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LETTERS TO THE EDITOR

Isolation from bovine plasma of a protein that enhances vascular permeability in rats

In 1969, Gecse, Karády & others isolated from bovine plasma a smooth muscle-stimulating substance which was predominantly α_2-globulin and had a molecular weight of about 800 000. It was obtained by fractionating the plasma proteins by Cohn's method and then identifying the active material in fraction IV–1 after purification using a Sephadex G 200 column. In the present study, the proteins of bovine plasma were fractionated by Cohn's method using cold ethanol and tested for their ability to increase vascular permeability in rat skin. It was found that only fraction II + III possessed this ability, the fraction being predominantly γ-globulin with a molecular weight of about 160 000.

Plasma was obtained from healthy oxen by centrifuging citrated blood at 4000 g for 15 min at 0° and then fractionating the proteins by Cohn's method and freeze-drying them. From each protein fraction, 250 μg in 0·1 ml saline was injected intradermally into the depilated skin of adult albino rats which had azovan blue dye (10 mg/kg) in their circulation. Thirty min later, the rats were killed, their dorsal skin was removed, cleaned and extracted with Germanin dissolved in methanol (Jancso-Gabor, Szolcsanyi & Jancso, 1967), and the amount of dye in solution determined in a spectrophotometer at 620 nm. Only fraction II + III exerted a pronounced effect on vascular permeability (49 $\pm$ 5 μg), the dye extracted being more than 5 times the control value of 9 $\pm$ 3 μg or any of the values obtained with the other fractions. γ-Globulin accounted for about 50% of the protein in fraction II + III when determined by gel electrophoresis. This fraction was then transferred to a Sephadex G 200 column (diameter 2 cm, length 63 cm) and eluted with tris buffer (pH 8·05) passing at a rate of 10 ml/h. Samples of eluate (1·5 ml) were tested for biological activity and for protein content by measuring light absorption at 280 nm and by studying gel electrophoresis. The active eluate enhancing vascular permeability contained more than 89% of γ-globulin and its rate of flow from the column corresponded with compounds of a molecular weight of about 160 000. The activity was significantly decreased 15 min after incubation with crystalline trypsin (1 mg/mg protein at 37° and pH 8·0) and was completely abolished at 50 min. Whereas soya bean trypsin inhibitor prevented the vascular permeability-increasing action of trypsin alone, it did not modify the activity of the Sephadex-separated active fraction from bovine plasma. When the active fraction was subjected to alkaline hydrolysis (pH 12, 100°), the activity significantly *increased* during the first 90 min of hydrolysis (exuded dye being 98 $\pm$ 8 μg) but then quickly decreased and by 180 min it was lost. Finally, on dilution 10-fold with saline, activity decreased and was finally lost when the fraction was diluted 100-fold, whereas undiluted guinea-pig serum was ineffective yet increased in activity as the serum was diluted (see Fig. 1).

At least two vascular permeability-enhancing factors have been previously isolated from the plasma and serum of guinea-pig, rat, rabbit and man. One of these has been shown to be identical with kininogenase whereas the other was called Permeability Factor (or PF, for short) and this was activated by dilution. According to Miles (1969) and Movat and his co-workers (1969 a, b), the activity of these two factors on

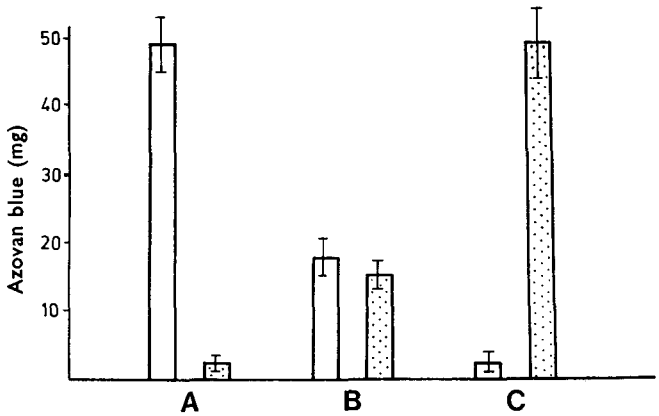

FIG. 1. The effect of dilution of the Sephadex-separated active fraction from bovine plasma (open columns) and of guinea-pig serum (dotted columns) on the vascular permeability action, as measured by the amount of azovan blue dye (μg, ordinate) exuded into rat skin after intradermal injections of 0·1 ml. Mean values $\pm$ s.e. of 20 determinations are recorded. A. Undiluted. B. Diluted 10-fold. C. Diluted 100-fold.

vascular permeability is suppressed by incubation with soya bean trypsin inhibitor, whereas in the present study that of the Sephadex-separated active fraction remained unchanged and further, it was not activated by dilution. On the basis of these findings, the plasma fraction that enhances vascular permeability is not kininogenase or PF. Its action, however, may lead to the leakage of plasma proteins into the extracellular compartments, thereby suggesting that kinin is being formed in the tissues. In pathological states where plasma protease levels are raised (such as in shock and anaphylaxis), the activity of the new permeability factor may be of importance, particularly in alkaline conditions. However, the optimum dose of the active protein was found to be in the range 100–200 μg, values which are to be compared with those of prostaglandin (10–100 ng) exerting a similar action.

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REFERENCES

GECSE, Á., KARÁDY, S., LÓZSA, A., ZSILINSZKY, E. & WEST, G. B. (1969). *J. Pharm. Pharmac.* **21**, 700–701.

JANCSO-GÁBOR, A., SZOLCSÁNYI, J. & JANCSO, N. (967). *Ibid.*, **19**, 486–487.

MILES, A. A. (1969). *Proc. Roy. Soc. Biol.*, **173**, 341–349.

MOVAT, H. Z., TRELOAR, M. P., DILORENZO, N. L., ROBERTSON, J. W. SPENCER, H. B. (1969 a). *Cellular and Humoral Mechanism in Anaphylaxis and Allergy.* pp. 215–223. Editor: Movat, H. Z., Basel Karger–New York.

MOVAT, H. Z., TRELOAR, M. P. & TAKEUCHI, Y. (1969 b). *J. Immun.*, **103**, 875–878.