The effects of neopyrithiamine on free ammonia and the amides are partially reversed by thiamine, while cocarboxylase is much more effective and brings about nearly complete reversal (Table II). On this basis, it is concluded that the changes observed with neopyrithiamine are *specifically* due to its "antivitamin" property in this species also. It has also been observed that while, at the dosage employed, cocarboxylase nearly completely reverses the changes in asparagine and free ammonia, it is only slightly effective in restoring the growth inhibited by neopyrithiamine. This shows that the changes observed in the nitrogeneous constituents are primarily due to a state of thiamine deficiency, and not due to growth inhibition. The striking changes produced by neopyrithiamine in the levels of glutamic acid, aspartic acid, asparagine and ammonia, substances which are all closely linked with the Krebs tricarboxylic acid cycle, seem to point significantly to the influence, direct or indirect, which thiamine exerts on nitrogen metabolism.

TABLE II

THE REVERSAL OF NEOPYRITHIAMINE-EFFECT BY COCARBOXYLASE AND THIAMINE

(Values in mg per 5 g seeds)

	Control	*Neopyrithiamine 1 mg*	*Neopyrithiamine 1 mg + cocarboxylase 1 mg*	*Neopyrithiamine 1 mg + thiamine 0.5 mg*
Free ammonia	0.67	2.41	0.6323	1.471
Glutamine	29.09	32.42	30.28	35.76
Asparagine	85.45	19.15	70.78	30.23

Indirect evidence for the absolute requirement of thiamine in glutamic acid metabolism has been obtained in animals also. While normally-fed rats tolerated well an intraperitoneal injection of sodium glutamate, neopyrithiamine-treated rats, showing no other symptoms of vitamin deficiency than weight loss, developed immediately after injection of glutamate all the symptoms of acute thiamine-deficiency—hunched back, staggered gait, severe paralysis of the limbs, violent convulsions, the animals rolling on their sides and flinging themselves in the air, ending finally in death, and all this occurring within an hour of the injection. The same rats had, three hours earlier, withstood well an injection of the same volume of sterile water, so that the effect is not due to the shock of the injection, but to the glutamate injected. This aggravation of thiamine deficiency symptoms by glutamic acid is interpreted as being due to a complete utilization, and hence exhaustion, of all the available thiamine for the metabolism of the glutamic acid injected.

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The estimation of cysteine and cystine by potentiometric titration with mercuric chloride

Methods have so far been published for the estimation of thiols and disulphides by amperometric titration with copper (II)[1], silver[2,3,4] and mercury (II)[4,5,6] and by potentiometric titration with silver[7]. It has now been found that potentiometric titration with mercuric chloride offers sufficient advantages to justify the introduction of yet another method. In this short communication the results with cysteine and cystine are described.

The apparatus and method described by CECIL AND MCPHEE[7] were used. The electrodes consisted of 28 s.w.g. gold wire which had been dipped in mercury for a few seconds. This should leave an even coating of mercury adhering to the end of the wire. These electrodes give the same potential as a mercury pool and are more convenient in use.

The initial reaction of cysteine with mercuric ion is given by the equation:

$$2RSH + Hg^{++} \rightleftharpoons (RS)_2Hg + 2H^+ \quad (1)$$

With an excess of mercuric ion the compounds $(RS)_2Hg_2$ and $(RS)_2Hg_3$ are formed[8]. Approximately 1 m*M* cysteine hydrochloride was titrated with 0.1 *M* mercuric chloride in buffered solutions over the range pH 1.2 to 6.7 using nitric acid, acetate, and phosphate as buffers. The end-points were sharp and the electrodes equilibrated rapidly so that a titration could be completed in 5 minutes. The results over this pH range were in agreement to $\pm$ 0.5% and were formed in accord with Eq. (1). The cysteine mercuric mercaptide formed is soluble under these conditions. When the same solution of cysteine hydrochloride was estimated by this method and by potentiometric silver titration[7] the results agreed to within the experimental error.

The mercury titrations are similar to the silver titrations in that, at pH $>$ 2.5, the electrodes behave as mercury thiol electrodes when cysteine is in excess (*i.e.* before the end-point) for the reasons discussed[7]. One difference is that mercury thiol electrodes are formed spontaneously and so the pre-treatment described for silver thiol electrodes is unnecessary. The potentials obtained in the presence of free thiol are more subject to variations with rate of stirring than those obtained with silver thiol electrodes. In this method, however, stirring is effected by rotating the titration vessel at steady speed by means of an air turbine[7,9] and reproducible potentials are obtained at pH 6.7 and below.

Chloride can be present in relatively large amounts without causing error. The limits depend on the pH, 0.2 *M* at pH 2.0 and 2 *M* at pH 6.7 being permissible. These concentrations caused a slight flattening of the titration curve but did not affect the end-points. Ethylenediaminetetraacetic acid (EDTA), which was used in the previous work[7] to reduce the rate of thiol oxidation, must not be used as it binds mercuric ion strongly.

Cystine is estimated by allowing it to react with sodium sulphite according to the equation:

$$RSSR + SO_3^{=} \rightleftharpoons RS^- + RSSO_3^- \quad (2)$$

and titrating the cysteine formed. The procedure differs from that used previously[7] in that the titrations must be carried out in acid solution because sulphite binds mercuric ion strongly at neutral pH. Approximately 1 m*M* cystine in 5 m*M* H_2SO_4 was mixed with a 15 to 25 molar excess of 0.1 *M* Na_2SO_3 in an evacuated Thunberg tube (since EDTA cannot be used). The pH must be between 6.5 and 7.0. The reaction is complete in approximately 30 minutes at room temperature after which a sample is acidified to pH $<$ 2 with dilute nitric acid and titrated for cysteine.

As with cysteine the results agree with those found by potentiometric silver titration.

The advantage of this method may be summarised as follows. The procedure is simple and rapid, and the electrodes are easy to prepare. The titrations can be carried out in the presence of both oxygen and chloride. Mercuric ion does not show the same tendency as silver ion to form complexes with the mercaptides formed.

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