LVI. THE GLUTATHIONE CONTENT OF ANTI-ANAEMIC SUBSTANCES USED IN THE TREATMENT OF PERNICIOUS ANAEMIA.

By ROBERT FLEMING.

From the Physiological Laboratory, University of Geneva.

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It is generally agreed that the daily ingestion of fresh liver in the treatment of pernicious anaemia gives better results than those obtained from the use of various extracts prepared specifically by the drug industry for use in this disease. There is hardly any doubt, however, as to the fact that both supply in some measure a biological principle or principles necessary to the well-being of the patient. The difference therefore between the therapeutic value of fresh liver on the one hand and of the commercial preparations on the other is probably one of degree only. This difference might be accounted for by a loss or a modification of the active substance or substances during the process of preparation. It was considered of interest to examine some of these reputed blood-formation stimulants with respect to certain definite chemical substances which have been isolated from fresh liver. In the present communication we propose to deal only with glutathione. It is well known that glutathione occurs in appreciable quantities in liver, kidney, placenta, bone marrow and red blood corpuscles. We have also found this substance present in the gastric mucosa of the pig.

During a series of experiments on experimental anaemic conditions in animals we have had evidence that the glutathione content of various organs varies considerably from the supposed normal.

Several workers have determined the percentage of glutathione in fresh liver and have given figures ranging from 0.15 % to 0.40 %. These figures vary with the species and probably with different members of the same species. Further, it seems that all the glutathione is present in the reduced state.

As a type of commercial liver preparation we selected Heparglandol prepared by Hoffmann-La Roche, Bâle, who kindly furnished us with quantities sufficient for our experiments. This product is widely used in Switzerland in the treatment of anaemic conditions. It is easily soluble in hot water except for a small amount of fat which can be separated by filtration in the cold. A 10 % aqueous solution of Heparglandol gives a feeble nitroprusside reaction indicating the presence of an —SH group. If, however, a part of this solution

be gently boiled for a minute with an equal volume of a 5 % solution of sodium cyanide the resulting solution on cooling gives a vivid nitroprusside reaction. It is known that disulphides are reduced by cyanides but in the cold reduction is very slow and uncertainty sometimes exists as to when reduction is complete. We found that the boiling of a solution of a disulphide such as cystine or oxidised glutathione with sodium cyanide brings about a rapid and complete reduction when the requisite quantities are present. In a part of the reduced solution we estimated the quantity of —SH as reduced glutathione by a colorimetric method elsewhere described by us [1930].

The mean of many observations gave a glutathione content of 0.17 % while the amount of reduced glutathione before reduction was not more than 0.03 %. Ventriculin, a desiccated extract of gastric mucosa widely used in America and Europe in anaemic conditions, was also examined as to its glutathione content. Quantities of this product were kindly placed at our disposal by Messrs Parke Davis and Co. It is a greyish white granular substance stated to be insoluble. If, however, it is ground to a very fine powder and extracted with boiling water the resulting solution gives a positive nitroprusside reaction. Extraction with 10 % trichloroacetic acid presents no advantage over extraction with water. If the aqueous extract be reduced as described above in the case of Heparglandol the resulting solution gives a very strong colour reaction with nitroprusside.

It is very necessary to grind the Ventriculin as finely as possible to ensure maximum extraction of the glutathione. Even with this precaution we believe that a certain amount still remains adherent to the extracted residue. In fact, if the nitroprusside reaction were applied directly to this residue it frequently showed a strong reddish-violet colour. In order to obtain the strongest extract we found that it was best to add the sodium cyanide during the operation of extraction so that reduction and extraction were accomplished at the same time. The mean of many colorimetric estimations of glutathione in the reduced solution gave a content of 0.24 % while the amount in the original extract did not exceed 0.05 %.

The iodimetric estimation was not applicable to these products. The aqueous extract of Ventriculin before reduction, while showing but a feeble coloration with the nitroprusside reagent, yet required a large amount of iodine before oxidation was complete as shown by starch as inside indicator. It seems highly probable that the substance responsible for the nitroprusside reaction in these two preparations is glutathione. By the application of the dimethyl-*p*-phenylenediamine test for cystine we assured ourselves that cystine was absent in both cases.

Estimations of glutathione were also made in the fresh gastric mucosa of the pig. The mucosa was separated from the muscular layer with the aid of a pair of scissors. The tissue was finely minced and ground with a 10 % solution of trichloroacetic acid in presence of fine washed sand. The mixture was boiled and filtered. In one part the reduced glutathione was estimated

colorimetrically. Another portion was neutralised and reduced as before and the glutathione estimated. The mean of several observations gave a total value of 0.12 % of which 75 % was in the reduced state. We are ignorant of the proportions of this tissue used by the manufacturers in the production of Ventriculin.

It would seem from our observations that glutathione enters into the composition of anti-anaemic substances and that in the commercial products it appears mostly in the oxidised form. As to whether it has of itself any ameliorating effect in anaemic conditions we are as yet unable to say. Experiments on animals are being conducted with the object of elucidating this question.

REFERENCE.

Fleming (1930). *Compt. Rend. Soc. Biol.* **106**, 259.