The Intracellular Ratio of Cysteine and Cystine in Various Tissues

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1. The cysteine-cystine ratio was measured in rat kidney cortex, diaphragm, jejunum, liver and brain. 2. This ratio was determined by incubating these tissues in buffer containing [³⁵S]cystine and then homogenizing the tissue in a buffered solution of *N*-ethylmaleimide. The products of this reaction were separated by high-voltage electrophoresis and the radioactivity in the cystine and 2-(L-2'-amino-2'-carboxyethylthio)-*N*-ethylsuccinimide regions was determined. 3. In these tissues cyst(e)ine was mainly present in the reduced form. 4. After incubation of [³⁵S]cystine with rat jejunal segments it was found that 36% of the cystine in the medium has been reduced. 5. Anaerobiosis, Na⁺-free media, glucose and high concentrations of cystine and lysine were found not to affect significantly the cysteine-cystine ratio in rat kidney-cortex slices.

Numerous studies of the kinetics of amino acid transport into various tissues *in vitro* have been described. One of the requirements of this type of study with radioactive isotopically labelled amino acids is that the radioactivity measured within the tissue corresponds to the amino acid being studied and not a metabolite of that amino acid. Investigations of cystine transport have so far not distinguished between the intracellular oxidized and reduced forms of the amino acid. This investigation was designed to use the active transport mechanism of various tissues to introduce radioactive isotopically labelled cystine into the tissue and then the extent of reduction of the labelled cystine was measured.

Previous studies of the ratio of reduced to oxidized glutathione and of cysteine to cystine in certain tissues by colorimetric methods have indicated that glutathione and cysteine are principally in the reduced state (Fujita & Numata, 1938*a,b*). More than one possible mechanism by which this reduction can be maintained have been described. Cystine reduction can occur by coupling through glutathione and glutathione reductase to NADH (Pihl, Eldjarn & Bremer, 1957). A direct cystine reductase has been described (Patrick, 1962) but not characterized and the existence of such a separate enzyme mechanism of this type has been questioned (Black, 1963).

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The method of measuring cystine-cysteine ratios described in this paper has been designed to remove all the cellular enzyme activity as rapidly as possible at the end of the tissue incubation period by precipitation of the enzymes with trichloroacetic acid. This prevents any further reduction of intracellular cystine during the isolation procedure. The reoxidation of cysteine was prevented by blocking the free thiol group with NEM.‡ It was found that this reagent reacted much faster with thiol groups than did iodoacetate. Before true intracellular cystine-cysteine ratios can be calculated from experiments in which tissues have been incubated in a buffer containing [³⁵S]cystine, it is necessary to make a correction for the amount of amino acid retained in the tissue that is in the extracellular fluid and has not entered the cells. This can be done by assuming that the concentration of amino acid in the extracellular fluid is the same as that in the incubating medium. This correction was carried out according to the procedure of Rosenberg, Blair & Segal (1961). We have measured the cystine-cysteine ratio in the medium as well as the tissue at the end of the incubation period. In those cases where no reduction of cystine in the medium occurred, it was assumed that all the cystine trapped in the ECF would be in the oxidized form. If some of the cystine in the medium was reduced it was assumed that the same cystine-cysteine ratio would be present in the ECF as was present in the medium.

‡ Abbreviations: NEM, *N*-ethylmaleimide; Cys-NEM, 2-(L-2'-amino-2'-carboxyethylthio)-*N*-ethylsuccinimide; ECF, extracellular fluid; ICF, intracellular fluid.

MATERIALS AND METHODS

Isotopically labelled compounds. [^{35}S]Cystine was purchased from Schwarz BioResearch Inc. (Orangeburg, N.Y., U.S.A.) and made up as a 2 mm solution in dilute NaOH containing about 10 $\mu\text{C}/\text{ml}$. Solutions were made frequently (about every 2 weeks) since analysis of these solutions by high-voltage electrophoresis revealed oxidation to cysteic acid to occur with time. Unless stated otherwise 100 μl . of this solution was added per flask to give a final concentration 0.1 mM. When cystine was converted into cysteine by dithiothreitol, 50 μl . was added to give 0.1 mM-cysteine.

[carboxy- ^{14}C]Inulin was obtained from New England Nuclear Corp. (Boston, Mass., U.S.A.).

Chemicals. Dithiothreitol and NEM were obtained from Calbiochem (Los Angeles, Calif., U.S.A.). Cys-NEM was prepared according to Smyth, Nagamatsu & Fruton (1960).

High-voltage paper electrophoresis. This was carried out with a Gilson Electrophoretic model D in 6.8% formic acid at 45° at 4000 v for 2 hr. with 43 in. Whatman 3MM papers (Rosenberg, Crawlhall & Segal, 1967).

Under these conditions, cystine migrates on the filter paper 2 in. further than Cys-NEM and these are both separated by 6 in. from the more acid products of cystine metabolism (cysteic acid, cysteinesulphinic acid and taurine).

Ninhydrin reagent used for staining marker amino acids was prepared as follows. Ninhydrin (4 g.) was dissolved in 400 ml. of acetone. Cadmium acetate (0.4 g.) was dissolved in a mixture of 8 ml. of acetic acid and 16 ml. of water. The cadmium acetate solution was then added to the ninhydrin solution. The resulting reagent could be used for about 3 weeks if stored in a dark bottle. Unstained areas corresponding to cystine and Cys-NEM were cut from the papers, placed in glass vials with 10 ml. of toluene-based phosphor (Liquifluor; New England Nuclear Corp.) and the radioactivity was determined by liquid-scintillation counting (Tri-Carb liquid-scintillation spectrometer; Packard Instrument Co.) at approx. 50% efficiency.

Preparation of tissues for studies in vitro. Tissues were obtained from 150 g. male Sprague-Dawley rats, which had been fed *ad libitum* on a Purina rat chow diet.

The technique for incubations and study of intracellular accumulation of isotopically labelled amino acids was essentially that described by Rosenberg *et al.* (1961) for kidney-cortex slices and by Marsh, Rosenberg & Segal (1962) for jejunal segments and diaphragm. Kidney and liver slices were prepared by a Stadie-Riggs microtome (0.4 mm. thick).

The following tissues were incubated in Krebs-Ringer bicarbonate buffer, pH 7.4 (2 ml.) (Krebs & Henseleit, 1932), in 25 ml. Erlenmeyer flasks at 37°: three kidney-cortex slices (one from each of three rats) weighing a total of about 60 mg., two liver slices (from a single animal) weighing 100 mg., nine jejunal segments (three from each of three rats) weighing 120 mg. and three quarter-diaphragms (one from each of three rats) weighing 120 mg. Brain slices, 0.2 mm. thick, from the cortex of rat cerebral hemispheres were prepared in a McIlwain microtome after the brains had been placed in a polythene bag and cooled in ice for about 10 min. Eight slices were transferred to each flask (approx. 50 mg.). It was not found possible to use forceps to remove the slices at the end of the incubation and therefore the medium was poured off and tissue rinsed twice with saline while still in the flask. With the last

washing the tissue was transferred to filter paper for blotting and weighing. Distribution ratios, the ratio counts/min./ml. of ICF to counts/min./ml. of medium, were calculated according to Rosenberg *et al.* (1961) after extracting the tissue amino acid pool with warm water.

Determination of total tissue water and extracellular space. Total tissue water was determined from the difference between tissue weight after blotting the tissue slices and the weight after drying them in a crucible at 105° for 24 hr. This value was expressed as per cent of wet-tissue weight. The extracellular space of the tissues was determined by the method of Rosenberg, Downing & Segal (1962) with [carboxy- ^{14}C]inulin.

Use of iodoacetate to block cysteine thiol groups and the rate of [^{35}S]cysteine reaction with iodoacetate. The method of Brigham, Stein & Moore (1959) was utilized. After incubation in [^{35}S]cystine rat kidney-cortex slices were dipped in 0.9% NaCl soln., blotted and homogenized in a Potter-Elvehjem homogenizer for 1 min. in 1 ml. of 0.1 M-phosphate buffer, pH 7.6, containing 100 mg. of iodoacetic acid, 42 mg. of NaHCO_3 and 20 mg. of disodium EDTA. A 0.1 ml. sample was removed at increasing times (5, 10, 20 and 30 min.) and mixed with 0.1 ml. of 10% trichloroacetic acid. Each of these fractions was then submitted to high-voltage electrophoresis and the radioactivity in the cystine and carboxymethylcysteine regions was determined. As a zero-time incubation control three kidney slices were added to 1 ml. of iodoacetate soln. containing 0.02 mM- ^{35}S]cystine and homogenized immediately. After 30 min. 1 ml. of trichloroacetic acid was added and samples of this mixture were submitted to electrophoresis.

The rate of reaction of [^{35}S]cysteine with iodoacetate was measured as follows: 50 μl . of [^{35}S]cystine (2 mM) and 50 μl . of dithiothreitol (10 mM) were added to 0.9 ml. of potassium phosphate buffer (0.1 M, pH 7.4). After 10 min. iodoacetate solution (1 ml.) was added, after which 0.1 ml. samples were removed at 0, 5, 10, 20 and 30 min., and added to 0.1 ml. of 6.8% formic acid. Samples (20 μl .) of each mixture were submitted to high-voltage electrophoresis and the cystine and carboxymethylcysteine regions counted for radioactivity.

Rate of reaction of cysteine with NEM and the effect of kidney slices on [^{35}S]cystine in the presence of NEM. The rate of reaction of cysteine with NEM was studied in a similar manner to the previous experiment. [^{35}S]Cystine (50 μl ., 2 mM), dithiothreitol (50 μl ., 10 mM) and potassium phosphate buffer (0.1 M, pH 7.4; 0.9 ml.) were mixed together. After 6 min., NEM (1 ml., 20 mM) in phosphate buffer (1 ml., 0.1 M, pH 7.4) was added and samples (0.1 ml.) were removed at increasing intervals of time (0, 1, 2.5, 10 min.) and added to 6.8% formic acid (0.1 ml.). Samples (20 μl .) of each were submitted to high-voltage electrophoresis and the radioactivity was determined in the cystine region and in the region corresponding to Cys-NEM.

To study the effect of kidney-cortex slices on [^{35}S]cystine in the presence of NEM, [^{35}S]cystine (10 μl ., 2 mM) was added to NEM solution (1 ml., 20 mM in 0.1 M-phosphate buffer, pH 7.4). Rat kidney-cortex slices were added and the tissue was immediately homogenized (Potter-Elvehjem). After 1 min. trichloroacetic acid (1 ml., 10%) was added. The protein was removed by centrifugation and the trichloroacetic acid removed by ether extraction (2 $\times$ 2 ml.). A sample of the aqueous phase was submitted to high-voltage electrophoresis. This experiment was carried out in duplicate.

RESULTS AND DISCUSSION

Reaction of cysteine with iodoacetate and NEM

Initial experiments were carried out with rat kidney-cortex slices incubated with [^{35}S]cystine for 30 min. at 37°. Under these conditions cystine is transported into the cell so that an isotope distribution ratio of about 1.7 was obtained. This tissue was then homogenized in phosphate buffer, pH 7.4, containing iodoacetate with the intention of blocking the reoxidation of cysteine to cystine and subsequently to separate these two forms by high-voltage electrophoresis. This procedure appeared satisfactory except that the iodoacetate-cysteine reaction required 30 min. to go to completion and this observation was confirmed by direct measurement of the rate of reaction between cysteine and iodoacetate when no tissue was present (Fig. 1). If intracellular enzymes for cystine reduction were present it would be possible for the cysteine-cystine equilibrium to be altered when the cysteine was removed from the reaction mixture by forming *S*-carboxymethylcystine. To test this possibility we took similar kidney slices and homogenized them in buffered iodoacetate with added [^{35}S]cystine. After $\frac{1}{2}$ hr. the proteins were precipitated with trichloroacetic acid, the mixture was centrifuged and the supernatant extracted with ether. A sample of the aqueous phase was then submitted

to high-voltage electrophoresis. By using the technique described in the Materials and Methods section it was found that 30% of the [^{35}S]cystine had been converted into cystine.

To overcome this long reaction period, which enabled the tissue to reduce the cystine after homogenization, we investigated the use of NEM as a blocking agent for the cystine SH group and confirmed spectrophotometrically the observation of Gregory (1955) that this reaction was complete within 1 min. We then investigated the rate of this reaction under our experimental conditions. [^{35}S]-Cystine was reduced to cysteine by dithiothreitol (Cleland, 1964). This reduction was found to be complete in 1 min. if a large excess of dithiothreitol was present. The mixture was then added to homogenized kidney slices and a solution of NEM in 0.1M-phosphate buffer, pH 7.4, was added. After 1 min. trichloroacetic acid was added, the precipitated protein removed by centrifugation, the excess of trichloroacetic acid was extracted with ether and a sample of the aqueous phase was submitted to high-voltage electrophoresis. It was found that all the [^{35}S]cystine was present as Cys-NEM. This product was found to run as a single band on high-voltage electrophoresis whereas the two possible isomers are separated by ion-exchange chromatography (Smyth, Blumenfeld & Konigsberg, 1964). If on the other hand no dithiothreitol was present in the reaction mixture all the ^{35}S as determined after high-voltage electrophoresis was present in the cystine region.

Fig. 1. Rate of reaction of iodoacetate with intracellular [^{35}S]cystine (■) and with [^{35}S]cystine reduced by dithiothreitol (▲).

Intracellular cystine-cysteine ratios

Rat kidney cortex in vitro. Having established that NEM reacted with cysteine in 1 min. and that it was then possible to remove the enzymes responsible for cystine reduction by precipitation of the proteins with trichloroacetic acid, we applied the procedure directly to rat kidney-cortex slices that had been incubated with L-[^{35}S]cystine. These results, which were reported in part in a short communication (Crawhall & Segal, 1966), showed (Table 1) that 90% of the radioactivity could be recovered as cystine or Cys-NEM. Radioactivity could not be recovered from the high-voltage electrophoresis in the regions corresponding to cysteic acid or taurine. Radioactivity in the protein precipitate accounted for only 4% of the intracellular radioactivity. It was then possible to calculate from the radioactivity in the cystine and Cys-NEM regions the cystine-cysteine ratio within the cell. To do this it was necessary to correct for cystine trapped in the ECF (27% of tissue weight for the rat kidney-cortex slice) (Rosenberg *et al.* 1961). After applying this correction it was found that 78% of the total cyst(e)ine was present in the

Table 1. *Transport of [³⁵S]cystine into rat jejunal segments, diaphragm, kidney cortex, liver and brain slices and the relative proportion of cysteine to cystine in these tissues*

Tissue	Tissue water (% of tissue wt.)	Inulin space (ECF) (% of tissue wt.)	Concn. of [³⁵ S]- cystine in media (mM)	Time of incubation (min.)	Isotope distribution ratio (ICF/ECF)	% of total radioactivity in tissue present as cyst(e)ine	Ratio cysteine to total cyst(e)ine (expressed as %)		
							In tissue		In media
							Corrected for cystine in ECF	Not corrected for cystine in ECF	
Jejunum	84	12	0.1	30	1.3	97	100	98	36
Diaphragm	78	26	0.1	30	0.3	93	100	68	6.6
Kidney	80	20	0.05	10	0.9	90	82	57	2
cortex	80	27	0.05	30	1.7	89	78	55	—
	80	27	0.05	90	3.3	78	63	51	5
Liver	75	27	0.1	30	1.5	72	100	85	15
Brain	89	10	0.1	30	3.0	54	87	82	15

Table 2. *Effect of anaerobiosis, Na⁺-free media, high cystine (1.2 mM) concentration and glucose (5.5 mM) on the uptake of [³⁵S]cystine and the intracellular cysteine-cystine ratio in rat kidney-cortex slices during 30 min. incubation*

Incubation conditions	Concn. of cystine in media (mM)	Isotope distribution ratio (ICF/ECF)	Ratio cysteine to total cyst(e)ine (expressed as %)	
			In tissue	
			for cystine in ECF	In media
Anaerobiosis	0.03	1.1	95	48
Na ⁺ -free	0.03	1.1	65	0
Aerobic	1.2	0.8	100	0
With glucose (5.5 mM)	0.05	1.7	80	—

reduced form after incubation for 30 min. This ratio was not affected by increasing the time of reaction of the homogenized tissue with NEM or by increasing the concentration of NEM. It was found that the proportion of cysteine altered a little depending on the duration of the incubation (Table 1) such that at 90 min. the proportion of cysteine had decreased to 63%. Similar analysis of the medium at the end of the incubation showed only 1% present as cysteine after 30 min. and less than 4% after 90 min. It was possible that the apparent decrease of intracellular reduction of cystine with time was caused by a shortage of energy for the reduction, but it was found that addition of glucose (final concn. 5.5 mM in Krebs-Ringer bicarbonate buffer) did not affect the cystine-cystine ratio at all (Table 2).

Normal human kidney cortex. After incubation of slices of human kidney cortex, obtained by sur-

gical biopsy, with [³⁵S]cystine in Krebs-Ringer bicarbonate buffer all of the radioactivity was recovered as cyst(e)ine. Only 8% of this was present as cystine and this is less than could be accounted for by cystine trapped in the ECF (56% of tissue weight). About 10% of cyst(e)ine in the medium was reduced to cysteine. Similar experiments were carried out with human jejunal mucosa obtained by peroral biopsy and these results have been reported elsewhere (Rosenberg *et al.* 1967).

Experiments on the effect of anaerobiosis, incubation in sodium-free medium or of high cystine or lysine concentrations on the cystine-cystine ratio in rat kidney cortex

Anaerobiosis. Incubation in an atmosphere of nitrogen + carbon dioxide (95:5) prevented cystine uptake by the tissue to a concentration ratio

greater than 1 in 30min. All the radioactivity was found to be present in the tissue as cyst(e)ine. About 17% of this was present as cystine without correction for cystine trapped in the ECF (27% of tissue weight). However, it was found that 48% of the radioactivity in the medium was present as cysteine. If it is assumed that after 30min. incubation the equivalent of one-half of the ECF was occupied by cystine and one-half by cysteine, that is that 13% of the tissue weight is cystine at an isotope distribution ratio of 1, then only 4% of the total intracellular cyst(e)ine was present as cystine. It had been shown (Segal, Schwartzman, Blair & Bertoli, 1967) that anaerobiosis inhibited active transport of cystine in the rat kidney-cortex slice. In this study we have found that cyst(e)ine was present in the tissue in the reduced state to an even greater extent in anaerobic medium than in aerobic medium. An additional observation was that under anaerobic conditions the cystine in the medium became reduced. It is not known whether this was caused by enzymic reduction by the tissue acting on the cystine in the medium or whether cysteine and glutathione were effluxing from the tissue and liberating $[^{35}\text{S}]$ cysteine by thiol-disulphide exchange with the $[^{35}\text{S}]$ cystine in the medium. It is probable that this phenomenon was occurring in aerobic medium also but could be obscured because of rapid reoxidation of the cysteine.

Incubation in sodium-free medium. Sodium ions were replaced in the incubation medium by triethylammonium ions (tris) (Fox, Thier, Rosenberg & Segal, 1964). As in the previous experiment, the isotope distribution ratio obtained at 30min. was only 1. In contrast with the anaerobic experiment no cysteine was present in the medium. Of the total cyst(e)ine 54% was present as cystine. Correction of this value for the ECF content (27% of the tissue weight) at an isotope distribution ratio of 1 leads to the result that 35% of the total cyst(e)ine is present as cystine or 65% as cysteine. This is identical with the result obtained in incubation experiments with Krebs-Ringer bicarbonate buffer, although in the latter medium an isotope distribution ratio of about 2 was obtained.

Incubation in medium containing 1.2 mM-cystine. Under this condition which is near the limit of solubility of cystine in buffer, an isotope distribution ratio of 0.8 was obtained after 30min. incubation.

This result is interpreted as meaning that the active transport mechanisms are becoming saturated at that concentration in relation to non-mediated transport so that no isotope distribution gradient can be maintained.

No cysteine was found to be present in the medium. Of the total cyst(e)ine in the tissue 38% was present as cystine. Correction for cystine

trapped in the ECF at an isotope distribution ratio of 0.8 gives a value of 100% for the true percentage of intracellular cysteine (Table 2).

Incubation in medium containing 30 mM-lysine. Schwartzman, Blair & Segal (1966) showed that 30 mM-lysine decreased cysteine efflux from the rat kidney-cortex slice. A possible explanation for this could have been that the intracellular cysteine-cystine ratio was altered by high concentrations of lysine. If more cystine was present, then this might efflux more slowly from the tissue and the rate of efflux of $[^{35}\text{S}]$ cyst(e)ine would decrease. Direct analysis of the intracellular cysteine-cystine ratio under these conditions showed that it was the same as that observed after incubation in the medium which did not contain lysine.

Intracellular cystine-cysteine ratios in rat tissues other than kidney cortex

Rat jejunal segments. The results obtained after incubation of jejunal segments with $[^{35}\text{S}]$ cystine were somewhat different from those found for the kidney-cortex slice. Of the intracellular radioactivity 97% was recovered as cystine or Cys-NEM and of this only 2% was present as cystine even without correction for cystine from the medium trapped in the ECF. The ECF for gut segments is 12% and calculation showed that about 8% of radioactivity could have been present as cystine for this reason alone. However, analysis of the medium showed that 36% of the medium radioactivity was present as cysteine. This reduction in the medium could possibly have occurred as the result of unidentified reductases from broken cells of the gut segments or from epithelial cells broken off during the incubation. Alternatively, considerable efflux of non-radioactive cysteine may have occurred from the tissue. This could undergo a thiol-disulphide exchange with the $[^{35}\text{S}]$ cystine in the medium with liberation of $[^{35}\text{S}]$ cysteine.

Rat diaphragm. The cut rat diaphragm is particularly poor at transporting cystine and after 30min. incubation an isotope distribution ratio of only 0.3 was obtained. It is not possible to say whether this represents active transport or non-mediated entrance of cystine into the cell. Direct analysis of the tissue cystine-cysteine ratio was found to be 1.2. However, the ECF for rat diaphragm is 26% and, with an isotope uptake of only 0.3, the amount of cystine found in the tissue was even less than could be accounted for after correction of the data for cystine trapped in the ECF and it appears that all the intracellular activity was present in the Cys-NEM regions. About 7% of the cystine in the medium had been reduced to cysteine.

Rat liver. In experiments in which rat liver slices

were incubated with [^{35}S]cystine an isotope distribution ratio of 1.5 was obtained after 30 min. The liver differed from other tissues studied on account of its relatively high rate of cystine catabolism. Only 72% of the intracellular radioactivity was accounted for as cystine or Cys-NEM. Most of the remaining radioactivity moved in a region corresponding to taurine on high-voltage electrophoresis. Analysis of the cystine-cystine ratio showed that 85% was present as cystine without correcting for any cystine trapped in the ECF and, if this correction was made, the intracellular form was entirely present as cystine (Table 1). The amount of cystine reduction occurring in the medium varied between experiments but was about 20%.

Rat brain. The measurement of transport of [^{35}S]cystine into rat cerebral cortex slices requires a few modifications to the experimental technique to enable the brain slices to be prepared and subsequently weighed. To obtain the value for total tissue water in brain it is necessary to incubate the slices in appropriate buffer for 30 min. before weighing the tissue and drying. This is required because the brain is unusual in that there is a gain of intracellular water during the period of incubation.

Sections of brain were taken mainly from the cortex of the cerebral hemispheres. The brain slices were found to be active in accumulating radioactive cystine, giving an isotope distribution ratio of 3.0 after 30 min.

As with the liver, the rate of metabolism of cystine in brain slices was quite high in relation to the rate of transport and after incubation for 30 min. only 54% of the total radioactivity in the tissue corresponds to cyst(e)ine. The remainder of the radioactivity migrated principally towards the cathode on high-voltage electrophoresis and was either cysteic acid or cysteinesulphinic acid, but its exact nature was not confirmed. The proportion of cystine found was 85% of the total cyst(e)ine after correction for cystine trapped in the ECF (10% of tissue weight). About 15% of the cystine in the medium was reduced to cystine.

Conclusion

After cystine has been transported into various tissues *in vitro* it becomes reduced to cysteine and probably endogenously synthesized cysteine would also remain in the reduced state so that free cystine itself may not have much importance within the cell. On the other hand, cystine predominates over

cystine in the plasma but will become reduced again after it has entered into the tissue cells. Kinetic studies of cystine transport *in vitro* will be less accurate than with certain other amino acids because the molecular form outside the tissue will be different from that inside the tissue.

A revival of interest in the reaction of cysteine with NEM since the publications of Gregory (1955) and Smyth *et al.* (1960) has recently occurred. Gorin, Martic & Doughty (1966) have studied the kinetics of the reaction of cysteine with NEM and find 0.7 sec. to be the half-reaction time at pH 7 and 1 mM reactant concentrations. Ellis (1966) has pointed out the usefulness of NEM in the study of sulphur metabolism in bacteria. Our results indicate that NEM has a role in the examination of cystine-cysteine interrelationships in mammalian tissue. One important area of investigation which may benefit by application of this technique is in tissue from human patients with cystine-storage disease, cystinosis.

REFERENCES

- Black, S. (1963). *Annu. Rev. Biochem.* **32**, 399.
- Brigham, M. P., Stein, W. H. & Moore, S. (1960). *J. clin. Invest.* **39**, 1633.
- Cleland, W. W. (1964). *Biochemistry*, **3**, 480.
- Crawhall, J. C. & Segal, S. (1966). *Biochem. J.* **99**, 19c.
- Ellis, R. J. (1966). *Nature, Lond.*, **211**, 1266.
- Fox, M., Thier, S., Rosenberg, L. E. & Segal, S. (1966). *Biochim. biophys. Acta*, **79**, 167.
- Fujita, A. & Numata, I. (1938a). *Biochem. Z.* **300**, 257.
- Fujita, A. & Numata, I. (1938b). *Biochem. Z.* **300**, 264.
- Gorin, G., Martic, P. A. & Doughty, G. (1966). *Arch. Biochem. Biophys.* **115**, 593.
- Gregory, J. D. (1955). *J. Amer. chem. Soc.* **77**, 3922.
- Krebs, H. A. & Henseleit, K. (1932). *Hoppe-Seyl. Z.* **210**, 33.
- Marsh, H. B., Rosenberg, L. E. & Segal, S. (1962). *Endocrinology*, **71**, 516.
- Patrick, A. D. (1962). *Biochem. J.* **83**, 248.
- Pihl, A., Eldjarn, L. & Bremer, J. (1957). *J. biol. Chem.* **227**, 339.
- Rosenberg, L. E., Blair, A. & Segal, S. (1961). *Biochim. biophys. Acta*, **54**, 479.
- Rosenberg, L. E., Crawhall, J. C. & Segal, S. (1967). *J. clin. Invest.* **46**, 30.
- Rosenberg, L. E., Downing, S. J. & Segal, S. (1962). *Amer. J. Physiol.* **202**, 800.
- Schwartzman, L. S., Blair, A. & Segal, S. (1966). *Biochem. biophys. Res. Commun.* **23**, 220.
- Segal, S., Schwartzman, L., Blair, A. & Bertoli, D. (1967). *Biochim. biophys. Acta*, **135**, 127.
- Smyth, D. G., Blumenfeld, O. O. & Konigsberg, W. (1964). *Biochem. J.* **91**, 589.
- Smyth, D. G., Nagamatsu, A. & Fruton, J. S. (1960). *J. Amer. chem. Soc.* **82**, 4600.