

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 eluated 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.

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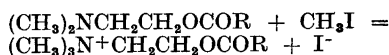
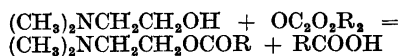
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Syntheses of Choline Esters of Monobasic Carbonic Acids

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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

Table 1.

Compound	Final b. p.	n_D^{20} at final b. p.
Acetic ester	68°/8 mm	1.4309
Propionic ester	70°/8 mm	1.4310
Butyric ester	81°/8 mm	1.4320

Table 2.

Compound	Grams of distillate	Grams of CH ₃ I	Yield in %, calc. on the alcohol
Acetylcholine	1	2.0	93
Propionyl »	1	1.8	92
Butyryl »	1	1.6	93

Table 3.

Formula	Mol. wt.	C		H		I		M.p. *
		calc	found	calc	found	calc	found	
C ₇ H ₁₆ O ₂ NI	273.12	30.8	30.8	5.9	5.8	46.5	47.0	161
C ₈ H ₁₈ O ₂ NI	287.15	33.5	33.4	6.3	6.3	44.3	44.7	130
C ₉ H ₂₀ O ₂ NI	301.18	36.0	35.8	6.7	6.6	42.3	42.7	87

* Determined with a "Kofler Heizbank".

under reduced pressure. It has been shown that all fractions up to a certain boiling point contain the dimethylaminoethyl ester. Thus all fractions are collected from the beginning of the distillation until temperature exceeds the boiling points given in Table 1.

Experimental: Dimethylaminoethylesters. One mole of acid anhydride was refluxed with 1 mole of dimethylaminoethanol on waterbath overnight. The reaction mixture was distilled at reduced pressure and all fractions collected up to and including the fraction at the temperature given in Table 1.

Choline esters. The combined fractions from the esterifications were dissolved in eight parts of dry ether. Methyl iodide was added in about 100 % excess and the reaction mixture

was allowed to stand for three days in a stoppered round-bottomed flask at 25° C. The iodides were obtained as colourless crystalline precipitates which were filtered off and dried in a desiccator. Recrystallisation of the products was found to be unnecessary. Data for the syntheses are given in Table 2 and the analyses are given in Table 3.

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