obtained. Recrystallisation from benzene gave a bright red, crystalline material, m.p. 140—156°. Paper chromatography (*iso*propylether, dimethyl sulphoxide, EDTA treated paper [3]) showed the presence of two phenolic components, $R_F$ 0.26 and 0.18. The mother liquors contained small amounts of a further slow-moving component ($R_F$ 0.15) and two rapid components ($R_F$ 0.57 and 0.38). The latter gave a brown colour with the benzidine reagent only after addition of sodium carbonate.

Separation of the mixtures as described for the dihydrochalcones [3] gave the following components: $R_F$ *0.57*, 0.3 g; *0.38*, 0.2 g; *0.26*, 0.3 g and *0.18*, 1 g.

*The $R_F$ 0.57 product* was recrystallised from methanol and sublimed, colourless plates, m.p. 100—102° identical with (±)-5-hydroxy-7-methoxyflavanone [3] (mixed m.p. and IR).

*The $R_F$ 0.38 product* was recrystallised from methanol and sublimed to give colourless needles, m.p. 119—120°, identical with (±)-5-hydroxy-4′,7-dimethoxyflavanone [3] (mixed m.p. IR).

*The $R_F$ 0.26 product* was recrystallised from benzene giving red prisms, m.p. 156—167°. The melting point range was not improved by repeated crystallisations. The infrared spectrum showed a broad band between 3 200 and 2 400 $cm^{-1}$ and absorptions at 1 635, 1 610 and 825 $cm^{-1}$. The ultraviolet spectrum showed a broad maximum at 355 m$\mu$ (log $\varepsilon$ 4.70). On attempted sublimation (130°, 0.005 mm) a colourless oil distilled. It solidified on cooling and proved to be identical with (±)-5-hydroxy-4′,7-dimethoxyflavanone (mixed m.p., IR). On standing, particularly in solution, the pigment was slowly converted into the flavanone.

*The $R_F$ 0.18 product* on recrystallisation from benzene gave red prisms with an indefinite melting point, 156—162°. Relevant infrared absorptions were observed at 3 200—2 400, 1 635, 745 and 685 $cm^{-1}$. The ultraviolet spectrum showed a broad absorption at 340 m$\mu$ (log $\varepsilon$ 4.74). Thermal rearrangement as described above gave (±)-5-hydroxy-7-methoxyflavanone in almost quantitative yield (identified by mixed m.p. and IR).

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1. Nilsson, M. *Acta Chem. Scand.* **13** (1959) 750.
2. Forsén, S. and Nilsson, M. *Acta Chem. Scand.* **13** (1959) 1383.
3. Nilsson, M. *Acta Chem. Scand.* **15** (1961) 154.
4. Zopf, W. *Ber. deut. botan. Ges.* **24** (1906) 264.
5. Lindstedt, G. *Acta Chem. Scand.* **4** (1950) 1042.

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## Gel Filtration Chromatography in the Separation of Human Serum

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It has been reported by Alridge [1], Mounter and Whittaker [2], Augustinsson [3], and Marton and Kalow [4] that human plasma contains arylesterase activity.

In connection with investigations of cytolytic serum factors [5,6], it was noticed that the arylesterase activity of various fractions obtained in continuous (courtain) flow electrophoresis of human serum had such values that the presence of two arylesterases is indicated.

It can now be reported that two different arylesterases have been partly separated in fractions from human serum. As found in the investigations cited [5,6], there is one heat labile (56°C for 30 min.) arylesterase in human serum which is not influenced by EDTA and one heat stabile arylesterase which is completely inactivated by EDTA.

*Experimental.* Normal human serum from healthy blood donors (10 ml) was fractionated at +4°C with ammonium sulphate at pH 5.2. The sediments from 0—50, 50—60, and 60—70 % saturation were dissolved in 5 ml portions and for desalting filtered through Sephadex [7] G-25 columns 2 × 28 cm, equilibrated with 0.9 % NaCl. The supernatant from 70 % saturation was filtered too. The different solutions were at the desalting by Sephadex filtration collected in 3 ml fractions and the optical density at 280 m$\mu$ was measured as well as protein nitrogen and arylesterase activity. All fractions filtered were free from ammonium

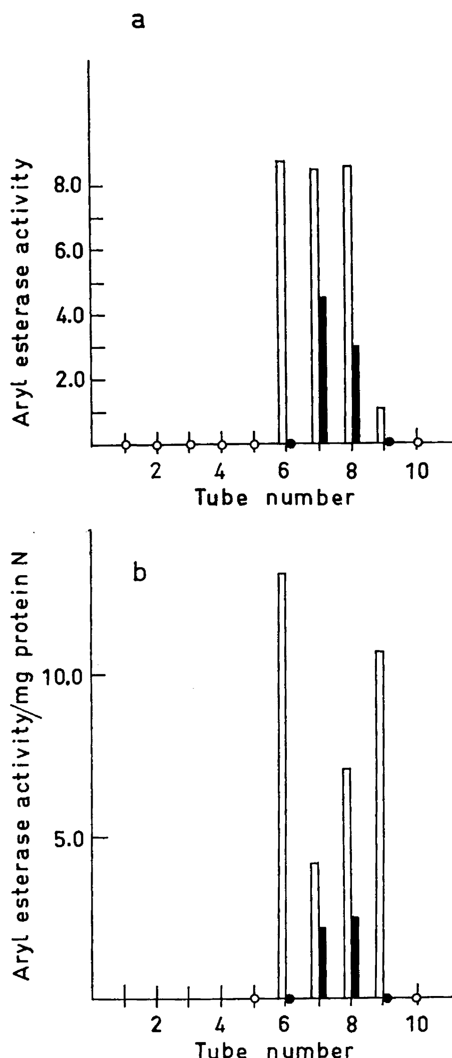


*Fig.* 1. Arylesterase activity in normal human serum fraction, precipitated at 60—70 % ammonium sulphate saturation. The precipitate was dissolved in 5 ml 0.9 % NaCl and filtered through a Sephadex G-25 column and collected in 3 ml fractions. Open areas: total arylesterase activity. Dark areas: heat stabile arylesterase activity. Substrate: phenylacetate in Tris buffer at pH 8.0. a: activity per volume; b: activity per mg protein nitrogen.

sulphate. Phenylacetate was used as substrate and the hydrolysis was measured by titration of liberated carboxyl groups. The assay mixture consisted of 0.50 ml sample and 3.00 ml 0.04 M phenylacetate in 0.1 M tris buffer at pH 8.0. The mixtures were incubated at 25°C for 60 min. The determinations were duplicates and controls for nonenzymatic hydrolysis were always run. The esterase activity was assayed in unheated samples as well as in samples heated at 56°C for 30 min.

*Results.* As can be seen in the figure, which shows the arylesterase activity of the precipitate from 60—70 % ammonium sulphate saturation, separation occurs after the gel filtration. In tube number 6 there is only the heat labile arylesterase. In tube number 7, however, more than 50 % of the activity remained after heating and in tube number 8 there is about 40 % of the heat stabile arylesterase present. In tube number 9 there is only a weak activity of the heat labile enzyme. The distribution of the two arylesterases was confirmed when the activity was tested in presence of EDTA in a final concentration of 0.001 M. Thus, it appeared that the esterase activity in tubes number 7 and 8 was completely removed with the agent but remained the same without EDTA in tubes number 6 and 9. Eserine sulphate (M $\times$ $10^{-4}$) had no effect, thus indicating the absence of cholinesterase. The precipitate from 50—60 % ammonium sulphate saturation and the supernatant after 70 % saturation had rather strong arylesterase activity, but only from the heat labile enzyme.

1. Alridge, W. N. *Biochem. J.* **53** (1953) 110.
2. Mounter, L. A. and Whittaker, V. P. *Biochem. J.* **54** (1953) 551.
3. Augustinsson, K.-B. *Nature* **181** (1958) 1786; *Acta Chem. Scand.* **13** (1959) 571, 1097.
4. Marton, A. and Kalow, W. *Can. J. Biochem. Physiol.* **37** (1959) 1367.
5. Paulsson, J.-E., Björklund, B. and Björklund, V. *Med. Riksstämman Stockholm* 1960; *Acta Pathol. Microbiol. Scand. In press.*
6. Lundblad, G., Paulsson, J.-E., von Zeipel, E. and Björklund, B. *Med. Riksstämman Stockholm* 1960; *Acta Pathol. Microbiol. Scand. In press.*
7. Porath, J. and Flodin, P. *Nature* **183** (1959) 1657.

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