then determines the amount of acids to be taken for the chromatography. The spots obtained were cut out, and the ferric complexes of the hydroxamic derivatives of the fatty acids were extracted with a FeCl_3 solution (5 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ + 25 ml conc. HCl + 575 ml water³). This solution was then analysed colorimetrically at a wavelength of 5200 Å. According to Goddu¹⁰ the accuracy of the measurement is $\pm 2\%$. The accuracy is somewhat reduced, however, because of the chromatographic procedure. In the conditions described the following R_F -values were obtained: formic 0.24, acetic 0.34, propionic 0.51, butyric 0.69, valeric 0.83, and caproic acid 0.90.

Separation and estimation of the acids from caproic to pelargonic acid. The analyses were carried out in the same conditions as those of the preceding group, except that the chromatographic solvent was the upper layer of the mixture benzene:formic acid:water (1:1:1)⁵. The following R_F -values were obtained: valeric 0.18, caproic 0.33, oenanthylic 0.49, caprylic 0.62, pelargonic 0.72, and capric acid 0.76.

Separation and estimation of the acids from capric to cerotic acid. The chromatography was carried out by the method previously described by the author⁸, using a paper saturated with hydrocarbon and as solvent a mixture of acetic acid and water (9:1) saturated with hydrocarbon. The hydrocarbon was evaporated and the paper treated for half an hour with an 1.5% aqueous solution of AgNO_3 . The excess of silver was removed by washing with deionized water, after which the paper was rapidly dried and sprayed with 0.05% ethanolic *p*-diethylaminobenzylidenrhodanine. After drying, the silver spots were conveniently visible in ultraviolet light. The spots were cut out and the paper was burned in the usual manner with sulfuric acid-perchloric acid-nitric acid. The amount of silver was determined colorimetrically by the dithizone method at a wavelength of 6250 Å¹¹. For qualitative detection with the silver reagent mentioned 0.05 μM of each acid is sufficient, but for quantitative estimation it is advisable to use 0.2–2 μM of each acid. According to several determinations the accuracy of the method is about $\pm 5\%$.

1. Reid, R. L. and Lederer, M. *Biochem. J. London* **50** (1951) 60.
2. Isherwood, F. A. and Hanes, C. S. *Biochem. J. London* **55** (1953) 824.
3. Stadtman, E. R. and Barker, H. A. *J. Biol. Chem.* **184** (1950) 769.
4. Block, R. J., Durrum, E. L. and Zweig, G. *A Manual of Paper Chromatography and Paper Electrophoresis*, Academic Press

Inc., Publishers, New York, N. Y. 1955, p. 162.

5. Thompson, A. R. *Australian J. Sci. Research B* **4** (1951) 180.
6. Wagner, H., Abisch, L. and Bernhard, K. *Helv. Chim. Acta* **38** (1955) 1536.
7. Kaufmann, H. P. and Budwig, J. *Fette u. Seifen* **53** (1951) 390.
8. Perilä, O. *Acta Chem. Scand.* **9** (1955) 864.
9. Inouye, Y. and Noda, M. *J. Agr. Chem. Soc. Japan* **25** (1952) 496.
10. Goddu, R. F., LeBlanc, N. F. and Wright, C. M. *Anal. Chem.* **27** (1955) 1251.
11. Sandell, E. B. *Colorimetric Determination of Traces of Metals*, Interscience Publishers, Inc., New York, N. Y. 1944, p. 394.

Received December 14, 1955.

Purification of Clearing Factor from Postheparin Plasma

ESKO A. NIKKILÄ and RAINER PESOLA

Department of Medical Chemistry, University of Helsinki, Finland

Since the original discovery by Hahn¹ of the lipemia clearing effect of heparin upon injection and the subsequent demonstration of an *in vitro*-active clearing principle in plasma withdrawn after heparin administration² much successful work has been done to elucidate the mechanism of this peculiar phenomenon. Evidence has accumulated to show that the clearing factor acts as a lipoprotein lipase³. In spite of these advances only a few attempts have so far been made for the purification of the active principle from postheparin plasma. Anfinsen *et al.*⁴ showed that the clearing factor falls in Fraction III-1,2,3 in the ethanol fractionation scheme of Cohn and they could thus achieve an about fourteen-fold purification of the activity. Later, Anfinsen and Quigley⁵ reported a purification up to 100- to 200-fold by a relatively laborious procedure based on a complex formation between the clearing factor and low density lipoproteins (enzyme-substrate complex). Earlier studies in this laboratory revealed that the clearing activity was precipitated quantitatively from plasma at a low ionic strength at pH 5.6 along with 1/20 to 1/25 of the plasma proteins⁶. This finding was confirmed by Hood *et al.*⁷ and the method has since been used by a

number of workers for obtaining a crude preparation of clearing factor.

Because of the irreversible inactivation of clearing factor in concentrated salt solutions its purification by the conventional salting out procedures is not possible. In the search for more effective methods of purification our attention was drawn to the recent findings of Nilsson and Wenckert⁸, who demonstrated that heparin is adsorbed to known prothrombin adsorbents and elutable with citrate and that a natural heparin-like anticoagulant occurs in plasma bound to prothrombin. It was thought that also the clearing factor, which most probably contains heparin, might behave like prothrombin against adsorbents, either due to the formation of a complex with the latter or due to its heparin content as such. Thus, the known prothrombin purification methods could possibly be advantageously applied for the purification of lipemia clearing activity as well. This proved in fact to be the case.

The clearing activity of postheparin plasma is completely adsorbed on calcium phosphate gel and can be eluted with 0.1 M citrate. By this means a 40-fold purification is achieved. Elution of the adsorbate with physiological NaCl removes about half of the proteins adsorbed but none of the clearing activity. Thus, washing of the precipitate with saline before the citrate elution increases the grade of purification up to about 85-fold. The supernatant liquid of the $\text{Ca}_3(\text{PO}_4)_2$ adsorbate has the same acceptor-protein activity as untreated plasma and can therefore be used in the assay of clearing activity. In the course of these experiments it was found that citrate is a powerful activator of the clearing reaction, also in oxalated plasma.

Further purification of the clearing factor could be attained by diluting the eluate, after removal of citrate by dialysis, with distilled water and adjusting the pH to 5.5. The precipitate formed contained about 60 % of the original clearing activity purified about 400-fold over the initial plasma on the protein-tyrosine basis.

Being undialysable the clearing factor differs from the natural heparin-like anticoagulant, which was reported by Nilsson and Wenckert⁸ to dissociate from its union with prothrombin during dialysis. The possible identity of clearing factor and the thermolabile anticoagulant described by Fiala⁹ remains to be established.

Oxalated plasma was found to have a lower clearing activity than the mere

postheparin plasma, thus suggesting that the active principle is adsorbed on calcium oxalate as well (like heparin and prothrombin).

The details of the purification procedure and a description of the properties of the active preparation thus obtained will be published shortly.

1. Hahn, P. F. *Science* **98** (1943) 19.
2. Anderson, N. G. and Fawcett, B. *Proc. Soc. Exptl. Biol. Med.* **74** (1948) 768.
3. Korn, E. D. *J. Biol. Chem.* **215** (1955) 15.
4. Anfinsen, C. B., Boyle, E. and Brown, R. K. *Science* **115** (1952) 583.
5. Anfinsen, C. B. and Quigley, T. W. *Circulation* **8** (1953) 435.
6. Nikkilä, E. *Scand. J. Clin. & Lab. Invest. Suppl.* **8** (1953).
7. Hood, B., Angerwall, G., Isaksson, B. and Welin, G. *Scand. J. Clin. & Lab. Invest.* **6** (1954) 1.
8. Nilsson, I. M. and Wenckert, A. *Acta Med. Scand.* **150** (1954) *Suppl.* 297.
9. Fiala, S. *Nature* **167** (1951) 279.

Received December 19, 1955.

Syntheses of Choline Esters of Monobasic Carbonic Acids

L.-E. TAMMELIN

Research Institute of National Defence,
Dept. I, Sundbyberg 4, Sweden

A convenient method which gives good yields of choline esters is shown by the following scheme:

The first step has been used by Campbell *et al.*¹ in theoretical studies of the amino alcohols. Using this method of synthesis acetylcholine, propionylcholine and butyrylcholine have been prepared in yields of more than 90 % of the theoretical, calculated on the dimethylaminoethanol.

The only step that requires special comment is the first. When the reaction between the alcohol and the anhydride is finished the reaction mixture is distilled