lability, pH optimum, and of the effect of storage on the enzyme in deficient subjects, for comparison with those of the enzyme in affected Negroes and Caucasians.

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¹ Marks, P. A., and Gross, R. T., *J. Clin. Invest.*, **38**, 2253 (1959).

² Motulsky, A. G., and Campbell, J. M., *Blood* (in the press).

³ Motulsky, A. G., Kraut, J. M., Thieme, W. T., and Musto, D. F., *Clin. Res.*, **7**, 89 (1959).

⁴ Swindler, D. R., *Human Biol.*, **27**, 284 (1955).

⁵ Walsh, R. J., and Cotter, H., *Austral. J. Sci.*, **17**, 175 (1955).

Isolation of Haptoglobin by Chromatography on Diethylaminoethyl- Cellulose (DEAE)

HAPTOGLOBIN was isolated first by Jayle *et al.*¹ using a technique which combined precipitation by ammonium sulphate with preparative starch-gel electrophoresis. More recently we found that haptoglobin could be obtained as a by-product during the routine fractionation of human plasma^{2,3}. This technique uses rivanol as a precipitating agent for the subfractionation of fraction IV, obtained at pH 5.8, and 35 per cent alcohol concentration, the final purification being achieved by chromatography on DEAE.

This technique gives pure haptoglobin suitable for metabolic research studies with and without addition of haemoglobin⁴. However, geneticists interested in the variation of haptoglobin groups often have only small samples of plasma at their disposal and they only need minute amounts of the isolated protein as test substances for electrophoretic work.

In our routine technique, the final purification of haptoglobin is obtained by adsorption of haptoglobin at pH 5 on DEAE using 0.03 M acetate buffer, whereas siderophilin, the main contaminant, is not adsorbed. Haptoglobin can be adsorbed under the same conditions from whole plasma, but is then accompanied by other proteins: ceruloplasmin, a second α_2 -glycoprotein, cholinesterase and some α_1 -globulins. Smithies⁵ already showed that the selective adsorption of haptoglobin needs a rather low pH. However, we noticed that haptoglobin at about 80 per cent purity can be obtained with a slightly modified elution technique.

Plasma is diluted to give a freezing point of -0.15° . The pH is then adjusted to 5 with acetic acid 0.03 M; DEAE-cellulose is equilibrated against acetate buffer 0.03 M, pH 5, a minimum of 25 gm. of dry cellulose being necessary for 1 l. of plasma. The adsorbent is poured into a column (the diameter being proportional to the volume handled) and the diluted plasma is passed by gravity flow. The column is then washed with acetate buffer, 0.03 M, until no more proteins appear in the effluent. Haptoglobin can then be eluted with 0.1 M acetate buffer, pH 5.

Fig. 1. *A*, Haptoglobin 2 : 1 separated by batch-adsorption from a small plasma sample. Eluate slightly concentrated. *B*, Haptoglobin 1 : 1 with and without added haemoglobin. Sample obtained by column chromatography of 250 ml. of plasma

The solution should contain 2–4 gm./l. of proteins; the total yield of haptoglobin based on the haemoglobin-binding capacity is about 50–60 per cent.

Ceruloplasmin together with some α_1 -globulin can be eluted at 0.2 M acetate buffer concentration and at the same pH. A second, not yet identified, α_2 -glycoprotein is eluted at 0.5 M acetate buffer.

Satisfying results have been obtained with small samples (2–4 ml.) as well as with volumes of 1 l. of plasma and more. Batch-adsorption can be used instead of the column adsorption. Since no dialysis is necessary, it is possible to achieve the isolation of haptoglobin a few hours after the collection of the blood sample.

The purity and the yield of the separated product seems to give data corresponding to those published by Smithies⁵ for haptoglobin obtained by chromatography on 'Dowex 2 X-10', pH 4.2.

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¹ Jayle, M. F., Boussier, G., and Tonnelat, *Bull. Soc. Chim. Biol.*, **38**, 343 (1956). Jayle, M. F., de Pallerets, G., and Moretti, J., *Bull. Soc. Chim. Biol.*, **41**, 1285 (1959).

² Steinbuch, M., and Pejaudier, L., *Nature*, **184**, 362 (1959).

³ Steinbuch, M., *Eighth Internat. Congr. Blood Transfusion, Tokyo* (1960).

⁴ Steinbuch, M., Blatrix, C., and Reuge, C., *Eighth Internat. Congr. Blood Transfusion, Tokyo* (1960).

⁵ Smithies, O., and Connell, G. E., *Biochem. J.*, **72**, 115 (1959).