

stances chemically like the physiological end-product of the previously defective metabolic process.

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¹ Garrod, A. E., *Lancet*, ii, 142 (1908).

² Hutchison, J. H., and McGirr, E. M., *J. Clin. Endocrinol. and Met.*, 14, 869 (1954).

³ Zondek, H., Leszynsky, H. E., and Zondek, G. W., *Acta Endocrinol.*, 29, 47 (1958).

⁴ Zondek, H., Leszynsky, H. E., and Zondek, G. W., *Brit. Med. J.*, i, 340 (1959).

dosage-levels. It may therefore prevent release of acetylcholine from the inactive bound state.

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¹ Frazer, A. C., Fletcher, R. F., Ross, Constance, A. C., Shaw, B., Sammons, H. G., and Schneider, R., *Lancet*, ii, 252 (1959).

² Anderson, C. M., Frazer, A. C., French, J. M., Gerrard, J. W., Sammons, H. G., and Smellie, J. M., *Lancet*, i, 836 (1952).

³ Billbring, E., Crema, A., and Saxby, O. B., *Brit. J. Pharmacol.*, 13, 440 (1958).

⁴ Paton, W. D. M., *Brit. J. Pharmacol.*, 12, 119 (1957).

⁵ Feldberg, W., and Lin, R. C. Y., *J. Physiol.*, 111, 96 (1950).

Effect of Wheat Gluten on the Peristaltic Reflex

SENSITIVITY to wheat or rye gluten is the essential mechanism in the pathogenesis of coeliac disease and idiopathic steatorrhoea. Such patients improve on a gluten-free diet and deteriorate on the re-introduction of gluten itself or its autoclaved and filtered peptic/tryptic digest into the diet¹. In these diseases a disturbance of small intestinal function is a major factor².

The peristaltic reflex of the isolated intestine³ was used to test the depressant activity of gluten and several of its fractions on intestinal motility. Perfusion of the autoclaved and filtered peptic/tryptic digest through the lumen did not produce any depression of this preparation. External administration, however, caused complete inhibition of longitudinal as well as circular muscle contraction. Movements returned while the material was still in the bath. This depression was also shown by an aqueous extract of gluten and was not affected by ultrafiltration. The ultrafiltrate of the aqueous extract was ten times as potent as the peptic/tryptic digest. All activity was abolished by acid hydrolysis. Incubation of the active material for a few minutes with small intestinal mucosa from the rat and with one specimen of human jejunal mucosa abolished the inhibitory effect.

The mechanism of this depressant effect of gluten fractions was studied with the help of the co-axially stimulated isolated guinea pig intestine⁴. The twitch response was markedly depressed for several minutes with either fraction. Assay of the acetylcholine output on the isolated guinea pig ileum treated with neostigmine and morphine⁵ showed a significant depression with the addition of either material. In preliminary experiments, the synthesis of acetylcholine by small intestinal cholinacetylase⁵ was not affected by the addition of the ultrafiltrate of the aqueous extract of gluten.

The conclusion is drawn that wheat gluten which is pathogenic in coeliac disease and idiopathic steatorrhoea contains a factor which depresses the peristaltic reflex of an isolated strip of intestine. This factor is heat-stable, resistant to peptic/tryptic digestion and not removed by ultrafiltration. It is sensitive to acid hydrolysis and digestion with small intestinal mucosa. It depresses acetylcholine output, but synthesis does not appear to be affected at moderate

HÆMATOLOGY

Substance from Erythrocytes of Blood Group A

BLOOD group substances from erythrocytes have been claimed to be glycolipids, while those from secretions were described as mucopolysaccharides. It has been suggested that glycolipids from erythrocytes owe their blood group activity to the contamination by secretions like blood group substances¹. The problem of identity or relationship between these two kinds of blood group substances may be solved only if their chemical and serological properties are directly compared.

The present communication describes the isolation and properties of a human blood group A substance from erythrocytes. Determinations of isoagglutination inhibition and hæmolysis (Förssman) inhibition tests were made using as a control the original sample of blood group A substance (kindly given by Prof. W. T. J. Morgan and Dr. W. M. Watkins of the Lister Institute, London) hereafter referred to as a 'standard A substance'.

Pooled, washed, human A erythrocytes were extracted with 4.05 vol. of 96 per cent w/w ethanol for 24 hr. at room temperature. The extract was Seitz-filtered and cooled to -7° C. A precipitate, 'crude A substance', which formed was centrifuged at -7° C., washed with acetone and dried in a vacuum. Its blood group activity, as measured by the isoagglutination inhibition test, was 2.5 per cent of the activity of the standard A substance; it did not contain O, H, Rh, M, N, P factors and exhibited no influenza virus hæmagglutination inhibition activity. Chemical analysis revealed 15.5 per cent reducing sugars as galactose, 2.6 per cent hexosamine, 3.4 per cent phosphorus, 2.0 per cent nitrogen; the glucosamine/galactosamine ratio² was 1:4.

The crude substance was extracted with acetone, ethyl ether, and petroleum ether successively, then dissolved in boiling methanol, filtered while hot, and the extract was cooled to 0° C. The precipitate formed ('precipitate M') contained 26.3 per cent reducing sugar, 5.0 per cent hexosamine, 1.4 per cent phosphorus. Its activity was about 10 per cent of that of the standard substance.

A sample of 'precipitate M' has been further purified by chromatography on a cellulose column. Two fractions emerged from the column: fraction I in