Fig. 2

few days the preparations lose their activity against monoamines, and as they do so they also lose their activity against serum vasoconstrictor.

These observations suggest that the serum vasoconstrictor contains a free terminal aliphatic amino-group which is oxidized by a monoamine oxidase in the lung preparation through the same mechanism as that suggested for tryptamine and tyramine.

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A New Method for Preparing Type-Specific Extracts from Haemolytic Streptococci of Group A, Type 2

HÆMOLYTIC streptococci of Lancefield's Group A (*Str. pyogenes*) can be classified into a number of types on the basis of precipitin reactions with an acid extract of the cocci and appropriate type-specific antisera; the type-specific material in the streptococci is a protein referred to as the *M*-substance¹. It has been a general experience that it is frequently difficult to obtain active extracts from strains of some types, including Type 2. I have found that, if extraction is carried out at neutral pH, and at 120° C. (in an autoclave) instead of at 100° C., satisfactory extracts are obtained with strains of Type 2.

Four extraction methods were compared, using in each case the deposit from 30 ml. of an 18-hr. culture in broth containing 10⁻⁵ *M* iodo-acetic acid². The methods were as follows: (1) Extraction at 100° C. with 0.3 ml. of *N*/5 hydrochloric acid for 10 min. followed by centrifuging, neutralization with *N*/5 sodium hydroxide and further centrifuging if necessary; this is the classical method. (2) The first method modified only in that the neutralization was carried out immediately after the extraction and before centrifuging. (3) The deposit was suspended in 0.5 ml. of saline, neutralized with *N*/5 sodium hydroxide using phenol red as indicator, and autoclaved at 120° C. for 5 min.; it was then centrifuged. (4) The same method as in (3) but heating to 100° C. for 10 min. instead of to 120° C. for 5 min. In each case the final supernatant was tested for type-specific antigen by the capillary method of Swift *et al.*³. The results are shown in the accompanying table.

Antigen obtained by method	Sera		
	Group specific	Type-specific	
		Type 2	Heterologous type
1	+++	0	0
2	+++	+	0
3	+++	+++	0
4	+++	+	0

The extract obtained by method (3) was clearly the best, and the fact that the reaction was lost when the extract was digested with trypsin for 24 hr. before testing indicates that it was most probably due to the *M* antigen. It is also noteworthy that neutralization before centrifuging gave a better extract than the classical method¹; it is probable that the *M* antigen is precipitated at the acid pH, and in the case of method (1) is removed in the centrifuging.

The experiment described above has been repeated with six other strains of Group A streptococci, considered on the basis of agglutination typing to be Type 2, with the same results.

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Free and Combined Riboflavin in the Blood Serum of Oestrogenized Fowls

THE serum riboflavin of the domestic fowl increases from an average of 0.07 p.p.m. to 0.86 p.p.m. when the bird comes into lay, and remains at this high level while laying; increases of the same order can be evoked by the administration of oestrogens to immature pullets¹.

Riboflavin has been shown to exist in the blood serum of rats in three forms: as free riboflavin, as flavin mononucleotide and as flavin adenine dinucleotide. In investigations into changes in serum riboflavin, it was therefore important to ascertain the relative concentrations of these three forms in both laying and non-laying pullets.

At this time, blood from oestrogenized immature pullets became available from other work in progress at this Centre, and this has been examined. The birds were Brown Leghorns bred at the Centre, and were ten weeks old at the start of the experiment. All birds were fed the same diet, and all received the injections on alternate days. They were killed on the day following the last injection, when they were twelve weeks old, and the bloods were examined by the method of Burch *et al.*² assuming all three forms to be present. The treatments and results are set out in the accompanying table.

Subsequent dissection indicated that increase in the dose of oestrogen had brought about hypertrophy

No. of bird	Dose of oestrodial benzoate (mgm.)	Free riboflavin (p.p.m.)	Flavin mono-nucleotide (p.p.m.)	Flavin adenine di-nucleotide (p.p.m.)
Y 1314	7 × 0.0	0.02	0.02	0.01
Y 1306	7 × 0.5	0.02	0.02	0.01
Y 1311	7 × 1.0	0.90	0.01	0.01
Y 1356	7 × 2.0	1.90	0.01	0.01
Y 1320	7 × 3.0	2.54	0.03	0.01