[CANCER RESEARCH 42, 2450-2456, June 1982]
0008-5472/82/0042-0000$02.00

Plasma Membrane Changes of Liver and Morris Hepatoma Induced by Retinol in Rats[1]

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ABSTRACT

The uptake and binding of p.o.-administered labeled all-*trans*-retinol was studied *in vivo* in rats bearing nine different types of Morris hepatomas. Radioactivity was found in acid-precipitable and acid-soluble fractions of serum, liver, and the respective tumor. In the different types of Morris hepatomas, the uptake of retinol was found to be decreased. Depending on their growth rates, the tumors accumulated 1.6 to 50% of the radioactivity determined in the respective host liver. A linear correlation was obtained when the increasing growth rates were plotted logarithmically *versus* the decreasing uptake rates of the vitamin. Evidently, the decreased uptake of retinol is a common feature of Morris hepatomas and seems to be related to malignant transformation and not to increased growth, because in the regenerating liver an increase of the uptake of p.o.-administered retinol was found.

A 67% decrease in the incorporation rate of labeled L-fucose and a simultaneously increased turnover of protein-bound L-fucose were the major alterations of the fucoprotein metabolism in the plasma membrane fraction of the liver induced by high doses of retinol (1.5 × 10^6 IU all-*trans*-retinol per kg of body weight). The half-life of protein-bound L-fucose was 23 hr in retinol-treated rats, whereas in pair-fed control animals it was 41 hr. However, in the cytosolic fraction, the half-life of protein-bound L-fucose increased from 36 hr to 110 hr by feeding retinol.

Protein synthesis in retinol-treated rats measured by labeled L-methionine incorporation was unchanged in liver, hepatoma, and serum protein during the first hr after the pulse. However, 2 hr after the L-methionine pulse, an additional increase of the incorporation rate was observed.

Fluorographic analysis of the plasma membrane polypeptides revealed characteristic changes in the labeling pattern after labeling with L-fucose *in vivo.* These alterations comprise shifts of bands in the apparent molecular weight range of 30,000 to 220,000. In the plasma membrane fraction of Morris hepatoma 9121, only minimal changes were seen, although 70 IU of vitamin A per mg protein were found in the tumor 24 hr after feeding 1.5 × 10^6 IU/kg of body weight.

This study indicates that the increased hepatic secretory activity after high doses of retinol leads to rapid turnover of incompletely fucosylated glycoproteins of the plasma membrane. The subsequent alterations in the glycosylation pattern as revealed by labeling with L-fucose *in vivo* are not detectable in the tumor plasma membrane. Therefore, the tumor plasma membrane is not the main target organelle of retinol. Possibly, changes in the host rather than in the tumor itself may be responsible for the anticarcinogenic effect of retinol and the retinoids.

INTRODUCTION

The various functions of the plasma membrane such as transport and permeability, cell-to-cell interaction and adhesion, recognition, and antigenicity are altered during increased proliferation and malignant transformation (30, 42). Glycoproteins and glycolipids are the structural components of the plasma membrane mediating these functions (24). Major structural changes of the membrane constituents during malignant transformation occur among their carbohydrate moieties (26, 52, 53, 67). In Morris hepatomas, specific alterations in the metabolism of protein-bound terminal sugars L-fucose (65), *N*-acetylneuraminic acid (27), and subterminal D-galactose (7) have been described. In the plasma membrane of Morris hepatomas, the content of protein-bound L-fucose is increased 4- to 6-fold (65). Correspondingly, 2 to 3 times enhanced fucosyltransferase activity was found in these experimental tumors (8) when compared to normal liver.

Retinol is well known as an important factor for epithelial growth and differentiation (37, 69). Its involvement in glycosylation reactions has recently been shown for retinyl phosphate, which acts as a lipid intermediate transferring mannosyl and galactosyl residues onto glycoconjugates (16, 21, 50, 56). The molecular structure of retinyl phosphate could allow glycosylation of glycoproteins within the plasma membrane (19). Thus, the monosaccharide donor may participate in the regulation of different glycosylation reactions which influence or even determine a variety of cellular functions (3, 32, 55, 57). Besides its involvement during glycosylation processes, retinol is considered as a surfactant of biological membranes including lysosomal membranes (13, 23). Plasma membrane alterations due to high retinol levels have been attributed to the action of liberated lysosomal enzymes (13, 23). By studying the incorporation of labeled L-fucose compared to L-methionine into different cell surface glycopolypeptides, it should be possible to distinguish between the different effects of vitamin A.

For the anticarcinogenic potency of retinoids (36, 59), both their interactions with the membranes and a steroid hormone-like action have been discussed (6, 43). These effects are mediated by CRBP[3] and CRABP (44, 45). These proteins have been detected in many tissues including different tumor cell lines (15); the binding of the retinoids to these proteins is

[1] Dedicated to Professor Dr. Helmut Holzer on the occasion of his 60th birthday.

This work was supported by the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg (SFB 46 and Bu 364/1), and by the Deutsche Forschungs- und Versuchsanstalt für Luft- und Raumfahrt, Köln, West Germany.

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Received August 19, 1981; accepted March 10, 1982.

[3] The abbreviations used are: CRBP, cellular retinol-binding protein; CRABP, cellular retinoic acid-binding protein and RBP, retinol-binding protein.

considered to be crucial for their potency to prevent or reverse tumor induction (34). Since Morris hepatomas have different degrees of differentiation, it is of interest to know a possible relationship between the growth rate of the hepatocellular carcinomas and their ability to bind retinol.

MATERIALS AND METHODS

Animals and Tumors. Male Wistar rats weighing 160 to 200 g each came from Ivanovas (Kisslegg, Germany); male ACI and Buffalo rats were bred in our laboratory. The animals were kept in a windowless room at 21° with constant humidity and light from 7:30 a.m. to 7:30 p.m. They were fed Altromin, a commercial diet (Altromin GmbH, Lage/Lippe, Germany) containing 18 to 20% (w/w) of protein and 15,000 IU of vitamin A per kg of the diet, and had free access to water. During the experiments in which high doses of retinol were additionally administered, the respective untreated control animals were pair-fed. Retinol was dissolved in commercially available wheat germ oil (Mazola) and given by stomach tube. The retinol doses administered did not significantly change the serum activities of lactate dehydrogenase (EC 1.1.1.27) and glutamic-pyruvic transaminase (EC 2.6.1.2). Morris hepatomas were transplanted i.m. into both hind legs of ACI and Buffalo rats. The properties of the different hepatocellular carcinomas are summarized in Table 1. The tumors were originally obtained from Dr. H. P. Morris, Howard University, Washington, D. C. Partial hepatectomy was performed according to the method of Higgins and Anderson (29), with removal of two-thirds of the liver. All operations, tumor transplantation, and killing were performed under ether anesthesia between 8 and 10 a.m.

Chemicals and Isotopes. L-[^{35}S]Methionine (specific activity, 1040 Ci/mol), L-[6-^{3}H]fucose (specific activity, 20 Ci/mol), and L-[1-^{14}C]fucose (specific activity, 50 Ci/mol) were obtained from Amersham-Buchler, Braunschweig, Germany. All-*trans*-[1-^{3}H]retinol (4.5 Ci/mol), [^{14}C]toluene, and $^{3}H_2O$ were from New England Nuclear (Dreieich, Germany). All-*trans*-retinol was bought from Fluka (Buchs, Switzerland) and was kept at −20° in the dark under nitrogen. Enzymes and the calibration proteins for polyacrylamide gel electrophoresis came from Boehringer Mannheim GmbH (Mannheim, Germany). Acrylamide, bisacrylamide, and *N,N,N',N'*-tetramethylethylenediamine were supplied by Serva (Heidelberg, Germany). All other chemicals of analytical grade were bought from E. Merck AG (Darmstadt, Germany) and C. Roth OHG (Karlsruhe, Germany).

Preparation of Cell Extracts. Blood was withdrawn, and the rats were perfused with 40 ml of a 0.9% NaCl solution containing 0.5 mM $CaCl_2$ via the inferior vena cava and the portal vein. Homogenates of liver and tumors were prepared as outlined previously (12). Liver plasma membranes were enriched by the method of Neville (40) with some modifications (4). For the isolation of hepatoma plasma membranes, a method was used yielding membrane fractions of comparable purity (27). Marker enzymes (7) were determined routinely to check the purity of the membrane fractions, which was the same in retinol-fed and control animals. Marker enzyme activities are published in a preceding paper (7). The plasma membrane fractions of highest purity, M1 and M2, were further analyzed. Cytosols were made by centrifugation (105,000 × *g*, 1 hr) of the respective homogenate.

Determination of Uracil Nucleotides. UDP-glucose, UDP-galactose, UTP+UDP, UMP, and the sum of the acid-soluble uracil nucleotides were determined enzymatically as described by Keppler *et al.* (31). Liver and hepatoma samples were obtained *in situ* by the freeze-clamp technique (70).

Assays. Radioactivity was determined in total tissue homogenates and in the respective acid-soluble extracts with internal standardization to correct for quenching (62). Protein-bound radioactivity was measured by a modified method of Mans and Novelli (62). The protein content was determined by the method of Lowry *et al.* (35) using bovine serum albumin as the standard. The determination of the retinol concentrations based on the antimony trichloride color reaction of Carr and Price (1, 14).

Polyacrylamide Gel Electrophoresis and Fluorography. Sample protein (60 µg) was solubilized as described previously (12) and was separated on a track of a 10% polyacrylamide slab gel in the presence of sodium dodecyl sulfate (62) using the apparatus designed by Studier (60). For fluorographic analysis, the gels were processed according to the method of Bonner and Laskey (9) and exposed for 4 to 6 weeks at −70° on Kodak RP Royal X-omat medical X-ray film.

Table 1

Properties of some Morris hepatomas

The relative growth rate is expressed as weeks between the transfer from one generation to another when the tumors have reached a diameter of 2.0 to 2.5 cm. Type 66 had been a highly differentiated and exceptionally slow-growing carcinoma (68). The properties of this tumor have been subject to substantial changing. Type 66 is now the most rapidly growing among the Morris hepatomas transplanted in our laboratory. An increase in the growth rate which is paralleled by a loss of differentiation has been described for other types as well (7) and may represent a general phenomenon of Morris hepatomas.

Type of hepatocellular carcinoma	Rat	Degree of differentiation	Relative growth rate (wk)
44	Buffalo	Highly differentiated	12.0
47C	Buffalo	Highly differentiated	8.0
7800	Buffalo	Intermediate between well and highly differentiated	6.0
5123tc	Buffalo	Well differentiated	5.0
9121	ACI	Predominantly well differentiated	3.8
3924A	ACI	Poorly differentiated	3.0
7777	Buffalo	Poorly differentiated	2.5
66	Buffalo	Poorly differentiated	2.0

RESULTS

Uptake and Binding of Retinol. Radioactivity derived from p.o. administered all-*trans*-[^{3}H]retinol was found in the acid-precipitable and acid-soluble fractions of the serum at a ratio of 5:1 (Chart 1). In liver, a similar ratio for specific radioactivity was detected. When compared to serum, the specific radioactivity was found to be 3-fold enriched in the total liver extract. However, in Morris hepatoma 9121, an acid-precipitable:acid-soluble ratioactivity ratio of 1:3 was detected after the feeding of labeled retinol. Only 6% of the radioactivity found in the host liver was found in the acid-precipitable fraction of this tumor (Chart 2). Using ethyl ether extraction (1), a concentration of 70 IU vitamin A per mg protein was found in Morris hepatoma 9121 24 hr after feeding of 1.5 × 10^{6} IU of the vitamin per kg of body weight.

In a number of other Morris hepatomas listed in Table 1, the protein-bound specific radioactivity was determined 24 hr after the p.o. administration of labeled retinol showing a linear correlation between the logarithmically plotted specific radioactivity and the growth rate of different tumors (Chart 3). In both strains, ACI and Buffalo, the binding of retinol was determined in regenerating liver 24 hr after resection of two-thirds of the liver and was compared to normal adult liver. In regenerating liver, the specific radioactivity in both the total liver extract and the acid-precipitable fraction was found to be 2-fold increased.

Fucoprotein Metabolism. The incorporation rates of L-fucose into different glycoprotein fractions of the liver are summarized in Table 2. No differences between retinol-treated and untreated rats were found in the cytosol and in the total extract of the liver. In the plasma membrane fraction, however, a 67%

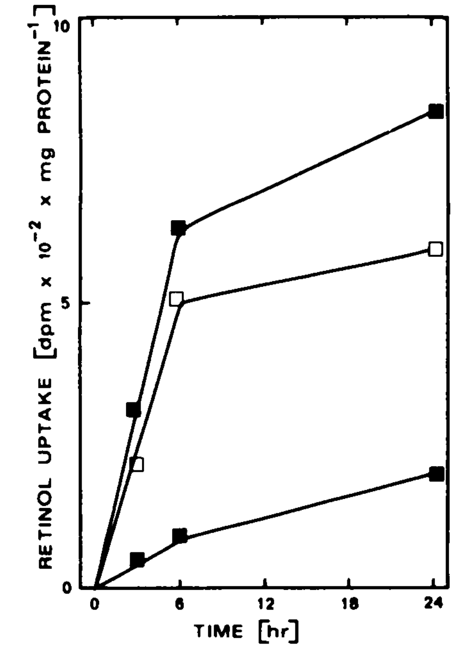

Chart 1. Uptake and binding of retinol to the serum of tumor-bearing rats. All-*trans*-[^{3}H]retinol (200 μCi) together with retinol (1.5 × 10^6 IU/kg of body weight) were administered p.o. to ACI rats bearing Morris hepatoma 9121. At different times after feeding the vitamin, the animals were killed, and the specific radioactivity was determined in the whole serum (■), in the acid-precipitable fraction (□), and in the acid-soluble serum fraction (■). Values are means of 3 rats.

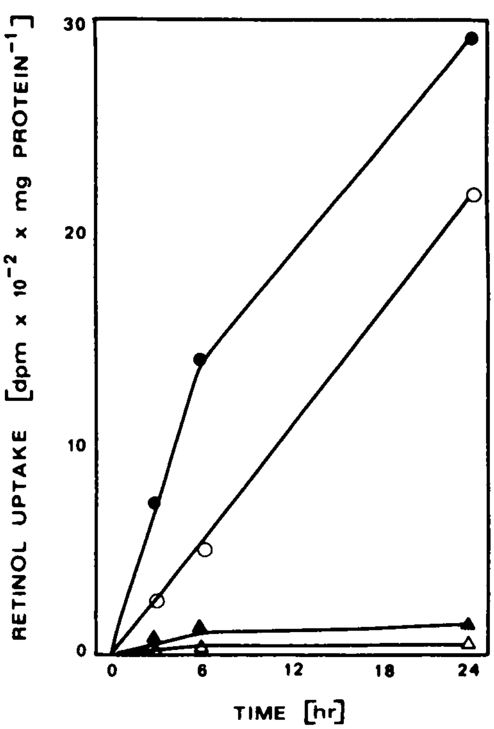

Chart 2. Uptake and binding of retinol to Morris hepatoma 9121 and host liver of ACI rats. All-*trans*-[^{3}H]retinol (200 μCi) together with unlabeled vitamin (1.5 × 10^6 IU/kg body weight) were given p.o. to ACI rats bearing Morris hepatoma 9121. At times indicated, the animals were killed, and the specific radioactivity was determined in the total cell extracts (●, ▲) and in the acid-precipitable fractions (○, △) of the host liver (●) and the Morris hepatoma (▲). Each *point* represents the mean of 3 rats.

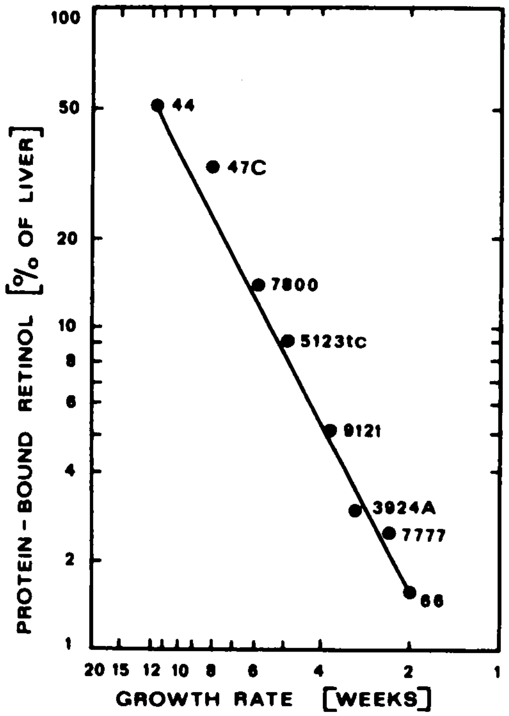

Chart 3. Correlation between the growth rate of different Morris hepatomas and their binding of retinol *in vivo*. ACI and Buffalo rats were used carrying different Morris hepatomas. When the tumor had reached a diameter of 2.0 to 2.5 cm (see *abscissa*), the animals were fed 200 μCi all-*trans*-[^{3}H]retinol together with 1.5 × 10^6 IU of the unlabeled vitamin per kg of body weight. Twenty-four hr later, the rats were killed, and the specific radioactivities were determined in the acid-precipitable fractions of the carcinomas and the respective host liver. The values represent the radioactivity found in the tumors, expressed as a percentage of that found in the respective host liver. The binding of labeled retinol in host livers and normal adult livers of ACI and Buffalo rats was in the same range (5000 cpm/mg protein). Each *point* represents the mean of 2 animals.

decrease was detected. The L-fucose incorporation into the serum glycoprotein fraction was enhanced by 34% (Table 2).

From the rate constants of degradation determined in these glycoprotein fractions, the half-lives of protein-bound L-fucose were calculated (Table 3). In the glycoproteins of the serum, the half-life of protein-bound L-fucose was found to be only slightly increased in retinol-fed animals. Probably due to the 3-fold prolonged half-life found in the cytosolic glycoprotein fraction, the half-life of protein-bound L-fucose in whole liver extract increased to 1.5-fold. However, L-fucose covalently bound to the plasma membrane glycoprotein fraction had a half-life reduced by 44% (Table 3). These results were confirmed by a double-label experiment (2) using L-[^{14}C]- and L-[^{3}H]fucose (data not shown).

Protein Synthesis. The incorporation rates of L-[^{35}S]methionine into the protein of serum, host liver, and hepatoma 9121 is shown by Table 4. One hr after the pulse, the incorporation of the labeled amino acid was found to be increased by 1.5- to 1.9-fold in the rats treated with high doses of vitamin A. A further increase of the incorporation rates is observed with time (data not shown). No changes were seen in the cytosolic fractions of liver and hepatoma. In hepatoma 7777, a smaller increase was observed than in hepatoma 9121 (data not shown).

Uracil Nucleotides. As shown in Table 5, the concentrations of the sum of the acid-soluble uracil nucleotides and UDP-

Table 2

Incorporation of L-fucose

Wistar rats were given all-*trans*-retinol (1.5 × 10^6 IU/kg body weight/day p.o. for 3 days). The control animals received the same volume of wheat germ oil daily. On Day 4, L-[^{14}C]fucose was injected i.p. at a dose of 100 μCi/kg body weight. One hr later, the animals were killed, and the radioactivity was determined in the acid-precipitable fractions.

	dpm/mg protein/hr	
Fraction	Retinol-treated	Untreated controls
Serum	6786 ± 363[a]	4507 ± 619
Whole liver	1135 ± 188	1193 ± 35
Cytosol	1170 ± 174	1074 ± 12
Plasma membrane	2329 ± 409	5861 ± 727

[a] Mean ± S.D. of 4 determinations.

Table 3

Half-life of protein-bound L-fucose

Wistar rats were treated p.o. with all-*trans*-retinol (1.5 × 10^6 IU/kg of body weight/day for 3 days). The control animals were pair fed and received the same volume of wheat germ oil. Twenty-four hr after the last retinol dose, 500 μCi of L-[^{3}H]fucose per kg of body weight were injected i.p. Six, 12, and 24 hr later, the animals were killed, and the specific radioactivity was determined. Rate constants of degradation (K_D) and the respective half-lives ($t_{1/2}$) were calculated from the decay of the specific radioactivity as outlined previously (62). Two animals were used per time point. The experiment was done in duplicate.

	Retinol-treated		Untreated controls	
Fraction	K_D (day^{-1})	$t_{1/2}$ (hr)	K_D (day^{-1})	$t_{1/2}$ (hr)
Serum	1.2323	13.5	1.3308	12.5
Whole liver	0.4378	38.0	0.6301	26.5
Cytosol	0.1512	110.0	0.4621	36.0
Plasma membrane	0.7233	23.0	0.4058	41.0

galactose were decreased in the livers of retinol-fed rats. The levels of other uracil nucleotides measured were found to be unchanged and were in the range reported previously (7, 31).

Fluorographic Analysis. Only minor differences could be revealed in the Coomassie-stainable polypeptide pattern of plasma membranes isolated from retinol-treated rats compared to that of control animals. In both the liver and hepatoma 9121 plasma membranes, the appearance of a band in the apparent molecular weight range of 100,000 can be seen (Fig. 1). Plasma membrane fluorograms of both liver and hepatoma show a general increase in the labeling of polypeptide bands as detected after the injection of labeled L-methionine. A major increase of L-methionine labeling occurs in 2 bands with apparent molecular weights of 160,000 and 70,000 in the plasma membrane of liver. The changes found in the serum, the range of prealbumin fraction, and the RBP are marked by *arrows* in Fig. 1. When comparing the fucopolypeptide pattern, characteristic changes were observed in plasma membranes of host liver. The *numbered arrows* indicate the alterations induced by retinol. *Arrow 1* points to a highly labeled fucopolypeptide band appearing after treatment with retinol. *Arrow 2* indicates shifts of 2 bands towards lower molecular weight. In the apparent molecular weight range of 70,000, a sharpening of a wider band to a lower- and higher-molecular-weight component can be seen after feeding retinol and is indicated by *Arrow 3*. *Arrow 4* points to a loss of the label, whereas *Arrow 5* indicates the appearance of a newly labeled band with an apparent molecular weight of 30,000. However, in the hepatoma plasma membrane polypeptides, only minimal differences in L-fucose labeling pattern could be found.

Table 4

Incorporation of L-methionine

ACI rats bearing Morris hepatoma 9121 either were pretreated p.o. with retinol (1.5 × 10^6 IU/kg body weight daily for 3 days) or received the same amount of wheat germ oil. On Day 4, L-[^{35}S]methionine (4.322 mCi/kg body weight) was injected i.p. One hr later, the animals were killed, and the radioactivity was determined in the acid-precipitable material of different fractions.

	dpm/mg protein/hr	
Fraction	Retinol-treated	Untreated controls
Serum	56,662 ± 6,676[a]	33,585 ± 1,215
Whole liver	85,424 ± 8,942	49,780 ± 13,074
Cytosol	64,272 ± 16,173	62,805 ± 7,651
Plasma membrane	106,303 ± 16,665	73,674 ± 21,948
Whole hepatoma	79,088 ± 13,824	49,890 ± 16,616
Hepatoma cytosol	99,914 ± 17,291	86,368 ± 23,359
Hepatoma plasma membrane	165,826 ± 43,637	83,611 ± 24,757

[a] Mean ± S.D. of 4 determinations.

Table 5

Concentration of uracil 5'-nucleotides

Wistar rats were given all-*trans*-retinol (1.5 × 10^6 IU/kg of body weight p.o. daily for 3 days). The respective control rats received the same volume of wheat germ oil. Twenty-four hr after the last feeding of retinol, the livers were obtained by the freeze-clamp technique (70) and were immediately transferred to liquid nitrogen. The uracil nucleotides were determined enzymatically (31).

	μmol/g wet liver wt	
Uracil nucleotide	Retinol-treated	Untreated controls
UDPG	0.310 ± 0.017[a]	0.340 ± 0.010
UDP-galactose	0.070 ± 0.006	0.097 ± 0.003
UTP + UDP	0.262 ± 0.019	0.235 ± 0.01
UMP	0.052 ± 0.023	0.090 ± 0.025
ΣUMP[b]	0.960 ± 0.049	1.235 ± 0.020

[a] Mean ± S.D. of 4 determinations.
[b] Sum of acid-soluble uracil nucleotides.

DISCUSSION

The present study shows that the radioactivity derived from p.o.-administered all-*trans*-retinol is readily bound to serum protein. The protein fraction has been identified as the RBP:prealbumin complex (25, 49). The ratios of protein-bound and free radioactivity are markedly different for liver, hepatoma, and serum. This may be due to the fact that some of the retinol administered may be in circulation or in the tissue in the free state and not bound to the various binding proteins. The hydrolysis of protein-bound retinol with the subsequent liberation of radioactivity into the acid-soluble fraction should be a minor contributing factor. A major finding is that the binding of retinol to Morris hepatomas is greatly reduced when compared to normal liver, even though a substantial amount of the vitamin reaches the carcinomas. The proteins capable of binding all-*trans*-retinol within the cell have been identified, purified, and characterized in various tissues (6, 15). Our data clearly show that the ability of the tissue to bind retinol varies depending on the degree of differentiation of the Morris hepatomas, indicating that either the concentration of binding proteins, *e.g.*, the surface receptor of RBP, the CRBP, or other binding proteins, or their capacity to bind retinol is greatly diminished. In a large variety of cultured tumor cell lines, the presence of CRBP has been studied (5, 34). From these data, no correlation between the degree of differentiation of the cells and the binding capacity of CRBP can be derived. Ong *et al.* (47) have found an increase of the retinol binding in colorectal adenomas. The general hyperplasia of the crypt provoked by the carcinogen

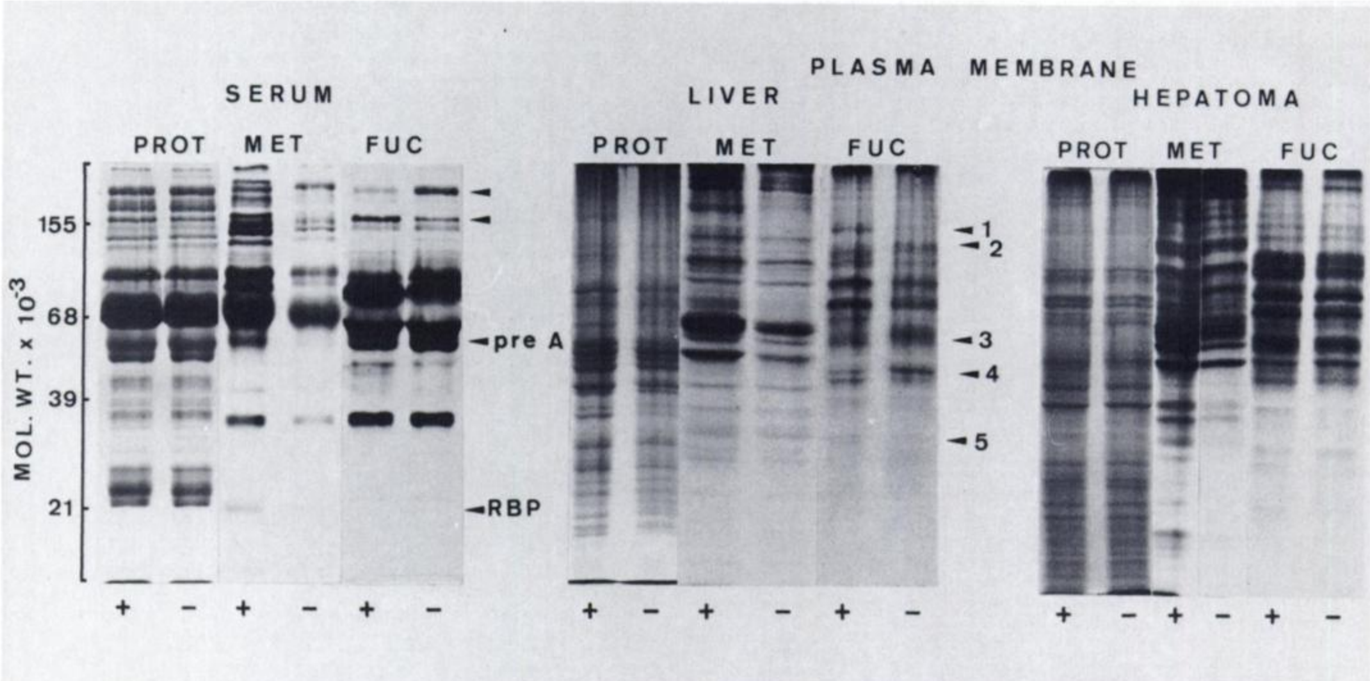

Fig. 1. Fluorographic analysis of plasma membranes and the serum of retinol-treated rats. ACI rats carrying Morris hepatoma 9121 were given 1.5×10^6 IU retinol per kg body weight p.o. per day for 3 days (+). Control animals received the same amount of wheat germ oil daily (−). At Day 4, either 4.32 mCi L-[^{35}S]methionine (*MET*) or 1.2 mCi L-[^{14}C]fucose (*FUC*) per kg body weight were injected i.p. One hr later, the animals were killed. Plasma membrane polypeptides of host liver and hepatoma as well as of serum were separated on 10% polyacrylamide gels by electrophoresis in the presence of sodium dodecyl sulfate. In addition to fluorograms, the protein patterns after staining with Coomassie Brilliant Blue (*PROT*) are shown. Calibration polypeptides of known molecular weight were used as markers. *Numbered arrows,* alterations in the fucopolypeptide composition of liver plasma membrane which are described under "Results." *pre A,* prealbumin.

1,2-dimethylhydrazine was not accompanied by an increase of the CRBP levels. In contrast, Palan and Romney (48), who investigated the CRBP in normal and dysplastic human cervix uteri tissue, have reported a significant decrease of the binding of [^{3}H]retinol *in vitro* paralleled by the loss of differentiation. Similarly, in intestinal carcinomas, a 20-fold decrease in the concentration of retinol when compared to the original tissue has been described by Sundaresan and DeLuca (61). In human hepatocellular carcinomas, a decreased CRBP concentration was found by Muto and Omori (39). Our data on the ability of different Morris hepatomas to bind [^{3}H]retinol *in vivo* to a cellular protein fraction are in accordance with the latter studies. It should be emphasized that the decrease of the binding of retinol is a common feature of Morris hepatomas and is evidently expressed simultaneously with uncontrolled proliferation, because in the rapidly growing liver after partial hepatectomy the binding of retinol *in vivo* was not lowered but enhanced. During the fetal development of the liver, which resembles the status of hepatic regeneration in many respects (10), increased CRBP levels were found (46).

There is accumulating evidence for the involvement of retinol into glycosylation processes, which is possibly a key role of the vitamin (16, 50, 56). However, it is still uncertain in which manner retinol is related to the process of differentiation. Our data clearly show that high levels of retinol influence the hepatic fucoprotein metabolism. Retinyl palmitate can stimulate the mannosylation of glycoproteins (28), thus altering the number or kind of L-fucose-binding sites. By increasing the number of high-mannose-type oligosaccharide side chains, the number of complex-type side chains may be reduced, thus reducing the number of L-fucose-binding sites. The resulting decreased fucosylation of cell surface glycoproteins after the administration of high doses of retinol could also be a consequence of an altered D-galactose content of the plasma membrane, because L-fucose is attached to subterminal D-galactose in glycoconjugates. However, high doses of retinyl palmitate did not affect the galactose incorporation (28).

The decreased concentration of UDP-galactose (Table 5) could be explained by a trapping of D-galactose by a retinyl derivative. Support comes from the recent finding that high doses of retinol activate galactosyltransferase activity (51). Since in rat liver membranes the transfer of galactose from UDP-galactose to retinyl phosphate is not catalyzed by retinyl phosphate (20), a different galactosyl transfer reaction may be operative.

The specific alterations, which were found in the fucopolypeptide pattern of the plasma membrane (Fig. 1), can be attributed to an increased hepatic secretory activity during retinol treatment. Both the increased protein secretion in the serum (Table 4) and the diminished half-life of protein-bound L-fucose in the plasma membrane allow the conclusion that the increased hepatic secretory activity induced by retinol leads to a rapid turnover of incompletely fucosylated glycoproteins. The described Golgi stacks (38) and increased number of vesicles fusing with the plasma membrane (33) could be signs of enhanced secretion. Different cells such as hamster embryo cells (64), rat intestinal cells (17), or mouse epidermal cells (72) show an increase in periodic acid-Schiff staining after treatment with retinol or retinoids, thus indicating an increased carbohydrate content. The reversal of keratinization to mucus secretion of hamster tracheal cells due to vitamin A deficiency is achieved by retinoids (41). However, the increased turnover can also be attributed to action of fucosidases liberated from lysosomes by retinol as described for other enzymes (23, 54). Brandes *et al.* (11) found a loss of cell surface coat material and a reduction of negative cell surface charge in L1210 leukemic cells after treatment with retinol. These observations were attributed to a loss of cell surface sialic acid because of their striking similarity to observations after treatment of cells with neuraminidase (11).

In both liver fractions, the total cell homogenate and the plasma membrane, the apparent half-lives of protein-bound L-fucose range from 13.5 to 45 hr and from 8.7 to 45 hr, respectively (63, 66). Our data are within this range. The half-lives of the control rats are relatively long because the pair-fed control rats were starved due to the decreased food intake of retinol-treated animals. Conversely, enhanced catabolism of cytoplasmic proteins during starvation was found by Dice and Walker (22). According to our knowledge, similar data on membrane-bound L-fucose are still lacking.

Our results show that during treatment with retinol the catabolism of plasma membrane-bound L-fucose is markedly enhanced if the same nutritional status is provided (Table 3).

The fucopolypeptide pattern of the plasma membrane of Morris hepatoma 9121 shows no changes after feeding retinol, which is in contrast to the liver. One explanation is the inability of transplanted hepatomas to secrete serum proteins (58). On the other hand, these results may also reflect the loss of some functions of the tumor cell membrane as contact inhibition or recognition, which are mediated or regulated by changes in the glycosylation of glycoconjugates (55, 57, 65, 67). Retinyl phosphate has been suggested (18, 19, 71) to represent the "membrane mediator for surface glycosylation" (71). It is evident that high levels of retinol modulate the glycosylation of membrane glycoproteins, thus becoming a possible regulatory tool of the eukaryotic cell. Similar changes are not provoked in the tumor cell membrane. The decreased binding of retinol to cellular proteins of Morris hepatomas may cause the lack of response of tumor cells. As already mentioned, the binding properties may be crucial for the anticarcinogenic action of retinol and the retinoids. Provided that the plasma membrane of the tumor cell is not the key target organelle for the antitumorigenic effect, it is worth studying whether by higher retinol levels the host gains the ability to suppress further tumor growth.

ACKNOWLEDGMENTS

The expert technical assistance of Barbara Hassels-Vischer and K. Happersberger is gratefully acknowledged. We thank Dr. L. M. DeLuca for critical reading of the manuscript.

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