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REFERENCES

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). *J. biol. Chem.*, **193**, 265–275.

NOWELL, P. T., SCOTT, C. A. & WILSON, A. (1962). *Br. J. Pharmac. Chemother.*, **18**, 617–624.

ROBERTS, J. B., THOMAS, B. H. & WILSON, A. (1968). *Biochem Pharmac.*, **17**, 9–12.

SOMANI, S. M., ROBERTS, J. B. & WILSON, A. (1972). *Clin. Pharmac. Ther.*, **13**, 393–399.

Resistance of rats to the potentiating action of phosphatidyl serine on dextran responses

One of the amines released from mast cells during the dextran anaphylactoid reaction in rats, is histamine and yet only trace amounts of this amine are released when isolated peritoneal mast cells are incubated with dextran (Selye, 1968). Recently, Goth, Adams & Knoohuizen (1971) reported that phosphatidyl serine (PS) selectively and markedly potentiates this histamine release from isolated mast cells and the potentiation has since been shown to be calcium-dependent, maximal enhancement occurring at physiological levels of 1·0 mM calcium when the concentration of PS is 10 μg ml^{-1} (Foreman & Mongar, 1972).

As a colony of Wistar rats genetically resistant to the dextran anaphylactoid reaction was available (Harris, Kalmus & West, 1963), it was of interest, therefore, to study the effect of PS on their isolated peritoneal mast cells as a deficiency in PS may be the cause of resistance to dextran. Furthermore, a study of the effects *in vivo* of PS on dextran in Wistar rats sensitive to dextran (ASH) as well as in Wistar rats resistant to dextran (NR) was considered important as the histamine release *in vitro* is so markedly potentiated.

Rats were anaesthetized with ether and decapitated. Four ml of Tyrode solution containing heparin (10 μg ml^{-1}) was injected intraperitoneally and after 1 min of abdominal massage the fluid containing the peritoneal mast cells was removed. Aliquots were diluted with Tyrode solution containing heparin (10 μg ml^{-1}) and different concentrations of dextran (average molecular weight 67 000), with or without PS (10 μg ml^{-1}).* After incubation at 37° for 15 min, the cells were separated from the fluid using Millipore filters, the protein in the fluid was precipitated using trichloroacetic acid and heat (100°, 10 min), and the precipitate was removed by centrifugation (1000 *g*, 5 min). Samples were then neutralized with NaOH and assayed using the fluorimetric method of Anton & Sayre (1969). To obtain the total histamine content of control samples, the above procedure was followed, except that the passage through Millipore filters was omitted. Values shown for histamine release have been corrected by subtraction of the spontaneous release.

Local anaphylactoid reactions were produced in rat paws by injecting 0·1 ml (s.c.) Tyrode solution containing either dextran or dextran with PS, and assessed by measuring the increase in volume 1 h later using a volume differential meter. Other animals were injected intradermally with 0·1 ml Tyrode solution containing either dextran or dextran with PS into the shaved skin of the back after pre-treatment with intravenous azovan blue dye (30 mg kg^{-1}). 30 min later, they were killed and the reaction assessed by measuring the amount of dye in each weal using the method of Harada, Takeuchi & others (1971).

* (Koch-Light, Folch fraction 3.)

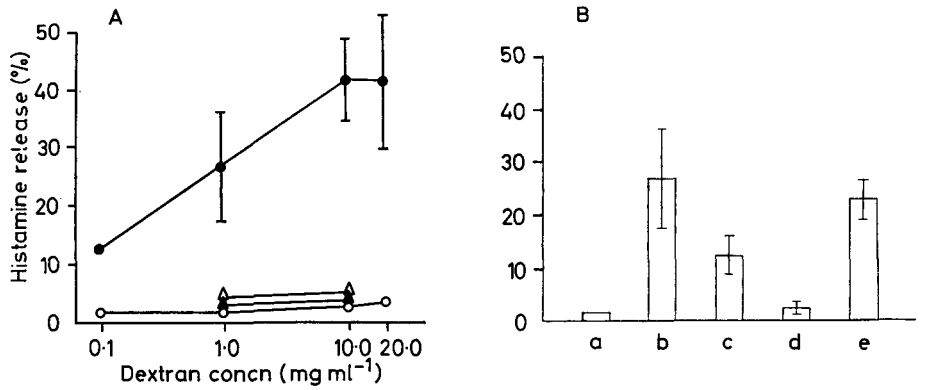

FIG. 1. A. Effect of PS (10 μg ml^{-1}) on the histamine release induced by dextran from isolated peritoneal mast cells. ASH rats—dextran alone ○—○, dextran + PS ●—●; NR rats—dextran alone △—△, dextran + PS ▲—▲. Note that PS potentiates the histamine release by dextran in ASH rats but not in NR rats. Vertical lines are s.e.

B. Effect of glucose and galactose on the potentiation by PS (10 μg ml^{-1}) of the histamine release induced by dextran (1 mg ml^{-1}) from isolated mast cells of ASH rats. (a) Dextran + 5·6 mM glucose; (b) dextran + PS + 5·6 mM glucose; (c) dextran + PS + 11·2 mM glucose; (d) dextran + PS + 28 mM glucose; (e) dextran + PS + 28 mM galactose. Note that the galactose does not inhibit the PS potentiation of histamine release by dextran in ASH rats. Vertical lines are s.e.

Spontaneous release of histamine from isolated peritoneal mast cells of ASH rats was about 10% of the total histamine content. Dextran alone released only up to 3% more, even when used in concentrations of 20 mg ml^{-1}. However, when PS was included in the incubation mixture up to 40% of the total was released (Fig. 1A), and maximal release occurred when the ratio of dextran to PS was 1000 (that is, when dextran at 10 mg ml^{-1} was used). This result agrees with that reported by Foreman & Mongar (1972). There was no release of histamine from mast cells when PS was used alone. In NR rats, on the other hand, although spontaneous release was slightly higher than in ASH rats and dextran released up to 4% of the total histamine, PS failed to enhance the histamine release by dextran (Fig. 1A), even when concentrations producing maximal release in ASH rats (10 mg ml^{-1}) were used. A structural or chemical difference in the cell membrane of mast cells from the two types of rat may therefore be considered.

In further experiments with isolated peritoneal mast cells from ASH rats, it was found that whole blood or plasma, unlike PS, did not potentiate histamine release by dextran. However the potentiating action of PS on dextran in these animals was partially inhibited by increasing the glucose concentration in the Tyrode solution used for incubation from 5·6 to 11·2 mM and was totally inhibited when 28 mM glucose was present (Fig. 1B). Poyser & West (1965) have already reported that glucose, but not galactose, inhibits the local response of rats to intradermal dextran, and a similar result was obtained in the present work, using isolated mast cells, when galactose (28mM) had no inhibitory effect on the PS potentiation (Fig. 1B).

When local anaphylactoid reactions were tested in the paws, a dose-dependent increase in paw volume was obtained in ASH rats using doses of dextran between 100 μg and 1 mg, and PS (in doses of 1, 5 and 50 μg) did not potentiate these responses. Thus, when the ratio of doses of dextran to PS was 1000, no potentiation was found; however, in this type of experiment only the *effects* of released mast-cell amines are measured. In NR rats, dextran alone was 250 times weaker than in ASH rats in producing a local subcutaneous response and PS did not potentiate this small increase in paw volume. Similarly, histamine release from the peritoneal cavity of ASH rats by dextran was not potentiated by PS.

Intradermal dextran in ASH rats (doses between 100 μg and 1 mg) produced a dose-dependent increase in dye leakage, but again PS in doses up to 50 μg failed to enhance these responses. In NR rats, dextran alone was 250 times weaker than in ASH rats and PS was ineffective. In this type of experiment, as in the paw experiments, only the *effects* of released amines on vascular permeability are being measured.

Thus, PS potentiates the action of dextran on isolated mast cells of ASH rats but does not enhance its action in the skin or subcutaneous tissues where enough PS may be normally present for the dextran reaction to occur. When mast cells are isolated for experimental work, a necessary factor for the dextran reaction may be lost, and the experiments with blood and plasma indicate that this factor is not contained in these intravascular fluids. However as PS does not potentiate dextran in isolated cells of NR rats, a deficiency of PS is unlikely to be the cause of failure of these animals to respond to dextran.

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REFERENCES

ANTON, A. H. & SAYRE, D. F. (1969). *J. Pharmac. exp. Ther.*, **166**, 285–292.
FOREMAN, J. C. & MONGAR, J. L. (1972). *Br. J. Pharmac.*, **46**, 767–769.
GOTH, A., ADAMS, H. R. & KNOOHUIZEN, M. (1971). *Science*, **173**, 1034–1035.
HARADA, M., TAKEUCHI, M., FUKAO, T. & KATAGIRI, K. (1971). *J. Pharm. Pharmac.*, **23**, 218–219.
HARRIS, J. M., KALMUS, H. & WEST, G. B. (1963). *Genet. Res.*, **4**, 346–355.
POYSER, R. H. & WEST, G. B. (1965). *Brit. J. Pharmac. Chemother.*, **25**, 602–609.
SELYE, H. (1968). In *Anaphylactoid Edema*, Warren H. Green Ind., St. Louis, Missouri, U.S.A., p. 14.

The influence of starch and lactose on the release rates of drugs from hard gelatin capsules

The influence of additives on the rates of release of drugs from hard gelatin capsules has been studied by Samyn & Jung (1970) and Newton, Rowley & Törnblom (1971a, b). Newton & others (1971b) examining the effect of a diluent, lactose, a lubricant, magnesium stearate, and a wetting agent, sodium lauryl sulphate, on the release of the hydrophobic drug, ethinamate, found the effect produced by each additive was dependent on the presence, and level, of the other two additives. If the diluent only was considered, a level of 50% was required to increase the drug release rate, lower levels being presumably insufficient to change the hydrophobic nature of the powder bed.

We have examined the effect of two commonly used diluents, lactose and starch (potato) on the release of a hydrophobic drug, phenobarbitone, and a hydrophilic drug, phenobarbitone sodium. Experiments were made with a single size fraction, 75–104 μm, of both drugs and diluents to eliminate the influence of particle size (Newton & Rowley, 1970). No 5 hard gelatin capsules were used and were hand filled to a constant weight of 100 mg $\pm$ 5 mg.

Dissolution testing was by the beaker method of Levy & Hayes (1960) adapted for capsules as described by Newton & Rowley (1970), using a stirrer speed of 80 rev min^{-1}. The dissolution fluid was distilled water (1900 ml) maintained at 37°. During a test, samples were taken at regular time intervals, filtered through a No. 5 sintered glass filter, and the amount of drug in solution estimated immediately by ultraviolet spectroscopy at 253 nm after suitable dilution to 0·1 N with sodium hydroxide solution.