Note on the Purification of Human Antihemophilic Globulin

BIRGER BLOMBÄCK, MARGARETA BLOMBÄCK and INGA MARIE NILSSON

Chemistry Department II, Karolinska Institutet, Stockholm, Sweden

Purification of the antihemophilic globulin in Cohn's fraction I (human) by different procedures in the presence of glycine has been reported earlier [1,2]. A fraction, denoted as I-1-A, was obtained by these procedures. The yield of antihemophilic globulin in this fraction was about 80 % of that in fraction I, and the activity per mg of protein was about 200 times that of plasma. The antihemophilic factor of fraction I-1-A probably forms a complex with fibrinogen. Approximately two-thirds of the fraction are coagulable with thrombin. Attempts to dissociate this complex by fractionation with organic solvents were unsuccessful.

It was, however, found that in contrast to the behaviour of the antihemophilic activity in plasma (*cf.* Ref.[3]), the antihemophilic activity in the purified fraction I-1-A could easily be adsorbed on barium sulphate, aluminium oxide and calcium phosphate (di- and tribasic) as well as on kaolin and bentonite. Barium sulphate adsorbed (from a 0.05 M sodium citrate solution) not only the antihemophilic activity, but also considerable amounts of fibrinogen. The other salts adsorbed more or less selectively the antihemophilic activity, leaving fibrinogen and other proteins in the supernatant. Attempts to elute the activity from all these salts were unsuccessful.

It was also found that the antihemophilic activity could conveniently be adsorbed on tricalcium citrate. Thus, 25 mg of tricalcium citrate adsorbed about 90 % of the antihemophilic factor from 1 ml of a 0.3 % protein solution of fraction I-1-A dissolved in 0.05 M sodium citrate, pH 6.8. After centrifugation and washing of the precipitate with 0.05 M sodium citrate, pH 6.8, the activity could be eluted by dissolving the tricalcium citrate precipitate in 2.0 ml of 0.1 M sodium-EDTA, pH 6.8.

Table 1.

Fraction	Protein		Activity	
	total, mg	yield, % of fr. I-1-A	yield, % of fr. I-1-A	relative, per mg prot.
I-1-A (10 ml)	33.1	100	100	1.0
Supernatant	30.0	90.6	7.3	0.08
			3.9 *	—
EDTA-eluate	3.0	8.5	99.0	11.0
			106.0	

* Values obtained in another experiment, using a different sample of fr. I-1-A.

It can be inferred from Table 1 that less than 10 % of the total protein in the solution of fraction I-1-A is adsorbed on tricalcium citrate. The fibrinogen in fraction I-1-A remained in the supernatant after adsorbtion. As judged by the protein determination procedure used [4], the antihemophilic activity per mg protein in the EDTA eluate is about 10 times higher than in the original fraction. Thus, purification of the antihemophilic factor as compared with plasma is about 2000 times on the protein basis.

Work is now in progress to recover and further study the antihemophilic factor in the EDTA solution. A complete report will be given in *Arkiv Kemi*.

1. Blombäck, B. and Blombäck, M. *Arkiv Kemi* **10** (1956) 415.
2. Blombäck, M. *Arkiv Kemi* **12** (1958) 387.
3. Wagner, R. H., and Thelin, G.M. *Hemophilia and Hemophilioid Diseases*, Chapel Hill, Ed. Brinkhous, 1957.
4. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. *J. Biol. Chem.* **193** (1951) 265.

Received October 16, 1958.