mucins[9]. The release of sialic acid from mucin should raise its *p*H of minimum solubility towards neutrality and under the conditions experienced in saliva would tend to cause the resultant mucin to precipitate out of solution. It has been found in this laboratory that there is a significant increase in the turbidity of saliva containing antibiotic on the addition of neuraminidase compared with a control saliva to which heat denatured enzyme has been added.

Reference to a typical oral organism that is known to produce neuraminidase could not be found in the literature. This may be because the enzyme is induced (adapted) by the organisms in the saliva because of the paucity of carbohydrate present. Recent work suggests that the neuraminidase of *Vibrio cholerae* is an induced enzyme[2]. Some indication that the enzyme is induced in saliva is shown by the fact that the sialic acid is released much more slowly from salivary mucin in the presence of an added 1 per cent buffered glucose. After 90-min incubation of fresh saliva containing glucose, the sialic acid content of the mucin was, in the majority of cases, more than 90 per cent its original value and in some instances over 50 per cent remained after 24 h incubation. This is in striking contrast to the results obtained in the absence of glucose (Fig. 1). The *p*H of the solutions in all instances were kept above 6 by the addition of phosphate buffer.

It is thought in this laboratory that the reaction involving the release of sialic acid from salivary mucin leading to its precipitation under mildly acid or neutral conditions plays an important part in the formation of dental plaque, and the lack of sialic acid and the presence of bound hexosamine in the plaque substantiate this view.

I thank Mrs. Mary Griffiths for assistance, Dr. A. J. Milne Gall and all members of the hospital staff who collected plaque and saliva, and the Medical Research Council for financial support.

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Isolation of Phosvitin from the Plasma of the Œstrogenized Immature Pullet

The presence of phosphoprotein in the plasma of the laying hen is widely held to be the result of the action of œstrogenic substances produced by the growing ova of the mature bird[1], though the precise nature and quantities of œstrogenic substances present in the blood stream have not yet been determined. Recently it has been shown that the phosphoprotein in the plasma of the laying hen is phosvitin, the principal protein of egg yolk[2], and it seemed desirable to examine the plasma of œstrogen-treated non-laying birds to determine whether or not such treatments could, in fact, lead to the formation of phosvitin.

Immature pullets 12–14 weeks old were injected intramuscularly with œstradiol benzoate (2 mg/day) on alternate days for 10 days. During this time the plasma phosphoprotein concentrations increased markedly. The birds were then bled, the plasma collected and the phosphoprotein precipitated as the calcium complex before working up as described by Heald and McLachlan[2]. The partially purified fraction was further purified by stepwise elution from diethylaminoethyl cellulose with sodium chloride at *p*H 8·6[3]. Two main fractions containing phosphorus were obtained: a major fraction eluted with 0·30 M sodium chloride amounting to 54–60 per cent of the phosphorus, and a second fraction eluted with 0·25 M sodium chloride amounting to 30 per cent of the phosphorus added. The remaining 10–15 per cent of the phosphorus was found in minor fractions eluted with 0·15 M and 0·20 M sodium chloride respectively. The major fraction contained 9·5 per cent phosphorus and 9·75 per cent nitrogen, yielding a nitrogen/phosphorus ratio of 2·22. It contained 3·66 moles serine/10^4 g protein yielding a serine/phosphorus ratio of 1·0. These properties are identical with those of phosvitin from egg yolk[4] and from the plasma of the laying hen[2]. The second peak contained 6·3 per cent phosphorus and 10·9 per cent nitrogen, yielding a nitrogen/phosphorus ratio of 3·83. It contained 2·03 moles serine/10^4 g protein, giving a serine/phosphorus ratio of 1·0.

The results clearly establish that administration of œstrogen to the immature pullet results in the appearance of phosvitin in the plasma. Since, in birds of this age, the ovary is not developed and largely non-functional[1] the results also provide strong evidence that the synthesis of phosvitin takes place elsewhere in the body. The second component, which contains phosphorus and serine in a ratio indicative of their existence as phosphorylserine, was not found in any quantity in the plasma of the laying bird[2]. Since the immature pullet has no developing ova capable of removing plasma phosphoprotein for yolk formation it is considered that this second component is either an accumulated intermediate of phosvitin synthesis or a product of its breakdown.

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Inhibition of Biological Synthesis of Acetylcholine by Triethylcholine

Bowman and Rand have suggested that the mechanism by which the triethyl analogue of choline causes failure of neuromuscular transmission is by interfering with synthesis of acetylcholine in the nerve endings[1]. If so, triethylcholine may be like HC-3 (hemicholinium), which has been found to inhibit the synthesis of acetylcholine by organized nerve tissue[2,3].

The experiments reported here were carried out to determine the effect of triethylcholine on synthesis of acetylcholine by sub-cellular fractions of rabbit brain homogenates.

The choline acetylase (acetyl-Co*A*: choline-*O*-acetyltransferase, EC 2.3.1.6) incubation system used was that described by Hobb, 1963[4]; acetyl-Co*A* is produced from acetylphosphate and coenzyme *A*, by phosphotransacetylase. The ACh synthesized was assayed biologically using the frog rectus abdominis muscle. Triethylcholine has been shown to be inactive on this assay preparation[1] and it was confirmed that triethylcholine does not affect contractions due to acetylcholine.

Table 1 shows the effect of triethylcholine on the synthesis of acetylcholine by the large granule or 'mitochondrial' fraction of rabbit brain (P_2 fraction) (ref. 4). When the concentration of choline (0·8 mg/tube) was optimal an equimolar concentration of triethylcholine (1·04 mg/tube) inhibited synthesis of acetylcholine by 11 per cent; doubling the triethylcholine concentration almost doubled the inhibition, and at the largest concentration of triethylcholine (10·4 mg/tube) the inhibition was 40 per cent.