| Table | <b>2</b> . | Analysis | of | 4 | hour | urine | samples | after | ingestion | of | pyridoxine | hydrochloride. |
|-------|------------|----------|----|---|------|-------|---------|-------|-----------|----|------------|----------------|
|-------|------------|----------|----|---|------|-------|---------|-------|-----------|----|------------|----------------|

| Amount of pyridoxine ingested | Volume of 4 hour urine sample | 4-Pyridoxic acid excreted |  |  |  |
|-------------------------------|-------------------------------|---------------------------|--|--|--|
|                               | -                             | Huff and Perlzweig method | $egin{array}{c} \mathbf{New} \\ \mathbf{method} \end{array}$ |  |  |
| 1 mg                          | 93 ml                         | 0.69 mg                   | 0 mg   |  |  |
| 2 mg                          | 270 ml                        | 0.84 mg                   | 0 mg   |  |  |
| 3 mg                          | <b>325</b> ml                 | 0.79 mg                   | 0.09 mg  |  |  |
| 4 mg                          | 260 ml                        | 2.14 mg                   | 0.73 mg  |  |  |
| 5 mg                          | 380 ml                        | 1.28 mg                   | 0.59 mg  |  |  |

surable excretion of 4-pyridoxic acid, 4 hour urine samples after intake of 1, 2, 3, 4 and 5 mg of pyridoxine were analysed with the new technique. The results are shown in Table 2 and compared with results obtained with the Huff and Perlzweig method.

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- Huff, J. W., and Perlzweig, W. A. J. Biol. Chem. 155 (1944) 345.
- Johnson, B. C., Hamilton, T. S., and Mitchell, H. H. J. Biol. Chem. 158 (1945) 619.
- Rabinowitz, J. C., and Snell, E. E. Proc. Soc. Exptl. Biol. Med. 70 (1949) 235.
- Mueller, J. F., and Vielter, R. W. J. Clin. Invest. 29 (1950) 193.
- Sarrett, H. P. J. Biol. Chem. 189 (1951) 769.
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## On the Hexosamine Component of Ovomucin

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In the course of a study on the carbohydrate groups of some protein constituents of hen's eggs and especially of the eggwhite proteins it was found that ovomucin gave the colour reactions characteristic for sialic acid. The other substances

investigated gave none or only weak reactions. Later it was shown that ovomucin as well as several other substances contain considerable amounts of this acid <sup>1</sup>. As recently reported sialic acid occurs together with chondrosamine in submaxillary mucin, in gangliosides, in an acid glycoprotein from plasma and in seromucoid <sup>2-4</sup>. The hexosamine of ovomucin has therefore been examined too. So far glucosamine only has been isolated from the total eggwhite <sup>5,6</sup> and yolk <sup>6,7</sup> and from some of their constituents, as ovomucoid <sup>6,8</sup> and ovalbumin <sup>6,9,10</sup>.



Fig 1. A. Powder diagram of a-glucosamine hydrochloride. B. Powder diagram of first hexosamine fraction from an acid hydrolysate of ovomycin,



Fig. 2. A. Powder diagram of β-chondrosamine hydrochloride. B. Powder diagram of last hexosamine fraction from the hydrolysate of ovomucin.

Ovomucin was prepared according to Sörensen  $^{11}$ . It contained 11.8 per cent hexosamine and 7-8 per cent sialic acid  $^{1}$ . The isolation and the identification of the hexosamine were performed mainly in the same way as described in an earlier paper  $^{2}$ .

One g of ovomucin was heated with 5 N hydrochloric acid under reflux on a boiling water bath for 18 hrs. After filtration and evaporation the material was boiled again with 1 N hydrochloric acid for 14 hrs. It was then treated with charcoal, filtered and brought to dryness. The residue was several times taken up in methanol and the extracts evaporated. Finally, the residue was dissolved in a small amount of dry methanol and the solution placed in an desiccator at room temperature. The crystalline precipitates obtained were identified by their x-ray diffraction patterns.

The x-ray powder diagrams of the first fractions were identical with that of a-

glucosamine hydrochloride (Fig. 1), those of the last fractions with that of  $\beta$ -chondrosamine hydrochloride (Fig. 2). The glucosamine hydrochloride formed the main part of the crystalline material.

- 1. Werner, I., and Odin. L. Arch. Biochem.
- Blix, G., Svennerholm, L., and Werner, I. Acta Chem. Scand, In press.
- 3. Odin, L., and Werner, I. Arch. Biochem. In press.
- 4. Werner, I. Arch. Biochem. In press.
- Fränkel, S., and Jellinek, C. Biochem. Z. 185 (1927) 392.
- Levene, P. A., and Mori, T. J. Biol. Chem. 84 (1929) 49.
- 7. Onoe, T. J. Biochem. (Japan) 24 (1936) 1.
- Stacey, M., and Woolley, J. M. J. Chem. Soc. (1940) 184.
- Langstein, L. Z. physiol. Chem. 31 (1900) 49.
- 10. Neuberger, A. Biochem. J. 32 (1938) 1435.
- 11. Sörensen, M. Biochem. Z. 269 (1934) 271.

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