

https://dspace.org/

Partial Purification Of And Attempted Monoclonal Antibody Production Against The Vervet Mokey Hepatic Oestrogen-binding Protein

Bornman, Liza

2015-01-09
http://hdl.handle.net/10539/16362

Downloaded from a DSpace repository. https://dspace.org/

receptor ligand conjugated to BSA will induce the production of anti-idiotypic antibodies to the combining sites of the antigen specific antibodies. The great advantage of this approach is that no purified receptor is required.

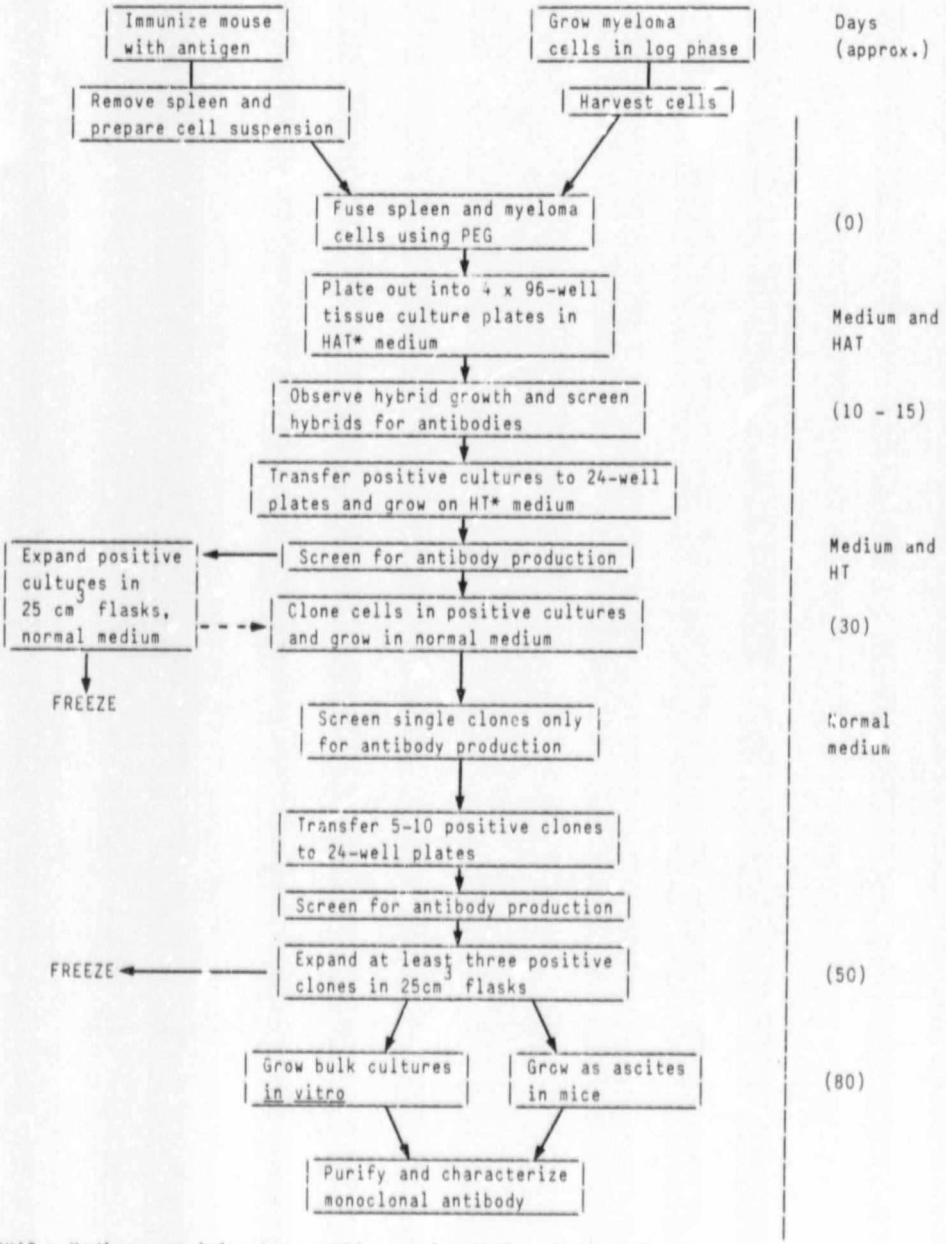
4.3.3 Fusion, selection and maintenance of hybrids

A number of fusion protocols have been documented using polyethylene glycol (PEG) as fusing agent (210, 215, 216). In addition to PEG, dimethyl sulphoxide (DMSO) is sometimes employed to improve fusion (217), although some authors reported a minimal effect with DMSO (215). A mixture consisting of a 10 : 1 ratio of spleen to myeloma cells is commonly used during fusions (209). The different stages during McAb production is summarized in Scheme 4.1 (reproduced from reference 209).

Spent medium from the polyclonal hybridoma cell cultures was assayed for antibody activity against partially purified HEBP (male and female) and uterine cytosol. From a total of 474 wells, 34 gave positive results against female HEBP (♀ - HEBP), 8 against male HEBP (♂ - HEBP) and one reacted positively against all three antigen preparations. These results are summarized in Table 4.3. Thus a 9% positive reaction against the partially purified HEBP was obtained.

Enrichment of the spleen cell culture for the B cell which is making the desired antibody may be a solution for the problems experienced in obtaining specific McAb from animals immunized with a weak immunogen or minute amounts of the antigen. French *et al.* (213) reported that a 50-fold increase in hybridomas making the desired antibody, compared to a standard fusion, was obtained when spleen cells harvested for fusion have been re-injected into irradiated recipients along with the antigen. Another approach to enrich the cells making the desired antibody is to immunize the spleen cells *in vitro* using spleen cells obtained from immunized animals or as a primary

SCHEME 4.1: PRODUCTION OF MONOCLONAL ANTIBODIES (TAKEN FROM REFERENCE 209)



*HAT = Medium containing hypoxanthine, aminopterin and thymidine.
*HT = Medium containing hypoxanthine and thymidine.

TABLE 4.3: RESULTS OBTAINED DURING THE ELISA EXECUTED ON THE SPENT MEDIUM OF POLYCLONAL CELL CULTURES.

Preparation used to coat microtiter plates	Reaction of spent medium from three types of Polyclonal cell cultures (% of total number of polyclonal cell cultures)		
	[c] A (0,2)	B (7,2)	C (1,7)
[a] VMUC	+	-	-
[b] HEBP (♀)	+	+	-
HEBP (♂)	+	-	+

[a] VMUC = vervet monkey uterine cytosol.

[b] HEBP used for coating was partially purified.

[c] A, B, C = different groups of polyclonal cell cultures.

immunization (213). Antibody producing hybridomas can be generated in this manner when only small amounts of the antigen is available. Thus, the hybridoma technique makes it possible to obtain McAb even from a weak immunogen available in small amounts.

4.3.4 Screening methods

The need for a screening procedure which is fast, reliable, sensitive and relevant, prior to fusion of cells, has been stressed by numerous workers (209, 210). With a proper screening assay, useful hybrids should be selected as early as possible to selectively clone the relevant hybrids. Cloning and freezing in of positve clones are time consuming steps in the process and it is therefore important to select only the most useful hybridomas. The high level of competition for growth between different clones in a polyclonal cell culture together with chromosome segregation that leads to instability of expression are very important reasons to clone and reclone as early as possible. Some care must be taken when developing a screening assay to detect McAb. Firstly the concentration of antibody in the culture filtrate is approximately 1 - 25 µg/mℓ compared to that of the hyperimmune serum 0,1 - 10 mg/mℓ (209). Immunoassays often rely on the polyvalent recognition of antigens as obtained by polyclonal antisera (209).

Antibody-secreting hybrids can be identified by the detection of antibody in the spent medium (binding, hemagglutination and lytic assays together with assays based on biological activity of the antigen) or the direct detection of antibody-secreting cells (plaque-forming clones) (210). Binding assays are markedly suitable for McAb work because they are highly specific, sensitive, simple and could be applied for all classes of antibodies and most antigens. The indirect binding solid phase assay and immunoprecipitation were two binding assays still under investigation for employment as screening

assays during the McAb production against the HEBP. In solid phase assays like ELISA, the antigen is bound to a solid support (plastic, filter paper, Sepharose, nitrocellulose). Antibodies in the tissue culture supernatants are bound to the antigen and measured by binding a second labelled (radio, enzyme or fluorescent) antibody. Although the use of a radio labelled second antibody is highly sensitive, an enzyme linked second antibody which excludes radioactive hazards, is inexpensive, stable and provides a fast visual result. Variation on the ELISA includes the use of a radioactive substrate to measure the second antibody-enzyme conjugate (218) or the use of radiolabelled protein-A instead of a secondary antibody (219). One should, however, note that the latter compound doesn't bind to Fc regions of all classes.

During immunoprecipitation the culture supernates are screened for their ability to precipitate a labelled protein (antigen) when a second antibody or protein-A is added. A pure iodinated or tritiated antigen must be used during this assay, unless the precipitate is to be analysed on a polyacrylamide gel (209). A pure antigen could not be employed in the immunoprecipitation assay employed during the production of McAb to the HEBP. However, distinction between specific and non-specific proteins was made possible by the addition of a 1 000-fold excess of unlabelled DES during the preparation of the radioactive hormone-receptor complex (Section 4.2.5). The immune complex obtained following incubation of the hybridoma culture supernates with immune and control sera should be precipitated by a second antibody using a quantity that gives maximum precipitation. Chicken anti-mouse Ig (IgY) and normal mouse serum were standardized in order to determine the optimal amounts required to give maximum precipitation of the immune complex with the minimum amount of IgY (Table 4.4 and Figure 4.3). Results obtained during screening of immune and control sera with the ELISA and immunoprecipitation assays are illustrated in Figures 4.1 and 4.2 and summarized in Table 4.2.

TABLE 4.4: A SUMMARY OF THE RESULTS OBTAINED DURING THE QUANTITATIVE PRECIPITATION REACTION BETWEEN NORMAL MOUSE SERUM (11 µℓ) AND DIFFERENT VOLUMES (5 - 35 µℓ) OF CHICKEN ANTI-MOUSE IMMUNOGLOBULIN (IgY).

IgY	Normal mouse serum	PBS	[a]Protein Content		
			Supn.	wash	Ppt.
(µℓ)	(µℓ)	(µℓ)	(µg)	(µg)	(µg)
5	11	984	472,9	2,57	1,38
10	11	979	492,3	2,63	1,60
15	11	974	519,1	3,23	2,17
20	11	969	514,3	3,41	3,18
25	11	964	459,8	3,29	6,32
30	11	959	508,6	2,66	4,68
35	11	954	517,1	2,69	4,73

[a] The protein content of the different samples was determined by the method of Bradford (152) as described in Section 3.2.10.

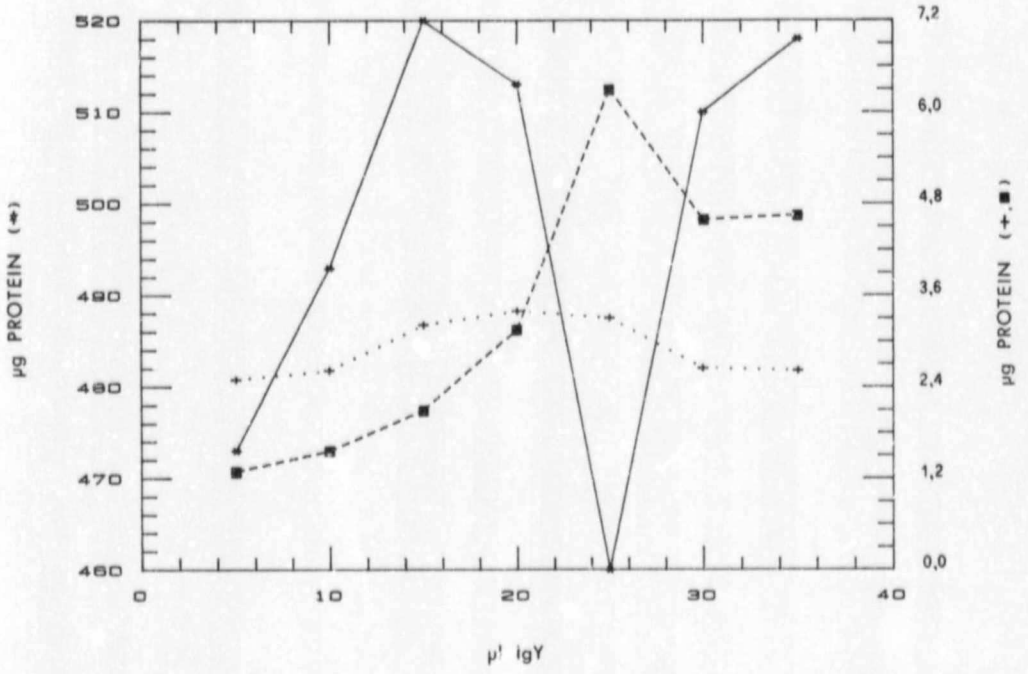


FIGURE 4.3

The quantitative precipitation reaction between normal mouse serum and chicken anti-mouse Ig (IgY). A constant volume of normal mouse serum (11 µℓ) were added to different quantities of IgY and incubated for 5 h at 4°C. Following centrifugation and washing with PBS the protein content of the supernatant (*—*), wash (+····+) and precipitate (■--■) was determined.

In Scheme 4.2 a comparison between immunoprecipitation and ELISA is illustrated. The double antibody precipitation assay for the detection of antibodies against the oestrogen receptor (204, 205, 212) appears to be the assay of choice in many laboratories. However, during the production of McAb to the HEBP, no positive reaction was obtained with this assay in contrast to the ELISA. Other disadvantages of the immunoprecipitation assay are that it is more laborious (220). The ELISA used for the detection of antibodies to the HEBP is a reliable and fast immuno assay, but it has numerous limitations. In this assay no distinction can be made between antibodies produced against specific and non-specific HEBP. Thus a great deal of the positive reaction observed during screening with the ELISA could be the result of antibodies produced against contaminants present in the partially purified HEBP preparation, used for immunization and microtiter plate coating.

4.3.5 Cloning

Results obtained for the screening of the monoclonal cell cultures for antibody activity against different antigen preparations are summarized in Table 4.5. Three polyclonal hybrid lines (A, B and C) were cloned. The antigen preparations (used for coating of the microtiter plates) against which these lines have shown antibody activity prior to cloning, are listed in Table 4.3. Polyclonal hybrid line A has shown antibody activity against all three antigen preparations (uterine cytosol, partially purified HEBP from male and female vervet monkey). This hybrid line was therefore more important and was subsequently cloned twice with a large number (96 and 120 respectively) of picked clones. The two other polyclonal hybrid lines (B and C) have shown antibody activity against one antigen preparation respectively. Cloning of a B-type polyclonal hybrid line was done once with 24 clones picked while two C-type hybrid lines were cloned and

SCHEME 4.2: A COMPARISON BETWEEN IMMUNOPRECIPITATION AND ELISA AS SCREENING METHODS TO DETECT ANTIBODY, PRESENT IN IMMUNE SERUM, AGAINST THE APPROPRIATE ANTIGEN.

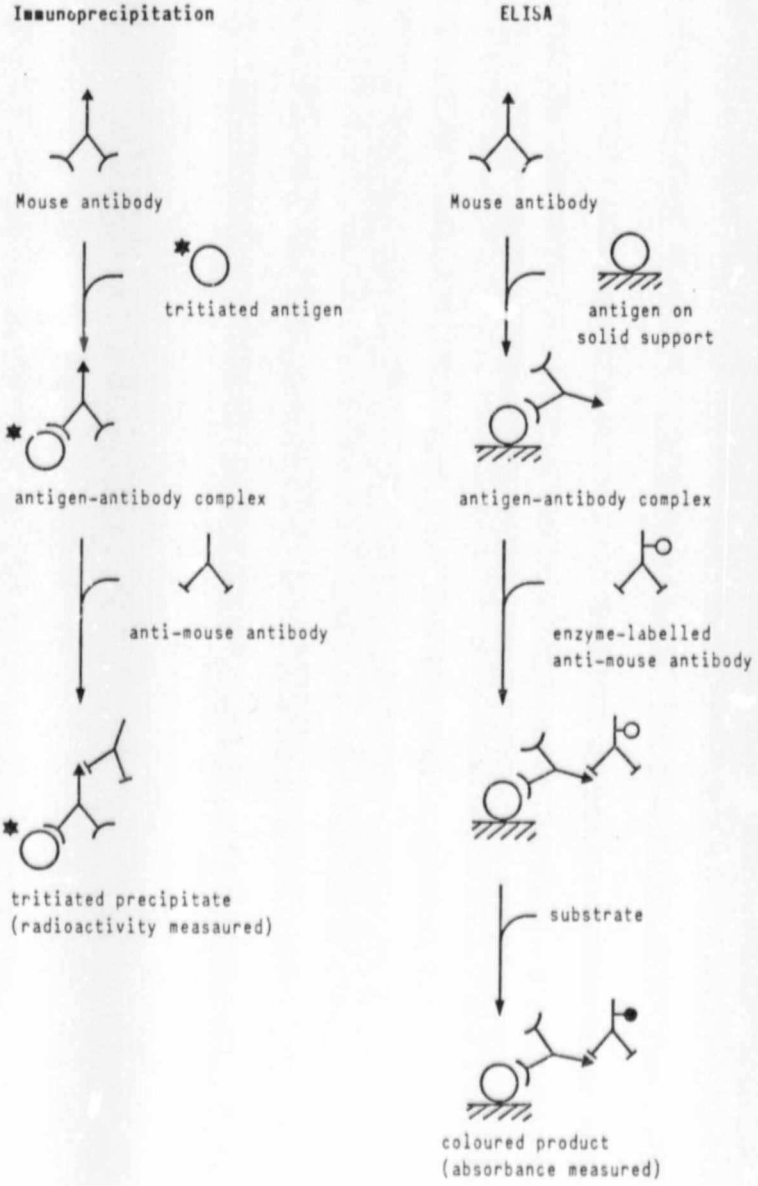


TABLE 4.5: A SUMMARY OF THE ELISA RESULTS OBTAINED DURING CLONING OF THE POLYCLONAL CELL CULTURES WHICH SHOWED A POSITIVE REACTION AGAINST DIFFERENT ANTIBODY PREPARATIONS.

	Postive controls	Polyclonal cell cultures					
		A		B	C		
Number of clonings done :		2		1	2		
Number of clones picked :		96	120	24	24	24	
Antibody reaction against [a]antigen preparation :							
	[b]VMUC :	-	-	-	-	-	
	[c]HEBP (♂) :	-	-	-	-	-	
	[c]HEBP (♀) :	-	-	-	-	-	
	[d]UEBP :	-	-	-	-	-	

[a] Antigen preparations used for coating of the microtiter plates.
[b] VMUC = vervet monkey uterine cytosol.
[c] HEBP = partially purified hepatic oestrogen-binding protein.
[d] UEBP = partially purified uterine oestrogen-binding protein.

24 clones selected with each cloning. A total of 288 monoclonal cell cultures were screened for antibody activity against four antigen preparations with the aid of the ELISA. No antibody activity against the four antigen perparations (Table 4.5) could be detected in the spent medium of any of the screened monoclonal cell cultures.

The development of a fast, sensitive and specific screening assay was seriously hampered by problems encountered in purifying the HEBP to homogeneity, with the aid of affinity chromatography. The need for an assay that can specifically identify the clones producing the desired antibody can not be over-estimated. The ELISA used in this study did not provide absolute specificity that resulted in the selection of a large number of positive cultures that could not be conveniently handled. Instability and clonal competition are particularly frequent in early stages of growth (209). Thus, the cloning of positive polyclonal cell cultures must be done as early as possible. Considering the fact that the selected polyclonal cell cultures were maintained for nearly two months prior to cloning, the above-mentioned problem could have been the main reason for the failure in obtaining the desired McAb. Other aspects that should have received greater attention were the regular screening of supernatants and the preparation of stocks of clones (freezing) before the culture became negative. Cloning can be done either in semi-solid agarose or by limiting dilution (221). Agarose is known to cause a lower cloning efficiency and this may result in the selection of vigorous but unwanted hybrids. Colonies of cells picked from agar are often not absolutely pure clones (210).

4.4 SUMMARY

A 6 091-fold purified HEBP was injected into three C57 black/6 mice (both sexes). Antibody activity could positively be identified in two of the three immune sera by an ELISA, seven days after a booster injection. The mice were given a final

injection of the antigen three months later and sacrificed. $Sp^{2/0}$ myeloma cells were fused with the mouse spleen cells (1 : 10) and plated out in 474 wells of microwell tissue culture plates. Antibody activity was detected by ELISA in 43% of the polyclonal cell culture supernates. The polyclonal cell cultures were cloned on agar and 288 clones selected. No antibody activity could, however, be detected in the spent media.

CHAPTER 5

CONCLUDING DISCUSSION

Over the past decade hepatic oestrogen-binding proteins have been demonstrated in a variety of species (103, 146, 147, 154). In some cases sex related differences have been illustrated (104, 106, 149). Male and female vervet monkey liver cytosol contains the same levels of high affinity oestrogen-binding proteins (Table 2.4). With the aid of multipoint saturation analyses an additional binding protein, exhibiting a high capacity and moderate affinity was identified in the liver cytosol from male vervet monkeys (Figures 2.6). SDG analyses, however, revealed the presence of two binding components (3,8S and 8,1S) in both sexes (Figure 2.7). A male-specific binding protein (male-SBP) (104), an unusual oestrogen-binding protein (UEBP) (106) and a high capacity, low affinity (HCLA) oestrogen-binding protein (112) have been described in the literature. This protein reportedly exhibits moderate affinity, a high capacity and sediments at 3-4S on SDG and can not be precipitated at 30% ammonium sulphate saturation (104, 106). According to the supporters of the male-specific binder, this protein is present in very low levels in the female liver but features prominently in the adult male liver (104, 106, 112). The data obtained in this study illustrated that the high capacity, moderate affinity binding protein was more abundant in the male monkey liver although it was present in significant amounts in the female liver (Figures 2.6 and 2.7). In this study it is clearly shown that this binder is not an exclusive property of the adult male liver.

The role of the molybdate oxyanion in the stablization of steroid hormone receptors features as an important topic over the last decade (155-159). It was previously postulated that the interaction of the molybdate oxyanion with the receptor might cause a positive cooperative effect on oestradiol binding. However, the opposite effect with the HEBP was observed (Table 2.5, Figure 2.8). This discrepancy might be the result of negative cooperative binding due to conformational changes in the HEBP or differences in the molecular properties of the hepatic and uterine

oestrogen-binding proteins. From the SDG profiles the 8,1S binding component appeared to be stabilized in the presence of molybdate at the cost of the 3,8S component (Figure 2.8).

Corticosteroid binding globulin (CBG) displays some affinity for oestrogens and is furthermore, abundantly present in the liver (160, 161). A possible contribution of hepatic CBG to the high levels of binding activity in the liver has therefore been investigated. However, the presence of a large excess of hydrocortisone during titration assays and SDG analyses did not have any effect on the levels of HEBP measured (Table 2.5, Figure 2.8).

Two specific binding components were observed with GPC of the HEBP from male and female vervet monkeys (Figures 2.9 and 2.10); 4×10^5 daltons and $4,2 \times 10^4$ daltons. High ionic strength conditions favour the $4,2 \times 10^4$ dalton component and more so the female 4.2×10^4 dalton component. Thus, sex related differences in vervet monkey hepatic oestrogen-binding protein levels and some molecular properties appear to be either absent or very small. Considering the result obtained during this investigation it can be inferred that the 8,1S oestrogen-binding protein is a typical oestrogen receptor and that in addition to this component there is a second, low affinity oestrogen-binding protein present in livers of both sexes. However, this binder reaches higher levels in the male than in the female monkeys.

In the effort to purify the HEBP from the vervet monkey, nuclear extraction and salt fractionation were initially examined as prepurification methods. However these methods were found to be insufficient for the purification of the HEBP (Tables 3.1 and 3.2). In contrast with these results Gschwendt *et al.* (172) reported the purification to homogeneity of the nuclear ER from chick liver by using a combination of ammonium sulphate precipitation and affinity chromatography.

Heparin, a highly sulphated glycosaminoglycan, dissociates aggregates of oestrogen-receptor complexes via a mechanism not exactly understood (177).

Heparin-Sepharose chromatography is a qualifying step during purification of the HEBP for the following reasons: (1) it allows partial purification of the HEBP (Table 3.3), (2) it serves as a concentrating procedure (Table 3.8), (3) the heparin used for the elution of protein adsorbed onto the heparin-Sepharose affinity matrix, prevents aggregation of the receptor and (4) heparin-Sepharose chromatography eliminates the excess unlabelled ligand employed during elution of specifically adsorbed protein from DES-Agarose; the preceding step (Table 3.7). The HEBP, partially purified by heparin-Sepharose chromatography (Figure 3.2) exists as two entities with sedimentation coefficients of 3,8S and 8,1S respectively (Figure 3.3). The latter is less prominent under high ionic strength conditions.

Specific affinity chromatography (DES-Agarose) proved to be the most powerful tool during the partial purification of the vervet monkey HEBP (Table 3.5, Figure 3.4). DES-Agarose binds oestradiol-binding proteins selectively with high capacity (173). The spacer arm between DES and agarose is stable and therefore not susceptible to cleavage by cytosolic enzymes. Derivatization of the matrix or ligands is not required for the covalent coupling reaction. Thus, the preparation of DES-Agarose is relatively easy. An enrichment of the vervet monkey HEBP of approximately 7 435-fold (30% recovery) was achieved following DES-Agarose and heparin-Sepharose affinity chromatography (Table 3.8), which compares favourably with the partial purification values reported for the chicken liver ER (168). The apparent loss of HEBP may be attributed to three factors: (1) non-specific interactions of the HEBP with the affinity matrix that result in the loss of this protein during the washing procedures (174), (2) the decreased stability of the protein when it is highly purified (174) and (3) the incomplete elution of specifically adsorbed HEBP from the affinity matrix (164).

The observed isoelectric values for the partially purified vervet monkey HEBP (6,8 and 6,9) correlate with those of 6,6 and 6,8 found for the calf uterine oestrogen receptor (170) and with those of 6,8, 6,2 and 5,9 reported for the vervet monkey uterine receptor in the absence of sodium molybdate. Various molecular masses for the isolated oestrogen receptor have been reported. Following SDS-PAGE of purified rabbit uteri ER, Van

Oosbree et al. (173) observed two oestrogen-binding proteins with molecular masses of 50 000 and 65 000 daltons. The purified chick liver ER of Gschwendt et al. (172) migrated as a single band on a SDS polyacrylamide gel with a molecular mass of 55 000 dalton. During this study clear evidence for three HEBPs were found with molecular masses of 30 000, 38 000 and 68 000 daltons.

For the preparation of monoclonal antibodies the purity of an immunogen employed during immunization is irrelevant if the method of assay distinguishes between antibodies to the specific component and antibodies to the impurities (210). The assay used during screening stages to detect and clone the hybrid secreting the desired antibody is the most important factor and should be given a great deal of attention (209, 210).

The vervet monkey HEBP, purified 6 091-fold (Table 4.1) with DES-Trisacryl GF 2000 affinity chromatography, was employed as immunogen. Following an immunization program and screening of immune sera (Figures 4.1 and 4.2), spleen cells of the immunized mice were fused with $Sp^{2/0}$ myeloma cells. A 43% positive reaction (ELISA) of the polyclonal hybridoma cell cultures against the antigen was obtained (Table 4.3). The double antibody precipitation assay (Scheme 4.2) for the detection of antibodies against the ER appears to be the assay of choice in many laboratories (105, 205, 212). However, this assay was found to be insufficient during this investigation (Figure 4.1 and Table 4.2). It was therefore decided to employ the ELISA (Scheme 4.2) while solving the problem with the more specific double antibody immune precipitation assay. Screening methods should, however, be well worked out before cell fusion (209). Since the problems experienced with the double antibody precipitation assay was not cleared up at the time the cell fusion was executed, the ELISA was employed as screening assay. However, this assay did not provide absolute specificity and a large amount of non-specific hybridoma cultures were therefore detected. The positive polyclonal cell cultures were cloned and a total of 288 clones selected. Unfortunately no antibody activity could be detected in the spent media of any of the monoclonal cell cultures (Table 4.5).

In conclusion, the main reasons responsible for the unfortunate outcome of this part of the study may be the following: (1) instability and clonal competition between polyclonal cell cultures that are particularly frequent in early stages of growth (209, 210), (2) the relatively long period preceding cloning of positive polyclonal cell cultures and finally (3) the insufficient specificity of the screening assay that resulted in a large number of positive cultures detected which could not be conveniently handled.

* * *

REFERENCES

1. Cooper, A.P.: Lectures on the principles and practice of surgery. 8th Edition. John Thomas, London, (1835) 333 pp.

2. Schinzinger, A.: Uber carcinoma mammae. Verhandlungen der Deutschen Gesellschaft für Chirurgie. 18 (1889) 28.

3. Beatson, G.T.: On the treatment of inoperable cases of carcinoma of the mamma: suggestion for a new method of treatment, with illustrative cases. Lancet II (1896) 104-107.

4. Thompson, A.: Analysis of cases in which oophorectomy was performed for inoperable carcinoma of the breast. Brit. Med. J. VI (1902) 1538-1541.

5. Clarke, B.W.: An analysis of 99 cases of inoperable carcinoma of the breast treated by oophorectomy. Lancet i (1905) 227-228.

6. De Courmelles, F.: Ley rayons x et le radium en the rapeutique gynecologique. Acta Radiologica 6 (1926) 322-326.

7. Huggins, C. and Dao, Y.: Adrenalectomy and oophorectomy in the treatment of advanced carcinoma of the breast. J. Am. Med. Assoc. 151 (1953) 1388-1394.

8. MacCorquodale, D.W., Thayer, S.A. and Doisery, E.A.: Isolation of principal estrogenic substance of liquor folliculi. J. Biol. Chem. 115 (1936) 435-443.

9. Huggins, C. and Clark, P.Y.: Quantative studies of prostatic secretion: effect of castration and of estrogen injection on the normal and on the hyperplastic prostate glands of dogs. J. Exp. Med. 72 (1940) 747-762.

10. Huggins, C and Hodges, C.V.: Studies on prostatic carcinoma of prostate. Cancer Res. 1 (1941) 293-297.

11. Huggins, C., Stevens, R.E. and Hodges, C.V.: Studies on prostatic cancer: effects of castration on advanced carcinoma of prostatic gland. Arch. Surg. 43 (1941) 209-223.

12. Huggins, C. and Bergenstal, D.M.: Inhibition of human mammary and prostatic cancers by adrenalectomy. Cancer Res. 12 (1952) 134-141.

13. Huggins, C.: Endocrine-induced regression of cancers. Science 156 (1967) 1050-1054.

14. Luyt, R. and Olivecrona, H.: Experiences with hypophysectomy in man. J. Neurosurg. 10 (1953) 301-316.

15. Pearson, D.H., Ray, B.S. and Harrold, C.C.: Hypophysectomy in treatment of advanced cancer. J. Am. Med. Assoc. 161 (1956) 17-21.

16. Glascock, R.F. and Hoekstra, W.G.: Selective accumulation of tritium labelled hexoestrol by the reproductive organs of immature female goats and sheep. Biochem. J. 72 (1979) 673-682.

17. Jensen, E.V. and Jacobson, H.I.: Basic guide to the mechanism of estrogen action. Rec. Prog. Horm. Res. 18 (1962) 387-414.

18. Mobbs, B.G.: The uptake of tritiated oestradiol by dimethyl= benzanthracene-induced mammary tumors of the rat. J. Endocrinol. 36 (1966) 409-414.

19. Jensen, E.V., De Sombre, E.R. and Jungblut, P.W.: Estrogen receptors in hormone responsive tissues and tumors. In:Endogenous Factors influencing Host-Tumour Balance (Edited by R.W. Wissler, T.L. Dae and S. Wood Jr.). University of Chicago Press, Chicago, (1967) pp. 15-30.

20. Terenius, L.: Selective retention of estrogen isomers in estrogen-dependent breast tumors of rats demonstrated by in vitro methods. Cancer Res. 28 (1968) 328-337.

21. Toft, D.O. and Gorski, J.: A receptor molecule for estrogens: isolation from the rat uterus and preliminary characterization. Proc. natn. Acad. Sci. U.S.A. 55 (1966) 1574-1581.

22. Jensen, E.V., Suzuki, T. and Kawashima, T.: A two step mechanism for the interaction of estradiol with rat uterus. Proc. natn. Acad. Sci. U.S.A. 59 (1968) 632-638.

23. Gorski, J., Toft, D., Shyamala, G., Smith, D., and Notides, A.: Hormone receptors: studies on the interaction of estrogen with the uterus. Rec. Prog. Hor. Res. 24 (1968) 45-80.

24. Stumpf, W.E.: Subcellular distribution of ^{3}H-estradiol in rat uterus by quantitative autoradiography: a comparison between ^{3}H-estradiol and ^{3}H-morethynodrel. Endocrinology 83 (1968) 777-782.

25. Erdös, T.: Properties of a uterine estradiol receptor. Biochem. Biophys. Res. Commun. 32 (1968) 338-343.

26. Jensen, E.V., Numato, M., Brecher, D.I. and De Sombre, E.R.: Hormone-receptor interaction as a guide to biochemical mechanism. Biochem. Soc. Symp. 32 (1971) 133-159.

27. Higgins, S.Y., Rousseau, G.G., Baxter, Y.D. and Tomkins, G.M.: Early events in glucocorticoid action. J. Biol. Chem. 248 (1973) 5866-5872.

28. Milgrom, E., Atger, M and Baulieu, E.E.: Acidophilic activation of steroid hormone receptors. Biochemistry 12 (1973) 5198-5205.

29. King, W.J. and Greene, G.L.: Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells. Nature 307 (1984) 745-747.

30. Press, M.F. and Greene, G.L.: An Immunocytochemical method for demonstrating estrogen receptor in human uterus using monoclonal antibodies to human estrophilin. Lab. invest. 50 (1984) 480-486.

31. King, W.J., De Sombre, E.R., Jensen, E.V. and Greene, G.L.: Comparison of immunocytochemical and steroid binding assays for estrogen receptor in human breast tumors. Cancer Res. 45 (1985) 293-304.

32. Poulsen, H.S., Ozzello, L., King, W.J. and Greene, G.L.: The use of monoclonal antibodies to estrogen receptors for immunoperoxidase detection of ER in paraffin sections of human breast cancer tissue. J. Histochem. Cytochem. 33 (1985) 87-92.

33. Welshons, W.V., Lieberman, M.E and Gorski, J.: Nuclear localization of unoccupied oestrogen receptors. Nature 307 (1984) 747-749,

34. Katzenellenbogen, J.A., Carlson, K.E., Heiman, D.F., Robertson, D.W., Wei, L.L. and Katzenellenbogen, B.S.: Efficient and highly selective covalent labelling of the estrogen receptor with [^{3}H]-tamoxifen aziridine. J. Biol. Chem. 258 (1983) 3487-3495.

35. Stumpf, W.E. and Sar, M.: Autoradiographic localization of estrogen, androgen, progestin and glucocorticosteroid in 'target tissues' and 'non-target tissues'. In:Receptors and Mechanism of Steroid Hormones (Edited by J.R. Pasqualini). Marcel Dekker, New York (1976) pp. 41-84.

36. Clark, C.R. and Hall, M.D.: Hormone receptor autoradiography: recent developments. TIBS II (1986) 195-199.

37. Firestone, G.L. and Yamamoto, K.R.: Two classes of mutant mammary tumour virus-infected HTC cell with defects. Mol. Cell. Biol. 3 (1983) 149-160.

38. Edwards, D.P., Adams, D.J., Savage, N. and McGuire, W.L.: Estrogen induced synthesis of specific proteins in human breast cancer cells. Biochem. Biophys. Res. Commun. 93 (1980) 804-812.

39. Nawata, H., Bronzert, D. and Lippman, H.E.: Isolation and characterization of a Tamoxifen-resistant cell line derived from MCF-7 human breast cancer cells. J. Biol. Chem. 256 (1981) 5016-5021.

40. Schrader, W.T.: New model for steroid hormone receptors? Nature 308 (1984) 17-18.

41. Clark, C.R.: The cellular distribution of steroid hormone receptors: have we got it right? TIBS 101 (1984) 207-208.

42. Jungblut, P.W., Kuweit, E., Sierralta, W., Truitt, A.J. and Wagner, R.K.: A proposal for assessment of hormone sensitivity and consequent endocrine therapy of breast cancer. J. Physiol. Chem. 359 (1978) 1259-1263.

43. Carlson, R.A. and Gorski, J.: Characterization of a unique population of unfilled estrogen-binding sites associated with the nuclear fraction of immature rat uteri. Endocrinology 106 (1980) 1776-1784.

44. Clark, C.R., MacLusky, N.J. and Naftolin, F.: Unfilled nuclear oestrogen receptors in the rat brain and pituitary gland. J. Endocrinol. 93 (1982) 327-338.

45. Sonnenschein, C., Soto, A.H., Colofiore, J. and Farookhi, R.: Estrogen target cells, estaolishment of a cell line derived from the rat pituitary tumour MtT/F4. Exp. Cell Res. 101 (1976) 15-22.

46. Edwards, D.D., Martin, P.M., Horwitz, K.B., Chamness, G.C. and McGuire, W.L.: Subcellular compartmentalization of estrogen receptors in human breast cancer cells. Exp. Cell Res. 127 (1980) 197-213.

47. Clark, J.H. and Peck, E.J.: Female sex steroids: receptors and function. Rec. Prog. Horm. Res. 36 (1979) 89-134.

48. Jackson, V. and Chalkley, R.: The binding of Estriol-17β to the bovine endometrial nuclear membrane. J. Biol. Chem. 249 (1974) 1615-1626.

49. Zava, D.J. and McGuire, W.L.: Estrogen receptor: unoccupied sites in nuclei of a breast tumor cell line. J. Biol. Chem. 252 (1977) 3702-3707.

50. Sheridan, P.J., Buchanan, J.M. and Anselmo, V.C.: Equilibrium: the intracellular distribution of steroid receptors. Nature 282 (1979) 579-582.

51. Raam, S.: Hormone-free estrogen receptors in target cell nuclei: a commentary. Steroids. 47 (1986) 337-340.

52. Raam, S. and Vrabel, D.M.: Evaluation of an enzyme-immunoassay kit for estrogen-receptor measurements. Clin. Chem. 32 (1986) 1496-1502.

53. Nelson, J., Clarke, R. and Murphy, R.F.: The unoccupied estrogen receptor: some comments on localization. Steroids. 48 (1986) 121-124.

54. Fuxe, K., Wikstrom, A.C., Okret, S., Agnati, L.F., Harfstrand, A., Yu, Z.Y., Granholm, L., Zoli, M., Vale, W. and Gustafsson, J.A.: Mapping of glucocorticoid receptor immunoreactive neurons in the rat tel - and diencephalon using a monoclonal antibody against rat liver glucocorticoid receptor. Endocrinology 117 (1985) 1803-1812.

55. Antakly, T. and Eisen, H.J.: Immunocytochemical localization of glucocorticoid receptor in target cells. Endocrinology 115 (1984) 1984-1989.

56. Perrot-Applanat, M., Groyer-Picard, M.T., Logeat, F. and Milgrom, E.: Ultrastructural localization of the progesterone receptor by an immunogold method. Effect of hormone administration. J. cell. Biol. 102 (1986) 1191-1199.

57. Gurpide, E. and Welch, M.: Dynamics of uptake of estrogens and androgens by human endometrium. J. Biol. Chem. 244 (1969) 5159-5167.

58. Szego, C.M.: The lysosome as a mediator of hormone action. Rec. Prog. Horm. Res. 30 (1974) 171-233.

59. Zanker, K.S., Proscha, O.W. and Blumel, G.: Plasma membrane-integrated estrogen receptors in breast tissue: possible modulator molecules for intracellular hormone level. J. Cancer Res. and Clin. Oncol. 100 (1981) 135-148.

60. Scatchard, G.: The attraction of proteins for small molecules and ions. Ann. N.Y. Acad. Sci. 51 (1949) 660-672.

61. Baulieu, E.E.: Aspects of steroid hormone-target cell interactions. In: Advances in Experimental Medicine and Biology. Steroid Hormone Receptor Systems (Edited by W.W. Leavitt and J.H. Clark). Plenum Press, New York, Vol. 117 (1979) pp. 377-398.

62. Hawkins, R.A., Roberts, M.M. and Forrest, A.P.: Estrogen receptors and breast cancer: Current status. Brit. J. Surg. 67 (1980) 153-169.

63. Sherman, M.R., Pickering, L.A., Rollwagen, F.M. and Miller, L.K.: Meroreceptors: proteolytic fragments of receptors containing the steroid-binding site. Fed. Proc. 37 (1978) 167-173.

64. Puca, G.A., Nola, E., Sica, V. and Bresciani, F.: Estrogen-binding protein of calf uterus. Interrelationship between various forms and identification of a receptor-transforming factor. Biochemistry 11 (1972) 4157-4165.

65. Hendry, W.J. and Danzo, B.J.: Further characterization of a steroid receptor-active protease from the mature rabbit epididymis. J. steriod Biochem. 25 (1986) 433-443.

66. Hazato, T. and Murayama, A.: Protection of steroid hormone receptors by protease inhibitors. Biochem. Biophys. Res. Commun. 98 (1981) 488-493.

67. Notides, A.C., Lerner, N. and Hamilton, D.E.: Positive cooperativity of the estrogen receptor. Proc. natn. Acad. Sci 78 (1981) 4926-4930.

68. Müller, R.E., Traish, A.M., Hirota, T., Bercel, E. and Wotiz, H.H.: Conversion of estrogen receptor from a state with low affinity for estradiol into a state of higher affinity does not require 4S to 5S dimerization. Endocrinology 116 (1985) 337-345.

69. Weichman, B.M. and Notides, A.C.: Estrogen receptor activation and the dissociation kinetics of estradiol, estriol and estrone. Endocrinology 106 (1980) 434-439.

70. Gehring, U.: Steroid hormone receptors: biochemistry, genetics and molecular biology. TIBS 12 (1987) 399-402.

71. Green, S., Walter, P., Kumar, V., Krust A., Bornert, J-M., Argos, P. and Chambon, P.: Human estrogen-receptor cDNA: sequence, expression and homology to v-erb-A. Nature 320 (1986) 134-139.

72. Greene, G.L., Gilna, P., Waterfield, M., Baker, A., Hort, Y. and Shine, J.: Sequence and expression of human estrogen-receptor complementary-DNA. Science 231 (1986) 1150-1154.

73. Krust, A., Green, S., Argos, P., Kumar, V., Walter, P., Bornert, J.M. and Chambon, P.: The chicken estrogen-receptor sequence: homology with v-erb-A and the human estrogen and glucocorticoid receptors. EMBO J. 5 (1986) 891-897.

74. Danielsen, M., Northrop, J.P. and Ringold, G.M.: The mouse glucocorticoid receptor: mapping of functional domains by cloning, sequencing and expression of wild-type and mutant receptor proteins. EMBO J. 5 (1986) 2513-2522.

75. Hollenberg, S.M., Weinberger, C., Ong, E.S., Cerelli, G., Oro, A., Lebo, R., Thompson, E.B., Rosenfeld, M.G. and Evans, R.M.: Primary structure and expression of a functional human glucocorticoid receptor cDNA. Nature 318 (1985) 635-641.

76. Miesfeld, R., Rusconi, S., Godowski, P.J., Maler, B.A., Okret, S., Wikström, A.C., Gustafsson, J.Å. and Yamamoto, K.R.: Genetic complementation of a glucocorticoid receptor deficiency by expression of cloned receptor cDNA. Cell 46 (1986) 389-399.

77. Loosfelt, H., Atger, M., Misrahi, M., Guiochon-Mantel, A., Meriel, C., Logeat, F., Benarous, R. and Milgrom, E.: Cloning and sequence-analysis of rabbit progesterone-receptor complementary-DNA. Proc. natn. Acad. Sci. U.S.A. 83 (1986) 9045-9049.

78. Jeltsch, J.M., Krozowski, Z., Quirin-Stricker, C., Gronemeyer, H., Simpson, R.J., Garnier, J.M., Krust, A., Jacob, F. and Chambon, P.: Cloning of the chicken progesterone-receptor. Proc. natn. Acad. Sci. U.S.A. 83 (1986) 5424-5428.

79. Conneely, O.M., Sullivan, W.P., Taft, D.O., Birnbaumer, M., Cook, R.G., Maxwell, B.L. Zarucki-Schulz, T., Greene, G.L., Schrader, W.T. and O'Malley, B.W.: Molecular-cloning of the chicken progesterone-receptor. Science 233 (1986) 767-770.

80. Berg, J.M.: Nucleic acid-binding proteins: more metal-binding fingers. Nature 319 (1986) 264-265.

81. Kumar, V., Green, S., Staub, A. and Chambon, P.: Localization of the estradiol-binding and putative DNA-binding domains of the human estrogen-receptor. EMBO J. 5 (1986) 2231-2236.

82. Rusconi, S. and Yamamoto, K.R.: Functional dissection of the hormone and DNA-binding activities of the glucocorticoid receptor. EMBO J. 6 (1987) 1309-1315.

83. Pagvar, F., Wrange, C. and Carlstedt-Duke, J.: Purified glucorticoid receptors bind selectively *in vitro* to a cloned DNA fragment whose transcription is regulated by glucocorticoids *in vivo*. Proc. natn. Acad. Sci. U.S.A. 78 (1981) 6628-6632.

84. Mulvihil, E., Le Pennec, J.P. and Chambon, P: Chicken oviduct progesterone receptor: Location of specific regions of high-affinity binding in cloned DNA fragments of hormone-responsive genes. Cell 24 (1982) 621-632.

85. Clark, J.H., Hardin, J.W. and McCormack, S.A.: Estrogen receptor binding and the stimulation of normal and abnormal growth. In: Steroid Receptors and Hormone-Dependent Neoplasia (Edited by J.L. Wittliff and O. Dapunt). Masson, New York, (1980) pp. 19-27.

86. Kallas, J., Fasy, T. and Hollander, V.P.: Assessment of estrogen receptor-histone interactions. Proc. natn. Acad. Sci. U S.A. 78 (1981) 2874-2878.

87. Clark, J.H., Markaverich, B. and Upchurch, S.: Nuclear binding of the estrogen receptor: heterogeneity of sites and uterotropic response. In: Advances in Experimental Medicine and Biology. Volume 117. Steroid Hormone Receptor Systems (Edited by W.W. Leavitt and J.H. Clark). Plenum Press, New York, (1979) pp. 17-46.

88. Horwitz, K.B. and McGuire, W.L.: Estrogen control of progesterone receptor in human breast cancer. Correlation with nuclear processing of estrogen receptors. J. Biol. Chem. 253 (1978) 2223-2228.

89. Siebert, K. and Lippman, M.: Hormone receptors in breast cancer. Clinics in Oncology 1 (1982) 735-793.

90. Auricchio, F., Migliaccio, A. and Rotondi, A.: Inactivation of ER in vitro by nuclear dephosphorylation. Biochem J. 197 (1981) 569-574.

91. Auricchio, F., Migliaccio, A., Castoria, G., Lastoria, S. and Schiavone, E.: ATP-dependent enzyme activating hormone binding of estradiol receptor. Biochem. Biophys. Res. Commun. 101 (1981) 1171-1178.

92. Auricchio, F., Migliaccio, A., Domenico, M.D. and Nola, E.: Oestradiol stimulates tyrosine phosphorylation and hormone binding activity of its own receptor in a cell-free system. EMBO J. 6 (1987) 2923-2929.

93. Menard, J., Corvol, P., Foliot, A. and Ragnaud, J.P.: Effects of estrogens on renin substrate and uterine weights in rats. Endocrinology 93 (1973) 747-751.

94. Glinoer, D., McGuire, R.A., Gershengorn, M.C., Robbins, J. and Berman, M.: Effects of estrogen on thyroxine-binding globulin matabolism in Rhesus monkeys. Endocrinology 100 (1977) 9-17.

95. Chamness, G.C., Costlow, M.E. and McGuire, W.L.: Estrogen receptor in rat liver and its dependence on prolactin. Steroids 26 (1975) 363-371.

96. Eisenfeld, A.J., Aten, R.F., Weinberger, M., Haselbacher, G., Halpern, K. and Krakoff, L.: Estrogen receptor in mammalian liver. Science 191 (1976) 862-865.

97. Danzo, B.J., Krishnamurthy, V. and Eller, B.C.: High affinity estrogen binding by rabbit liver. Biochim. Biophys. Acta. 500 (1977) 310-321.

98. Powell-Jones, W., Nayfeh, S.N. and Lucier, G.W.: Translocation of 4S and 5S forms of estrogen receptor into rat liver nuclei in vitro. Biochem. Biophys. Res. Commun. 85 (1978) 167-173.

99. Laragh, J.H., Bauer, L., Brunner, H.R., Buhler, F.R., Sealey, J.E. and Vaughn, E.D.: Renin, angiotensin and aldosterone system in pathogenesis and management of hypertensive vascular disease. Am. J. Med. 52 (1972) 633-652.

100. Corvol, P.L., Chrambach, A., Rodbard, D. and Bardin, C.W.: Physical properties and binding capacity of testosterone-estrodiol-binding globulin in human plasma determined by polyacrylamide gel electrophoresis. J. Biol. Chem. 246 (1971) 3435-3443.

101. Song, C.S., Rifkind, A.B., Gillette, P.N. and Kappas, A.: The effect of estrogens, progestins and pregnancy on hepatic function. Am. J. Obstet. Gynecol. 105 (1969) 833-847.

102. Aten, R.F. Dickson, R.B. and Eisenfeld, A.J.: Female and male green monkey liver estrogen receptor. Biochem. Pharmac. 28 (1979) 2445-2450.

103. Eisenfeld, A.J. and Aten, R.F.: Estrogen receptor in the mammalian liver. In: Advances in Steroid Biochemistry and Pharmacology (Edited by M.H Briggs and A. Corbin). Academic Press, New York, Vol. 7 (1979) pp. 91-117.

104. Powell-Jones, W., Thompson, C., Nayfeh, S.N. and Lucier, G.W.: Sex differences in estrogen binding by cytosolic and nuclear components of rat liver. J. steroid Biochem. 13 (1980) 219-229.

105. Eagon, P.K., Fisher, S.E., Imhoff, A.F., Porter, L.E., Stewart, R.R., Van Thiel, D.H. and Lester, R.: Estrogen-binding proteins of male rat liver: influences of hormonal changes. Arch. Biochem. Biophys. 201 (1980) 486-499.

106. Smirnova, O.V., Vishnyakova, T.G., Smirnov, A.N. and Rozen, V.B.: The unusual estrogen-binding protein (UEBP) of rat liver: the role of sex steroids and hypophysis in its regulation. J. steroid. Biochem. 23 (1985) 461-468.

107. Eagon, P.K., Porter, L.E., Gavaler, J.S., Egler, K.M. and Van Thiel, D.H.: Effect of ethanol feeding upon levels of male-specific hepatic estrogen binding protein: a possible mechanism of feminization. Clin. Exp. Res. 5 (1981) 183-187.

108. Lax, E.R., Tamulevicius, P., Müller, A. and Schriefers, H.: Hepatic nuclear estrogen receptor concentrations in the rat - influence of age, sex, gestation, lactation and estrous cycle. J. steroid Biochem. 19 (1983) 1083-1088.

109. Dickson, R.B., and Eisenfeld, A.J.: Estrogen receptor in liver of male and female rats: endocrine regulation and molecular properties. Biol. Reprod. 21 (1979) 1105-1114.

110. Thompson, C., Powell-Jones, W. and Lucier, G.W.: Sex differences in hepatic estrogen binding proteins. Biochem. J. 194 (1981) 1-8.

111. Lucier, G.W., Slaughter, S.R., Thompson, C., Lamartiniere, C.A. and Powell-Jones, W.: Selective actions of growth hormone on rat liver estrogen binding proteins. Biochem. Biophys. Res. Commun. 103 (1981) 872-879.

112. Rumbaugh, R.C., Clark, J.C., McDaniel, O.S. and Lucier, G.W.: Feminization of the concentrations of hepatic estrogen-binding proteins by ectopic pituitary. Endocrinology 112 (1983) 1363-1369.

113. Eriksson, H.A.: Estrogen-binding sites of mammalian liver: endocrine regulation of estrogen receptor synthesis in the regenerating rat liver. J. steroid Biochem. 17 (1982) 471-477.

114. Powell-Jones, W., Thompson, C., Raeford, S. and Lucier, W.: Effect of gonadectomy on the ontogeny of estrogen-binding components in rat liver cytosol. Endocrinology 109 (1981) 628-636.

115. Norstedt, G., Wrange, Ö. and Gustafsson, J-Å.: Multi-hormonal regulation of the estrogen receptor in rat liver. Endocrinology 108 (1981) 1190-1196.

116. Winborn, W.B., Sheridan, P.J. and McGill, H.C.: Sex steroid receptors in the stomach, liver, pancreas and gastrointestinal tract of the baboon. Gastroenterology 92 (1987) 23-32.

117. Tong, J.H., Layne, D.S., Dostaler, S. and Williamson, D.G.: Comparative studies of the 17β-estradiol receptors in rabbit liver, kidney and uterus. J. steroid Biochem. 18 (1983) 273-279.

118. Wolfson, A.J.: Identification of a large form of the chick liver estrogen receptor. J. Recept. Res. 2 (1981-82) 317-330.

119. Beers, P.C. and Rosner, W.: The binding of estrogens in the liver of the rat: demonstration and endocrine influences. J. steroid Biochem. 8 (1977) 251-258.

120. Aten, R.F., Dickson, R.B. and Eisenfeld, A.J.: Estrogen receptor in adult male rat liver. Endocrinology 103 (1978) 1629-1635.

121. Edmondson, H.A. and Henderson, B.: Liver-cell adenomas associated with use of oral contraceptives. N. Engl. J. Med. 294 (1976) 470-472.

122. Knowles, D.M. and Wolff, M: Focal nodular hyperplasia of the liver. A clinicopathologic study and review of the literature. Hum. Pathol. 7 (1976) 533-545.

123. Christopherson, W.M., Mays, E.T. and Barrows, G.H.: A clinicopathologic study of steroid related tumors. Am. J. Surg. Pathol. 1 (1977) 31-41.

124. Francavilla, K., DiLeo, A., Van Thiel, D.H., Ove, P., Wu and Starzl, T.E.: Estrogen binding protein activity for hepatoma 7777 compared with normal rat liver. Gastroenterology 87 (1984) 1410-1416.

125. Porter, L.E., Elm, M.S., Van Thiel, D.H. and Eagon, P.K.: Hepatic estrogen receptor in human liver disease. Gastroenterology 92 (1987) 735-745.

126. Ohnishi, S., Murakami, T., Moriyama, T., Mitamura, K. and Imawari, M.: Androgen and estrogen receptors in hepatocellular carcinoma and in the surrounding non-cancerous liver tissue. Hepatology 6 (1986) 440-443.

127. Molteni, A., Bahu, R.M., Battifora, H.A., Fors, E.M., Reddy, J.K., Rao, M.S. and Scarpell, D.G.: Estradiol receptor assays in normal and neoplastic tissues. Ann. Clin. Lab. Med. 9 (1979) 103-108.

128. Friedman, M.A., Demanes, D.J. and Hoffman, P.G.: Hepatomas: hormone receptors and therapy. Am. J. Med. 73 (1982) 362-366.

129. Iqbal, M.J., Wilkinson, M.L., Johnson, P.J. and Williams, R.: Sex steroid receptor proteins in foetal, adult and malignant liver tissue. Br. J. Cancer 48 (1983) 791-796.

130. Nagasue, N., Ito, A., Yukaya, H. and Ogawa, Y.: Estrogen receptors in hepatocellular carcinoma. Cancer 57 (1986) 87-91.

131. Porter, L.E., Elm, M.S., Van Thiel, D.H. and Eagon, P.K.: Estrogen receptor in human liver disease (abstr.). Clin. Res. 31 (1983) 287A.

132. Mays, E.T. and Christopherson, N.: Hepatic tumors induced by sex steroids. Semin. Liver Dis. 4 (1984) 147-157.

133. Reznik-Schuller, H.: Carcinogenic effects of diethylstilbestrol in male Syrian golden hamsters and European hamsters. J. Natl. Cancer Inst. 62 (1979) 1083-1088

134. Schardein, J.L., Kaump, D.H. and Woosley, E.T.: Long term toxicologic and tumorogenesis studies on an oral contraceptive agent in albino rats. Toxicol. Appl. Pharmacol. 16 (1970) 10-23.

135. Reuber, M.D.: Influence of hormones on N-2-fluorenyldiacetamide-induced hyperplastic hepatic nodules in rat. J. Natl. Cancer Inst. 43 (1969) 445-452.

136. Taper, H.S.: The effect of estradiol-17-phenylpropionate and estradiol benzoate on N-nitrosomorpholine-induced liver carcinogens in ovariectomized female rat. Cancer 42 (1978) 462-467.

137. Yager, J.D. and Yager, R.: Oral contraceptive steroids as promoters of hepatocarcinogenesis in female Sprague-Dawley rats. Cancer Res. 40 (1980) 3680-3685.

138. Wanless, I.R. and Medline, R.: Role of estrogens as promoters of hepatic neoplasia. Lab. invest. 46 (1982) 313-320 .

139. Baum, J.K., Holtz, F., Bookstein, J.J. and Klein, E.W.: Possible association between benign hepatomas and oral contraceptives. Lancet ii (1973) 926-929.

140. Neuberger, J., Portmann, B., Nunnerley, H.B., Laws, J.W., Davis, M. and Williams, R.: Oral contraceptive associated liver tumors: occurence of malignancy and difficulties in diagnosis. Lancet. i (1980) 273-276.

141. Goodman, Z.D. and Ishak, K.G.: Hepatocellular carcinoma in women: probable lack of etiologic association with oral contraceptive steroids. Hepatology 2 (1982) 440-444.

142. Fisher, B., Gundez, M., Saffer, E.A. and Shu, Z.: Relation of estrogen and its receptors to rat liver growth and regeneration. Cancer Res. 64 (1984) 2410-2415.

143. Mishkin, S.Y., Farber, E., Kun, H.R., Mulag, S. and Mishkin, S.: Evidence for the hormone dependency of hepatic hyperplastic nodules: inhibition of malignant transformation after exogenous 17α-estradiol and Tamoxifen. Hepatology 3 (1983) 308-316.

144. Heston, W.E., Vlahakis, G. and Desmukes, B.: Effects of the antifertility drug Enovid in five strains of mice, with particular regard to carcinogenesis. J. Natl. Cancer Inst. 51 (1973) 209-224.

145. Metzler, M. and McLachlan, J.A.: Oxidative metabolites of diethyl-stilbestrol in fetal, neonatal and adult mouse. Biochem. Pharmacol. 27 (1978) 1087-1094.

146. Porter, L.E., Elm, M.S., Van Thiel, D.H., Dugas, M.C. and Eagon, P.K.: Characterization and quantitation of human hepatic estrogen receptor. Gastroenterology 84 (1983) 704-712.

147. Duffy, M.J. and Duffy, G.J.: Estradiol receptors in human liver. J. steroid Biochem. 9 (1978) 233-235.

148. Dickson, R.B., Aten, R.F. and Eisenfeld, A.J.: An unusual sex steroid-binding protein in mature male rat liver cytosol. Endocrinology 103 (1978) 1636-1646.

149. Miroshnichenko, M.L., Smirnova, O.V., Smirnov, A.N. and Rozen, V.B.: The unusual estrogen-binding protein (UEBP) of male rat liver: structural determinations of ligands. J. steroid Biochem. 18 (1983) 403-409.

150. Wrange, O., Norstedt, G. and Gustafsson, J.: The estrogen receptor in rat liver: quantitative and qualitative analyses by isoelectric focussing in polyacrylamide gel. Endocrinology 106 (1980) 1455-1462.

151. Potgieter, H.C., Spies, J.H., Klein, T., Thierry, M. and Van der Watt, J.J.: Estrogen and progesterone receptors in the uterus of the vervet monkey. J. Recept. Res. 5 (1985) 193-218.

152. Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt. Biochem. 72 (1976) 248-254.

153. Rodbard, D.: Mathematics of hormone receptor interaction. Adv. Exp. Med. Biol. 36 (1973) 289-326.

154. Eisenfeld, A.J., Aten, R.F., Haselbacher, G.K. and Halpern, K.: Specific macromolecular binding of estradiol in the mammalian liver supernatant. Biochem. Pharmac. 26 (1977) 919-922.

155. Noma, K., Nakao, K., Sato, B., Nishizawa, Y., Matsumoto, K., and Yamamura, Y.: Effect of molybdate on activation and stabilization of steroid receptors. Endocrinology 107 (1980) 1205-1211.

156. Chen., T.J., MacDonald, R.G. and Leavitt, W.W.: Uterine progesterone receptor: stabilization and physicochemical alterations produced by sodium molybdate. Biochemistry 20 (1981) 3405-3411.

157. Mauck, L.A., Day, R.N. and Notides, A.C.: Molybdate interaction with estrogen receptor: effect on estradiol binding and receptor activation. Biochemistry 21 (1982) 1788-1793.

158. Potgieter, H.C., Klein, T., Magagane, F., Savage, N. and Wittliff, J.L.: Molybdate and the molecular properties of the vervet monkey estrogen receptor. J. steroid Biochem. 25 (1986) 951-956.

159. Klein, T., Potgieter, H.C., Spies, J.H., Van der Watt, J.J. and Savage, N.: Reversibility of the stabilization effect of sodium molybdate on uterine estrogen and progesterone receptors of the vervet monkey. J. Recep. Res. 5 (1985) 267-295.

160. Strel'chyonok, O.A. and Avvakumov, G.V.: Evidence for the presence of specific binding sites for transcortin in human liver plasma membranes. Biochim. Biophys. Acta. 755 (1983) 514-517.

161. Mickelson, K.E., Forsthoefel, J. and Westphal, U.: Steroid-protein interaction. Human corticosteroid binding globulin: some physicochemical properties and binding specificity. Biochemistry 20 (1981) 6211-6218.

162. Puca, G.A., Nola, E., Sica, V. and Bresciani, F.: Purification of estrogen receptors. In Methods in Enzymology (Edited by B.W. O'Malley and J.G. Hardman). Academic Press, New York, Vol. 36 (1975) pp. 331-349.

163. King, R.J.B.: Mini review; Receptor structure: a personal assessment of the current status. J. steroid Biochem. 25 (1986) 451-454.

164. Greene, G.L. and Press, M.F.: Steroid receptor structure (including monoclonal antibodies and new methods of determination). J. steroid Biochem. 24 (1986) 1-7.

165. Chong, M.T. and Lippman, M.: Purification of estrogen receptors from MCF-7 human breast cancer cells. Cancer Res. 40 (1980) 3172-3176.

166. Puca, G.A., Nola, E., Sica, V. and Bresciani, F.: Purification of oestrogen receptors. I. Biochemistry 10 (1971) 3769-3780.

167. Sica, V and Bresciani, F.: Estrogen-binding proteins of calf uterus. Purification to homogeneity of receptor from cytosol by affinity chromatography. Biochemistry 18 (1979) 2369-2378.

168. Schneider, W. and Gschwendt, M.: Partial purification of the nuclear estrogen receptor from chicken liver by affinity chromatography. Biochim. Biophys. Acta. 633 (1980) 105-113.

169. Madhok, T.C. and Leung, B.S.: Purification of rabbit uterine cytosolic estrogen receptors by affinity chromatography. J. steroid Biochem. 15 (1981) 299-305.

170. Sica, V., Parikh, I., Nola, E., Puca, G. and Cuatrecasas, P.: Affinity chromatography and the purification of estrogen receptors. J. Biol. Chem. 248 (1973) 6543-6558.

171. Hubert, P., Mester, J., Dellacherie, E., Neel, J. and Baulieu, E.: Soluble biospecific macromolecule for purification of estrogen receptor. Proc. natn. Acad. Sci. U.S.A. 75 (1978) 3143-3147.

172. Gschwendt, M., Hähnel, R. and Ratajczak, T.: Purification to homogeneity of the nuclear estrogen receptor from chick liver. Biochim. Biophys. Acta. 760 (1983) 238-245.

173. Van Oosbree, T.R., Kim, U.H. and Mueller, G.C.: Affinity chromatography of estrogen receptors on diethylstilbestrol-Agarose. Analyt. Biochem. 136 (1984) 321-327.

174. Truong, H., Geynet, C., Millet, C., Soulignac, O., Bucourt, R., Vignau, M., Torelli, V. and Baulieu, E.: Purification of estradiol receptor by affinity chromatography. Representative experiments. FEBS LETTERS 35 (1973) 289-294.

175. Greene, G.L., Nolan, C., Engler, J.P. and Jensen, E.V.: Monoclonal antibodies to human estrogen receptor. Proc. natn. Acad. Sci. U.S.A. 77 (1980) 5115-5119.

176. Molinari, A.M., Medici, N., Moncharmont, B. and Puca, G.A.: Estradiol receptor of calf uterus: interaction with heparin-agarose and purification. Proc. natn. Acad. Sci. U.S.A. 74 (1977) 4886-4890.

177. Ratajczak, T. and Hähnel, R.: Investigation of methods of estradiol receptor purification prior to affinity chromatography. J. steroid Biochem. 13 (1980) 439-444.

178. Burton, K.: Study of conditions and mechanisms of diphenylamine reaction for colorometric estimation of deoxyribonucleic acid. Biochem. J. 62 (1956) 315-323.

179. Ultrogel, Magnogel and Trisacryl, Practical guide for affinity chromatography and related techniques, Réactifs IBF ed., 1983.

180. Budowle, B.: Increasing the sensitivity of protein detection of a silver stain for agarose gels. Electrophoresis 5 (1984) 174-175.

181. Wyckoff, M., Rodbard, D. and Chrambach, A.: Polyacrylamide gel electrophoresis in sodium dodecyl sulfate containing buffers using multi-phasic buffer systems: Properties of the stack, valid R_f-measurement and optimized procedure. Analyt. Biochem. 78 (1977) 459-482.

182. Jovin, T.M.: Multiphasic zone electro-phoresis. I. Steady-state moving boundary systems formed by different electrolyte combinations. Biochemistry 12 (1973) 871-890.

183. De Gruyter, W.: Component visualization. In: Gel Electrophoresis and Isoelectric focussing of Proteins (Edited by R.C. Allen, C.A. Saravis and H.R. Maurer). Berlin, New York, (1984) pp. 181-183.

184. Hamilton, T.H.: Steroid hormones, ribonucleic acid synthesis and transport, and the regulation of cytoplasmic translation. In: The Biochemistry of Steroid Hormone Action (Edited by R.M.S. Smellie). Academic Press, New York and London, (1971) pp. 49-84.

185. Smith, R.G. and Schwartz, R.J.: Isolation and purification of a hen nuclear oestrogen receptor and its effect on transcription of chick chromatin. Biochem. J. 184 (1979) 331-343.

186. Moudgil, V.K., Eesalu, T.E., Buchou, T., Renoir, J.M., Mester, J. and Baulieu, E.E.: Transformation of the chick oviduct progesterone receptor in vitro: effects of hormone, salt, heat and adenosine triphosphate. Endocrinology 116 (1985) 1267-1274.

187. La Forest, A.C., Pratt, W.B.: ATP-dependent ac of L cell glucocorticoid receptors to the steroid bind.. .orm. J. Biol. Chem. 254 (1979) 4772-4778.

188. Singh, V.B. and Moudgil, V.K.: Phosphor.tion of rat liver glucocorticoid receptor. J. Biol. Chem. 260 (1985) 3684-3690.

189. Al-Nuaimi, N., Davies, P. and Griffiths, K.: Purification of the cytoplasmic oestrogen receptor from mammary tumours induced in rats with dimethylbenzanthracene. J. Endocr. 81 (1979) 119-130.

190. Puca, G.A., Medici, N., Molinari, A.M., Moncharmont, B., Nola, E. and Sica, V.: Estrogen receptor of calf uterus: an easy and fast purification procedure. J. steroid Biochem. 12 (1980) 105-113.

191. Sica, V., Puca, G.A., Molinari, A.M., Buonaguro, F.M. and Bresciani, F.: Effect of chemical perturbation with NaSCN on receptor-estradiol interaction. A new exchange assay at low temperature. Biochemistry 19 (1980) 83-88.

192. Sawyer, W.H. and Puckridge, J.: The dissociation of proteins by chaotropic salts. J. Biol. Chem. 248 (1973) 8429-8433.

193. Omukai, Y., Nohno, T., Ikeda, M., Watanabe, S., Senoo, T., Saito, T. and Hosokawa, K.: Effects of thiocyanate on cytosol androgen receptor from Shionogi carcinoma 115. J. steroid Biochem. 19 (1983) 1055-1059.

194. Bond, J.P. and Notides, A.C.: Estrogen receptor purification by affinity chromatography using an orange triazine dye. Analyt. Biochem. 163 (1987) 385-390.

195. Wittliff, J.L., Feldhoff, P.W., Fuchs, A. and Wehle, R.D.: Polymorphism of estrogen receptors in human breast cancer. In Physiopathology of Endocrine Diseases and Mechanisms of Hormone Action (Edited by R.J. Solo, A. Nicola and J. Blaquier). Alan R. Liss, New York, (1981) pp. 375-396.

196. Gibbons, W.E., Higashi, M. and O'Brien, T.: Isoelectric heterogeneity of human uterine estrogen binding proteins. J. steroid Biochem. 19 (1983) 1291-1295.

197. Mueller, G.C., Van Oosbree, T.R. and Kim, U.H.: Differential release of estradiol from immobilized estrogen receptors by p-sec-amylphenol: evidence for a functional microheterogeneity of estrogen receptors. J. Recep. Res. 4 (1984) 773-785.

198. Monsma, F.J., Katzenellenbogen, B.S., Miller, M.A., Ziegler, Y.S. and Katzenellenbogen, J.A.: Characterization of the estrogen receptor and its dynamics in MCF-7 human breast cancer cells using a covalently attaching antiestrogen. Endocrinology 115 (1984) 143-153.

199. Lubahn, D.B., McCarty Jr., K.S. and McCarty, K.S.: Electrophoretic characterization of purified bovine, porcine, murin , rat and human uterine estrogen receptors. J. Biol. Chem. 260 (1985) 2515-2526.

200. Miller, M.A., Mullick, A., Greene, G.L. and Katzenellenbogen, B.S.: Characterization of the subunit nature of nuclear estrogen receptors by chemical crosslinking and dense amino acid labelling. Endocrinology 117 (1985) 515-522.

201. Sakai, D. and Gorski, J.: Reversible denaturation of the estrogen receptor and estimation of polypeptide chain molecular weight. Endocrinology 115 (1984) 2379-2383.

202. Olsen, M.R., Van Oosbree, T.R. and Mueller, G.C.: Characterization of rabbit uterine estrogen receptor proteins by radioiodination and partial peptide mapping. Endocrinology 119 2177-2185.

203. Greene, G.L., Closs, L.E., De Sombre, E.R. and Jensen, E.V.: Estrophilin: pro and anti. J. steroid Biochem. 12 (1980) 159-167.

204. Greene, G.L., Closs, L.E., De Sombre, E.R. and Jensen, E.V.: Antibodies to estrophilin: comparison between rabbit and goat antisera. J. steroid Biochem. 11 (1979) 333-341.

205. Greene, G.L., Closs, L.E., Fleming, H., De Sombre, E.R. and Jensen, E.V.: Antibodies to estrogen receptor: Immunochemical similarity of estrophilin from various mammalian species. Proc. natn. Acad. Sci. U.S.A. 74 (1977) 3681-3685.

206. Fernö, M., Borg, Å. and Sellberg, G.: Enzyme immuno assay of the ER in breast cancer biopsy samples. A comparison with isoelectric focussing. Acta Radiologica Oncologica 25 (1986) 171-175.

207. Fishman, J.H. and Fishman, J.: Copper and the estradiol receptor. Biochem. Biophys. Res. Commun. 144 (1987) 505-511.

208. Okulicz, W.C. and Johnson, L.D.: The relative binding affinity of diethylstilbestrol to uterine nuclear estrogen receptor: effect of serum and serum albumin. Proc. Soc. exp. Biol. Med. 185 (1987) 478-483.

209. Mayer, R.J. and Billet, E.: Techniques in the production and application of monoclonal antibodies. In Techniques in the life sciences, B1/II Supplement (Edited by K.F. Tipton). Elsevier Scientific Publishers, Ireland Ltd., (1985) pp. 1-55.

210. Galfre, G. and Milstein, C.: Preparation of monoclonal antibodies: strategies and procedures. Methods Enzymol. 73 (1981) 1-45.

211. Lacobelli, S., Natoli, V., Scambia, G., Santeusanio, G., Negrini, R. and Natoli, C.: A monoclonal antibody (AB/3) reactive with human breast cancer. Cancer Res. 45 (1985) 4334-4338.

212. Greene, G.L., Fitch, F.W. and Jensen, E.V.: Monoclonal antibodies to estrophilin: probes for the study of estrogen receptors. Proc. natn. Acad. Sci. U.S.A. 77 (1980) 157-161.

213. French, D., Fischberg, E., Buhl, S. and Scharff, M.D.: The production of more useful monoclonal antibodies; 1. Modifications of the basic technology. Immunology Today 7 (1986) 344-346.

214. Cleveland, W.L., Wassermann, N.H., Sarangarajan, R., Penn, A.S. and Erlanger, B.F.: Monoclonal antibodies to the acetylcholine receptor by a normally functioning auto-anti-idiotypic mechanism. Nature 305 (1983) 56-57.

215. Fazekas de St., Groth, S. and Scheidegger, D.: Production of monoclonal antibodies: strategy and tactics. J. Immunol. Methods 35 (1980) 1-21.

216. Oi V.T. and Herzenberg, L.A.: Immunoglobulin-producing hybrid cell lines. In Selected Methods in Cellular Immunology (Edited by B.B. Mishell and S.M. Shiigi). Freeman, San Francisco, (1980) pp. 351-372.

217. Borrebaeck, C.A.K. and Etzler, M.E.: Production and characterisation of a monoclonal antibody against the seed lectin of the Dolichol biflorus plant. J. Biol. Chem. 256 (1981) 4723-4725.

218. Hsu, I.C., Yolken, R.H. and Harris, C.C.: Ultrasensitive enzymic radioimmunoassay. In: Methods in Enzymology (Edited by J.J. Langone and H. van Vunakis). Academic Press, New York, Vol. 73 (1981) pp. 383-394.

219. Surolia, A., Pain, D. and Khan, M.I.: Protein A: nature's universal anti-antibody. TIBS Feb (1982) 74-76.

220. Noonan, K.D., Starling, J.J. and Seitz, T.L.: Application of monoclonal antibodies to the study of the distribution of cell surface proteins: prospects and problems. In Monoclonal Antibodies in Endocrine Research (Edited by R.E. Fellows and G.S. Eisenbarth). Raven Press, New York, (1981).

221. Kennett, R.M., McKearn, T.J. and Bechtol, U.B. (Eds.): Monoclonal Antibodies. Hybridomas: A new dimension in biological analysis. Plenum Press, New York and London, (1981).

22. Phillips, H.J.: Dye exclusion test for cell viability. In: Tissue Culture Methods and Applications (Edited by P.F. Kruse, Jr. and M.K. Patterson, Jr.) Academic Press, New York, (1973) pp. 406-408.

Author Bornman Liza
Name of thesis Partial Purification Of And Attempted Monoclonal Antibody Production Against The Vervet Mokey Hepatic Oestrogen-binding Protein. 1988

PUBLISHER:
University of the Witwatersrand, Johannesburg
©2013

LEGAL NOTICES:

Copyright Notice: All materials on the University of the Witwatersrand, Johannesburg Library website are protected by South African copyright law and may not be distributed, transmitted, displayed, or otherwise published in any format, without the prior written permission of the copyright owner.

Disclaimer and Terms of Use: Provided that you maintain all copyright and other notices contained therein, you may download material (one machine readable copy and one print copy per page) for your personal and/or educational non-commercial use only.

The University of the Witwatersrand, Johannesburg, is not responsible for any errors or omissions and excludes any and all liability for any errors in or omissions from the information on the Library website.