Production and characterization of a monoclonal antibody to biotin

Krishnamurti DAKSHINAMURTI,*† Rajinder P. BHULLAR,* Anne SCOOT,* Edward S. RECTOR,† Guy DELESPESE† and Alec H. SEHON†

Departments of *Biochemistry and †Immunology, Faculty of Medicine, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Canada R3E 0W3

Monoclonal antibodies to biotin have been prepared by using biotin linked to keyhole limpet haemocyanin (KLH) as the antigen. Spleen cells obtained from mice immunized with biotin–KLH were fused with the myeloma cell line NS-1. The resulting hybridomas were screened for the production of antibodies to biotin using an enzyme-linked immunosorbent assay. Clones producing antibodies to biotin were isolated by limiting dilution methods. Four cell lines, each derived originally from a different fusion, were chosen for the production of monoclonal antibodies. The monoclonal antibodies obtained have been characterized with respect to their ability to interact with biotin, biotin–bovine serum albumin, biotin–KLH and biocytin as well as to inhibit biotin-dependent enzymes. They have been used to produce cellular biotin deficiency *in vitro* for studies of biotin function.

INTRODUCTION

Biotin is an essential requirement for cells in culture (Dakshinamurti & Chalifour, 1981). Its obligatory involvement in carbohydrate and lipid metabolism is related to its role as the prosthetic group of acetyl-CoA carboxylase, propionyl-CoA carboxylase, β -methylcrotonyl-CoA carboxylase and pyruvate carboxylase (Mistry & Dakshinamurti, 1964; Wood & Bardeen, 1977). A stimulatory role for biotin in RNA and protein synthesis (Boeckx & Dakshinamurti, 1974, 1975) as well as in enhancement of guanylate cyclase (Vesley, 1982) and glucokinase (Dakshinamurti & Cheah-Tan, 1968; Spence & Koudelka, 1984) activity have been reported. Addition of biotin to biotin-deficient HeLa cell cultures results in an increase in the rate of DNA and protein syntheses and also cell growth (Bhullar & Dakshinamurti, 1985a). The possibility that biotin fulfils a role in metabolism other than as the prosthetic group of biotin-containing carboxylases has been examined (Dakshinamurti *et al.*, 1985). The high affinity between the glycoprotein avidin and biotin has been exploited in various areas of biological research, in particular, affinity cytochemistry (Bayer & Wilchek, 1980; Korpela, 1984). The restrictions of this method have also been recognized (Bayer & Wilchek, 1980). Various cell lines, such as HeLa, baby hamster kidney and human skin fibroblasts, have been shown to bind and internalize avidin (Dakshinamurti & Chalifour, 1981; Chalifour & Dakshinamurti, 1982). In view of this we have explored the possibility of producing monoclonal antibodies to biotin for use in studies on the cellular functions of biotin. Antibodies produced by the traditional methods are heterogenous and hence are not true analytical reagents. We report here the production and characterization of monoclonal antibodies to biotin.

EXPERIMENTAL

Materials

Eagle's Minimal Essential Medium (EMEM), fetal bovine serum, penicillin G, streptomycin, fungizone, hypoxanthine and Freund's complete adjuvant were purchased from Grand Island Biological Company (St. Louis, MO, U.S.A.). L-[4,5-³H]Leucine (62 Ci/mmol) was purchased from Amersham Corp. (Oakville, Ontario, Canada). KLH, biotin, carbodi-imide, acetyl-CoA, *N*-hydroxysuccinimidobiotin, ATP, BSA and propionyl-CoA were obtained from Sigma Chemical Co. [³H]Biotin (10 Ci/mmol) was a gift from Hoffman–LaRoche Inc. (Nutley, NJ, U.S.A.). Affi-Gel Protein A MAPS kit and reagents for polyacrylamide-gel electrophoresis were obtained from Bio-Rad Laboratories (Mississauga, Ontario, Canada). NaH¹⁴CO₃ (40–60 mCi/mmol) and Aquasol-2 scintillant were purchased from New England Nuclear Corp. (Montreal, Canada). Horseradish peroxidase-conjugated affinity-purified goat anti-[mouse IgG + IgM (H + L)] was obtained from Kirkegaard & Perry Labs (Gaithersburg, MD, U.S.A.). Pristane (1,6,10,14-tetramethylpentadecane) was purchased from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). All other chemicals used were of reagent grade.

Antigen preparation

Biotin was covalently attached to KLH. Activated biotin (*N*-hydroxysuccinimidobiotin; 10 mg) was added to 62 mg of KLH dissolved in 5.0 ml and 0.2 M-borate buffer (pH 9.4). This was stirred for 6 h at room temperature and dialysed for 24 h against four changes of 4 litres each of 0.9% NaCl. The biotin–KLH was used as the antigen.

Abbreviations used: KLH, keyhole limpet haemocyanin; BSA, bovine serum albumin; EMEM, Eagle's Minimal Essential Medium; PBS, phosphate-buffered saline; e.l.i.s.a., enzyme-linked immunosorbent assay; IBS, imidazole-buffered saline.

† To whom correspondence and reprint requests should be addressed.

Biotin was covalently attached to BSA according to the procedure of Berger (1979). Biotin (500 mg) and 5 μ Ci of [3 H]biotin were suspended in 7.5 ml of 50% aqueous pyridine with constant stirring at room temperature. Carbodi-imide (2.5 g) was dissolved in 12.5 ml of 50% aqueous pyridine, and this mixture was added dropwise to the biotin suspension. Stirring was continued for an additional 30 min at room temperature during which time a clear solution was formed. Albumin (250 mg) dissolved in 6.25 ml of water was added dropwise to the biotin/carbodi-imide reaction mixture and stirring was continued at room temperature for 4.5 h. The mixture was dialysed for 24 h against four changes of 4 litres each of 0.9% NaCl. Determination of the radioactivity before and after dialysis indicated approx. 8 mol of biotin bound/mol of bovine serum albumin.

Immunization

Female BALB/c mice were immunized by two subcutaneous injections of 5 μ g of biotin-KLH emulsified in Freund's complete adjuvant (0.1 ml of emulsion/injection per mouse) at a 1 month interval. At 3 weeks after the second injection and 3 days before fusion, the mice were injected intraperitoneally with 5 μ g of biotin-KLH in 0.5 ml of PBS. At the time of killing, serum was collected to provide a positive control in subsequent immunoassays.

Cell fusion and cloning procedures

The cell fusion procedure using NS-1 myeloma cells and poly(ethylene glycol) as the fusing agent has been described in detail elsewhere (Rector *et al.*, 1985). Six individual fusions were performed, generating a total of 576 primary cultures. Supernatants were tested for the mouse anti-biotin antibodies 11 days following fusion. Selected cultures (150) were subsequently expanded and retested for antibody activity. Of these, four cultures were selected for the derivation of monoclonal cell lines. Using the limiting dilution procedure a total of 188 clones were obtained subsequently, of which 20 were selected for expansion and storage in liquid N₂. Four of these cell lines, each derived originally from a different fusion, were chosen for the production of the monoclonal antibodies reported in this paper.

E.l.i.s.a.

The initial screening and selection of culture supernatants containing antibodies to biotin was undertaken by employing an e.l.i.s.a. Biotin-BSA, 0.1 ml, at a concentration of 10 μ g/ml in Na₂CO₃ buffer (pH 9.0), was used to coat the wells of 96-well flat-bottomed polystyrene microtitre plates (Costar, Cambridge, MA, U.S.A.). After incubation at 4 °C overnight, the wells were washed once and incubated for 5 min with a solution of 1% BSA in PBS (PBS/BSA). The plates were then inverted, shaken, and immediately thereafter culture supernatants or control serum samples were placed into the wells (0.1 ml/well) and the plates incubated at room temperature for 2 h. Following removal of the samples and one wash with 0.02 M-IBS containing 0.02% Tween 20 (IBS/Tween), peroxidase-conjugated affinity-purified goat anti-[mouse IgG+IgM (H+L)] diluted 1:100 in PBS/BSA was placed into the wells (0.1 ml/well). The plates were then incubated at room temperature for 2 h with gentle shaking. The wells were washed five times with IBS/Tween and a substrate solution containing 2,2'-

azino-di-(3-ethyl-benzthiazoline sulphonate) and H₂O₂ in cacodylate buffer was added (0.1 ml/well). The apparent absorbance at 405 nm generated in each well was determined after a final incubation of 10 min at room temperature. Antiserum obtained from immunized mice provided a positive control for all assays. Normal mouse serum and tissue culture medium were used as negative controls.

Saturation analysis by e.l.i.s.a.

Monoclonal antibody-containing supernatants from hybridoma cultures were mixed with 100 μ l of varying concentrations of biotin-BSA (0–1000 μ g/ml), biotin-KLH (0–1000 μ g/ml), biotin (10⁻²–10⁻¹⁰ M) and biocytin (10⁻²–10⁻¹⁰ M). After incubation at 4 °C for 24 h, the antibody/antigen mixtures were transferred into 96-well microtitre plates that had been previously coated with biotin-BSA. After incubation for 24 h at 4 °C, unbound proteins were removed by three 2 min washes with IBS/Tween. The second antibody was added and the e.l.i.s.a. completed as described above.

Production and purification of monoclonal antibodies

Female BALB/c mice were primed by intraperitoneal injection (0.5 ml/mouse) of pristane. After 2 weeks, monoclonal hybridoma cells (5 $\times$ 10⁶) were injected intraperitoneally in 0.5 ml of culture medium. Ascites fluids were collected after approx. 10 days, placed in a tube containing heparin and clarified by centrifugation.

Immunoglobulins from the ascites fluid were purified using Affi-Gel Protein A MAPS system. The Affi-Gel Protein A column (0.5 cm $\times$ 10 cm) was equilibrated with 5 bed vol. of binding buffer. Ascites fluid (2 ml) was diluted with an equal volume of binding buffer and applied to the column. The column was washed with 10 vol. of binding buffer. The IgG was eluted by washing the column with 15 vol. of elution buffer. Protein-containing fractions were pooled and dialysed against 10 mM-Tris/HCl (pH 7.5) buffer. Purity of the antibody was estimated by electrophoresis on 10% (w/v) SDS/polyacrylamide gels according to the method of Laemmli (1970).

Inhibition of acetyl-CoA carboxylase and propionyl-CoA carboxylase by monoclonal antibodies to biotin

The ability of monoclonal antibodies raised against biotin to inhibit the activity of the biotin-containing enzymes, acetyl-CoA and propionyl-CoA carboxylases, was investigated by using a 30% (NH₄)₂SO₄ fraction of rat liver cytosol (Gillevet & Dakshinamurti, 1982). A sample of rat liver cytosol was incubated for 15 min at 37 °C with ascites fluid containing monoclonal antibody to biotin. At the end of this time, enzyme activity was determined. Acetyl-CoA carboxylase activity was determined by the H¹⁴CO₃-fixation assay (Dakshinamurti & Desjardins, 1969). Propionyl-CoA carboxylase activity was measured according to procedure of Lane & Halenz (1962).

One unit of enzyme activity is defined as that amount which catalyses the carboxylation of 1 μ mol of substrate/min at 37 °C into acid-stable product.

Protein measurement

Protein content was determined according to the procedure of Lowry *et al.* (1951). Bovine serum albumin was used to construct the standard curve.

Cells and culture method

HeLa 229 cells (Nelson-Rees & Flandmeyer, 1976) were routinely cultured as a monolayer in EMEM supplemented with 5% fetal bovine serum, 100 units of penicillin G/ml, 100 μ g of streptomycin/ml and 2.5 μ g of fungizone/ml. HeLa cells were made biotin-deficient by passaging in EMEM supplemented with 5% biotin-deficient fetal bovine serum. Fetal bovine serum was rendered biotin-deficient by using Sepharose-avidin gel (Dakshinamurti & Chalifour, 1981).

Uptake of monoclonal antibody by HeLa cells in culture

Two different procedures were used to investigate the uptake of monoclonal antibody by HeLa cells in culture. In the first procedure the ability of monoclonal antibody to inhibit acetyl-CoA carboxylase activity *in vivo* was monitored. HeLa cells were subcultured and placed in EMEM supplemented with 5% fetal bovine serum for 24 h. At the end of this time period, medium was poured off and cells rinsed with warm EMEM. The cells were then placed in EMEM supplemented with 5% lipid-free fetal bovine serum (Bhullar & Dakshinamurti, 1985b). After 2 days, monoclonal antibody was added to the cell culture medium, and 6 h later cells were removed from the culture plates and washed three times with PBS (pH 7.5). Acetyl-CoA carboxylase activity was determined in a portion of the cell sonicate according to the procedure previously described (Bhullar & Dakshinamurti, 1985b).

In the alternative procedure, monoclonal antibody was reacted at 37 °C with [³H]biotin. Unbound [³H]biotin was removed by extensive dialysis of antibody-biotin complex at 4 °C against 0.15 M-NaCl. A portion (40 μ g) of antibody-biotin complex was added to the cell culture plates; 6 h later, cells were removed from the plates with a scraper and washed three times with cold PBS (pH 7.5). The cells were suspended in 10 mM-Tris/HCl (pH 7.2) containing 0.15 M-NaCl and sonicated in a Bronwill sonicator. A portion (25 μ l) of the sonicate was transferred to a scintillation vial containing 10 ml of Aquasol-2 and radioactivity was determined in a Beckman LS 2800 liquid-scintillation spectrometer.

Effect of monoclonal antibody on cellular protein synthesis in HeLa cells

Biotin-deficient HeLa cell cultures, after the second passage in the deficient medium, were supplemented with monoclonal antibody for 6 h while identical cell cultures were exposed to non-immune γ -globulin. An identical cell culture was left in the biotin-deficient medium. After 6 h, cells were removed from the respective culture dishes, washed three times with PBS (pH 7.5) and resuspended in 1 ml of leucine-free EMEM. The cells were then incubated for 20 min at 37 °C with L-[4,5-³H]leucine (10 μ Ci/ml). At the end of this incubation period, cells were washed three times with cold PBS, resuspended in 0.3 ml of 10 mM-Tris/HCl (pH 7.2) containing 0.15 M-NaCl and sonicated in a Bronwill sonicator. Aliquots of the sonicate were placed on Whatman 3 MM filter papers and washed successively with cold 10% (w/v) trichloroacetic acid, 5% trichloroacetic acid (twice) and ethanol. The filter papers were dried, transferred to scintillation vials containing 10 ml of Aquasol-2 and radioactivity was determined in a Beckman LS-2800 liquid-scintillation spectrometer.

RESULTS

Development of monoclonal antibodies

Fusion of spleen cells obtained from mice immunized with biotin-KLH with the murine myeloma cell line, NS-1, yielded a highly successful fusion. The supernatants from initial fusion cultures were all strongly positive for anti-biotin antibodies, and of the crude cultures chosen for secondary screening all retained the ability to produce anti-biotin antibodies. Limiting dilutions of crude cultures yielded approx. 50% of the possible number of single clones (188/384) and of these 75% were positive for anti-biotin antibodies. Monoclonal antibody was obtained by ascites production in mice using four different clones (nos. 28, 33, 38, 142). One clone, no. 33, produced antibody of high affinity that bound both free and haptenic biotin antigens (Figs. 1a-1c) as well as biocytin (Fig. 1d). It is seen from Fig. 1 that both biotin-BSA and biotin-KLH inhibit antibody binding to solid phase several orders of magnitude more than does free biotin. The profiles of the binding of biotin-BSA and biotin-KLH are similar and on that basis they should have an equal number of binding sites. It is not possible to calculate the molar ratios of binding between free biotin and biotin-KLH as can be done for free biotin and biotin-BSA, in view of the wide range (3×10^5 - 9×10^6) given for the molecular mass of haemocyanin. However, the fact that protein-bound biotin inhibits antibody binding to the solid phase more effectively, on a molar basis, than does free biotin should facilitate biological use of this antibody, since cellular and circulating biotin is mostly protein-bound. Antibody produced by clone no. 33 was also highly effective at inhibiting the activity of the biotin-containing enzyme, propionyl-CoA carboxylase (Table 1). The other clones produced antibody of much lower affinity than that from clone 33 and were 1000-fold less effective at inhibiting propionyl-CoA carboxylase activity. The activity of acetyl-CoA carboxylase was inhibited in a fashion similar to that for propionyl-CoA carboxylase (4.25×10^3 m-units of acetyl-CoA carboxylase inhibited by 1 ml of ascites fluid from clone number 33). There was no inhibition of either carboxylase when they were incubated with ascites fluid which did not contain anti-biotin antibody.

Purification of antibody from ascites fluid

Monoclonal antibody was purified from ascites fluid produced from clone number 33. Affi-Gel Protein A MAPS system (Bio-Rad laboratories) was utilized. This system resulted in a one-step purification of antibody from the ascites fluid. SDS/polyacrylamide-gel electrophoretic analysis showed only two major protein-staining bands corresponding to the immunoglobulin heavy and light chains respectively, thus confirming the high purity of the final antibody preparation (Fig. 2). The faint band adjacent to the heavy chain band seen on this slightly overloaded gel may reflect (a) low levels of an unrelated protein, (b) low levels of heavy chains derived from normal immunoglobulins present in ascites fluid, or (c) post-translational changes of the monoclonal heavy chain. The purified antibody was effective at inhibiting both acetyl-CoA carboxylase and propionyl-CoA carboxylase activities [2.59 m-units of acetyl-CoA carboxylase and 2.33 m-units of propionyl-CoA carboxylase respectively inhibited per μ l of antibody (0.13 μ g of protein)].

Fig. 1. Relative affinities of monoclonal anti-biotin antibodies for biotin, biotin-BSA, biotin-KLH and biocytin

The relative affinity of monoclonal antibodies (clone nos. indicated on the Figure) for different biotin-containing antigens was determined by competition saturation analyses. The monoclonal antibodies were incubated overnight at 4 °C with varying concentrations of antigen prior to performing an e.l.i.s.a. with biotin-BSA as the plate-coating antigen. B/B_0 represents the amount of monoclonal antibody bound to the plates in the presence of competing antigen (B) relative to the amount bound in the absence of competing antigen (B_0).

Table 1. Inhibition of propionyl-CoA carboxylase by monoclonal antibodies directed toward biotin

Rat liver propionyl-CoA carboxylase was incubated for 15 min at 37 °C with ascites fluid containing monoclonal antibody to biotin. At the end of the incubation, enzyme activity was measured as described in the Experimental section.

Clone no.	Propionyl-CoA carboxylase (m-units) inhibited by 1 ml of ascites fluid
28	14.6
33	15.2×10^3
38	10.4
142	14.9

Uptake of antibody by HeLa cells in culture

When purified monoclonal antibody was added to culture medium of HeLa cells it was taken up as indicated by the inhibition of acetyl-CoA carboxylase activity (Table 2). Control immunoglobulin did not affect the activity of acetyl-CoA carboxylase; 6 μ g of [3 H]biotin-antibody complex was taken up per mg of cell protein.

Effect of antibody on protein synthesis

When purified monoclonal antibody was added to the culture medium of second passage biotin-deficient HeLa cells, the rate of protein synthesis as measured by [4,5- 3 H]leucine incorporation was significantly decreased as compared with control cells (Table 3). The rate of protein synthesis in control cells remained unaffected by the addition of nonimmune serum to the culture medium (Table 3).

Fig. 2. Analysis by SDS/polyacrylamide-gel electrophoresis

Purified monoclonal antibody (10 μ g) (lane 2) and ascites fluid (10 μ g) (lane 3) was denatured by heating at 100 °C for 2 min in the presence of 1% SDS and 5% 2-mercaptoethanol. Lane 1 shows the polypeptide standards: phosphorylase *b* (M_r 97400), glutamate dehydrogenase (55400), lactate dehydrogenase (36500) and trypsin inhibitor (20100).

DISCUSSION

Monoclonal antibody raised against biotin was effective in binding biotin, haptenic biotin and biocytin (Fig. 1). The monoclonal antibody was purified from the ascites fluid in a single step by using the Affi-Gel Protein A MAPS system. When the final preparation was analyzed by SDS/polyacrylamide-gel electrophoresis, only the light and heavy immunoglobulin chains were observed (Fig. 2). Immune serum containing antibodies to the haptenic group biotin have been prepared by immunization of rabbits with biotin-BSA (Ahmad *et al.*, 1981). However, the multivalent specificity of these antibodies makes them a less attractive reagent in

Table 2. Inhibition of acetyl-CoA carboxylase *in vivo* by monoclonal antibody to biotin

HeLa cells cultured in lipid-free serum were exposed to monoclonal antibody or nonimmune serum for 6 h. At the end of this time period, cells were removed from culture dishes, washed with PBS and acetyl-CoA carboxylase activity was assayed as described in the Experimental section. Results represent mean $\pm$ S.D. for four separate determinations.

Status	Acetyl-CoA carboxylase activity (m-units/mg of protein)
Control	2.13 $\pm$ 0.20
Control + nonimmune serum	2.04 $\pm$ 0.15
Control + monoclonal antibody to biotin	1.04 $\pm$ 0.10

Table 3. Effect of monoclonal antibody on protein synthesis in HeLa cells

HeLa cells cultured in biotin-deficient medium for two passages were exposed to nonimmune serum or monoclonal antibody to biotin for 6 h. At the end of this time period cells were removed from culture dishes and rate of protein synthesis were determined as described in the Experimental section. The results represent means $\pm$ S.D. for three separate determinations in each group.

Status	[3 H]Leucine incorporated (pmol/ μ g of protein)
Control	2.20 $\pm$ 0.12
Control + nonimmune serum	2.34 $\pm$ 0.18
Control + monoclonal antibody to biotin	1.44 $\pm$ 0.16

assessing the role of biotin in metabolism. This led us to develop a monoclonal antibody to biotin.

The monoclonal antibody can be used in assessing the role of the biotinyl group in biotin-containing enzymes. Acetyl-CoA carboxylase of mammalian origin exists in the cell as an inactive protomer, which is converted into the enzymically active form in the presence of citrate (Lane *et al.*, 1974). As the monoclonal antibody to biotin can be taken up by HeLa cells in culture, the antibody can be used to investigate the cellular protomer/polymer ratio of acetyl-CoA carboxylase under different nutritional and hormonal conditions. Assay *in vitro* for acetyl-CoA carboxylase has shown that the polymerized enzyme is not inactivated by the monoclonal antibody whereas it is inactivated when treated with antibody prior to polymerization [acetyl-CoA carboxylase + citrate (polymerized enzyme), 8.95 m-units/mg of protein; acetyl-CoA carboxylase + anti-biotin antibody, added prior to polymerization, + citrate, 0 m-units/mg of protein; acetyl-CoA carboxylase + citrate + anti-biotin antibody, added after enzyme polymerization, 8.92 m-units/mg of protein].

Monoclonal antibody to biotin was able to decrease the rate of protein synthesis in HeLa cells in culture. Previously, we have shown that the rate of protein synthesis is decreased in biotin-deficient HeLa cells as compared with cells cultured continuously in the presence of biotin (Bhullar & Dakshinamurti, 1985a). The effect of monoclonal antibody is observed rapidly even though cells were not completely biotin-deficient before addition of the antibody to the medium (Table 3). The function of biotin in cellular processes can be studied using monoclonal antibody.

The high affinity between biotin and avidin has been utilized in various areas of biological research. Of much significance is the use of the avidin-biotin complex for the localization and evaluation of cell surface receptors. In these studies, biotin is attached to cell surface functional groups through an appropriate reactive derivative. Subsequent incubation with ferritin-avidin conjugate has been used for the ultrastructural visualization of the cell surface receptor. Alternatively, biotinylated membrane sites are saturated with free avidin. Subsequent interaction with biotin-conjugated ferritin has also been used for the specific labelling of the cell surface receptor. It has been assumed that coupling a small molecule such as biotin to the receptor would only slightly affect binding characteristics. In some instances this has been verified prior to the use of this technique. However, this is not always so. Thus, the addition of avidin to the medium of biotinylated lymphocytes has been used to mimic lectin-induced stimulation. Whereas the lectin-induced stimulation of lymphocytes is reversible, the stimulation observed with avidin was irreversible (Bayer & Wilchek, 1980). Before using the versatile avidin-biotin complex method, it must be determined whether this interaction in the experimental system under study comprises a biotin-containing, biotin-recognizing or biotin-free system. Avidin is a basic glycoprotein (Green, 1975). The oligosaccharide moiety or its ion-exchange properties might contribute to side reactions. It has been shown that avidin, perhaps mimicking a natural ligand, binds to isolated rat liver plasma membrane (Chalifour & Dakshinamurti, 1983) and to a variety of cells studied (Dakshinamurti & Chalifour, 1981; Chalifour & Dakshinamurti, 1982). This emphasizes the need for proper controls for applying the avidin-biotin complex technique to affinity cytochemical studies. The use of monoclonal antibody in these studies would obviate such problems. Biotin transport as well as the non-prosthetic group function of biotin could be studied by using the specific antibody. Antibiotin antibody could also be used in isolation and purification procedures where the affinity between biotin and avidin prove to be too strong to be useful.

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REFERENCES

- Ahmad, F., Ahmad, P. M., Dickstein, R. & Greenfield, E. (1981) *Biochem. J.* **197**, 95-104
- Bayer, E. A. & Wilchek, M. (1980) *Methods Biochem. Anal.* **26**, 1-45
- Berger, M. (1979) *Methods Enzymol.* **62**, 319-326
- Bhullar, R. P. & Dakshinamurti, K. (1985a) *J. Cell. Physiol.* **123**, 425-430
- Bhullar, R. P. & Dakshinamurti, K. (1985b) *Biosci. Rep.* **5**, 491-496
- Boeckx, R. L. O. & Dakshinamurti, K. (1974) *Biochem. J.* **140**, 549-556
- Boeckx, R. L. O. & Dakshinamurti, K. (1975) *Biochim. Biophys. Acta* **383**, 282-289
- Chalifour, L. E. & Dakshinamurti, K. (1982) *Biochem. Biophys. Res. Commun.* **104**, 1047-1053
- Chalifour, L. E. & Dakshinamurti, K. (1983) *Biochem. J.* **210**, 121-128
- Dakshinamurti, K. & Chalifour, L. E. (1981) *J. Cell. Physiol.* **107**, 427-438
- Dakshinamurti, K. & Cheah-Tan, C. (1968) *Arch. Biochem. Biophys.* **127**, 17-21
- Dakshinamurti, K. & Desjardins, P. R. (1969) *Biochim. Biophys. Acta* **176**, 221-229
- Dakshinamurti, K., Chalifour, L. E. & Bhullar, R. P. (1985) *Ann. N.Y. Acad. Sci.* **445**, 38-55
- Gillevet, P. M. & Dakshinamurti, K. (1982) *Biosci. Rep.* **2**, 841-848
- Green, N. M. (1975) *Adv. Prot. Chem.* **29**, 85-133
- Korpela, J. (1984) *Med. Biol.* **62**, 5-26
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680-685
- Lane, M. D. & Halenz, D. R. (1962) *Methods Enzymol.* **5**, 576-581
- Lane, M. D., Moss, J. & Polakis, S. E. (1974) *Curr. Top. Cell. Regul.* **8**, 139-195
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Mistry, S. P. & Dakshinamurti, K. (1964) *Vitam. Horm.* **22**, 1-55
- Nelson-Rees, W. & Flandmeyer, R. R. (1976) *Science* **191**, 96-98
- Rector, E., Nakajima, T., Rocha, C., Duncan, D., Lestourgeon, D., Mitchell, R. S., Fischer, J., Schon, A. H. & Delespesse, G. (1985) *Immunology* **55**, 481-488
- Scoot, A., Dakshinamurti, K., Rector, E., Delespesse, G. & Schon, A. (1985) *Ann. N.Y. Acad. Sci.* **445**, 423-424
- Spence, J. T. & Koudelka, A. P. (1984) *J. Biol. Chem.* **259**, 6393-6396
- Vesley, D. L. (1982) *Science* **216**, 1329-1330
- Wood, H. G. & Bardeen, R. F. (1977) *Annu. Rev. Biochem.* **46**, 385-414