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The action of some synthetic lysolecithins and lecithins on erythrocytes and lipid bilayers

GOTTFRIED AND RAPPORT[1] have investigated the hemolytic action of a series of lyso phosphoglycerides. The nature of the linkage of the hydrocarbon chain did not appreciably affect lytic activity, but unsaturation in the parafinnic chain of lysolecithin was found to reduce the hemolytic action. In order to obtain additional information on the relevance of chemical structure to hemolytic activity we assayed a number of synthetic lysolecithins and lecithins containing fatty acid constituents of different chain lengths. In addition, the action of some of these compounds on lipid bilayers was studied.

The following lecithins were prepared by acylation of glycero-3-phosphoryl choline[2,3]: 1,2-dimyristoyl-glycero-3-phosphoryl choline ((dimyristoyl)lecithin), 1,2-didecanoyl-glycero-3-phosphoryl choline ((didecanoyl)lecithin) and 1,2-diheptanoyl-glycero-3-phosphoryl choline ((diheptanoyl)lecithin). The synthesis of 1-oleoyl-2-butyryl-glycero-3-phosphoryl choline ((1-oleoyl-2-butyryl)lecithin) was as described by BIRD *et al.*[4]. Hydrolysis of lecithins with snake venom phospholipase A furnished 1-stearoyl-glycero-3-phosphoryl choline ((stearoyl)lysolecithin), 1-oleoyl-glycero-3-phosphoryl choline ((oleoyl)lysolecithin), 1-myristoyl-glycero-3-phosphoryl choline ((myristoyl)lysolecithin) and 1-decanoyl glycero-3-phosphoryl choline ((decanoyl)-lysolecithin). The purity of the phospholipids was confirmed by thin-layer and paper chromatography. Known amounts of these compounds were emulsified in 0.9% saline by vigorous shaking. Beef erythrocytes were washed 3 times with 0.9% saline and the cells were suspended in 0.9% saline to give suspensions with a 50% hematocrit. The lysis test was carried out by adding a small volume of the lysin solution to 5 ml of 0.9% saline in the cuvette of a Vitatron spectrophotometer, and the transmission was adjusted at 100%. Subsequently 50 μl of the erythrocyte suspension was added and the alteration of transmission at 625 mμ was recorded as a function of time. The time required to give 50% hemolyses at various concentrations of lytic agent is represented in order to facilitate a comparison with the bilayer experiments. Lipid bilayers were generated from a solution of total lipid from beef erythrocytes (1% lipid in decane) in an apparatus essentially the same as that described by THOMPSON[5].

Measurements were made of the average survival time of these films at various concentrations of lytic agent injected into one of the compartments.

Lysis of erythrocytes was affected by (stearoyl)lysolecithin, (myristoyl)lyso-lecithin and (oleoyl)lysolecithin in that order (Fig. 1A). However, in comparison to these compounds (decanoyl)lysolecithin revealed a lytic action of a much lower order, 2.0 μmoles/ml being necessary to give an onset of lysis under the conditions used. The experiments indicate that the hemolytic activity of lysolecithin is highly dependent on the chain length and further experiments with lysolecithin analogs with fatty acid constituents between C_{10} and C_{14} can give us further information about this relation-ship. In agreement with the observations of GOTTFRIED AND RAPPORT[1] (oleoyl)-lysolecithin was found to be less active as compared with (stearoyl)lysolecithin; moreover, the time required to give 50% hemolysis was increased for the unsaturated

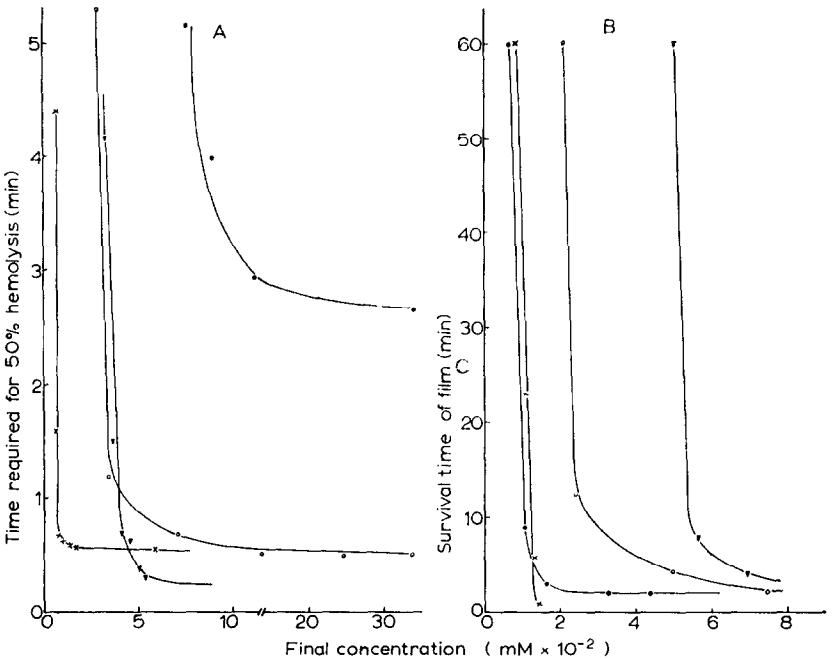

Fig. 1. Lytic action on beef erythrocytes (A) and bilayers of lipids from beef erythrocytes (B) of (stearoyl)lysolecithin (×——×), (myristoyl)lysolecithin (▼——▼), (oleoyl)lysolecithin (●——●) and (didecanoyl)lecithin (○——○).

compound. Not only monoacyl phosphoglycerides, but also certain diacyl derivatives can exert hemolytic action as shown by the results with (didecanoyl)lecithin (Fig. 1A) the action of which equals that of (myristoyl)lysolecithin. It is interesting to note that the activity of this lecithin is of a much higher magnitude than that of its so-called lyso derivative. As regards the other lecithins tested, (diheptanoyl)lecithin required a minimum concentration of 2.2 μmoles/ml to cause lysis whereas (dimyristoyl)-lecithin caused no lysis at concentrations up to 1.5 μmoles/ml. These observations have to be extended to other lecithins with chain length between C_7 and C_{14} in order to assess whether (didecanoyl)lecithin is the most lytic lecithin of the saturated series. A mixed acid lecithin, *viz.* (1-oleoyl-2-butyryl)lecithin, was found to be weakly hemolytic (lysis at 0.6 μmole/ml). This compound has been found to give micelles of a type similar to lysolecithin–lecithin mixtures[6,7]. Further studies on the size and shape of micelles of various synthetic lysolecithins and lecithins are in progress. The lipid bilayers were not lysed by (dimyristoyl)lecithin at the relatively high concentrations which were sublytic to beef erythrocytes. On the other hand, the lysolecithins containing stearic, myristic and oleic acid as well as the lytic (didecanoyl)lecithin rapidly disrupted these lipid bilayers at concentrations which are of the same order of magnitude as those found for the lysis of erythrocytes (Fig. 1B). These results support the view that the hemolysis of erythrocytes by lysolecithins has to be attributed largely to an interaction with the lipid constituents of the red cell[8]. On the other hand, the lipid bilayers showed some interesting differences in susceptibility as compared with erythrocytes, *viz.* in the membrane (stearoyl)- and (oleoyl)lysolecithins gave a nearly

similar behaviour whereas these compounds exhibited different lytic activities for erythrocytes. It appears that further studies on the action of lysolecithins and lecithins of different chemical constitution on erythrocytes and lipid bilayers may give useful information about the mechanism of lysis. In this context it is of interest to note that VAN ZUTPHEN AND VAN DEENEN[9] observed that, in membranes generated from lecithin–lysolecithin mixtures, a most significant decrease in the electrical resistance of the lipid bilayer occurred at a critical concentration of a given lysolecithin.

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