Short Communications

The Intracellular Cysteine/Cystine Ratio in Kidney Cortex

By JOHN C. CRAWHALL and STANTON SEGAL

*Clinical Endocrinology Branch, National Institute of Arthritis and Metabolic Diseases,
National Institutes of Health, Bethesda, Md., U.S.A.*

(Received 3 March 1966)

Little direct evidence is available on the intracellular proportions of cysteine and cystine. We have investigated this problem by employing the cystine-transporting mechanism of the kidney cortex to label the intracellular amino acid pool with [³⁵S]cystine *in vitro*. Cystine was then separated from cysteine after use of the thiol-blocking reagent NEM,* which reacts with cysteine to form Cys-NEM (Smyth, Nagamatsu & Fruton, 1960) and the respective radioactivities were measured. In addition, direct analysis of intracellular cystine and cysteine was also carried out by using an automatic amino acid analyser.

Three rat kidney-cortex slices were incubated in Krebs-Ringer bicarbonate buffer (2ml.) (Rosenberg, Blair & Segal, 1961) containing L-[³⁵S]cystine (0.1mM). At the completion of the incubation the slices were removed, washed in 0.9% sodium chloride, dried by blotting, weighed and then homogenized (Potter-Elvehjem) in a solution of 20mM-NEM (1ml.) in 0.1M-potassium phosphate buffer, pH 7.4 (Gregory, 1955). After 1min. the protein was precipitated with 10% (w/v) trichloroacetic acid (1ml.). A 0.2ml. sample of the medium was also treated with the same quantity of NEM.

After precipitation of the protein, the supernatant from the transport experiment was extracted with ether to remove the trichloroacetic acid, and 60 μ l. and 20 μ l. portions of the NEM-treated tissue extract and incubation media respectively were submitted to high-voltage electrophoresis (Gilson High Voltage Electrophorator model D) at 4000 v at 45° in formic acid (6.8%) for 2hr. No detectable amount of radioactive cystine or Cys-NEM was removed from the aqueous phase by this ether extraction. Standard marker spots containing taurine, Cys-NEM, cystine and glycine were also applied to the paper. At the completion of the electrophoresis, these marker channels were stained with ninhydrin. The experimental channels were cut into sections that were then immersed in a toluene-based phosphor and counted in a liquid-scintillation counter. It was found that Cys-NEM ran as a single spot in this system whereas it

separated into two isomers on ion-exchange chromatography (Smyth, Blumenfeld & Konigsberg, 1964). In a typical experiment with rat kidney-cortex slices after incubation with [³⁵S]cystine for 30min., 348 counts/min. were recorded in the cystine region and 660 counts/min. in the Cys-NEM region before correction for [³⁵S]cystine trapped in the extracellular fluid. This correction was made by calculating the proportion of total counts/min. observed in the tissue that could be accounted for by the known contribution of the extracellular fluid, assuming that the radioactivity in the extracellular fluid was the same as that in the medium. This correction has to be applied to the cystine value only as it was shown that only cystine and not cysteine was present in the medium.

We have confirmed both spectrophotometrically and by use of [³⁵S]cystine that the reaction between cysteine and NEM was complete within 1min. No reduction of [³⁵S]cystine occurred if this was added to rat kidney slices that were then homogenized in NEM and 10% trichloroacetic acid added after 1min.

The results in Table 1 show that in the rat kidney cortex the cysteine/cystine ratio is 4:1 after 10min. incubation decreasing to about 2:1 after 90min. The reason for this changing ratio is not known but it is possible that the intracellular reductive mechanisms are becoming less effective with increasing time of incubation. By using the same electrophoretic technique it was found that cystine in the media remained at least 95% in the oxidized form.

Similar experiments, carried out with normal human kidney cortex obtained at open biopsy, showed that all the intracellular cyst(e)ine was present in the reduced state. The extracellular fluid for human kidney cortex as determined by inulin space is 54% of the total weight of tissue (Fox, Thier, Rosenberg, Kiser & Segal, 1964) whereas that for the rat kidney cortex is 26.7% (Rosenberg, Downing & Segal, 1962). After the transport of L-[³⁵S]cystine into the human kidney cortex the cystine that was observed in the tissue could be entirely accounted for by the cystine that would be expected to be trapped in the extracellular fluid.

* Abbreviations: NEM, *N*-ethylmaleimide; Cys-NEM, 2-(L-2-amino-2-carboxyethylthio)-*N*-ethylsuccinimide.

Table 1. *Distribution of intracellular cysteine and cystine after the transport of L-[³⁵S]cystine into kidney cortex*

The procedure of Rosenberg *et al.* (1961) was used for the rat kidney-cortex experiments. The medium consisted of L-[³⁵S]cystine (0.1 mM) in Krebs-Ringer bicarbonate buffer, pH 7.4 (2 ml., approx. 1 μ C of ³⁵S/flask). Human kidney cortex was sliced in a Stadie-Riggs microtome and incubated in a similar manner. The isotope distribution ratio is given by (counts/min./ml. of intracellular fluid)/(counts/min./ml. of extracellular fluid). The numbers of experiments on which these results are based are given in parentheses. Cysteine was separated as Cys-NEM by high-voltage electrophoresis.

Tissue	Time of incubation (min.)	Isotope distribution ratio between the intracellular fluid and medium	Cysteine (% of total intracellular cysteine + cystine)
Rat kidney	10	0.9 (4)	81.2 (S.D. $\pm$ 4.5)
	30	1.7 (4)	76.8 (S.D. $\pm$ 4.5)
	90	3.3 (4)	62.2 (S.D. $\pm$ 2.9)
Human kidney	30	2.6 (2)	100
	45	4.7 (2)	100

An alternative method for determining the intracellular cysteine/cystine ratio has been used for rat kidney cortex. The tissue was homogenized in water and deproteinized and the intracellular amino acids were determined by ion-exchange chromatography by using a gradient elution procedure on a Beckman 120B amino acid analyser (Crawhall, Thompson & Bradley, 1966). Paired tissue from the same rat renal cortex was homogenized in NEM in 0.1 M-phosphate buffer, pH 7.4. After 1 min. the protein was precipitated and the intracellular amino acids were again determined. The apparent cystine content of the tissue without the addition of NEM was 1.6 μ moles of half-cystine/g. wet wt. of tissue whereas only 0.05 μ mole/g. was found when NEM was present, and two new peaks corresponding to 2.7 μ moles of Cys-NEM/g. had appeared. No change was observed in the concentration of other amino acids. It is thought that the cystine found after homogenization in water was formed from intracellular cysteine and that this oxidation was blocked by NEM. Absolute values for cystine or cysteine concentrations within the tissue cannot be calculated from these results because no correction has been made for any mixed disulphide formation between cysteine and glutathione (Tallan, Moore & Stein, 1954). However, most of the cystine found in the first analysis must have been present in the tissue as cysteine.

There is some difference between the result obtained by isotope analysis and that obtained by direct amino acid analysis in that by the former method 20–40% of the total free cyst(e)ine was found to be present as cystine depending on the duration of the incubation. However, this difference

may not be significant if the latter form of analysis is considered as zero time of the incubation experiment.

Additional studies with the transport of [³⁵S]-cystine into rat jejunum, brain, liver and diaphragm also show a high intracellular cysteine/cystine ratio.

In a recent study of cystine transport in human kidney slices no distinction was made between cystine and cysteine in the tissue (Fox *et al.* 1964). In view of these present findings it becomes difficult to discuss cystine uptake in terms of a concentration gradient as the amino acid is in the oxidized form in the medium and principally in the reduced state in the tissue. These observations must therefore be taken into account in any kinetic investigation of cystine-transport mechanisms and in studies of disorders of cystine metabolism and transport.

J. C. C. is at present Visiting Scientist at the National Institutes of Health, Bethesda, on leave from the Medical Unit, St Bartholomew's Hospital, London.

- Crawhall, J. C., Thompson, C. J. & Bradley, K. H. (1966). *Analyt. Biochem.* **14**, 405.
 Fox, M., Thier, S., Rosenberg, L. E., Kiser, W. & Segal, S. (1964). *New Engl. J. Med.* **270**, 556.
 Gregory, J. D. (1955). *J. Amer. chem. Soc.* **77**, 3922.
 Rosenberg, L. E., Blair, A. & Segal, S. (1961). *Biochim. biophys. Acta*, **54**, 479.
 Rosenberg, L. E., Downing, S. J. & Segal, S. (1962). *Amer. J. Physiol.* **202**, 800.
 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.
 Tallan, H. H., Moore, S. & Stein, W. H. (1954). *J. biol. Chem.* **211**, 927.