Fig. 1. Projection of $S(S_2O_5CH_3)_2$ along the b axis.

refinements the electron density map shown in Fig. 1 was obtained. The reliability factor $\Sigma||F|_{obs} - |F|_{calc}|/\Sigma|F|_{obs}$ is at this stage 0.22, and the structure is being further refined. The present sulphur parameters are:

	S_I	S_{II}	S_{III}	S_{IV}	S_V
x	0.129	0.133	0.234	0.403	0.428
z	0.119	-0.015	-0.053	-0.047	-0.159

Fig. 1 shows that the compound possesses an unbranched chain structure, the distances S_I-S_{III} , $S_{II}-S_{IV}$ and $S_{III}-S_V$ in the projection being 2.91 Å, 3.11 Å and 2.93 Å, respectively, *i.e.*, too large for bonds to exist between those atoms.

This is the first structure determination reported for a pentathionic compound. Details of the complete structure analyses of the sulphur, selenium and tellurium methanethiosulphonates will be published in due course.

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The Fluorometric Measurement of 4-Pyridoxic Acid in Normal Urine

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Huff and Perlzweig¹ have isolated and identified the excretion product 4-pyridoxic acid (2-methyl-3-hydroxy-4-carboxy-5-hydroxymethylpyridine), which is supposed to be the main excretion product of vitamin B₆. In the same work they described a fluorometric method for quantitative determination of 4-pyridoxic acid in urine. The fluorometric determination is based on the fact that the lactone form of 4-pyridoxic acid gives a fluorescent intensity 25 times that of the free acid, which in itself is highly fluorescent. In human urine the product occurs in the free acid form. By heating the urine in acid solution this compound is converted into its lactone form. The blank is an untreated urine sample. With this method it has been found that the average 24 hour urinary excretion in man is from 3 to 4 mg²⁻⁴.

In a critical study of the Huff and Perlzweig method Sarrett⁵ has shown that the blank is not reliable in measurements of normal urine. Sarrett reports of unsuccessful attempts to separate the 4-pyridoxic acid from interfering substances by means of adsorption or precipitation.

Using the same method we have found that other substances than 4-pyridoxic acid present in the urine increase in fluorescence by treatment with acid. We have therefore used a blank prepared in a different way. Our technique is based on the re-conversion of the lactone into the free acid by heating the urine in alkaline solution. The first step is to heat the urine with acid. By this treatment the free acid is converted into its lactone form and the fluorescence of this sample containing the

Table 1. Fluorescence measurement of 4-pyridoxic acid in normal urine and in urine after ingestion of 25 mg pyridoxine hydrochloride.

		Fluorometer- reading
4-Pyridoxic acid standard solution	μg 0.3	31
4-Pyridoxic acid standard solution	μg 0.6	57
Blank for the standard		5
Standard treated with NaOH	μg 0.6	5
Normal urine (final dilution 1—200)		
Sample A		72
Blank B		43
Blank C		74
Blank B treated with NaOH without preliminary treatment with acid		47
Urine after ingestion of pyridoxine (final dilution 1—1 000)		
Sample A		51
Blank B		10
Blank C		12

lactone is measured. Thereafter the sample is heated with alkali. By this procedure the lactone ring opens again. The fluorescence of this sample is taken as the blank. When this new blank is used in the determination of 4-pyridoxic acid in normal urine the values obtained are considerably smaller. This seems to indicate that the previous findings of rather high amounts of 4-pyridoxic acid in normal urine are incorrect.

Technique: Sample A and blank B are prepared according to an unpublished modification of the technique described by Huff and Perlzweig (from Duke University, Dept. of Biochemistry, 1946, personal communication). Blank C is the new blank. Fluorescence measurements were done with a Farrand microphotofluorometer with filters: primary Corning 5 860, secondary Corning 4 308 + 3 389.

Sample A: Normal urine is diluted 10 to 20 times and 1.00 ml of the diluted urine is measured into a test tube graduated at 10 ml. 1.00 ml of 2 N HCl is added and test tube placed in a boiling bath for 20 minutes. After cooling 1.00 ml of 2 N NaOH is added and the sample is diluted to the 10 ml mark with a saturated borax solution.

Blank B: Consists of the same reagents, but heating is omitted,

Blank C: After heating with acid and neutralization as described with sample A 1.00 ml of 2 N NaOH is added and the tube again immersed in a boiling bath for 15 minutes. After cooling and addition of 1.00 ml of 2 N HCl saturated borax is added to 10 ml mark.

Table 1 shows the results with normal urine and with urine after ingestion of 25 mg pyridoxine hydrochloride. It is seen that the increase in fluorescence after acid treatment of normal urine *does not* disappear by alkali treatment. The increased fluorescence does disappear in the standard solution of 4-pyridoxic acid and in the urine obtained after ingestion of pyridoxine. (Alkali treatment in itself produces only a slight increase in fluorescence intensity.)

The conclusion is that the increase in fluorescence of normal human urine after treatment with acid must be caused by substances other than 4-pyridoxic acid as this increase in fluorescence is unremovable by alkali treatment. According to this free 4-pyridoxic acid is not excreted on a normal diet and occurs only after test doses of vitamin B₆. In order to determine the least possible quantity of pyridoxine which after ingestion will produce a mea-

Table 2. 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	New method
1 mg	93 ml	0.69 mg	0 mg
2 mg	270 ml	0.84 mg	0 mg
3 mg	325 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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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 acid¹. As recently reported sialic acid occurs together with chondrosamine in submaxillary mucin, in gangliosides, in an acid glycoprotein from plasma and in seromucoid²⁻⁴. The hexosamine of ovomucin has therefore been examined too. So far glucosamine only has been isolated from the total eggwhite^{5,6} and yolk^{6,7} and from some of their constituents, as ovomucoid^{6,8} and ovalbumin^{6,9,10}.

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