N-acetyl neuraminic acid, kindly supplied by Prof. G. Blix, was used as a standard to determine the sialic acid quantitatively. The results are presented in Table 1.

The results indicate that the content of sialic acid in a granuloma pouch is much greater than in a corresponding abdominal region not involved in the pathological process.

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Content of Sialic Acid in Serous Glands of Equines

In histochemistry it is well known that an objection to the specificity of the periodic acid–Schiff reaction for mucopolysaccharides is due to the possible interference of some amino-acids. This objection is particularly important when the periodic acid–Schiff reaction is applied to serous glands, in which the secretion is essentially proteins. However, Junqueira *et al.*[1] found material giving a positive reaction in the parotids of many mammals, and concluded that substances other than proteins were present in the secretion of parotid gland. This observation opened up an interesting problem of cellular biology, regarding the possibility of a multiple secretion from serous glands.

In order to confirm this observation, a comparative histochemical investigation of material positive to the periodic acid–Schiff reagent and the ribonucleic acid content of some serous glands was carried out by Bignardi *et al.*[2]. This investigation, using pancreas, parotid and orbital glands of equines, demonstrated that the intensity of periodic acid–Schiff reaction varied inversely with the ribonucleic acid content. On the basis of general views on the function of ribonucleic acid in protein biosynthesis, this result was interpreted as indirect evidence that positive reaction to the periodic acid–Schiff reagent was really due to carbohydrate material.

Since more direct evidence for this view could be obtained by chemical analysis of some constituent of mucopolysaccharides, we have investigated the content of sialic acid in serous glands. The emphasis on sialic acid was due to its presence in submaxillary mucin[3] and to its strong reactivity for periodic acid. (In a very recent paper of Quintarelli *et al.*[4], a relation between sialic acid and positive periodic acid–Schiff reaction has been reported.)

Sialic acid has been determined in orbital glands (lacrimal gland and gland of the third eyelid), parotid and pancreas of ass and horse.

The glands were removed and stored under acetone. About 50 mgm. (dry tissue) of each gland was used for sialic acid determination, which was carried out by Svennerholm's method[5]. The tissue was homogenized with 5 ml. of 0·1 *N* sulphuric acid by Potter–Elvehjem homogenizer and refluxed at 80° C. for 1 hr. The hydrolysate was centrifuged and the supernatant chromatographed on 'Dowex 2 × 8'; the sialic acid was eluted by acetate buffer 0·1 *M*, *p*H 4·6, and sialic acid determined by the resorcinol reaction.

Table 1. Content of Sialic Acid in Serous Glands of Equines
(Values are referred to 100 mgm. of dry tissue)

Animals	Lacrimal gland	Gland of the third eyelid	Parotid	Pancreas
Ass 1	500 μgm.	410 μgm.	195 μgm.	118 μgm.
2	420 ,,	362 ,,	167 ,,	127 ,,
3	430 ,,		181 ,,	
Horse*				
1	621 μgm.	408 μgm.	157 μgm.	134 μgm.
2	778 ,,	427 ,,	138 ,,	168 ,,

* The presence of sialic acid in parotid gland of the horse has been reported by Svennerholm (ref. 5).

The standard curve was obtained with N-acetyl-neuraminic acid isolated from sheep submaxillary gland and kindly supplied to us by Prof. G. Blix. The results are reported in Table 1.

The results show that the highest content of sialic acid is in the orbital glands, a low content in parotid and the lowest in pancreas. Since positive periodic acid–Schiff reaction is also highest in glands of the orbit, is less in the parotid and is weakly positive in the pancreas, there seems to be a relation between the periodic acid–Schiff reaction and sialic acid content.

The presence of sialic acid and, probably, of a sialopolysaccharide, in serous glands shows that these glands elaborate not only protein but also carbohydrate material.

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In vivo Metabolism of N-Acetylhexosamine ^{35}S-Sulphate Esters

In a previous report[1] evidence was presented indicating that the monosulphate ester obtained as the principal product of the direct sulphation of N-acetyl-D-glucosamine with chlorosulphonic acid was identical with N-acetyl-D-glucosamine 6-O-sulphate prepared by definitive synthesis. In addition, following preliminary observations on the structure of the monosulphate ester prepared by the direct sulphation of N-acetyl-D-galactosamine[1], independent results from work on tritylation (triphenylmethylation)[2], and infrared spectroscopy[3,4], indicate that this material is also a 6-O-sulphate ester. Since available information suggests that N-acetyl-D-glucosamine 6-O-sulphate and N-acetyl-D-galactosamine 6-O-sulphate exist *in vivo* as part of the polymer chains of keratosulphate and chondroitin sulphate-C respectively[5,6], it was of interest to examine the *in vivo* metabolism of these N-acetylhexosamine sulphate esters.

The direct action of chloro-^{35}S-sulphonic acid on N-acetyl-D-glucosamine and N-acetyl-D-galactosamine yielded in each case a mixture of the mono-^{35}S-sulphate derivative together with the di-^{35}S-sulphate and unchanged parent compounds[1]. These preparations were fractionated by zone electrophoresis on