present use is rather limited. Biochemical data collected on retinoids suggested to us that toxic effects of retinoids occur when the concentration of these very lipophilic compounds is in excess of the concentration of their specific carrier protein, i.e., when there are free retinoids in contact with cells. Consequently, we attempted to create synthetic "carriers" for retinoids. The most successful system we prepared (4) was composed of retinal, bound chemically to semicarbazide groupings which were

attached to dextran; the system furthermore required the presence of β-cyclodextrin for full solubilization. The structure of one unit of such a polymer is illustrated below. When the effects of such solubil-



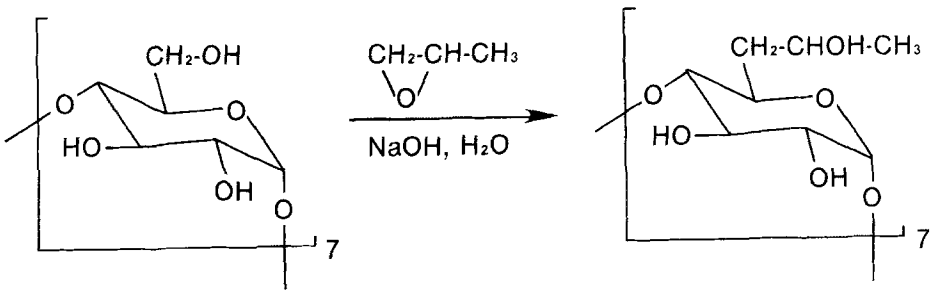
ized retinal were compared with the effects of retinal alone, it was obvious that the inhibitive effects on the growth of cancer cells were decreased by about one order by solubilization; fortunately the toxic effects were diminished by two orders of magnitude. Thus, the solubilization of retinal improves about ten times its usefulness; better inhibition of growth of transformed cells can be obtained without onset of non-discriminative toxicity.

Retinoids were used also in our subsequent work on cyclodextrins. β-cyclodextrin has rather low water solubility and that limits its ability to form soluble complexes and is probably also the cause of its toxicity when applied parenterally. We previously had a good experience with 2,6-O-dimethyl-β-cyclodextrin as a solubilizing agent; consequently we wanted to continue with that compound and introduce it into biotests we had available (5). The preparation and structure of 2,6-O-dimethyl-β-cyclodextrin is illustrated in the scheme below. The problem of



interest was the effects exerted by the solubilizer in circulating blood on the toxicity of retinoic acid in mice (6). Results were rather confusing for some time, but eventually a clear and beautiful pattern emerged. The solubilizer affected the toxicity of retinoic acid in a manner principally depending on the method of its administration. In experiments described subsequently lethal doses of retinoic acid were used; such doses induce well defined symptoms of hypervitaminosis A and deaths occur within a couple of weeks. When the solubilizer was administered parenterally and simultaneously with retinoic acid, the toxic effects were increased and animals died within a couple of days; no symptoms of hypervitaminosis A were observed. Obviously the solubilizer helped to spread toxin through the organism and the lethal effects on tissues were immediate. The other series of experiments were performed as follows: mice were treated with high doses of retinoic acid alone and hypervitaminosis A was left to establish itself; then after about one week the solubilizer was applied parenterally. This type of administration of the solubilizer improved the survival rate of poisoned animals. Obviously, the solubilizer again helped to distribute retinoic acid through the organism, but this time to lifesaving ends. The solubilizer in that case extracted the toxin from the poisoned organs, a process which helped to clear the toxin from the organism.

Another group of highly lipophilic compounds of biological interest are sex hormones. These steroids have important therapeutical applications. Testosterone is required as supplementation therapy for males who, as a result of endocrinological defect or an injury, have a low level of this hormone. Progesterone and estradiol may benefit postmenopausal females who suffer from decreases in production of these hormones, a process which may result in disturbances of the calcium balance in the body and osteoporosis. Furthermore, administration of sex hormones can manipulate the menstrual cycles in females; a measure of birth control and measure of diminution of premenstrual tension can be obtained in that manner. These benefits are nevertheless difficult to achieve by oral administration of sex hormones. Sex hormones are rapidly metabolized and thus their administration of the p.o. route has very low efficiency. Parenteral administration of suitably esterified hormones is used instead, but that is a rather inconvenient method for protracted usage. Alternatively, synthetic analogs of hormones have been in use, in which rapid metabolism of sex hormones is prevented by introduction of specific chemical groups into their molecule. This approach has been very successful but again it taxes the metabolism. In our work we have systematically studied the co-administration of sex hormones and non-toxic solubilizers; our aim was to overcome the problems with absorption and degradation of these hormones. Eventually a method using the condensation product of β-cyclodextrin with epichlorohydrin or with propylene oxide was worked out which enabled efficient administration of sex hormones to humans by the oral route (Josef Pitha, unpublished results). The condensation reaction of β-cyclodextrin with propylene oxide, leading to the desired derivative, is depicted in the following scheme.



6 O : main reaction site
2 O & 3 O : minor reaction sites

Non-toxic solubilizers obviously may find many applications in the biomedical field. Our experience indicates that the desired bio-effects may possibly be obtained with solubilizers from any of the classes evaluated, but considerable chemical work and tests are often required. The proper choice of a suitable class of solubilizers can obviously shorten the chemical work and tests; basic level research on various solubilizers may considerably improve our ability to make this choice.

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