Androgen-binding Protein

PURIFICATION FROM RAT EPIDIDYMIS, CHARACTERIZATION, AND IMMUNOCYTOCHEMICAL LOCALIZATION*

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Androgen-binding protein (ABP) was purified from caput epididymis of the rat by sequential chromatography on DEAE-Sepharose, hydroxylapatite, dihydroluteosterone-17β-hemisuccinyl-1,6-diaminohexane-Sepharose, and Sephadex G-150. The final product migrated as a single band corresponding to a peak of protein-bound [3H]dihydroluteosterone on polyacrylamide gel electrophoresis. A molecular weight of 100,000 was estimated by sedimentation equilibrium. On sodium dodecyl sulfate-polyacrylamide gel electrophoresis, subunits of M, = 47,000 and 41,000 were observed. Amino acid analysis indicated ABP to be rich in leucine while nonpolar aminoacids totaled only 51%. Its carbohydrate content is 25%. Antibodies to purified ABP were raised in a rabbit and evaluated by immunodiffusion, immunoelectrophoresis, blading inhibition, radioimmunossay, and immunocytochemistry. Immunoperoxidase staining localized ABP in the basal and adluminal regions of seminiferous tubules of rat testis and in secretory granules of cultured Sertoli cells. In principal cells of caput epididymis, ABP is concentrated in the supranuclear region known to contain morphological specializations for absorption. These immunocytochemical results confirm that ABP synthesized and secreted by Sertoli cells in the testis is transported to the epididymal duct via testicular fluid and is taken up by epithelial cells of the proximal segments.

The discovery of androgen-binding protein in epididymis and testicular fluid (1–3) and its subsequent identification as a specific Sertoli cell secretory protein (4–6) has provided a valuable marker for studies on the hormonal regulation of Sertoli cell function (7–11). Until recently, however, ABP1 was identified solely by its androgen-binding activity.

We have purified ABP from rat epididymis.2 The purified protein has been characterized and antisera has been raised in rabbits. Immunocytochemical studies show specific staining of Sertoli cells, the site of ABP production, and of epithelial cells in the caput epididymis, where ABP is absorbed from the testicular fluid.

METHODS

Chemical Analysis—Protein (100 µg) was hydrolyzed in 6 N HCl in evacuated, sealed tubes for 24 h. Hydrolysate was analyzed in a Bio-Cal BC 200 automatic amino acid analyzer equipped with an Auto- lab integrator. Cystine was also determined as cysteic acid in an independent run following performic acid oxidation. Tryptophan was not analyzed. Carbohydrate composition was determined by gas-liquid chromatography (12).

Analytical Ultracentrifugation—Equilibrium sedimentation was carried out at 20 °C for 22 h at 15,000 rpm in an MSE Centriscan 75 centrifuge equipped with UV scanning optics. Sample consisted of a 0.04% solution of ABP in 50 mM Tris-HCl buffer, pH 7.4, containing 1M KCl, 10% glycerol, and 0.01% sodium azide.

SDS-Polyacrylamide Gel Electrophoresis—Cylindrical gels (5 × 60 mm) were prepared according to Laemmli (13) with the exception that gels contained 4 mM urea and 1 mM EDTA. Stacking and running gels were 3.6 and 8% acrylamide, respectively. Electrophoresis buffer contained 0.096 M glycine, 0.025 M Tris, and 0.1% SDS. Protein samples were suspended in 1% sodium dodecyl sulfate, 2.5 mM EDTA, 2.75 mM urea, 50 mM Tris-HCl, pH 6.7, 3% β-mercaptoethanol, 0.05% bromphenol blue and solubilized by heating 10 min at 90 °C. Samples were electrophoresed for 2 h at 1.5 mA/tube. Protein was stained with 0.25% Coomasie blue in 50% methanol, 10% acetic acid and destained with 20% methanol, 10% acetic acid.

Preparation of Antiserum—Lyophilized antigen (100 µg) was dissolved in 2 ml of saline (0.9% NaCl) solution and emulsified with an equal volume of Freund’s complete adjuvant and 10 mg of mycobacterium butyricum. A New Zealand white rabbit was immunized as described by Vaitukaitis et al. (14). Booster injections of antigen (100 µg) were given 2 and 4 months following the primary immunization, and the rabbit was bled 10 days following each booster.

Evaluation of Antiserum—ABP antibodies were detected by double immunodiffusion (15) against purified ABP. Specificity was further examined by crossed immunoelectrophoresis according to Laurell (16) and by inhibition of ABP binding of [3H]dihydroluteosterone on polyacrylamide gel electrophoresis (17).

RESULTS

ABP was purified by column chromatography on DEAE-Sepharose, hydroxylapatite, [3H]dihydroluteosterone hemisuccinate coupled to 1,6-diaminohexane Sepharose, and Sephadex G-150 (Fig. 1). Purified ABP formed a single band (RF = 0.5 relative to bromphenol blue) on polyacrylamide gel electrophoresis and corresponded to a peak of bound radio-

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¶ The abbreviations and trivial names used are: ABP, androgen-binding protein; SDS, sodium dodecyl sulfate; dihydroluteosterone, 17β-hydroxy-5α-androstane-3-one.

¶ Portions of this paper (including some "Methods," Figs. 1–5, Table 1, and additional references) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20014.
activity (Fig. 2). Equilibrium centrifugation of intact ABP indicated a single component system. Observed data fit well into the Lamm equation (Fig. 3), yielding a molecular weight estimate of 100,000 using a partial specific volume of 0.71 cm$^3$/g as determined from the amino acid composition. The homogeneity of ABP was also examined by electrophoresis in a 10% SDS-polyacrylamide gel (Fig. 4). ABP migrated as two major bands corresponding to molecular weights of 41,000 and 47,000 in a ratio of approximately 1:3, respectively.

The chemical composition of ABP is shown in Table I and some of its physicochemical properties are listed in Table II. ABP is exceptionally rich in leucine, even though the content of nonpolar amino acids (51%) is not extraordinary. Its hydrophobicity is average whereas fractional charge is relatively high. The carbohydrate content of ABP is unique in that it is high in glucose. The amount of N-acetylglucosamine recovered in the amino acid analyzer (12.2 nmol) was in acceptable agreement with the amount obtained by carbohydrate analysis (14.9 nmol).

Immunization of a rabbit with ABP raised precipitating antibodies, the highest titer being reached following the second booster. Antiserum was judged to be monospecific by immunodiffusion (not shown) and crossed immunoelectrophoresis (Fig. 5) against purified ABP. Single precipitin lines were observed with both methods. No precipitation was observed with preimmunization serum from the same rabbit.

Further evidence that the immune serum contained antibodies against ABP was obtained by quantitation of $[^3H]$-dihydrotestosterone binding during polyacrylamide gel electrophoresis (Fig. 6). Incubation of epididymis cytosol with...
ABP antiserum prior to electrophoresis decreased ABP binding of \(^{1}H\)dihydrotestosterone. This method of detecting antibodies was described previously (17). We have not determined whether the decrease in binding resulted from antibody inactivation of the binding site on ABP or from failure of the antigen-antibody complex to enter the gel.

Antibody was evaluated further by immunocytochemical staining using the peroxidase technique (20). Specific staining for ABP was observed in cultured rat Sertoli cells, seminiferous tubules of rat testis, and in the epithelium of caput epididymis (Fig. 7). "Method specificity" (21) was tested by staining with increasing dilutions of anti-ABP serum. Optimal staining was obtained with dilutions from 1:1,000 to 1:10,000. No staining was detectable at dilutions of 1:100,000 or higher, indicating that staining depended on the primary antiserum and not on subsequent reagents or on endogenous peroxidase. As an indication of antibody specificity, staining was significantly reduced when anti-ABP serum was preabsorbed in the testis and totally eliminated in the epididymis with purified ABP or epididymis cytosol.

### Table I

| Residue          | g/100 g peptide | Moles/mol protein* |
|------------------|-----------------|--------------------|
| Aspartic acid    | 15.25           | 88                 |
| Threonine        | 5.37            | 34                 |
| Serine           | 8.32            | 59                 |
| Glutamic acid    | 10.28           | 52                 |
| Proline          | 5.88            | 38                 |
| Glycine          | 5.00            | 50                 |
| Alanine          | 3.85            | 32                 |
| Half-cystine     | 1.22            | 8                  |
| Valine           | 4.48            | 29                 |
| Methionine       | 1.37            | 7                  |
| Isoleucine       | 2.06            | 12                 |
| Leucine          | 19.00           | 109                |
| Tyrosine         | 0.55            | 2                  |
| Phenylalanine    | 4.19            | 19                 |
| Histidine        | 3.49            | 17                 |
| Lysine           | 3.89            | 20                 |
| Arginine         | 5.62            | 25                 |

* Adjusted to the nearest integer.

### Table II

| Physicalchemical properties of ABP |
|-----------------------------------|
| Molecular weight                  |
| Dimer*                            | 100,000                        |
| Monomers*                         | 47,000                         |
| Non-polar residues                | 41,000                         |
| Carbohydrate                      | 51.3%                          |
| Partial specific volume           | 25.0%                          |
| Average hydrophobicity            | 0.71 cm²/g                     |
| Fractional charge                 | 1013 cal/residue               |

* Data treated according Lamm (19).

\[
\text{Molecular weight} = \frac{2RT \cdot \frac{dfnc}{d\ln c}}{1 - \bar{p} \cdot \bar{c} - \bar{r} \cdot \bar{c}}
\]

where \(\bar{p}\) is partial specific volume, \(\bar{c}\) is concentration, \(\bar{r}\) is the distance from center of rotation.

* Determined by SDS-polyacrylamide gel electrophoresis.

* Calculated from the amino acid composition.

* Analyzed by the method of Chambers and Clamp (12).

Specific staining was localized to secretory granules of cultured Sertoli cells (Fig. 7A). In seminiferous tubules, immunoreactive ABP staining was observed in clusters of small granules at the basal and adluminal regions of Sertoli cell cytoplasm (Fig. 7B). Different segments of the same tubule varied greatly in their content of immunoreactive ABP. In epididymis (Fig. 7C), ABP was localized largely in the caput, where it was found to coat the microvilli and to be concentrated within the Golgi region of principal cells. These cells are known to contain numerous coated vesicles, secondary lysosomes, and multivesicular bodies which constitute morphological evidence for the function of absorption (23).

**DISCUSSION**

Purification of ABP to homogeneity from rat epididymis cytosol is made possible by affinity chromatography coupled with conventional methods of protein separation. Similar procedures have been used to purify steroid-binding proteins from plasma (24, 25). In preparing the affinity column, the 17β-hemisuccinate ester of dihydrotestosterone is readily synthesized. We found the mixed anhydride method of coupling the steroid ester to 1,6-diaminohexane Sepharose more efficient and reproducible than the conventional carbodiimide method. Following the affinity chromatography step, albumin is the major contaminant and can be separated from ABP by gel filtration chromatography. ABP appeared to be homogeneous by electrophoresis in polyacrylamide gels of different pore size and by analytical ultracentrifugation. The molecular
weight of purified ABP as determined by sedimentation equilibrium is in reasonable agreement with previous estimates (90,000) on [3H]dihydrotestosterone-labeled ABP in crude preparations, either by Ferguson plots of electrophoretic mobility in gels of different pore size (26) or by measurements of Stokes radius and sedimentation coefficient (27). Musto et al. (28) have recently reported a molecular weight of 85,000 for purified rat ABP as determined by sedimentation equilibrium, but obtained molecular weights of 111,000 to 154,000 from Ferguson plots of electrophoretic mobility.

Electrophoresis on SDS-polyacrylamide gels yielded two bands corresponding to molecular weights of 41,000 and 47,000 in a ratio of approximately 1:3. These two dissimilar subunits are identical in size and mass ratio to those reported by Musto et al. (28). ABP isolated from the rat testis has recently been shown to consist of the same subunits which, by peptide mapping, appear to be identical with the subunits of epididymal ABP (29). Moreover, it has been shown by photoaffinity labeling that ABP isolated from the medium of cultured Sertoli cells is composed of the same subunits (30). ABP purified from rabbit epididymis is reported to have a similar subunit structure (31). These findings suggest that the different subunits may result from posttranslational processing of ABP in Sertoli cells. Differences in the carbohydrate content of ABP subunits might account for their unequal size; however, this remains to be demonstrated. Our finding that ABP is a glycoprotein is in contrast to a report in which sugars could not be demonstrated by periodic acid-Schiff staining or amino acid analysis of purified ABP (28). Others, however, have recently reported that purified ABP does stain with periodic acid-Schiff stain and binds to concanavalin A (32). This property of rat ABP is shared by testosterone-binding globulin (24), a serum protein not present in the rat, but found in numerous other mammalian species (33). Indeed, it appears likely that ABP and testosterone-binding globulin are very similar proteins when they coexist in the same species (34).

ABP is secreted by Sertoli cells into the seminiferous tubular fluid and is carried through the efferent ducts into the caput epididymis where most of its binding activity is lost (1). Immunocytochemical studies indicate that ABP is largely absorbed by epithelial cells of the caput epididymis, where it has been shown previously that most of its binding activity is destroyed (1). A smaller portion of secreted ABP enters the blood stream (35); however, its route of entry has not yet been established. The striking accumulation of ABP in the basal portions of Sertoli cells suggests that its entry into blood may be by way of the interstitial fluid of the testis. Immunoperoxidase staining of cultured Sertoli cells indicates that ABP is packaged in secretory granules of variable diameter. Secretion of ABP containing granules occurs in response to follicle-stimulating hormone and is associated with striking changes in shape from flat to stellate or elongate morphology (22, 36, 37). In vivo ABP production is regulated by follicle-stimulating and androgenic hormones (7, 10); however, a variable content of immunoreactive ABP has been found in different segments along the same seminiferous tubule (38). Recent studies on isolated segments of seminiferous tubules have shown that Sertoli cell secretion of ABP varies in association with different stages of the spermatogenic cycle, being highest during the late stages of spermatid maturation (38). These observations suggest that hormonal stimulation of spermatogenesis is modulated by variation in functional activities of Sertoli cells (39). Control mechanisms intrinsic to the seminiferous epithelium may influence Sertoli cell responses to
folic acid-stimulating hormone and androgens. The biological function of ABP remains to be determined; however, it could have local effects in the germinal epithelium associated with androgen action on spermatogenesis and more distal effects on epithelial cells in the caput epididymis. It has been suggested that ABP taken up by epithelial cells of the caput epididymis might serve to transport androgen from the testicular fluid to the cytoplasmic androgen receptor protein (1, 40).

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REFERENCES
1. French, F. S., and Ritzén, E. M. (1973) Endocrinology 93, 88–96
2. Vernon, R. G., Kopeć, B., and Fritz, I. B. (1974) Mol. Cell. Endocrinol. 1, 167–187
3. Hanson, V., Ritzén, E. M., French, F. S., and Nayef, S. N. (1975) Handb. Physiol. Sect. 7, Endocrinol. 5, 173–201
4. Hagenas, L., Ritzén, E. M., Ploen, L., Hanson, V., French, F. S., and Nayef, S. N. (1975) Mol. Cell. Endocrinol. 2, 339–350
5. Steindel, J. J., Dietze, J. E., Elkington, F. H., and Zanborn, B. (1975) Endocr. Res. Commun. 2, 261–272
6. Fritz, I. B., Rommerts, F. G., Louis, B. G., and Dorrington, J. H. (1976) J. Reprod. Fertil. 46, 17–24
7. Hanson, V., Weddington, S. C., McLean, W. S., Smith, A. A., Nayef, S. N., French, F. S., and Ritzén, E. M. (1975) J. Reprod. Fertil. 44, 363–375
8. Means, A. R., Fakunding, J. L., Hucks, C., Tindall, D. J., and Vitale, R. (1976) Recent Prog. Horm. Res. 32, 472–522
9. Tindall, D. J., Cunningham, G. R., and Means, A. R. (1978) J. Biol. Chem. 253, 166–169
10. Fritz, I. B. (1979) in Biochemical Actions of Hormones (Litwack, G., ed.) pp. 249–281, Academic Press, New York
11. Ellerking, J. S. H., Sanborn, B. M., and Steinberger, E. (1975) Mol. Cell. Endocrinol. 2, 157–170
12. Chambers, R. E., and Clamp, J. R. (1971) Biochem. J. 125, 1009–1018
13. Laemmli, U. K. (1970) Nature (Lond.) 227, 680–685
14. Vaitukaitis, J. L., Robbins, J. B., Nickelag, E., and Ross, G. T. (1970) J. Clin. Endocrinol. Metab. 31, 586–591
15. Ouchterlony, O. (1958) Progr. Allergy 6, 1–78
16. Laurell, C. B. (1966) Anal. Biochem. 15, 45
17. Weddington, S. C., Brandzaeg, P., Hanson, V., French, F. S., Petrusz, P., Nayef, S. N., and Ritzén, E. M. (1975) Nature 258, 257–259
18. Ritzén, E. M., French, F. S., Weddington, S. C., Nayef, S. N., and Hansson, V. (1974) J. Biol. Chem. 249, 6597–6604
19. Lamm, O. Z. (1929) Hoppe-Seyler's Z. Phys. Chem. A 143, 177–190
20. Petrusz, P., DiMeo, P., Ordonneau, P., Weaver, C., and Keefer, D. A. (1975) Histochemistry 46, 9–26
21. Petrusz, P., Sar, M., Ordonneau, P., DiMeo, P. (1976) Histochem. CO 24, 1110–1119
22. Kierszenbaun, A. L., Feldman, M., Lea, O., Spruill, W. A., Tres, L. L., Petrusz, P., and French, F. S. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 5322–5326
23. Hoffer, A. P., Hamilton, D. W., and Fawcett, D. W. (1973) Anat. Rec. 176, 199–202
24. Meckel, K. E., and Petà, P. H. (1975) Biochemistry 14, 957–963
25. Rosner, W., and Smith, R. N. (1975) Biochemistry 14, 4813–4820
26. Ritzén, E. M., Dobkins, M. C., Tindall, D. J., French, F. S., and Nayef, S. N. (1973) Steroids 21, 593–607
27. Hansson, V. (1972) Steroids 20, 575–596
28. Musto, N. A., Gunsalus, G. L., and Bardin, C. W. (1980) Biochemistry 19, 2953–2960
29. Larrea, F., Musto, N. A., Gunsalus, G. L., Mather, J., and Bardin, C. W. (1980) Endocrinology 106 (suppl), 136
30. Schmidt, W. N., Taylor, C. A., Jr., and Danzo, B. J. (1980) Endocrinology 106 (suppl), 136
31. Cheng, S. L., Musto, N. A., Gunsalus, G. L., and Bardin, C. W. (1980) Endocrinology 106 (suppl), 136
32. Tindall, D. J., Cheng, C. H., and Means, A. R. (1980) Endocrinology 106 (suppl), 148
33. Corvol, P., and Bardin, C. W. (1973) Biol. Reprod. 8, 277–282
34. Hanson, V., Ritzén, E. M., French, F. S., Weddington, S. C., and Nayef, S. N. (1975) Mol. Cell. Endocrinol. 1, 1–20
35. Gunsalus, G. L., Musto, N. A., and Bardin, C. W. (1980) in Testicular Development, Structure and Function (Steinberger, A., and Steinberger, E., eds) pp. 291–297, Raven Press, New York
36. Tung, P. S., Dorrington, J. H., and Fritz, I. B. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 1838–1842
37. Weiss, M. Jr., VanSickle, M., and Means, A. (1980) in Testicular Development, Structure and Function (Steinberger, A., and Steinberger, E., eds) pp. 89–98, Raven Press, New York
38. Ritzén, E. M., Boitani, C., Parvinen, M., French, F. S., and Feldman, M. (1981) Mol. Cell. Endocrinol., in press
39. Kierszenbaun, A. L. (1974) Biol. Reprod. 11, 365–376
40. Lobl, T., Campbell, J. A., Tindall, D. J., Cunningham, G. R., and Means, A. R. (1980) in Testicular Development, Structure and Function (Steinberger, A., and Steinberger, E., eds) pp. 323–330, Raven Press, New York

Additional references are found on p. 5175.
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Purification of ABP. Fractions were obtained at 0.4-mL volumes from column elution and concentrated to 200 mL using an ISCO 1500-12-A absorbance monitor equipped with a 0.5-nL flow cell.

Ion exchange chromatography was carried out on a DEAE-Sepharose column (50 x 3 cm) equilibrated with 10 mM Tris buffer. The DEAE-Sepharose column (500 mL) was packed at a flow rate of 100 mL/h. The column was equilibrated with 100 mM Tris (pH 8.0) buffer and absorbed ABP was eluted with a linear gradient from 0 to 0.2 M KCl. Fractions of 20 mL each were collected and absorbance monitored at 280 nm. Protein was monitored by absorbance at 280 nm. Fractions of 20 mL were collected and assayed for measurement of cholesterol and radioactivity.

Hydroxyapatite chromatography was performed on a 4 x 5-cm column containing 100 mL of hydroxyapatite. Eluted column fractions were collected with 10 molar KCl buffer (0.1 M Na2HPO4/0.1 M NaH2PO4) and equilibrated with 10 mM buffer. Fractions were collected and analyzed by SDS-polyacrylamide gel electrophoresis. Protein with a gel electrophoresis peak was further purified by polyacrylamide gel electrophoresis [4].

Affinity chromatography was performed on an 8 x 30-cm column containing approximately 0.5 g of avidin- labeled hydroxyapatite. The column was equilibrated in 10 mM buffer containing 100 mM KCl. Filled ABP fractions (20 mL) from the hydroxyapatite column were loaded onto the 8 x 30-cm column. The column was washed with 10 mM buffer containing 100 mM KCl and 100 mM EDTA/20 mM sodium phosphate buffer (pH 7.0) in 0.5-ml fractions. A constant zero baseline was observed with 100 mM buffer containing 0.5% SDS. Fractions of 20 mL were collected and assayed for measurement of radioactivity.

Protein eluting from the affinity column was dialyzed and concentrated. ABP was eluted on a Sephadex G-50 column (2.5 x 1.5 cm) equilibrated with 10 mM buffer. Fractions of 3 mL were collected and assayed for protein and radioactivity (Fig. 2). ABP eluted in a commercial peak corresponding to a radius of 40,400. The solid phase bound aggregated ABP was purified by DEAE-Sepharose chromatography.

In a purification carried out subsequent to obtaining ABP antigen, nonradioactive ABP was measured by antigen-antibody [5]. Assay of immunoreactive ABP is shown in Fig. 3. The nonradioactive product is likely underrepresented by the assay due to the low titrable content of ABP.

Figure 1. Ion exchange chromatography of rat epididymal cytosol on DEAE-Sepharose after incubation with 0.1 M (pH 7.0) Tris buffer. The DEAE-Sepharose column (500 mL) was packed at a flow rate of 100 mL/h followed by 1 x 400 MM Tris buffer. Fractions of 20 mL were collected and assayed for measurement of cholesterol and radioactivity.

Figure 2. Hydroxyapatite chromatography of rat epididymal cytosol and [3H] DHT bound. Fractions were collected with 10 mM KCl buffer (0.1 M Na2HPO4/0.1 M NaH2PO4) and equilibrated with 10 mM buffer. Fractions were collected and assayed by SDS-polyacrylamide gel electrophoresis. Protein with a gel electrophoresis peak was further purified by polyacrylamide gel electrophoresis [4].

Figure 3. Elution profile on hydroxyapatite of proteolytic [3H] DHT eluted in 40 mM KCl buffer (0.1 M Na2HPO4/0.1 M NaH2PO4) and applied to a 15 x 7-cm column of Bio-Gel HTP. Following sample application, the column was washed with 10 mM buffer. A protein adsorbed to the column was eluted in 0.1 M Na2HPO4/0.1 M NaH2PO4 containing 0.001 M EDTA at pH 7.0. Fractions of 20 mL were collected and assayed for measurement of radioactivity.

Figure 4. Affinity chromatography of ABP on a 4 x 5-cm column of [3H] DHT with 20-mM Tris buffer containing 0.5% SDS. Fractions from the affinity column were obtained at 0.4-mL volumes and assayed for radioactivity. Fractions of 20 mL were collected and assayed for measurement of radioactivity.

Figure 5. Gel filtration of partially purified ABP on Sephadex G-150. Fractions from the affinity column were pooled, eluted on Sephadex G-150 and concentrated on a small DEAE-Sepharose column. The sample (4 mL) was eluted with 10 mM KCl, 100 mM phosphate buffer (pH 7.0) and applied to a Sephadex G-150 column (2.5 x 1.5 cm) equilibrated with 10 mM buffer. Fractions of 2 mL were collected and assayed for measurement of radioactivity.

Table I. ABP Purification from Rat Epididymis

| Step | Volume | Total Protein | ABP Specific Activity | Yield |
|------|--------|---------------|----------------------|-------|
| (a)  |        | (mg)          | (mg)                |       |
| (b)  |        | (mg)          | (mg)                |       |
| Cyto	sol | 1000 | 10,100 | 1.5 | 0.004 | 100 |
| DEAE Sepharose | 700 | 7,000 | 1.2 | 0.0015 | 70 |
| Hydroxyapatite | 750 | 1,700 | 1.2 | 0.0013 | 50 |
| Affinity | 120 | 0.11 | 0.05 | 0.0002 | 12 |
| G-150 Sephadex | 27 | 0.41 | 0.40 | 1.200 | 9 |

1. Measured by the method of Lowry et al. [3] using bovine serum albumin as the reference standard.
2. Measured by radiodensity assay [5].
3. Measured by radiodensity assay [5].

References
1. Eneroth, B.F., Bore, P., Bagnall, S.P. and Liderman, S. (1955) J. Biol. Chem. 220, 1093-1096.
2. Vaux, J.R. and Osta, R.L. (1952) J. Am. Chem. Soc. 74, 1954-1958.
3. Lowry, O.H., Rosebrough, J.R. and Farr, A.L. (1951) J. Biol. Chem. 193, 265-275.
4. Pitzer, E.M., French, F.S., Whittington, S.L., Kapfer, S.H. and Hanson, W. (1974) J. Biol. Chem. 249, 6597-6604.
5. Hanks, S.K., Gunalas, C.C., Miljkovic, M. and French, F.S. (1975) Biochemical and Biophysical Research Communications 61, 145-152.
6. Kienitz, A.A., Feldman, M., Luttrall, D., Orelli, W., and Kruit, L.E. (1975) Proc. Nat. Acad. Sci. 72, 3532-3536.