cently by GLYNN[5]. He also reported that the decrease in taking up 5-HT could be partly restored by the addition of gangliosides. A similar reactivation of the 5-HT receptor complex in smooth muscle by gangliosides was described by BORN[6]. To ensure that the added C^{14}-5-HT was really transported into the platelets and not merely adsorbed to the surface of the cells, we isolated the monoamine storage organelles by the method of DA PRADA et al.[7]. About 60% of the C^{14}-5-HT content of the intact platelets could be recovered from the isolated organelles. The supernatant, after lysis of the organelles in 2 ml 1 *N* NaOH for 3 h at 25 °C, neutralization and centrifugation at 105,000 × *g* in a Beckman ultracentrifuge (Spinco L 2-50) for 30 min at 4 °C, was concentrated and analyzed on a thin-layer plate (Silica Gel H, Merck, Darmstadt) in the developing system: methylacetate-isopropanol-25% NH_4OH (9:7:4)[8]. The radioactive spots were localized with a Berthold thin-layer scanner, model LB 2722.

Our results indicate that the neuraminic acid of the outer layer of the platelet surface membrane, which is easily released by neuraminidase, is probably not involved in the 5-hydroxytryptamine receptor. It seems that the partial loss of the peripherical neuraminic acid, and the resulting decrease of the negative charge of the platelet surface, facilitates the taking up of 5-HT.

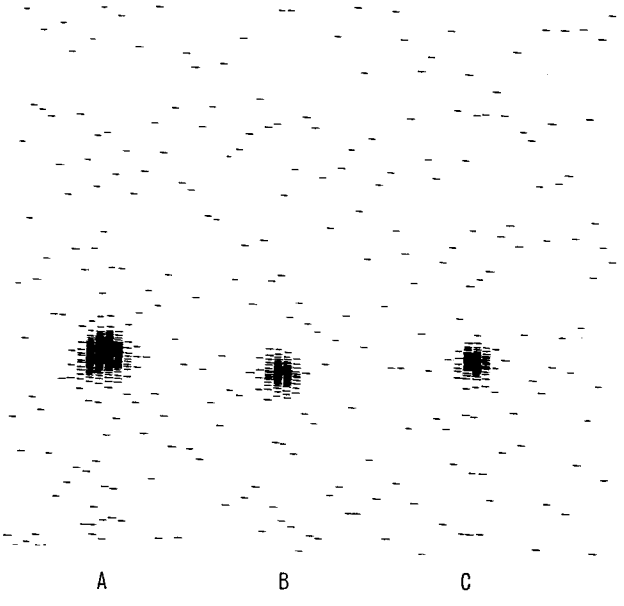

Fig. 3. Radio thin-layer chromatogram of C^{14}-5-HT. A) Test: C^{14}-5-HT. B) C^{14}-5-HT, lysed from isolated platelets. C) C^{14}-5-HT, lysed from isolated storage organelles.

The increase of the negative charge on the platelet membrane, by additional incorporation of neuraminic acid transfered by a sialyltransferase from rat liver homogenate, resulted in a reduction of the uptake of potassium ions[9]. The addition of 5-HT always enhanced the taking up of K^+ markedly. From these results it is highly evident that the extent of taking up K^+ by platelets is charge dependent, and that the diminution of the negative charge on the platelet surface by short neuraminidase treatment should accelerate the K^+-uptake into the platelets, too.

In our experiments, with low neuraminidase concentrations and a short incubation time, the neuraminidase of *Vibrio cholerae* did not liberate the neuraminic acid of glycolipids in the interior regions of the platelet membrane. The complete ganglioside pattern could be extracted after this treatment, but also neuraminic acid-containing glycoproteins were still present.

From the results after prolonged incubation with neuraminidase, it can be concluded that the recognition site, the absorption and the active transport mechanism of 5-HT is located within the platelet membrane and that the neuraminic acid, bound to glycoproteins or glycolipids within the membrane, participates in this process.

Zusammenfassung. Am 5-Hydroxytryptaminrezeptor der Thrombozyten ist die periphere Glykoprotein-Neuraminsäure nicht beteiligt. Der Verlust dieser peripheren Glykoprotein-Neuraminsäure fördert den Einstrom von 5-HT in die Thrombozyten. Der Rezeptor und der Transportmechanismus werden im Innern der Membran vermutet. Sie sind durch kurzfristige Neuraminidaseeinwirkung nicht erreichbar.

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The Inhibitory Effect of Toluidine Blue and Methylene Blue on Rat Mast Cell Damage by Promethazine, Chlorpromethazine and Chlorpromazine. Its Reversal by Glucose

In a previous paper it was shown that phenothiazine stains prevent rat mesentery mast cell damage induced by compound 48/80 and by the antihistamine drugs, diphenhydramine and chlorcylizine. It was also shown that this blocking effect was similar to that of metabolic inhibitors[1]. The present report describes the inhibitory effect of toluidine blue and methylene blue on rat mesentery mast cell damage by phenothiazine compounds with antihistamine activity: promethazine, chlorpromethazine, and chlorpromazine. The anti-enzymatic mechanism postulated to explain the inhibitory effect of these stains is substantiated.

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Materials and methods. The experimental procedure was similar to that previously described[2]. Pieces of rat mesentery were pre-incubated for 20 min in Tyrode in which the bicarbonate-phosphate buffer was substituted by *Tris* HCl (trihydroxymethylaminomethane) 0.005 *M*, pH 8.4, and containing either toluidine blue or methylene blue. Following the addition of the antihistamine, the incubation was carried on for 30 min more. All experiments were performed at 37°C. Fixation and staining of the pieces of mesentery and assessment of mast cell damage were performed as described by MOTA and DIAS DA SILVA[3]. Results are given as percentages of mast cells showing granule extrusion.

Drugs used. Toluidine blue (Harleco), methylene blue (Grubler). Promethazine (Phenergan, Rhodia), Chlorpromazine (Amplictil, Rhodia), and chlorpromethazine (Chlorphenergan, Rhone Poulenc) were kindly supplied by the manufacturers.

Results and discussion. At a concentration of 48 µg/ml, toluidine blue inhibited almost totally rat mesentery mast cell damage induced by promethazine (0.1 m*M*), but even at 4.8 µg/ml a 70% inhibition was found. A concentration of the stain 10 times higher (480 µg/ml) was required to abolish the damaging action either of chlorpromethazine (0.1 m*M*), which differs from promethazine by the presence of a Cl at the 2 position in the phenothiazine ring, or of chlorpromazine (0.05 and 0.1m*M*) (Figure 1). Methylene blue showed an inhibitory effect upon rat mast cell degranulation induced by the above antihistamine compounds similar to that of toluidine blue. However, it was distinctly less potent, for the action of promethazine (0.1 m*M*) was abolished only at a concentration of 160 µg/ml of the stain, while that of chlorpromethazine (0.1 m*M*) and chlorpromazine (0.1 m*M*) required 8 times more, 1280 µg/ml (Figure 2).

The blocking effect of toluidine blue (120 µg/ml) and of methylene blue (320 µg/ml) on rat mast cell degranulation by chlorpromazine (0.1 m*M*) was reversed partially when glucose (4.4 m*M*) was present in the incubation fluid throughout the experiment. It was found that in the presence of glucose the inhibitory effect of toluidine blue was reduced from 82 to 62%, and that of methylene blue from 75 to 33% (Figure 3).

In Figure 4 it can be seen that glucose (4.4 m*M*) also reversed the inhibitory action of toluidine blue (12 µg/ml) on the action of promethazine (0.1 m*M*) on rat mast cell degranulation from 87.5 to 49.2%. However, glucose had no effect whatsoever when toluidine at twice the concentration (24 µg/ml) was used.

The present results indicate that the 3 phenothiazine antihistamine compounds studied might act upon rat mesentery mast cells through a mechanism similar to that of diphenhydramine and chlorcyclizine, which also have antihistamine activity but have a different chemical structure. The anti-inhibitory effect of glucose suggests strongly that toluidine blue and methylene blue prevented mast cell damage by blocking Krebs cycle enzymes, an action similar to that of other anti-metabolic drugs[4,5]. Since a non-energy-requiring mechanism of histamine

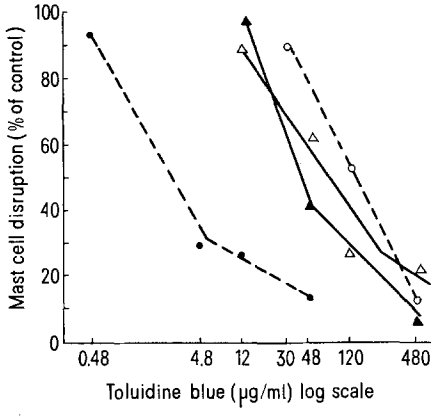

Fig. 1. Effect of toluidine blue on rat mesentery mast cell damage by promethazine (●---●, 0.1 m*M*), chlorpromethazine (○---○, 0.1 m*M*) and clorpromazine (▲—▲, 0.05 m*M*; △—△, 0.1 m*M*). Mast cell damage caused by toluidine blue itself was subtracted. Means of 2–8 experiments performed at pH 8.4.

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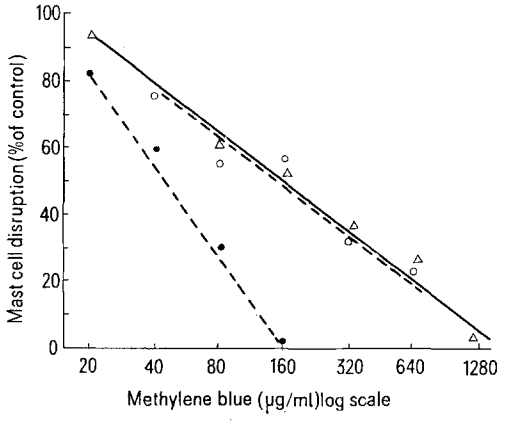

Fig. 2. Effect of methylene blue on rat mesentery mast cell damage by promethazine (●---●, 0.1 m*M*), chlorpromethazine (○---○, 0.1 m*M*), and chlorpromazine (△—△, 0.1 m*M*). Mast cell damage caused by methylene blue itself was subtracted. Means of 2–4 experiments performed at pH 8.4.

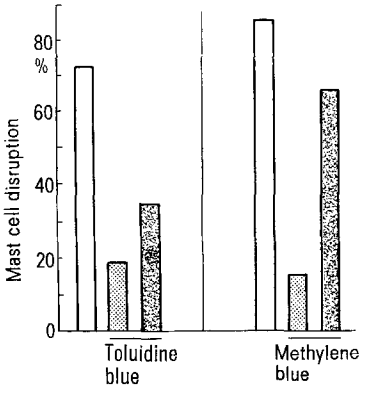

Fig. 3. Effect of toluidine blue (120 µg/ml) and methylene blue (320 µg/ml) on rat mast cell damage by chlorpromazine (□, 0.1 m*M*) in the absence (□) or presence (■) of glucose (4.4 m*M*). Means of 4 experiments (toluidine blue) and 2 experiments (methylene blue) performed at pH 8.4.

and 5-hydroxytryptamine release from rat peritoneal mast cells by chlorpromazine was described by FRISK-HOLMBERG[6] and JANSSON[7], we suggest that antihistamines act upon rat mast cells both by a lytic and by an energy-requiring mechanism. It is possible that the preparation procedures for isolating mast cells used by these authors may have enhanced the lytic effect, for the use of a Ficoll gradient has been described as causing cellular damage, and even a single washing of the cells with Hanks solution removes a surface protective material[8]. Moreover toluidine blue itself causes damage to rat peritoneal mast cells and releases histamine by a mechanism which is also dependent on cell metabolism[9]. Glucose was ineffective when toluidine blue was used at a concentration higher than that necessary to prevent the damaging action of promethazine. This same observation has been made with compound 48/80, chlorcyclizine and diphenhydramine, but the concentrations of toluidine blue at which glucose was ineffective varied with the different drugs[1]. These results suggest that toluidine blue inhibits rat mast cell damage by another mechanism, besides blocking Krebs cycle enzymes. The fact that chlorpromazine and chlorpromethazine required higher concentrations of the stains than promethazine for their action to be inhibited may be due to their higher lipophilic nature[10].

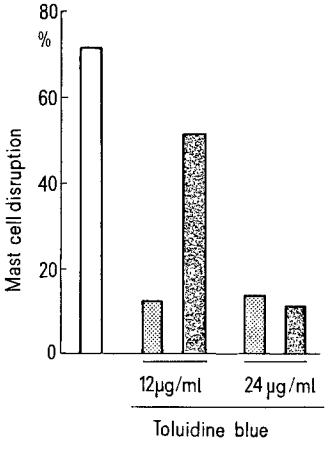

Fig. 4. Effect of toluidine blue on rat mast cell damage caused by promethazine (□, 0.1 m*M*) in the absence (▨) or presence (■) of glucose (4.4 m*M*). Mean of 9 experiments performed at pH 8.4.

Resumen. Se demuestra que la ación de los antihistamínicos prometazina, clorprometazina, y clorpromazina sobre los mastocitos de la rata es inhibida por el azul de toluidina y el azul de metileno. La glucosa revierte la acción inhibitoria de estos colorantes sobre la clorpromazina y la prometazina, sugeriendo que ellos actúan bloqueando el metabolismo celular. Se concluye que además de su acción lítica sobre los mastocitos de la rata, los antihistamínicos también actúan por un mecanismo que necesita de energia.

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In vitro Effect of a 5-Fluorodeoxyuridine Albumin Conjugate on Tumour Cells and on Peritoneal Macrophages

Conjugation with albumin gives to amanitin and to phalloidin a selective toxicity for cells having a high protein uptake[1-4]. The ultrastructural lesions produced in these cells by amanitin-albumin and by phalloidin-albumin conjugates are identical with those caused in hepatocytes by free amanitin and by free phalloidin respectively[1,4]. Since proteins after penetration into the cells are rapidly broken down by lysosomal enzymes[5], it is likely that the conjugates exert their toxic action within the cells after digestion of the protein moiety and release of the free toxins from conjugates. It was suggested[3] to try conjugating albumin with compounds which selectively damage cells in proliferation. If a compound with such a property is released in active form after penetration of the carrier protein into the cells (as in the case of amanitin and phalloidin), the resulting conjugate should specifically damage the dividing cells with a high protein uptake and could be useful in the therapy of histiocytic tumours. It is worth noting that 1 normal cells with high protein uptake (cells of macrophage system, granulocytes and kidney proximal tubule cells) do not proliferate[6,7]; 2. neoplastic proliferating histiocytes were found to be highly endocytic[8-10].

Besides neoplastic histiocytes, other types of tumour cells seem to display a high uptake of macromolecules[11,12]. Following this evidence, TROUET et al.[13] attempted to increase Daunorubicin penetration into tumour cells by administering the drug in a complex with DNA. En-

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