# The Fractionation of the Phosphorus-containing Proteins of Rat Liver Cell Sap

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The occurrence of phosphoserine and phosphothreonine in partial hydrolysates of liver protein has been demonstrated in previous reports from this laboratory [1,2]. Moreover, phosphoserine was also found in hydrolysates of rat liver nuclear, mitochondrial, microsomal and supernatant protein. Attempts are now in progress to isolate the native, phosphorus-containing proteins of these liver cell fractions. The fractionation of the proteins of the rat liver supernatant fraction is the subject of this communication.

2—4 hours after the intraperitoneal injection of radioactive phosphate into non-fasted male, Wistar rats, the animals were sacrificed by cervical dislocation. The livers were perfused with ice-cold isotonic saline followed by 0.25 M sucrose *via* the hepatic portal vein. The pH of both solutions had been adjusted to 7.4 and both contained 0.01 M versene. After mincing and homogenizing the livers in the versene-sucrose solution the supernatant fraction was prepared essentially according to Hogeboom [3]. The proteins of this fraction were then subjected to a preliminary fractionation by precipitation with either a modification of the method of Cohn *et al.*[4] or with ammonium sulfate. The various precipitates were subsequently dissolved in tris buffer (tris hydroxymethyl aminomethane) pH 7.8, dialyzed at 0—1° for 1—2 hours and then further fractionated by zone electrophoresis in tris buffer according to Porath [5] on 100—150 cm cellulose powder columns. After the completion of the electrophoresis the effluent from the column was followed by spectrophotometric reading at 260 and 280 mμ and by measurement of the $^{32}P$ content of the effluent tubes. The identity of the phosphoprotein fractions was established by hydrolysis followed by the chromatographic isolation of phosphoserine using the methods described previously [1,2]. A detailed paper will be published shortly.

1. Ågren, G., de Verdier, C.-H. and Glomset, J. *Acta Chem. Scand.* **8** (1954) 503.
2. Ågren, G., de Verdier, C.-H. and Glomset, J. *Acta Chem. Scand.* **8** (1954) 1570.
3. Hogeboom, G. H. in *Methods in Enzymology*, Vol. 1 p. 16 Academic Press Inc., New York, 1955.
4. Cohn, E. J. *et al.*, *Enzymes and Enzyme Systems*, Harvard University Press 1951.
5. Porath, J. *Biophys. et Biochim. Acta. In press.*

# Partition Chromatography of some $C_{27}$-Steroids

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With reversed-phase partition chromatography using *iso*propanol/water (165/135 ml) as mobile and chloroform/heptane (10/40 ml) as stationary phase, it is possible to separate hydroxy- and keto-$C_{27}$-steroids. Substances with hydroxyls in the 3-, 7- and 12-position and 3-ketones have been used and are separated into groups of compounds containing 3, 2 and 1 hydroxyl, respectively, and one with 3-ketones. Those with a double bond in the 5,6-position can be separated from the saturated ones. Furthermore, by increasing the content of water in the mobile phase a separation of 3α,7α-dihydroxy from 3α,12α-dihydroxy coprostane is obtained.

Table 1 shows the result of a run with the phases mentioned above. 4 ml of the stationary phase was supported on 4.5 g of Hyflo Supercel, made completely hydrophobic with dichlorodimethylsilane [1].

| Compound | Appearance of compound Beginning | Peak | End |
|---|---|---|---|
| 3α,7α,12α-Trihydroxy-coprostane | 17 | 22 | 26 |
| 7α-Cholesterol | 22 | 28 | 38 |
| 3α,7α-Dihydroxy-coprostane | 36 | 45 | 70 |
| 3α,12α-Dihydroxy-coprostane | 36 | 48 | 70 |
| Cholesterol | 75 | 89 | 110 |
| Cholestenone | 118 | 132 | 143 |
| Cholestanone | 150 | 175 | 200 |

Some other separations will be discussed.

1. Howard, G. A. and Martin, A. J. P. *Biochem. J. London* **46** (1950) 532.