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Formation of Methylene Blue-Lipid Complexes[1, 2]

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It is known that lesions from patients with lepromatous leprosy are able to fix methylene blue (MB) in its oxidized form after the intravenous administration of a 1% solution of the dye. Lesions from patients with dimorphous leprosy also retain the dye ([2]), but lesions from patients with tuberculoid leprosy do not fix MB. Previous reports from this laboratory ([1]) have shown that MB (in its oxidized form) is capable of forming complexes with mixtures of lipids extracted from several tissues. Three possible explanations are compatible with the positivity of the MB test in lepromatous leprosy lesions.

There may be an alteration in the intracellular oxido-reduction system favoring oxidation; the dye may combine with a peculiar lipid of lepromatous lesions; or both these possibilities may be active.

A benzene-water biphasic system, utilizing the insolubility of free MB in benzene, was used for the study of the second possibility, as previously described ([1]).

The contact between free MB in the aqueous phase and the lipids present in the organic phase results in the incorporation into the benzene phase of a variable proportion of the MB present in the aqueous phase. This can be measured.

This technic was directed at a study of the lipids capable of forming complexes with MB and the determination of the optimal conditions for the formation of these complexes.

MATERIALS AND METHODS

Pure lipids commercially obtained were used as were also lipids extracted from brains and livers of albino mice, by the method of Folch *et al* ([3]).

TABLE 1. *Amounts of methylene blue transported to the benzene phase by different lipids in a biphasic benzene-water system.*

Material studied	μg methylene blue/mg lipid
Lipid extract from brain of albino mouse	27.40
Lipid extract from liver of albino mouse	18.53
Pure lecithin	6.35
Pure cholesterol	0.00
Pure triolein	0.00
Pure oleic acid	0.00
Pure linoleic acid	0.00
Pure palmitic acid	0.00
Pure tripalmitin	0.00
Pure glycerol	0.00
Olive oil	0.00

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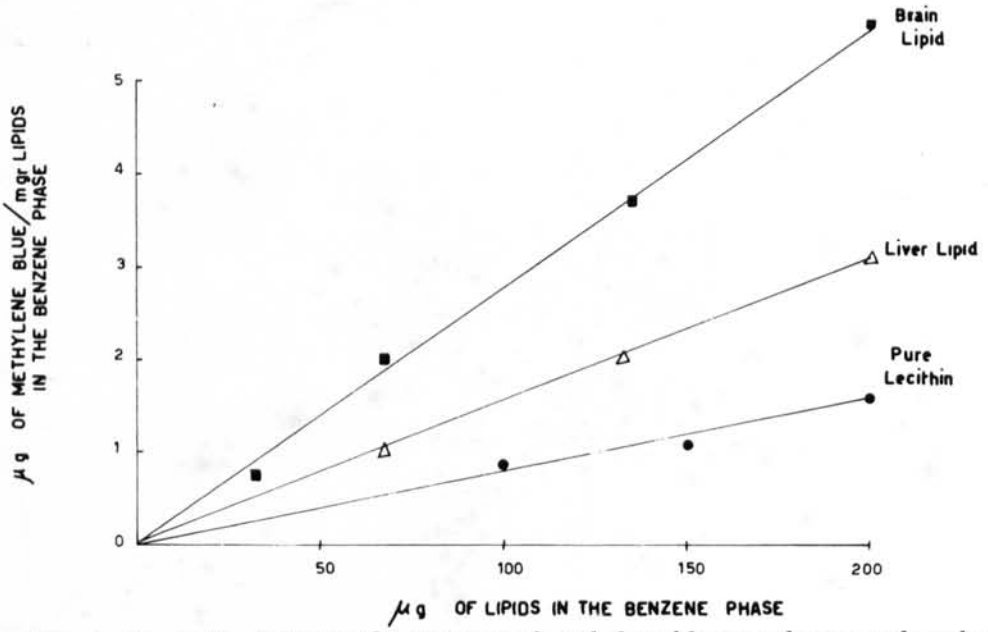

FIG. 1. Comparison between the transport of methylene blue to a benzene phase by lipids extracted from mouse brain and liver, and pure lecithins.

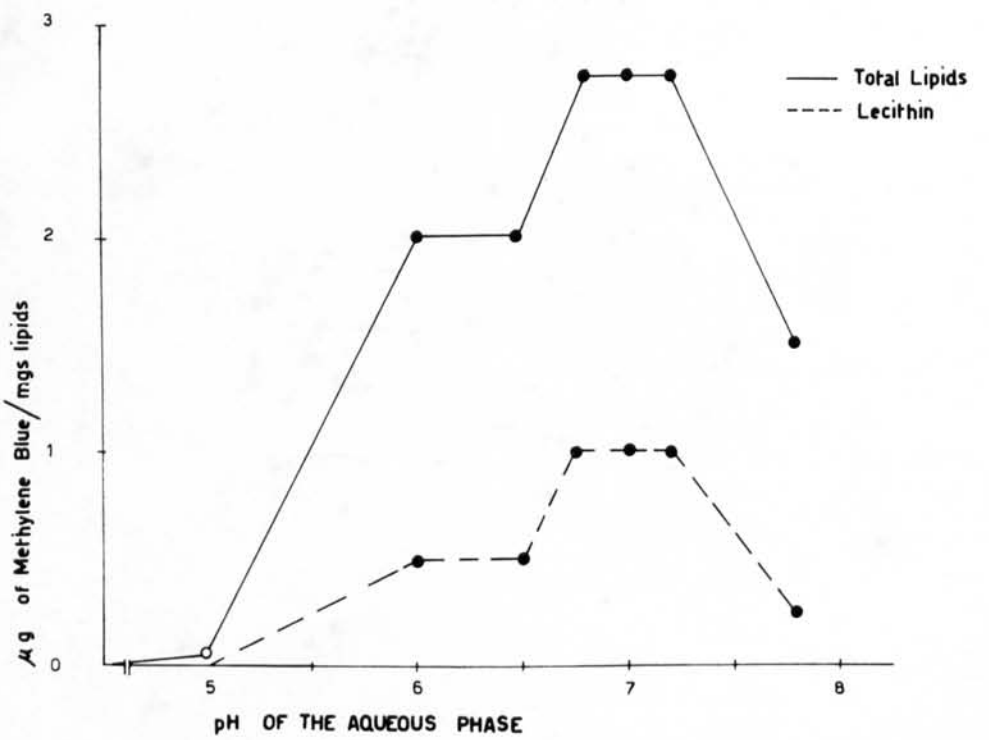

FIG. 2. Transport of methylene blue to a benzene phase in relation with the pH of the aqueous phase. o———o Acetate buffer; •———• Phosphate buffer.

RESULTS

Table 1 shows the transference of MB from the aqueous to the benzene phase by lipids extracted from brains and livers of albino mice and by pure lipids. Apart from the extracts mentioned, only lecithin was capable of transporting MB to the benzene phase. Although, the other phospholipids studied were capable of forming complexes with MB, it was not possible to accurately measure these because of the limited solubility of these lipids in benzene.

Of the lipids studied, phospholipids were the only ones capable of forming complexes with MB. Figure 1 shows that the transportation of MB by the lipids studied increases proportionally to the concentration of lipids in the benzene phase, at least in the concentration range used.

Figure 2 shows that the transference of MB to the benzene phase by the lipids under study is markedly influenced by the pH of the aqueous phase in the range of 6.0 to 7.4. This effect is the same with total extracts and with pure lecithins.

Since the formation of complexes between MB and phospholipids depends on the pH of the aqueous phase, we studied the effect of mono- and divalent cations on the transportation of MB to a benzene phase, finding that this transportation is strongly blocked by the presence of divalent cations in the aqueous phase (see Fig. 3). This effect is not seen in benzene with monovalent cations at the same ionic concentrations.

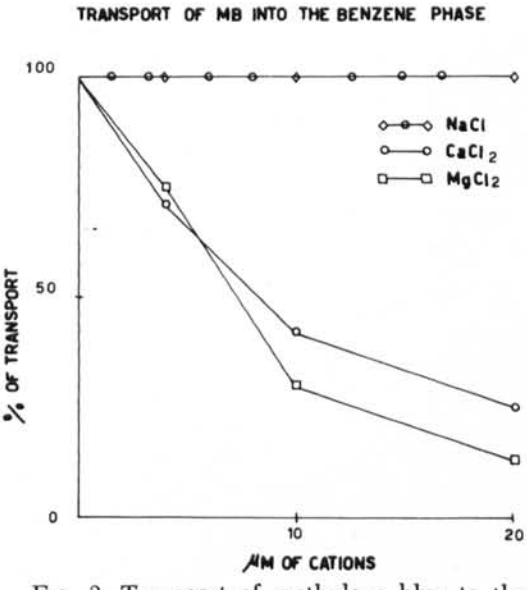

FIG. 3. Transport of methylene blue to the benzene phase of the biphasic benzene-water system in the presence of divalent and monovalent cations.

DISCUSSION

Of the various lipids studied, only phospholipids were capable of transporting MB to the benzene phase of the biphasic benzene-water system. Additional evidence for this is found in the fact that lipids extracted from the brain, which have a higher concentration of phospholipids than the total lipids extracted from the liver, showed a stronger transfer capacity than total liver lipids. The fact that the triglycerides studied, as well as the unsaturated fatty acids, are not capable of forming complexes with MB suggests that the phosphate group of the lecithins (or perhaps other negatively charged groups apart from the phosphate group in other phospholipids) is important in the formation of complexes with MB, thus eliminating any possible effect of the unsaturated fatty acids. This, together with the fact that the linkage between MB and the lecithins takes place at a given pH range and is blocked by divalent cations, strengthens the idea that the union takes place through an electrostatic interaction between the positive charge of MB and the negatively charged group(s) of lipids. The divalent cations may act by competing with the MB molecule in such a way that they prevent the union of the dye with the lipid molecule. Nevertheless, when the transport in the presence of lipids extracted from brain and liver were studied at the same concentration of lipid phosphorus, it was seen that the tissue extracts had a stronger transport capacity per milligram of phospholipid than the lecithins. This suggests that some part of the molecule of the phospholipids which is not present in the lecithin molecule plays an important role in the formation of these MB-phospholipids complexes.

SUMMARY

It is known that lesions from lepromatous leprosy fix methylene blue (MB) in its oxidized form after the intravenous administration of a 1% solution of the dye. The formation of complexes between MB and various lipids has been studied, on the basis of the great insolubility of this dye in benzene when in free form. Of the different lipids studied only lecithins were capable of forming complexes with the dye. The formation of these complexes is strongly dependent on the pH of the aqueous phase and it is blocked by the presence of divalent cations, which suggest an electrostatic interaction in the formation of these complexes.

RESUMEN

Se sabe que las lesiones de lepra lepromatosa fijan el azul de metileno (AM) en su forma oxidada después de la inyección intravenosa de una solución al 1% del colorante. Se estudió la formación de complejos entre el AM y varios lípidos, en base a la gran insolubilidad de este colorante en benceno cuando está en su forma libre. De los diferentes lípidos estudiados, sólo las lecitinas fueron capaces de formar complejos con el colorante. La formación de estos complejos depende acentuadamente del pH de la fase acuosa y se bloquea con la presencia de cationes divalentes, lo que sugiere una interacción electrostática en la formación de estos complejos.

RÉSUMÉ

On sait que les lésions de lèpre lépromateuse fixent le bleu de methylène dans sa forme oxydée, après administration intraveineuse d'une solution à 1 pour cent de ce colorant. La formation de composés entre le bleu de méthylène et divers lipides a été étudiée, en se basant sur la grande insolubilité de ce colorant dans le benzène, lorsqu'il est sous une forme libre. Parmi les différents lipides qui ont été étudiés, seules les lécithines étaient capables de former des composés avec le colorant. La formation de ces composés dépend fortement du pH de la phase aqueuse. Cette formation est empêchée par la présence de cations divalents, ce qui suggère qu'il existe une interaction électrostatique dans la formation de ces composés.

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