und ein Zwischengetriebe von 4 U/Min. auf 1 U/Std. angetrieben wird. Die Rückstellung zur Gewinnung der Ausgangsposition erfolgt über eine Zahnkuppelung (Figs. 2a und 2b).

Zur Nivellierung des Flüssigkeitsspiegels im Zentrifugengefäss im Bezug zur Auslauföffnung wird die Kunststoffhalterung der Gefässe bei gleitender Führung durch ihr Eigengewicht gegen die Programmierscheibe mit linearem Abfall des Durchmessers gedrückt und entsprechend des Flüssigkeitszuflusses so gesenkt, dass sich die Auslauföffnung des Mischteiles immer über dem Flüssigkeitsspiegel befindet. Die Programmierscheiben zum Nivellieren der Gefässhalterungen sind auf die gleiche zentrale Achse montiert. Die Mischteile sind beweglich auf Stifte gelagert und enthalten meanderförmig eingefräste Kanäle, die die Mischung der spezifisch schwereren und leichteren Lösung gewährleisten. Ihr Eigengewicht drückt deren Auslauföffnung gegen die Wandung des Zentrifugenröhrchens, wodurch die Bildung von Flüssigkeitswirbeln vermieden werden soll (Fig. 3). Durch Anschluss einer Kontaktuhr kann nach vorgewählter Zeit der Antriebsmotor abgeschaltet werden.

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Preparation of deproteinised tissue extracts for chromatography and assay of compounds related to glutathione

When extracting tissue for assay of glutathione and other thiols, the chief problem is to prevent losses either by oxidation of the thiol to disulphide or by binding to protein in thiol–disulphide exchanges. The extractants recommended for high recovery are salt-saturated 4 % (w/v) metaphosphoric acid or 3 % (w/v) sulphosalicylic acid[1]. Such extracts are not immediately suitable for chromatography because of the content of salts and involatile acids. Also direct colorimetric assays of thiol in extracts from brain or slightly fatty livers are ruined by opacity introduced by dispersed lipid material.

During studies on the metabolism of some foreign compounds in the rat, I wished to measure the effect of doses of these compounds on liver GSH and also to separate derivatives of GSH which had been formed *in vivo*. Extraction of components from tissues with ethanol is quite common and this paper describes the modifications of the technique needed to obtain good recoveries of GSH and its derivatives

in a clear solution sufficiently free of salt for high-resolution chromatography without further processing.

The extracting solution is prepared fresh by mixing 3 vol. ice-cold ethanol with 1 vol. ice-cold KH_2PO_4 (24 m*M*; pH 5.5) containing EDTA (0.1 m*M*). 2 g liver is homogenised in 25 ml of mixture with a high-speed smooth pestle homogeniser[2] and the homogenate centrifuged at 5,000 *g* for 10 min at 0°. The phosphate content of the clear pale yellow extract is less than the nominal 6 m*M* since crystallisation occurs from the initially supersaturated solution and the crystals are removed during the centrifugation.

At least 30 ml of extract can be applied without further processing to a Dowex column (15 × 1.3 cm) in the carbonate form for separation of basic, neutral and acidic amino acids as described by GAITONDE[3]. Alternatively the extract can be quickly concentrated to one tenth volume in a rotary evaporator at 35°: the opalescent concentrate is easily applied to chromatography paper and the resolution of the amino-acid constituents is not affected by other material in the extract.

The GSH content of liver extracted as described, and determined according to BEUTLER[4] *et al.*, is 85 % of that determined after deproteinisation with 3 % (w/v) sulphosalicylic acid. Recovery is decreased by the following:

(a) Alteration of the extraction pH outside the range 5–7. Since atmospheric oxidation in subsequent processing would be favoured by higher pHs, pH 5.5 was chosen as optimum.

(b) Increase of alcohol:buffer ratio above 3:1. Decreasing the ratio also proved unsatisfactory because it caused incomplete deproteinisation. Use of acetone–buffer in the proportion 2:1 gave 100 % recovery but the suitability of the extract for chromatography has not been assessed.

(c) Decrease of the extractant vol:tissue wt. ratio below 10:1. A similar effect was observed when 3 % sulphosalicylic acid was used as extractant.

There is no specific assay for S-derivatives of GSH. Standards were added to liver extract before centrifugation. The deproteinised extract was concentrated and subjected to paper chromatography. Ninhydrin-positive spots were cut out and eluted with ethanol (60 % v/v in water) and compared spectrophotometrically (565 mμ) with standards subjected only to chromatography. Recoveries ranged from 83–95 %.

Although of particular value for glutathione the method seems generally useful, particularly in view of the large volume of extract which can be applied to ion-exchange columns.

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