10810

Isolation of Glutathione from Thymus Glands.*

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Glutathione, like the thymus extract, has been found to stimulate the growth and development of the offspring of treated rats.[1] Also its presence in the extract was indicated by glutathione determinations.[2] However, glutathione is believed to play but a subsidiary rôle in the activity of the thymus extract.[1] † The present communication deals with the presence of glutathione in the thymus gland by direct isolation.

Procedure. Two to four kg of calf thymus glands, obtained immediately after killing, were ground and deproteinized with 3 to 4 volumes of 5% CCl_3COOH. After filtration through infusorial earth under vacuum the filtrate was brought to about pH 6 with concentrated NaOH and refiltered. The mercury salt was precipitated with either mercuric acetate or mercuric sulfate.[3] It was separated, washed, decomposed with H_2S in the presence of N/10 H_2SO_4 and treated with CO_2 The cuprous salt was next precipitated according to Hopkins' method.[4] It was necessary to add the Cu_2O slowly in order to separate the grayish white precipitate of glutathione, which appeared first, from the red precipitate of purine-like material which appeared on the addition of excess Cu_2O. The glutathione was separated when no more grayish white precipitate was formed and before the red precipitate appeared. It was centrifuged, washed once with N/2 H_2SO_4 and then with water until the washings were sulfate-free. Several of these copper precipitates from different preparations were combined and reprecipitated with Cu_2O. The decomposed salt was evaporated in a vacuum desiccator over P_2O_5 to a heavy

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1 Rowntree, L. G., Steinberg, A., Einhorn, N. H., and Schaffer, N. K., *Endocrinology*, 1938, **23**, 584.

2 Schaffer, N. K., Ziegler, W. M., and Rowntree, L. G., *Endocrinology*, 1938, **23**, 593.

† An ether extract of the thymus extract has recently been found active.

3 Kendall, E. C., McKenzie, B. F., and Mason, H. L., *J. Biol. Chem.*, 1929, **84**, 657.

4 Hopkins, F. G., *J. Biol. Chem.*, 1929, **84**, 269.

syrup. It was placed in the refrigerator for several hours, stirred occasionally, and after it had crystallized was transferred to a porous plate for several more hours. The crystalline mass was ground in a mortar with 95% alcohol, filtered, washed with alcohol and dried *in vacuo*. This material gave a satisfactory analysis, but a low melting point (162°).

Further purification was obtained by precipitation of the silver salt[5] with silver lactate. Three more precipitations, copper, silver and copper, were made in the order given. This material was recrystallized by dissolving in water, filtering and placing in the refrigerator. After several hours when no crystals had formed, alcohol was added to the appearance of cloudiness. The next day the crystals were separated by filtration, washed with alcohol and dried. The melting point was 191.0° (corrected), that for glutathione[6] being 190°.

The material at the second copper precipitate stage gave the following analysis:

	Calculated	N 13.68	S 10.44
$C_{10}H_{17}N_3SO_6$			
	Found	13.26	10.72

Iodine Titration. 15.26 mg dissolved in 50 cc of N/12 HCl were titrated with N/100 iodine. Found 4.79 cc. For glutathione[7] 4.98 cc.

Specific rotation. Found $[\alpha]_{D}^{24.5} = -19.8°$ (2% solution in water)

For glutathione[6] $[\alpha]_{D}^{27} = -21.3°$

The material at the first copper precipitate stage in one preparation had a copper content of 16.6% (calculated 17.2%). It represented a yield of 0.28 g of glutathione per kg of thymus glands.

Summary. Glutathione has been shown to be present in the thymus gland by direct isolation.

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5 Sullivan, B., and Howe, M., *J. Am. Chem. Soc.*, 1937, **59**, 2742.

6 du Vigneaud, V., and Miller, G. L., *J. Biol. Chem.*, 1936, **116**, 469.

7 Borger, G., Peters, T., and Kurz, M., *Z. physiol. chem.*, 1933, **217**, 255.