hormone secretion. The in vivo interaction between VIP-containing nerve fibers and other factors, neural as well as nutritional, which influence hormone secretion, is not known. The present study suggests that a potentiating synergism might be exerted through an interaction between cholinergic nerve fibers and VIP-containing nerve fibers. VIP and carbachol both displayed a marked glucagon secretory effect. The importance of these actions on the glucagon cell might well be expressed through an interactive neural regulation of glucagon secretion exerted by acetylcholine and VIP.

1 We thank Professor V. Mutt, Karolinska Institutet, Stockholm, for giving us VIP. The skilful technical assistance of Lena Kvist and Peter Okmark is gratefully acknowledged. This study was supported by grants from the Swedish Medical Research Council (14P-4289, 14X-4286) and the Medical Faculty, University of Lund, Sweden.

2 S.I. Said, in: Gastrointestinal Hormones, p.245. Ed. G.B.J. Glass. Raven Press, New York 1980.
3 J. Fahrenkrug, Digestion *19*, 149 (1979).
4 L.I. Larsson, J. Fahrenkrug, O. Schaffalitzky de Muckadell, F. Sundler, R. Håkanson and J. Rehfeld, Proc. natl Acad. Sci. USA *73*, 3197 (1976).
5 S.I. Said and R.N. Rosenberg, Science *192*, 907 (1976).
6 L.I. Larsson, Histochemistry *54*, 173 (1977).
7 B. Ahrén, J. Alumets, M. Ericsson, J. Fahrenkrug, L. Fahrenkrug, R. Håkanson, P. Hedner, I. Lorén, A. Melander, C. Rerup and F. Sundler, Nature *287*, 343 (1980).
8 F. Sundler, J. Alumets, R. Håkanson, J. Fahrenkrug and O. Schaffalitzky de Muckadell, Histochemistry *55*, 173 (1978).
9 A.E. Bishop, J.M. Polak, I.C. Green, M.S. Bryant and S.R. Bloom, Diabetologia *18*, 73 (1980).
10 J.E. Gerich and M. Lorenzi, in: Frontiers in Neuroendocrinology, vol.5, p.265. Eds W.F. Ganong and L. Martini. Raven Press, New York 1978.
11 I. Lundquist, B. Ahrén, R. Håkanson and F. Sundler, in: Diabetes 1979, p.57. Ed. W.K. Waldhäusl. Excerpta Medica, Amsterdam, Oxford, Princeton 1980.
12 L.G. Heding, Diabetologia *7*, 10 (1971).
13 M.L. Bruss and A.L. Black, Analyt. Biochem. *84*, 309 (1978).
14 J. Szecówka, E. Sandberg and S. Efendić, Diabetologia *19*, 137 (1980).
15 B. Ahrén and I. Lundquist, Diabetologia *20*, 54 (1981).

Sertoli cells of adult rats in vitro. III. Purification of androgen-binding protein from the culture medium[1]

G. Citro, R. Zito, M.L. Marcante, M. Galdieri, A. Floridi and C. De Martino[2]

Regina Elena Institute for Cancer Research, Viale Regina Elena 291, I-00161 Rome (Italy), and Institute of Histology and Embriology 2°, University of Rome, I-00161 Rome (Italy), 9 January 1981

Summary. A method giving a high yield for the isolation and purification of the androgen-binding protein (ABP) from the nutritional medium of cultured Sertoli cells from adult rats is described.

Androgen-binding protein (ABP) is a secretory product of Sertoli cells which has been purified and characterized from the testis and the epididymus of several mammals[3-10]. Moreover, it is known that Sertoli cells in vitro are still able to synthesize ABP and release it into the medium[11,12]. This capacity makes them a useful source for the purification of this protein.

This paper describes a method for isolating ABP from the medium of cultured rat Sertoli cells and confirms previous findings[13] on the possibility of making cultures of Sertoli cells from adult rats.

Materials and methods. Sertoli cells of 4-month-old Long Evans rats were isolated and cultured as previously described[13]. The cells were subcultured into 10 roller-bottles, each containing 150 ml of Eagle's medium (Flow Lab., Rockville) supplemented with 10% foetal calf serum (Eurobio Lab., Paris). When monolayers became confluent, the nutritional medium was removed and replaced by an equal volume of fresh medium. After 10 days of culture the pooled media (1500 ml) were used for ABP purification. Protein concentration was evaluated according to Lowry et al.[14]. Proteins were precipitated with 40% saturated ammonium sulphate at 0 °C. The sediment, recovered by centrifugation, was dissolved in 5 ml of 0.1 M tris-HCl buffer, pH 7.4, with 1 M NaCl and dialyzed against the same buffer. The chromatography was performed by a 2089 Uvicord III, 280 nm filter, 11300 Ultrograd (LKB, Sweden). The gel filtration was performed at 0 °C on a column (2.5 × 100 cm) of superfine Sephadex G-200 eluted with 0.1 M tris-HCl buffer pH 7.4 containing 1 M NaCl. The flow rate was 10 ml/h and fractions of 2 ml were collected, starting from 100 ml of eluent. The ion exchange chromatography was performed according to Cervone et al.[15]. Immunoelectrophoresis, using anti-whole rat and anti-bovine sera (Behring) and normal rat and bovine sera as controls was carried out according to Sega et al.[16]. Isoelectric focusing on polyacrylamide gel was performed by BIO-RAD equipment according to Wringley[17]. The pH gradient was measured by a BIO-RAD profiler. Gels were scanned at 590 nm with PMQ3 Zeiss spectrophotometer. ABP activity was determined on 0.3 ml (40 mg/ml) of fraction A from DEAE cellulose chromatography with ^{3}H-testosterone (2×10^{-9} M, average sp. act. 44Ci/mmole) purchased from New England Nuclear Co.[11].

Results. The fractions between the 2 peaks[7,10] were collected, concentrated and refiltered on Sephadex G-200 (fig. 1).

Purification procedure for ABP from Sertoli cell culture medium

Step	Total protein (mg)	Total pmoles bound	Sp. act. pmoles (mgprotein)$^{-1}$	Yield %	Purification (fold)
Whole medium	8000	31,818	3.9		
$(NH_4)_2SO_4$	50	30,860	617	97	158
1st Sephadex G200	10	22,274	2227	70	571
2nd Sephadex G200	3	20,683	6894	65	1767
DEAE-cellulose	1.5	17,500	11,667	55	2991

The specific activity of ABP after each step was determined according to Fritz et al.[11].

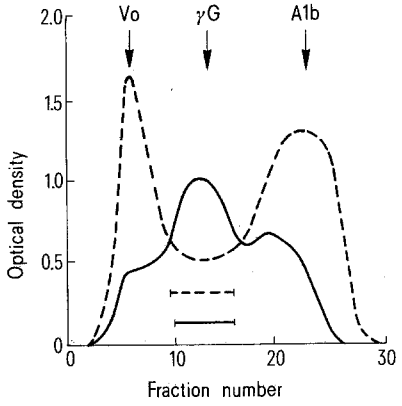

Figure 1. Filtration on Sephadex G-200 of 40% $(NH_4)_2$ SO_4 precipitated (dashed line). The fractions from 10 to 16 were collected, concentrated and refiltered on Sephadex G-200 (continuous line). The elution volumes of blue dextran (V_0), Globulin (γ-G) and albumin (Alb) are indicated by arrows.

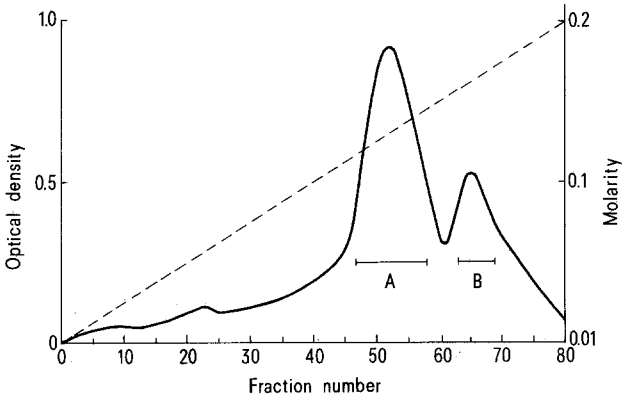

Figure 2. Ion-exchange chromatography on spherical DEAE-cellulose of the peak (10-16) pooled from the 2nd filtration on Sephadex G-200.

The peak between fractions 10 and 16 did not appear in the first gel filtration, due to the amount of proteins in the 2 major peaks (fig. 1). The fractions 10-16 were processed exactly as above and 1 ml (3 mg/ml) was dialyzed against 0.01 M phosphate buffer, pH 8. The patterns of DEAE cellulose chromatography is shown in figure 2. Peaks A and B were collected and concentrated to 4 mg/ml. Immunoelectrophoretic analysis demonstrated that the fractions A and B did not contain rat serum proteins. Fraction B showed a mild precipitin line with anti-whole foetal calf serum. These results excluded ABP presence in peak B.

The isoelectro-focusing of the proteins eluted in peak A gave a single band p. I=4.7 in the pH range 3-10 (fig. 3). The ^{3}H-testosterone binding activity (0.14 p-moles) was identical with the amount of fraction A present in the dialysis tube, assuming a molecular weight of 90,000 daltons, in agreement with the elution zone in the gel filtration on Sephadex G-200[9,10]. The purification steps for ABP are summarized in the table.

Discussion. The main advantage of the employment of Sertoli cell cultures, rather than epididymis and testes, in order to purify ABP is the possibility of minimizing the presence of extracellular components which contain large amounts of testosterone-estrogen binding albumin and globulin (TeBG)[17,21]. Because of the presence of foetal calf serum in the medium of Sertoli cell cultures, we chose to isolate ABP by classical methods rather than by affinity chromatography which retains bovine serum proteins

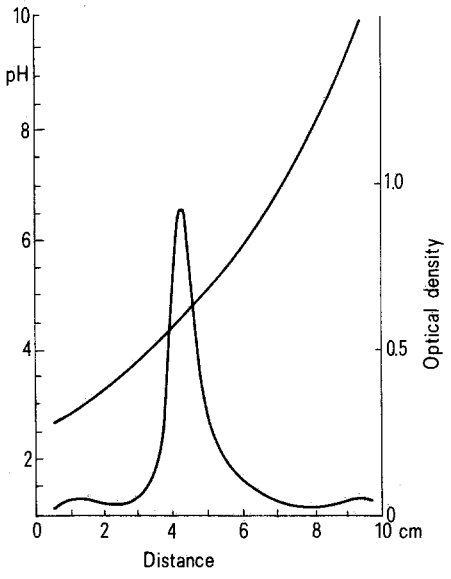

Figure 3. Isoelectric focusing on polyacrylamide gel of fraction A (30 µg) collected from DEAE-cellulose chromatography, pH gradient 3-10.

(TeBG). It has been shown that TeBG possesses not only a high binding capacity for steroids, but also antigenic determinants and physico-chemical properties, common to the rat ABP[20]. Nevertheless, with the above-described method these contaminations are excluded as demonstrated by isoelectrofocusing on polyacrylamide gel which shows a single protein that does not cross-react with normal rat and bovine sera.

1 This work was supported by grants No. 80.01547.96 and No. 80.01582.96 from the Consiglio Nazionale delle Ricerche.
2 Reprint requests to: C. De M., Regina Elena Institute for Cancer Research, Viale Regina Elena 291, I-00161 Rome (Italy).
3 V. Hansson and O. Djöseland, Acta endocr. *71*, 614 (1972).
4 F.S. French, S.N. Nayfeh and E.M. Ritzen, Biol. Reprod. *9*, 82 (1973).
5 F.S. French and E.M. Ritzen, J. Reprod. Fert. *32*, 479 (1973).
6 E.M. Ritzen and F.S. French, J. Steroid Biochem. *5*, 151 (1974).
7 V. Hansson, O. Djöseland, E. Reusch, A. Attramadal and O. Torgersen, Steroids *21*, 457 (1973).
8 An-F Hsu and P. Troen, J. clin. Invest. *61*, 1611 (1978).
9 V. Hansson, E.M. Ritzen, F.S. French and S.N. Nayfeh, Handb. Physiol. Endocr. *5*, 173 (1975).
10 N.A. Musto, G.L. Gunsalus and C.W. Bardin, Biochemistry *19*, 2853 (1980).
11 I.B. Fritz, F.F.G. Rommerts, B.G. Louis and J.H. Dorrington, J. Reprod. Fert. *46*, 17 (1976).
12 A.F. Karl and M.D. Griswold, Cell Biol. intern. Rep. *4*, 669 (1980).
13 C. De Martino, M.L. Marcante, A. Floridi, G. Citro, M. Bellocci, A. Cantafora and P.G. Natali, Cell Tissue Res. *176*, 69 (1977).
14 O.K. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randal, J. biol. Chem. *193*, 265 (1951).
15 F. Cervone, J.D. Brito, G. Di Prisco, F. Garofano, L.G. Norona, S. Traniello and R. Zito, Biochim. biophys. Acta *295*, 555 (1973).
16 E. Sega, G. Citro and P.G. Natali, J. natn. Cancer Inst. *62*, 1125 (1979).
17 C. Wringley, Sci. Tools *15*, 17 (1968).
18 E.M. Ritzen, V. Hansson and F.S. French, Endocrinology *92*, A-129 (1973).
19 K.E. Mickelson and P.H. Petra, Biochemistry *14*, 957 (1975).
20 W. Rosner and R.N. Smith, Biochemistry 14, 4813 (1975).
21 Y. Suzuki, E. Itayaki, H. Mori and T. Hosoya, J. Biochem., Tokyo *81*, 1721 (1977).
22 K.E. Mickelson and P.H. Petra, J. biol. Chem. *253*, 5293 (1978).