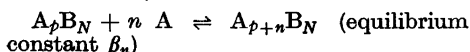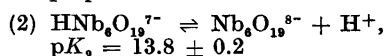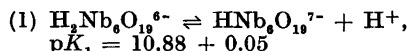values than those obtained for the forward titrations.

The data (Fig. 1) show that the Z (log $[OH^-]$) curves for different B values coincided, indicating that all species taking part in the hydrolysis should have the same nuclearity N. The reactions can be written

$$A_p B_N + n\ A \rightleftharpoons A_{p+n} B_N \quad \text{(equilibrium constant } \beta_n)$$

where $A = OH^-$, $B = Nb(OH)_5$ and p and n are integers. If we assume three species with $n = 0$, 1, and 2 we obtain $Z_{calc} = 1 + (\beta_1[OH^-] + 2\beta_2[OH^-]^2)/NB$. When comparing Z with Z_{calc} excellent agreement is obtained with $N = 6$, $\log \beta_1 = 3.28 \pm 0.05$ and $\log \beta_2 = 3.7 \pm 0.2$ (graphical estimation of errors). However, due to analytical errors in the stock solutions a correction $\Delta Z = (Z_{calc} - Z)$ had to be applied to the data. This correction has been carried out in Fig. 1. ΔZ equalled -0.002, -0.006, -0.002, and -0.010 for the solutions $I-IV$, respectively. This corresponds to a mean error in the ratio K/Nb of 0.3%. On the other hand if $N = 5$ agreement between Z_{calc} and Z seems impossible unless $\Delta Z = 0.030$ (see Fig. 1). In the stock solutions, $\Delta Z = 0.030$ corresponds to an error in the ratio K/Nb equal to 2.5% (approx.). Such a large systematic error is very unlikely.

From β_1, β_2, and pK_w ($= 14.16$) the acidity constants for the reactions are obtained in the usual way

(1) $H_2Nb_6O_{19}^{6-} \rightleftharpoons HNb_6O_{19}^{7-} + H^+$,
  $pK_1 = 10.88 \pm 0.05$

(2) $HNb_6O_{19}^{7-} \rightleftharpoons Nb_6O_{19}^{8-} + H^+$,
  $pK_2 = 13.8 \pm 0.2$

The formulas of the species are thus partly in agreement with the findings of Jander and Ertel.[1] However, the irreversibility in the most alkaline solutions and the precipitates observed must be subjected to further investigation.

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On the Occurrence of Free Selenium-Containing Amino Acids in Onion *(Allium cepa)*

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The incorporation of selenium — mostly as ^{75}Se — into living systems has been investigated to some extent. McConnell and Roth,[1] for instance, have shown that after administration of ^{75}Se it was present in all the rat liver fractions studied. The uptake of Se was greatest in the soluble fraction, followed by the mitochondria, the microsomes and the nuclei. Tuve and Williams[2] have identified selenomethionine and selenocystine in a hydrolysate of the proteins of *E. coli* grown in a sulfur-deficient medium containing radioactive selenite. Cowie and Cohen[3] found that selenomethionine could completely replace methionine for the normal growth of a methionine-requiring mutant of *E. coli*. Selenocystine but no selenoglutathione could be found in the hydrolysate. McConnell and Cooper[4] found that selenium was present in the albumin, globulin, euglobulin, and pseudoglobulin fractions and also in crystalline hemoglobin and in hemin and globin after subcutaneous injections of sodium selenate.

However, the part that selenium plays in metabolic reactions remains obscure. It seems to be a necessary element at least for cattle, where deficiency can cause heart-muscle degeneration.[5] The fact that selenium can substitute for vitamin E in preventing some, but not all, of the symptoms in vitamin E deficiency has led

Green[6] to believe that two parallel mechanisms for the biosynthesis of CoQ, the one involving vitamin E, the other selenium, exist in animals. No work on the possible selenium-containing free amino acids in plants has, however, come to our attention.

In view of the possible role that such compounds may play in nutrition we undertook the following investigation. Because the onion shows a rich variety of interesting sulfur-containing amino acids and oligopeptides as evidenced by work in this laboratory [7-9] and elsewhere, it seemed promising as a test plant.

Onions were grown for 25 days and then injected with 0.2 ml each of a neutral solution containing 300 μC[75] Se as selenite. After 2 days the free amino acids were prepared in the usual

Fig. 1. Paper chromatogram of ethanol extract of onion injected with ^{75}Se labelled selenite. White spots represent amino acids coloured with ninhydrin: 1 gly, 3 val, 4—5 leu, ileu, 6 phe, 8 ser, 13—15 his, arg, lys, 16 asp, 17 glu, 18 met, 25 asp—NH₂, 60 pipecolic acid, 131 cycloalliin, 138 methylcysteine sulphoxide. Spots $a-m$ (diagonal ruling) are radioactive Se-compounds. Spot b is probably Se-cystine and spot c Se-methionine. Spot a is tentatively estimated to be Se-propenylselenocysteine selenoxide and spot d Se-methyl-selenocysteine selenoxide. All Se-containing cysteine derivatives are not seen on this chromatogram.

way through freezing in solid carbon dioxide and extraction with cold 70 % ethanol and subsequent absorption and elution of the amino acids with IR—120. Two-dimensional paper chromatograms were prepared from these amino acids. Autoradiograms were then prepared from the chromatograms before spraying with ninhydrin, because spraying was found to volatilize some of the radioactivity. Fig. 1 represents such an autoradiogram. The lined spots are the radioactive areas, none of which became visible with ninhydrin owing to the very minute amounts involved. The other spots are ordinary ninhydrin-developed amino acid spots.

In order to identify the radioactive spots we decided to synthesize some Se-derivatives of selenocysteine as model and reference substances. As a starting compound we employed selenocystine, synthesized according to Zdansky.[10] This was reduced with sodium in liquid ammonia and coupled to the corresponding alkyl bromides yielding Se-methyl-, Se-propyl, and Se-allyl-selenocysteine.

These were white, crystalline compounds travelling on paper chromatograms in butanol-acetic acid exactly like their sulphur analoga. We have, however, not succeeded in our attempts to oxidize the above selenides to the corresponding selenoxides although we have tried a rather broad spectrum of oxidizing agents, solvents and temperature conditions. The attempted oxidations of seleno-homocystine and -methionine, synthesized according to Painter,[11] were also not successful.

Because of the difficulties in the preparation of model substances for identification, we decided to try an isolation of the selenium-containing amino acids on a scale large enough to make chemical identification possible. For this purpose preliminary experiments were conducted aiming at the production of living onion tissue with a maximal selenium content. Because the growth of seeds in various seleniferous soils was very poor, injection of selenium was tried. That the composition of the selenium-containing amino acids is identical when the selenite is injected and when it is absorbed through the roots in the normal way was proved in a separate preliminary experiment.

Next the maximal amount of selenium that could be injected into an onion *in vivo* without serious damage was investigated. The result showed that onions can absorb 10 ppm of injected selenium without any damage. 30 ppm caused a red color (elementary Se?) to develop along the injection holes. 100 and 300 ppm caused severe damage, the inside of the bulb being red, soft and evil-smelling. The chromatograms prepared from these
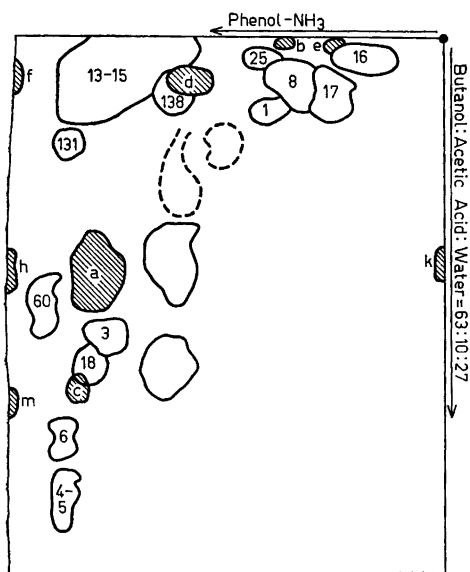
different onions did not, however, show any great differences.

The γ-amino-butyric acid spot and another blue spot, probably due to β-carboxy-propyl-cysteine, grow with increasing selenium concentration.

After these preliminary experiments, 18 onions (1750 g total fresh weight) were injected with inactive selenite to a selenium concentration of 15 ppm. This selenium contained 10.4 mC ^{75}Se in order to make the detection of selenium compounds formed and their isolation easier. After one week of additional growth the amino acids were extracted and isolated as usual. They were then chromatographed on Dowex-1-acetate first with water and then with increasing concentrations of acetic acid. The radioactivity of each fraction was measured and the amino acid content ascertained by one-dimensional chromatography. The fractions containing the most active substance (spot a in Fig. 1) were pooled and taken to dryness *in vacuo*. The residue was then chromatographed on a cellulose column with 2-butanone: acetic acid: water = 3:1:1 (v/v/v) in order to purify it from other constituents especially the lachrymatory precursor of onion,[7] which was eluted in almost exactly the same range of fractions. The radioactive spot disintegrated almost completely in this operation, however, making a chemical identification from this batch impossible.

It is, however, on the basis of chromatographic and ion-exchange behaviour similarity and also because of the relative abundancies tentatively estimated that this major radioactive spot is the selenium analogue of the lachrymatory precursor or Se-propenyl-selenocysteine selenoxide. The batch also contained many other radioactive spots, most of the compounds of which in very minor amounts. Some spots are also thought to be disintegration products.

Two spots were chromatographically compared with authentic synthesized selenium compounds in three solvents and were, on the basis of almost identical behaviour, identified as selenocystine and -methionine (spots b and c in Fig. 1). The solvents were BuOH:HOAc:H$_2$O = 12:3:5 (v/v/v), BuOH:Pyr:H$_2$O = 1:1:1 (v/v/v) and PhOH:H$_2$O = 1000:365 (w/w) (ammonia atmosphere). Three other radioactive spots were, on the basis of the proximity to the sulphur analoga on two-dimensional paper chromatograms, thought

perhaps to be Se-methyl-selenocysteine selenoxide (spot d in Fig. 1), Se-[β-carboxy-propyl]-selenocysteine and γ-glutamyl-Se-propenyl-selenocysteine.

The results show that the selenized onion contains a considerable number of selenium-containing amino acids, albeit some in very low concentration. Owing partly to the unstability of the selenium compounds and partly to the minor amounts involved, the attempted isolation and chemical identification of the selenium-containing amino acids from onion did not succeed. However, by co-chromatography two of the selenium-containing amino acids of the onion have been identified with a reasonable degree of certainty: selenomethionine and selenocystine. In addition three tentative identifications have been made: Se-methyl-selenocysteine-selenoxide, Se-[β-carboxypropyl]-selenocysteine and γ-glutamyl-Se-propenyl-selenocysteine.

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