tive organs supports the concept that virus titers are enhanced only in organs that already contain some free virus. Further studies are needed to determine whether any latently infected cells were converted to producer cells. The possibility that skin allograft placement somehow permitted increased seeding of MCMV from other sites such as the salivary glands, where the virus is present in high titer, is considered unlikely. Viremia was rare at this stage of infection and virus titers in other organs, such as the liver and the lung, were not enhanced.

Recently, Olding and his co-workers (*13*) activated and recovered MCMV from the spleen cells of 2- and 5-month-old mice infected in utero or at birth by cocultivation with allogeneic fibroblasts. No virus was detected when disrupted spleen cells were cultured. The latently infected cells were apparently B lymphocytes. These experiments showing activation of MCMV by allogeneic reaction in vitro may represent the counterpart of our findings in the animal.

Mouse leukemia virus has been activated in inapparently infected mice by the transfer of parental spleen cells to an F_1 recipient, producing a graft-versus-host reaction (*14*). Later, Hirsch and his associates (*15*) found that latent leukemia virus was activated by a combination of skin allograft and administration of antilymphocyte serum. The mechanism by which mouse leukemia virus is activated is not known, but there is evidence that activation is not solely a function of lymphocyte blast transformation (*16*).

Lopez and co-workers (*4*) found that CMV infections in renal transplant recipients are temporally associated with graft rejection episodes. They proposed that either the renal allograft activates a latent CMV infection or that the viral infection in some way promotes graft rejection. Evidence indicating that CMV enhances cell-mediated immune responses would support the latter hypothesis. Actually, the opposite appears to be the case in mice. Howard and associates (*17*) found that skin grafts made across strong (H-2) and weak (H-Y) histoincompatibility barriers had prolonged survival times when the recipient mice were acutely infected with MCMV. Graft survival time was significantly increased in mice infected up to 7 days before grafting. In addition, spleen cells harvested from mice infected with MCMV showed decreased uptake of tritiated thymidine in response to phytohemagglutinin and in mixed lymphocyte culture. Our studies show that a skin allograft from a histoincompatible donor enhances MCMV infection in recipient mice. These two observations taken together support the concept that host response to the allograft activates CMV infection in renal transplant recipients, rather than the reverse.

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References and Notes

1. D. Rifkind, T. E. Starzl, T. L. Marchioro, W. R. Waddell, D. T. Rowlands, Jr., R. B. Hill, Jr., *J. Am. Med. Assoc.* **189**, 808 (1964).
2. J. E. Craighead, J. B. Hanshaw, C. B. Carpenter, *ibid.* **201**, 725 (1967).
3. H. K. Andersen and E. S. Spencer, *Acta Med. Scand.* **186**, 7 (1969).
4. C. Lopez, R. L. Simmons, S. M. Mauer, J. S. Najarian, R. A. Good, *Am. J. Med.* **56**, 280 (1974).
5. V. A. Fulginiti, R. Scribner, C. G. Groth, C. W. Putnam, L. Brettschneider, S. Gilbert, K. A. Porter, T. E. Starzl, *N. Engl. J. Med.* **279**, 619 (1968).
6. P. Neiman, P. B. Wasserman, B. B. Wentworth, G. F. Kao, K. G. Lerner, R. Storb, C. D. Buckner, R. A. Clift, A. Fefer, L. Fass, H. Glucksberg, E. D. Thomas, *Transplantation* **15**, 478 (1973).
7. L. Kääriäinen, J. Paloheimo, E. Klemola, T. Mäkelä, A. Koivuniemi, *Ann. Med. Exp. Biol. Fenn.* **44**, 297 (1966); D. P. Stevens, L. F. Barker, A. S. Ketcham, H. M. Meyer, Jr., *J. Am. Med. Assoc.* **211**, 1341 (1970); A. M. Prince, W. Szmuness, S. J. Millian, D. S. David, *N. Engl. J. Med.* **284**, 1125 (1971).
8. D. J. Lang, *Arch. Gesamte Virusforsch.* **37**, 365 (1972).
9. R. N. Fine, C. M. Grushkin, M. Malekzadeh, H. T. Wright, Jr., *Arch. Surg.* **105**, 564 (1972); A. S. Coulson, Z. J. Lucas, M. Condy, R. Cohn, *West. J. Med.* **120**, 1 (1974).
10. D. Rifkind, N. Goodman, R. B. Hill, *Ann. Intern. Med.* **66**, 1116 (1967).
11. R. L. Simmons, R. Weil, III, M. B. Tallent, C. M. Kjellstrand, J. S. Najarian, *Transplant. Proc.* **2**, 419 (1970); D. S. Davis, S. J. Millian, J. C. Whitsell, G. H. Schwartz, R. R. Riggio, K. H. Stenzel, A. L. Rubin, *Ann. Surg.* **175**, 257 (1972); J. D. Briggs, M. C. Timbury, A. M. Paton, P. R. F. Bell, *Br. Med. J.* **4**, 520 (1972).
12. J. E. Osborn and D. L. Walker, *Infect. Immun.* **3**, 228 (1970).
13. L. B. Olding and M. B. A. Oldstone, *Fed. Proc.* **33**, 788 (1974); L. B. Olding, F. C. Jensen, M. B. A. Oldstone, *J. Exp. Med.* **141**, 561 (1975).
14. M. S. Hirsch, P. H. Black, G. S. Tracy, S. Leibowitz, R. S. Schwartz, *Proc. Natl. Acad. Sci. U.S.A.* **67**, 1914 (1970).
15. M. S. Hirsch, D. A. Ellis, P. H. Black, A. P. Monaco, M. L. Wood, *Science* **180**, 500 (1973).
16. M. S. Hirsch, S. M. Phillips, C. Solnik, P. H. Black, R. S. Schwartz, C. B. Carpenter, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1069 (1972); S. M. Phillips, M. S. Hirsch, J. André-Schwartz, C. Solnik, P. H. Black, R. S. Schwartz, J. P. Merrill, C. B. Carpenter, *Cell. Immunol.* **15**, 169 (1975).
17. R. J. Howard, J. Miller, J. S. Najarian, *Clin. Exp. Immunol.* **18**, 119 (1974).
18. Supported by NIH research grant 5R01-AI 11798. Preliminary results of this investigation were presented at the Fourteenth Annual Interscience Conference on Antimicrobial Agents and Chemotherapy in San Francisco, California, 11 to 13 September 1974.

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Increased Urinary Excretion of Cyclic Guanosine Monophosphate in Rats Bearing Morris Hepatoma 3924A

Abstract. *Urinary excretion of cyclic guanosine monophosphate (GMP) increased in rats bearing Morris hepatoma 3924A, and a correlation coefficient of .842 was observed comparing nucleotide excretion and tumor size. Irradiation of tumor or 5-fluorouracil administration delayed the increases in urinary cyclic GMP and tumor size. Surgical removal of tumors resulted in a rapid decline in cyclic GMP excretion to baseline levels. Cyclic adenosine monophosphate excretion was not altered by implantation, irradiation, or excision of tumor.*

The urinary excretion of cyclic guanosine monophosphate (GMP) increased significantly in rats bearing explants of Morris hepatoma 3924A. The increased excretion of cyclic GMP was diminished with local irradiation of tumor, 5-fluorouracil administration, or after tumor excision; however, the excretion of cyclic adenosine monophosphate (AMP) was not altered. These studies are of interest in view of a previous report describing increased levels of cyclic GMP in hepatomas in vivo (*1*). Rapidly growing hepatomas (3924A and 7288 ctc) contain very high concentrations of cyclic GMP (20- to 200-fold greater than normal liver or slower growing hepatomas). In other studies, the addition of exogenous cyclic GMP or its analogs to cell cultures increased DNA, RNA, and protein synthesis and cell proliferation (*2*). Rudland *et al.* (*3*) also described an activation of particulate guanylate cyclase from cell cultures of mouse fibroblasts with a fibroblast growth factor. In addition, Voorhees *et al.* (*4*) reported increases in cyclic GMP in rapidly growing epidermis from psoriatic lesions. In contrast, increased cyclic AMP in cell cultures has been associated with decreased proliferation and morphological changes resembling differentiated functions (*5*). However, studies with hepatomas in vivo have demonstrated increased cyclic AMP compared to liver (*1, 6*). Thus, the in vivo studies with cyclic GMP are consistent with cell culture studies and suggest that increased cyclic GMP is associated with proliferation. There have been no reports describing altered urinary excretion of cyclic nucleotides with tumors other than several hormone secreting tumors (*7*). Some of the observations reported here have been presented in abstract form (*8*).

Suspensions of Morris hepatoma 3924A were injected subcutaneously into the backs of female ACI rats weighing 150 to 180 g (*9*). Throughout the study rats were provided free access to laboratory chow

and water. From 2 to 5 weeks after implantation of tumor, rats were placed in metabolic cages for daily urine collections. Urine was also collected from control animals without implanted tumors. Urines were collected at room temperature, and in most experiments collecting containers had 10 ml of 0.1*N* HCl. Urinary cyclic nucleotide levels were similar whether HCl was used as a preservative or not. At the end of each daily collection period portions of urine were stored at −20°C until they were assayed. With some animals, 14 days after tumor implantation the tumors were x-irradiated locally with 250 kv-peak at the rate of 800 roentgens per minute with a 0.5-mm copper and 1.0-mm aluminum filter by a 2-cm cone, for a total dose of 3750 roentgens. The rest of the body was shielded and received about 0.5 percent of the dose delivered to the irradiated tumor (*10*). Some animals were given 5-fluorouracil (150 mg/kg) intraperitoneally 21 days after tumor implantation (*9*). In other experiments, 38 days after tumor implantation rats were anesthetized with ether; one group of animals had a sham incision, and in a second group of animals the tumor was excised.

All animals were weighed daily, and tumor volume was estimated three times weekly with calipers (*9, 11*). Tumor volume was calculated on the assumption that tumors were hemiellipsoids where volume is one-half the product of length times width times height (*11*). Appropriately diluted urines were assayed for cyclic GMP with a modification (*12*) of the radioimmunoassay method of Steiner (*13*); cyclic AMP was assayed with a protein binding method (*14*). Urines were also assayed for creatinine content with an automated picric acid method. Values for urinary cyclic nucleotides are mean values ± the standard error of three to six rats in each group and are expressed as micromoles excreted per day per gram of urinary creatinine.

Daily urinary excretion of cyclic GMP and cyclic AMP in normal ACI rats was 3.20 ± 0.11 and 10.77 ± 0.85 μmole per gram of urinary creatinine, respectively. Within several weeks after tumor inoculation, cyclic GMP excretion increased significantly, while excretion of cyclic AMP was unaltered. With progressive tumor growth (Table 1), excretion of cyclic GMP continued to increase. After 46 days of tumor implantation, the longest time examined, urinary cyclic GMP was 68.5 ± 15.5 μmole/g and was, thus, increased 21-fold over normal values. Irradiation with 3750 roentgens locally to tumors 14 days after implantation delayed both tumor growth and the increase in cyclic GMP excretion (Table 1). Excretion of cyclic AMP was unaltered in nonirradiated or irradiated animals. In other experiments (not shown) the administration of 5-fluorouracil (150 mg/kg, intraperitoneally) also resulted in (i) delayed tumor growth (*9*) and (ii) decreased cyclic GMP excretion without alteration of cyclic AMP excretion. Urinary cyclic nucleotides were also examined after excision of tumors from animals. Within 1 day after tumor excision cyclic GMP excretion decreased to the normal range, while cyclic AMP excretion was unaltered (Table 2). Cyclic GMP excretion continued to increase in sham-operated animals. Cyclic AMP excretion was unaltered with a sham operation or tumor removal.

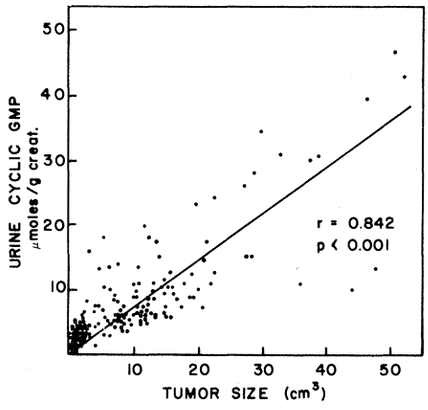

Fig. 1. Relation of urinary cyclic GMP to hepatoma 3924A size. The correlation coefficient (*r*) for cyclic GMP excretion and hepatoma 3924A implant size is .842 ($P < .001$; 186 determinations were made in 33 animals).

Studies from several laboratories have demonstrated that increased cyclic GMP or its exogenous administration correlates with increased cell culture proliferation (*2, 3*) and rates of tumor growth in vivo (*1*). It has also been postulated that the effect of a fibroblast growth factor on 3T3 cell culture proliferation is due to increased cyclic GMP synthesis and accumulation (*3*). The very high levels of cyclic GMP in Morris hepatoma 3924A, about 200-fold greater than in normal liver, led us to consider that cyclic GMP may be increased in the urines of rats bearing these tumors. The increased urinary excretion of cyclic GMP in ACI rats bearing this hepatoma implant (Fig. 1)

Table 1. Effect of local irradiation on urinary cyclic nucleotides in rats with hepatoma 3924A. Fourteen days after ACI rats were inoculated with suspensions of Morris hepatoma 3924A, 3750 R was administered locally to some tumor implants as indicated. Urinary cyclic nucleotides and tumor volume were determined as described. Values presented are means ± the standard error. There were six animals in each group.

Time (days) after:		Cyclic GMP (μmole/g creatinine)		Cyclic AMP (μmole/g creatinine)		Tumor volume (cm^3)	
Implantation	X-ray	No x-ray	X-ray	No x-ray	X-ray	No x-ray	X-ray
15	1	4.24±0.90	4.56 ±1.09	13.09±3.84	13.30±2.38	1.19± 0.23	1.32 ±0.24
16	2	6.15±0.64	3.67*±0.49	14.39±1.37	14.11±3.94		
19	5	7.57±1.54	2.82*±0.45	17.56±5.08	12.67±2.33		
21	7	9.40±1.87	2.98*±0.49	17.89±4.43	15.06±2.75	4.58± 0.78	0.89*±0.23
23	9	10.31±2.64	2.74*±0.26	12.41±1.26	10.28±1.06	7.32± 1.05	1.00*±0.22
29	15	17.17±1.03	4.13*±1.12	12.13±0.44	12.45±1.32	13.89± 2.01	1.37*±0.46
34	20	25.01±4.40	4.56*±1.05	13.69±0.47	12.89±1.24	27.39± 3.46	3.12*±1.42
41	27	31.79±5.80	8.63*±3.17	11.43±1.71	11.83±1.33	56.27±10.29	7.45*±3.33
46	32	68.55±15.5	13.89*±5.40	14.74±2.36	13.85±0.92	108.17±20.88	11.09*±7.27

*$P < .02$.

Table 2. Urinary cyclic nucleotides before and after surgical removal of hepatoma. Morris hepatoma 3924A was implanted in ACI rats. Thirty-eight days after tumor implantation either a sham incision or excision of tumor was performed as indicated. Values presented are means ± S.E. (three sham or six tumor-excised animals). The values for cyclic GMP excretion in this experiment are less than those in the experiment of Table 1. This is due to smaller inoculations of tumor; animals with tumor implants of similar sizes in both experiments had comparable excretions of cyclic GMP. Abbreviations: SO, sham operated; TE, tumor excised.

Time days after:		Cyclic GMP (μmole/g creatinine)		Cyclic AMP (μmole/g creatinine)	
Implantation	Surgery	SO	TE	SO	TE
35	−3	5.08 ± 0.34	6.04 ± 0.50	12.29 ± 1.50	10.40 ± 0.78
36	−2	5.62 ± 0.57	6.59 ± 0.34	11.16 ± 2.16	10.25 ± 0.97
37	−1	6.13 ± 0.78	7.04 ± 0.62	11.31 ± 2.72	10.09 ± 0.83
39	1	6.59 ± 0.58	1.87*† ± 0.15	11.50 ± 1.98	11.26 ± 1.07
40	2	8.57 ± 1.15	1.91*† ± 0.22	11.03 ± 1.70	11.14 ± 1.28
42	4	10.80† ± 1.63	2.21*† ± 0.19	13.46 ± 1.70	11.96 ± 1.11
43	5	12.21† ± 1.32	2.15*† ± 0.21	14.43 ± 3.27	13.09 ± 2.14

*$P < .02$ compared to SO. †$P < .03$ compared to nonoperated.

correlated with tumor size ($r = .842$). When tumor growth was inhibited with either local irradiation or administration of 5-fluorouracil, the increase in cyclic GMP excretion was also delayed. Furthermore, within 1 day of tumor excision cyclic GMP excretion returned to control values. No alterations in cyclic AMP excretion were observed under any of the conditions examined.

The increased excretion of cyclic GMP may result directly from the release or secretion of cyclic GMP by the tumor or an effect of the tumor on other tissue or tissues. Thus, the tissue source for the increased urinary cyclic GMP has not been established, and additional studies are needed. Nevertheless, the correlation of cyclic GMP excretion and tumor size in our study has important implications for basic research and diagnostic and therapeutic studies. If increased cyclic GMP excretion is observed with any types of tumors in patients, it could be useful for tumor diagnosis. The correlation of cyclic GMP excretion and tumor size and its alteration with irradiation, chemotherapy, or tumor removal also suggest that cyclic GMP excretion may be useful clinically in the assessment of therapeutic response and tumor recurrence in some patients. In any event, cyclic GMP excretion in rats bearing hepatomas can be used as an index of tumor growth and regression and should prove useful in examining this model system. Cyclic nucleotide excretion in animals and patients with other tumors and the source of the cyclic GMP in urine remain to be determined.

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References and Notes

1. E. W. Thomas, F. Murad, W. B. Looney, H. P. Morris, *Biochim. Biophys. Acta* **297**, 564 (1973).
2. J. W. Hadden, E. M. Hadden, M. K. Haddox, N. D. Goldberg, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3024 (19 ➡ R. Kram and G. M. Thomkins, *ibid.* **70**, 1659 (1973); W. Seifert and P. S. Rudland, *Nature (Lond.)* **248**, 138 (1974).
3. P. S. Rudland, D. Gospodarowicz, W. Seifert, *Nature (Lond.)* **250**, 741 (1974).
4. J. Voorhees, M. Stawiski, E. Duell, M. Haddox, N. Goldberg, *Life Sci.* **13**, 639 (1973).
5. W. L. Ryan and M. L. Heidrick, *Science* **162**, 1484 (1968); J. R. Sheppard, in *Cyclic AMP, Cell Growth and the Immune Response*, W. Braun, L. M. Lichtenstein, C. W. Parker, Eds. (Springer-Verlag, New York, 1974), p. 290.
6. R. Chayoth, S. Epstein, J. B. Field, *Biochem. Biophys. Res. Commun.* **49**, 1663 (1972).
7. F. Murad, *Adv. Cyclic Nucleotide Res.* **3**, 355 (1973).
8. ———, H. Kimura, H. P. Hopkins, W. B. Looney, *Proc. Am. Assoc. Cancer Res.* **16**, 138 (1975).
9. D. H. Russell, W. B. Looney, C. J. Kovacs, H. A. Hopkins, L. J. Morton, S. M. LeGendre, H. P. Morris, *Cancer Res.* **34**, 2382 (1974); C. J. Kovacs, H. A. Hopkins, R. M. Simon, W. B. Looney, *Br. J. Cancer*, in press.
10. W. B. Looney, J. S. Trefil, J. C. Schaffner, C. J. Kovacs, H. A. Hopkins, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2662 (1975).
11. W. B. Looney, A. A. Mayo, P. M. Allen, J. Y. Morrow, H. P. Morris, *Br. J. Cancer* **27**, 341 (1973).
12. H. Kimura, E. Thomas, F. Murad, *Biochim. Biophys. Acta* **343**, 519 (1974).
13. A. L. Steiner, C. W. Parker, D. M. Kipnis, *J. Biol. Chem.* **247**, 1106 (1972).
14. A. Gilman and F. Murad, *Meth. Enzymol.* **C 38**, 49 (1974).
15. We thank J. Holmes, J. Krisanda, J. Wakefield, and R. Aldred for technical assistance. Supported by PHS grants AM 15316, CA 12758, and CA 10729, a grant from the Virginia Heart Association, and a PHS research career development award (AM 70456) to F.M.

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Retinoic Acid Binding Protein: Occurrence in Human Tumors

Abstract. *Extracts of human carcinomas from lung and breast contain a protein which binds retinoic acid (vitamin A acid) with high specificity. The binding protein was not detected in normal lung or breast tissue from the same patients.*

The importance of vitamin A (retinol) for normal cellular differentiation of epithelial cells is firmly established (*1*). Vitamin A acid (retinoic acid) is produced in small quantities in vivo from retinol (*2*) and, if administered in the absence of retinol, can support growth and development of the rat but will not maintain vision (*3*) or reproductive function (*4*). The molecular mechanism of action of these compounds is obscure. Recently we have described the presence of two cellular binding proteins in rat tissue, one specific for retinol (*5*) and the other specific for retinoic acid (*6*). They are present in organs in very small quantities (0.3 to 1.5 μg per gram, wet weight) and possess high specificity for their respective ligands. We have found that the ability of *cis*-isomers of retinol to bind to the cellular retinol binding protein parallels their potency in promoting growth in the retinol-deficient rat (*7*), suggesting the involvement of the binding proteins in the mechanism of vitamin A action. Here we report the occurrence of the specific binding protein for retinoic acid in extracts from a limited number of human carcinomas. We could not detect the binding protein in extracts of nonmalignant tissue of the same organ from the same patients.

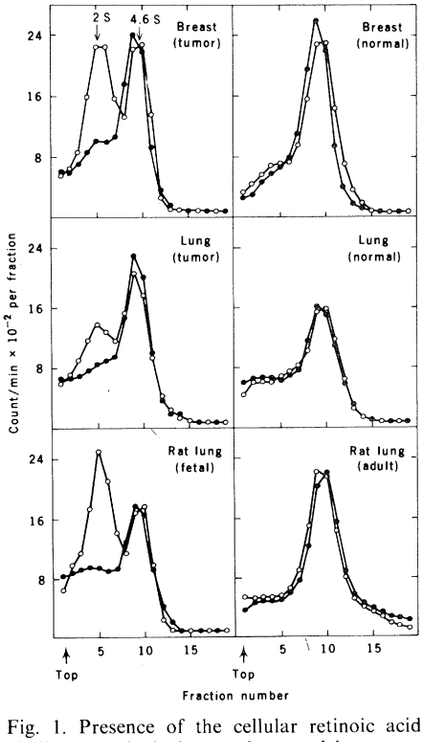

Fig. 1. Presence of the cellular retinoic acid binding protein in human lung and breast tumors and rat fetal lung. Portions of the tissue extracts were incubated with 40 nmole of [^{3}H]-retinoic acid (○) and with an additional 8 μmole of unlabeled retinoic acid (●). Portions (0.2 ml) of the incubation mixtures were subjected to sucrose gradient centrifugation for 20 hours at 148,000*g*.

Malignant and normal tissue were obtained from specimens removed surgically at Vanderbilt University Hospital. Routine diagnostic techniques of the surgical pathology laboratory were used to assign the designation malignant or normal to the tissue specimens (*8*). Fresh samples were stored at −20°C until assay. Extracts were prepared by homogenization of the tissue in two volumes of 0.05*M* tris(hydroxymethyl)aminomethane hydrochloride, *p*H 7.5, using a Polytron PT 10 homogenizer, followed by centrifugation at 31,000*g* for 10 minutes. The supernatant liquid was collected and titrated to *p*H 5 at 4°C with 1*M* acetic acid. The precipitated protein was removed by centrifugation at 31,000*g* for 10 minutes and the supernatant liquid (extract) retitrated to *p*H 7.5 with 1*M* NaOH.

The extracts were tested for the presence of a binding protein for retinoic acid by incubating portions with 40 nmole of all-*trans*-[11,12-^{3}H]retinoic acid (1.45 c/mmole) in the dark at 4°C for 4 to 5 hours. In order to determine the specific binding, duplicate portions containing 8 μmole of unlabeled retinoic acid were used. The ligands were added in 5 μl of ethanol containing 1 mg of α-tocopherol per milliliter. Immediately before sucrose gradient centrifugation, incubation mixtures were treated by adding 0.2 ml of a charcoal dextran solution (*9*) to absorb free retinoic acid. The charcoal-coated dextran was removed after a 5-minute incubation by centrifugation at 1000*g* for 5 minutes. A portion (0.2 ml)