The taurine content of skeletal muscle in genetically dystrophic chickens is reported to be higher than in normal chickens[4]. The dystrophic animals excrete this amino acid and its metabolite in the urine[5,6]. Dystrophy induced by drugs or X-irradiation also enhanced excretion of taurine in urine[7]. Reports have indicated the involvement of a presynaptic defect in muscular dystrophy[8–10]. On the results of cross innervation studies in the mouse, Law and Atwood have proposed that a "trophic" factor which is released from a presynaptic site may be absent in dystrophic muscle[9].

In our experiments, cats were anaesthetized with 'Dial-urethan' (0.6 ml kg^{-1}, i.p.) and a sciatic nerve–anterior tibialis muscle preparation made. The sciatic nerve was stimulated supramaximally at a rate of 6.4 Hz with square waves of 0.5 ms duration. The contractile force was recorded with a Grass FT03 Force-displacement transducer. Taurine was dissolved in Krebs-Henseleit solution (*p*H 7.4)[11].

When taurine was injected into the femoral vein on the contralateral side from the monitored anterior tibialis muscle, no effect on twitch tension at any concentration (0.1 mm–10 mm kg^{-1}) was seen. It is possible that taurine did not reach the neuromuscular junction, because of its rapid binding to platelets (J. Gorden, personal communication).

Intra-arterial, sequential injections of taurine (1 mmol kg^{-1}) close to the monitored neuromuscular junction, however, produced a dose-dependent decline in the amplitude of the muscular twitch response.

When the sciatic nerve was tetanically stimulated, the depression of muscle contraction was temporarily reversed. Direct skeletal muscle stimulation also reversed the depression of contractility for the duration of the direct stimulation. These studies indicate that taurine depresses transmission at the neuromuscular junction. Studies described here, however, do not adequately pinpoint a presynaptic or postsynaptic action of the drug. Further studies will have to be made to delineate its precise site of action. The depressed neuromuscular transmission caused by increasing taurine concentrations in the vicinity of the neuromuscular junction is compatible with the demonstration of increased levels of this amino acid in skeletal muscle and urine of man and animals with muscular dystrophy.

Because taurine depressed neuromuscular transmission in the cat and changes in taurine metabolism have been reported in muscular dystrophic animals, we propose that changes in this amino acid may be related to muscular dystrophy or may be the biochemical basis of lesions caused by this disease.

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1 Jacobsen, J. G., and Smith L. H., jun., *Physiol. Rev.*, **48**, 424 (1968).
2 Davison, A. N., and Kaczmarek, L. K., *Nature*, **234**, 107 (1971).
3 Hammerstad, J. P., Murray, J. E., and Cutler, R. W. P., *Brain Res.*, **35**, 357 (1971).
4 Wilson, B. W., Peterson, D. W., and Lillyblade, A. L., *Proc. Soc. expl. Biol. Med.*, **119**, 104 (1965).
5 Bank, W., Rowland, L., and Ipson, J., *Trans. Am. neurol. Assn.*, **93**, 185 (1968).
6 Jacobsen, J. G., Collins, L. L., and Smith, L. H., jun., *Nature*, **214**, 1247 (1967).
7 Boyer, R. A., Yin, M. N., and Bowden, D. H., *Proc. Soc. expl. Biol. Med.*, **116**, 534 (1964).
8 Botez, M. I., *Nature new Biol.*, **238**, 285 (1972).
9 Law, P. K., and Atwood, H. L., *Nature*, **238**, 287 (1972).
10 Rolson, J. L. L., *Arch. Neurol.*, **26**, 258 (1972).
11 Winegrad, S., and Shanes, A. M., *J. gen. Physiol.*, **45**, 371 (1962).

Salting-out in Amphiphilic Gels as a New Approach to Hydrophobic Adsorption

HYDROPHILIC gels such as those formed from cross-linked dextran–Sephadex[-1], agar[2] and agarose[3] are extensively used for molecular-size dependent fractionation (such as gel filtration and gel permeation chromatography). Although solute-gel interaction other than, or in addition to, molecular sieving are often evident[4–6], such interactions have seldom been deliberately exploited for fractionation of protein mixtures. We describe below a method for protein fractionation based on hydrophobic salting-out adsorption in non-ionic amphiphilic gels.

Amphiphilic gels are produced by introducing a limited number of hydrophobic groups into hydrophilic gel-forming substances, yielding cross-linked polymers which are highly permeable and capable of extensive swelling in water as well as in many organic solvents. In aqueous solutions they show affinity for hydrophobic solutes or solutes with hydrophobic groups. The adsorbents, the use of which we advocate, do not contain ionic groups, in contrast to those obtained by coupling aliphatic amines, tryptophan, tryptophan esters and so on, to cyanogen bromide activated agarose[7–9]. Non-ionic amphiphilic gels, therefore, should not exhibit mixed ionic-hydrophobic adsorption but, instead, "pure" hydrophobic interaction with hydrophobic regions on the molecular surface of proteins and other solutes. The adsorption capacity for proteins can be very high in certain conditions, providing the fundamental basis for a promising new type of chromatography, which we shall term hydrophobic salting-out chromatography.

The gel used here to demonstrate the new fractionation technique, a benzyl ether of cross-linked agarose, can be prepared as follows: agarose gel of about 6% matrix content, cross-linked with epichlorohydrin or 2,3-dibromopropanol[10], preferably in the form of spherical beads, is swollen in water. To a gel volume of 200 ml including the interstitial water, 200 ml of 5 M NaOH is added, followed by 50 ml benzyl chloride and 1 g sodium borohydride. The suspension is heated to 80° C with stirring and reflux. After 5 h the reaction is discontinued and the product transferred to a Büchner funnel and washed with ethanol and water. The concentration of benzyl groups is estimated at around 1.8 mmol g^{-1} of dry gel substance.

Table 1 Concentration and Group Separation of Kidney Bean Proteins by Benzylated Cross-linked Sepharose 6B*

Fraction	Total protein (%)	Volume (ml)
A	58	4,000
B	5	60
C	15	120
D	10	150
E	4	20
Intermediate fractions	8	490

* See Fig. 2.

To illustrate the general phenomena, cytochrome *c* was selected as a convenient model substance. Several frontal chromatograms were developed on a column of benzylated cross-linked Sepharose 6B (1.0×19.5 cm) using solutions containing 1 mg of protein ml^{-1}. In one set of experiments the chromatograms were developed with solutions of 0.5 M sodium phosphate buffer (*p*H 7) containing different concentrations of sodium chloride. Chromatograms were also developed in 0.1 M sodium formate buffer (*p*H 3) with and without sodium chloride (Fig. 1). Adsorption increases strongly with increasing concentration of salt and is further enhanced by lowering the

*p*H from 7 to 3. Unsubstituted cross-linked Sepharose 6B does not adsorb cytochrome *c* in these conditions, which clearly demonstrates the importance of benzyl groups for the hydrophobic adsorption.

To illustrate concentration and group separation of proteins a diluted sample of kidney bean extract[11], containing 0.8 g of freeze-dried protein dissolved in 4 l of 0.1 M sodium formate buffer (*p*H 3) and 3 M in NaCl, was introduced into a benzyl agarose column. Elution was performed by increasing the *p*H, decreasing the ionic strength and lowering the polarity of the solvent (Fig. 2). Trypsin inhibitor activity[12] and haemagglutinin activity were measured on aliquots[13] (Fig. 2 and Table 1). Phytohaemagglutinins are found in Fractions C, D and E. Fraction E contains 95% of the trypsin inhibitor activity 200-fold concentrated on a volume basis. The purification factor based on adsorbance at 280 nm was 25 for trypsin inhibitor. In an experiment performed as above, except that sodium chloride was omitted from the sample and the starting buffer, only 12% of the protein material was adsorbed to the gel.

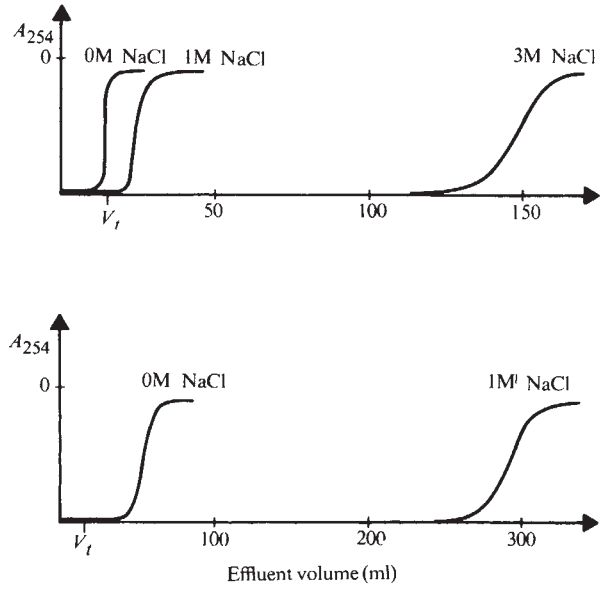

Fig. 1 Frontal chromatograms of cytochrome *c* in 0.05 M sodium phosphate buffer, *p*H 7.0 (above), and 0.1 M sodium formate buffer (below), obtained with and without sodium chloride included in the solutions. Transmission plotted against effluent volume. Adsorbent: benzyl ether of cross-linked 'Sepharose' 6B. Total volume of the column: 15.2 ml. See also text.

The bed used in these bean extract experiments has been repeatedly used over a period of more than three months. More than 20 chromatograms have been made and the gel has been exposed to amounts of protein corresponding to more than 50 times its own dry weight. No progressive change in its chromatographic properties has been observed. The gel is extremely durable and the adsorption-desorption processes are highly reproducible.

The following conclusions can be drawn from our present experience. (1) At high concentrations of salts and at *p*H values in the acid range a very high capacity can be attained. For example, benzylated agarose can adsorb amounts of proteins corresponding to several times its own dry weight. (2) High salt concentration often promotes such strong adsorption that, once adsorbed, a protein may be desorbed only by severe reduction in ionic strength and/or lowering of the polarity of the medium and/or elevation of the *p*H. In contrast to the other chromatographic methods based on agarose containing hydrophobic groups[7–9], desorption is thus achieved by decreasing the ionic strength, while in the former

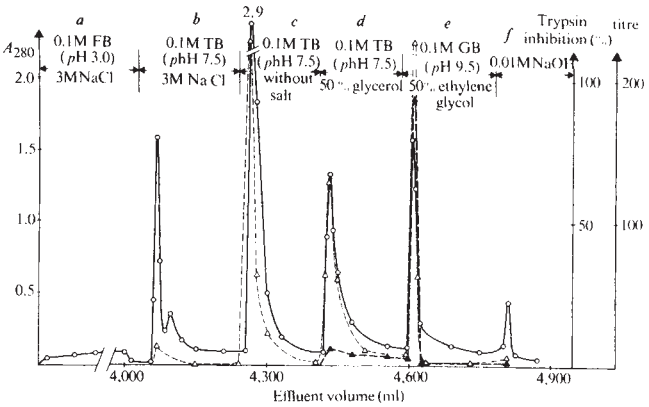

Fig. 2 Chromatogram of kidney bean extract on benzyl ether–'Sepharose' 6B. Column size: 0.9×11.5 cm; flow rate: introduction of sample 90 ml h^{-1}; flow rate: elution 18 ml h^{-1}; buffer systems: formate buffer (FB); Tris-HCl buffer (TB); glycine buffer (GB). Trypsin inhibition was measured on 40 times diluted material (5 min incubation with 0.1 mg trypsin ml^{-1}). Absorbancy at 280 nm: ○—○; trypsin inhibition (%): ▲—▲; agglutinating activity (titre) of human erythrocytes as inverted dilutions: △ - - - △. See also Table 1.

methods desorption is attempted with increasing salt concentrations (which promote hydrophobic adsorption). Furthermore, according to our method, desorption can be accomplished increasing rather than lowering the *p*H of the eluent. (3) Effective group separations and concentration of protein solutions can be accomplished easily.

The underlying principle of salting-out adsorption is not clearly understood, but presumably the driving force is the entropy gain arising from structure changes in the water surrounding the interacting hydrophobic groups.

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1 Porath, J., and Flodin, P., *Nature*, **183**, 1657 (1959).
2 Polson, A., *Biochim. biophys. Acta*, **50**, 565 (1961).
3 Hjertén, S., *Biochim. biophys. Acta*, **79**, 393 (1964).
4 Porath, J., *Biochim. biophys. Acta*, **39**, 193 (1960).
5 Gelotte, B., *J. Chromatogr.*, **3**, 330 (1960).
6 Eaker, D., and Porath, J., *Separation Sci.*, **2**, 507 (1967).
7 Yon, R. J., *Biochem. J.*, **126**, 765 (1972).
8 Er-el, Z., Zaidenzaig, Y., and Shaltiel, S., *Biochem. biophys. Res. Commun.*, **49**, 383 (1972).
9 Hofstee, B. H. J., *Biochem. biophys. Res. Commun.*, **50**, 751 (1973).
10 Porath, J., Janson, J.-C., and Låås, T., *J. Chromatogr.*, **60**, 167 (1971).
11 Rigas, D., and Johnsson, E. A., *Ann. N.Y. Acad. Sci.*, **113**, 800 (1964).
12 Fritz, H., Hartwich, G., and Werle, E., *Z. physiol. Chem.*, **345**, 150 (1966).
13 Salk, J. E., *J. Immun.*, **49**, 87 (1944).

Reference Abbreviations

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