Jpn. J. Cancer Res. (Gann)
79, 775–784 ; July, 1988

REVIEW

# CHEMICAL CARCINOGENESIS IN ANALBUMINEMIC RATS

Tadao KAKIZOE*[1] and Takashi SUGIMURA*[2]
*[1]*Urology Division, National Cancer Center Hospital and* *[2]*National Cancer Center, 1-1, Tsukiji 5-Chome, Chuo-ku, Tokyo 104*

## PROLOGUE

There are many examples of rapid progress in medical research on a particular disease after development of a suitable animal model. For instance studies on hypertension were promoted by use of spontaneous hypertensive rats,[1] studies on immunology by use of athymic nude mice[2] and cancer research by various animal models. Analbuminemic rats are another example of mutant animals that have contributed greatly to our understanding of the physiological function of albumin, together with cancer research. This review describes experiments on carcinogenesis in analbuminemic rats, which are useful in studies on the mechanism of carcinogenesis.

### *Analbuminemia in Humans and Rats*

The first finding of a human case of analbuminemia by Bennhold[3] in 1954 was very unexpected, because albumin normally constitutes 50–60% of the total serum protein and was thought to be essential for life. Up to 1983, 21 cases of analbuminemia had been reported.[4] Patients with analbuminemia show only mild symptoms such as slight edema, easy fatigability and occasional mild diarrhea. The decrease in serum albumin is associated with increases of serum globulins and cholesterol.

Nagase *et al.*[5] found an analbuminemic rat among a population of hypercholesterolemic Sprague-Dawley (SD) rats in 1977, and established a mutant strain with analbuminemia by breeding. Analbuminemic rats are apparently normal but are about 10% smaller in body weight than normal SD rats. The electrophoretic pattern of their serum proteins (Fig. 1) indicates a barely detectable level of albumin, in sharp contrast to the pattern of serum proteins from normal SD rats. These rats are characterized by both analbuminemia and hyperlipidemia with greatly increased serum cholesterol and triglyceride concentrations. Their serum osmolality, blood glucose level and electrolyte concentrations are almost the same as those in SD rats. The analbuminemia is inherited as an autosomal recessive trait. The rat albumin gene was mapped on chromosome 14 by analyzing a panel of mouse-rat hybrid cells.[6]

### *Molecular Mechanism of Analbuminemia of Rats*

Esumi *et al.*[7–9] studied the mechanism of lack of albumin in these animals. First they examined albumin synthesis in the liver of analbuminemic rats *in vivo* by intraperitoneal injection of L-[$^3$H]leucine. It was found that albumin was not synthesized in the liver of analbuminemic rats, whereas its synthesis amounted to about 14% of the total protein synthesis in the liver of normal rats.[7] Almost the same level of albumin mRNA precursors was detected in the nuclei of analbuminemic rat liver as in normal liver nuclei, but no albumin mRNA in the cytoplasm, indicating that analbuminemic rats have a unique type of mutation(s) affecting albumin mRNA maturation.[8] They cloned genomic DNA of serum

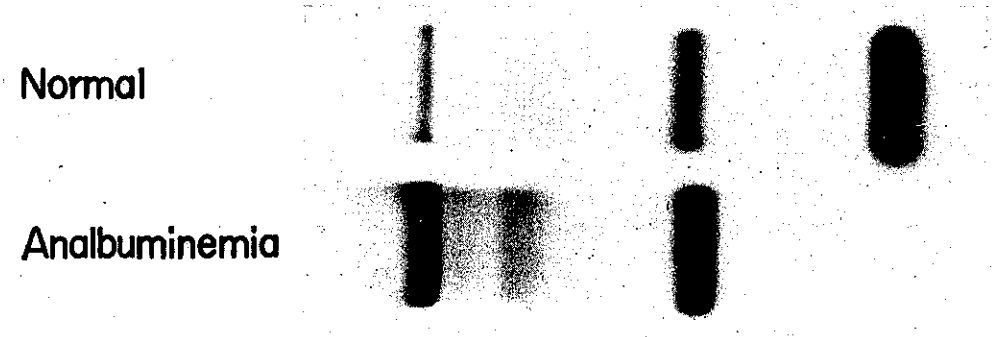


Fig. 1. Polyacrylamide gel electrophoretic pattern of the serum proteins of normal Sprague-Dawley and analbuminemic rats. Albumin was not detected in the serum of analbuminemic rats.

albumin from analbuminemic and normal rats. On structural analyses of these two albumin genes, they found that the albumin gene from analbuminemic rats had a deletion of seven base pairs from base 5 to base 11 from the 5′ end of intron HI of the gene. The nucleotide sequence of the 5′ end of intron HI is 5′G-T-A-G-G-T-T-T-C-C-G-C-G-A-G3′ in the normal rat gene, but is 5′G-T-A-G-C-G-A3′ in the analbuminemic rat gene. Therefore, 5′G-T-T-T-C-C-G3′ is deleted in the gene of analbuminemic rats. RNA blot hybridization of nuclear RNA of analbuminemic and normal rat liver using a DNA fragment containing intron HI as a probe showed that this abnormal intron sequence persisted in albumin mRNA precursors of analbuminemic rats.[9] These results indicated that a defect in splicing of albumin mRNA precursors due to the deletion of 7 bases in intron HI was responsible for absence of albumin production in this mutant animal.

*Carcinogenesis Experiments in Analbuminemic Rats*

Serum albumin is known to be a carrier of endogenous and exogenous compounds, such as bile acids, hormones, toxins, drugs and probably carcinogens.[10, 11] A series of carcinogenesis experiments has been conducted on analbuminemic rats. Since some carcinogens and their metabolites may be carried to their target organs by albumin, it seemed interesting to determine whether the distributions of target organs were the same in analbuminemic rats as in normal SD rats. Another interesting problem was whether the incidence of cancer was different in analbuminemic rats as a result of their abnormal lipid metabolism, altered serum protein composition or alteration of their cellular membrane and/or nutritional status due to lack of albumin.

1) Bladder Carcinogenesis in Analbuminemic Rats

Groups of 16 male analbuminemic rats and 15 male SD rats, initially 8 weeks old, were given CE-2 pellet diet and water containing 0.045% to 0.05% N-butyl-N-(4-hydroxybutyl)nitrosamine (BHBN) for the first 8 weeks. Since the daily water intakes of the two strains of rats were different, the concentration of BHBN was adjusted so that both strains of rats received equal amounts of BHBN per kg body weight. The experimental period was originally planned to be 40 weeks, but after 17 weeks, gross hematuria was observed in the analbuminemic rats and this increased in severity, so the experiment was stopped after 20 weeks. The incidences of bladder carcinomas induced in analbuminemic rats and SD rats were 16/16 (100%) and 3/18 (17%), respectively (Fig. 2). The average weight of the bladder including tumors was 2.90 g in analbuminemic rats and 0.19 g in SD rats (15:1). The tumors were all transitional cell carcinomas associated with squamous metaplasia. Invasion to the muscular layer was observed in 3/16 (20%) of the analbuminemic rats and in 1/18 (6%) of the SD rats.[12]

The urinary excretion of N-butyl-N-(3-carboxypropyl)nitrosamine (BCPN), a proximate carcinogen of BHBN, was similar in

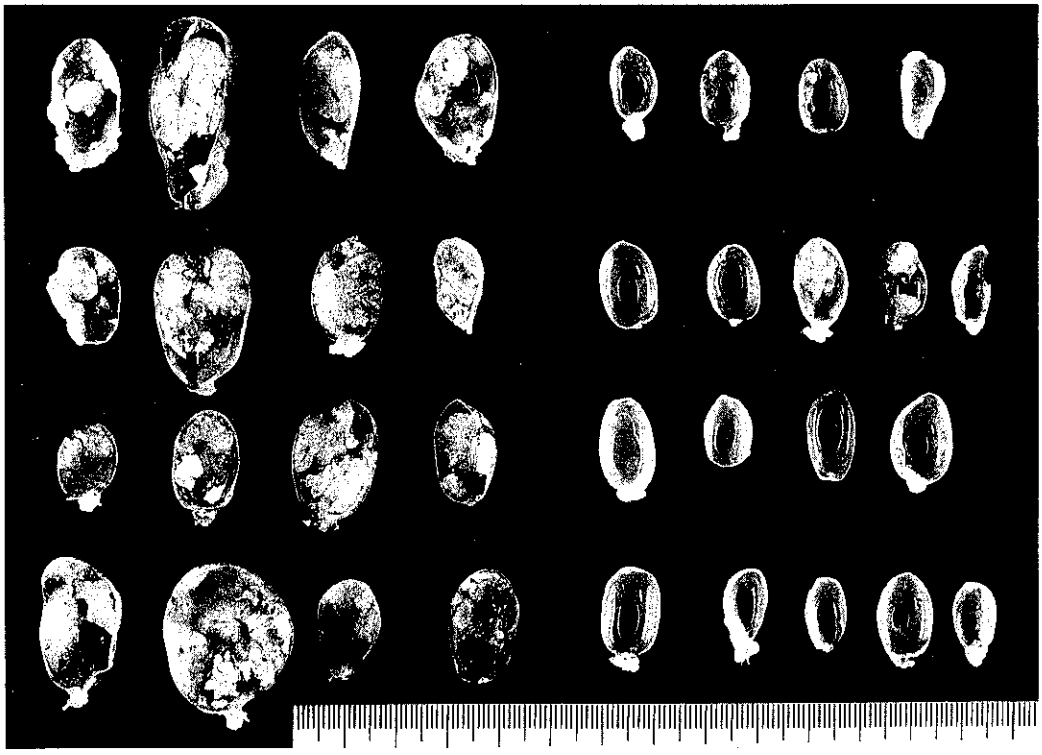

Fig. 2. Increased susceptibility of analbuminemic rats to induction of bladder cancer by N-butyl-N-(4-hydroxybutyl)nitrosamine. Left, bladders of analbuminemic rats. Right, bladders of Sprague-Dawley rats. (Cited from *Br. J. Cancer*, **45**, 474–476 (1982) with permission)

analbuminemic and SD rats (personal communication from Drs. M. Okada and E. Suzuki). The reason for the high susceptibility of analbuminemic rats to BCPN is unknown. The serum level of tryptophan in analbuminemic rats was 1/4 to 1/5 of that of control rats[13] and tryptophan was reported to be a promoter of bladder carcinogenesis in rats.[14] In the experiment, analbuminemic rats exhibited extraordinarily high susceptibility to bladder cancer induced by BHBN, much higher than expected from previous studies with tryptophan.

The susceptibilities of analbuminemic rats to other bladder carcinogens were examined by testing the agglutinability of their isolated bladder cells by concanavalin A.[15] The agglutinability of bladder cells of male analbuminemic rats, but not SD rats increased after one week of treatment with 0.001% BHBN in the drinking water or 0.01% N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT), 0.01% and 0.1% 4-aminobiphenyl, 0.1% 4-nitrobiphenyl, 0.1% benzidine, 0.1% 2-naphthylamine or 5% sodium saccharin in the diet. After treatment with 0.001% BHBN and 0.01% FANFT, which are non-carcinogenic doses in normal rats,[16, 17] the agglutination values were significantly high only in analbuminemic rats, suggesting that these doses of BHBN and FANFT are carcinogenic to analbuminemic rats, although not to SD rats. These results suggest that male analbuminemic rats are very susceptible to bladder carcinogens in general and therefore should be useful in screening tests for bladder carcinogens.

As far as FANFT is concerned, Hosaka *et al.*[18] conducted a carcinogenesis experiment in analbuminemic rats and SD rats using

0.2% FANFT in the diet for 10 weeks. After 52 weeks, the incidence of bladder cancer in analbuminemic rats was 0/18, while in SD rats it was 5/24 (21%), suggesting lower susceptibility of analbuminemic rats to FANFT. There is no clear explanation for the discrepancy of FANFT effects observed in the short-term assay and long-term carcinogenesis experiment.

An analbuminemic congenic strain of rats was established from ACI rats (ACI-alb).[19] These rats were also found to be highly susceptible to urinary bladder carcinogenesis induced by BHBN. The hyperlipidemia observed in analbuminemic rats was also seen in ACI-alb, probably due to the analbuminemia induced in ACI rats.

2) Induction of Hepatoma by 3′-Methyl-4-dimethylaminoazobenzene

Analbuminemic and SD rats were given 0.06% 3′-methyl-4-dimethylaminoazobenzene in CE-2 pellet diet for 195 days. The incidences of hepatocellular carcinomas induced in male and female analbuminemic rats were 25/32 (78%) and 23/35 (66%), whereas those in male and female SD rats were 28/30 (93%) and 16/29 (55%), respectively.[20] Thus there was no significant difference in the incidences in analbuminemic and normal rats. Moreover in this experiment, there was no significant difference in the induction of hepatocellular carcinomas in terms of their time of appearance, size or location in analbuminemic and SD rats.

3) Induction of Renal Tumors by N-Dimethylnitrosamine (DMN)

Analbuminemic and SD rats were given a single intraperitoneal injection of DMN at a dose of 50 mg/kg body weight. The incidences of renal tumors and the average weights of the kidneys including tumors 39 weeks later were, respectively, 76.0% and $10.8 \pm 4.1$ g in male analbuminemic rats, and 37.1% and $3.5 \pm 0.1$ g, in male SD rats.[21] Thus the analbuminemic rats showed significantly increased susceptibility to renal tumors. These tumors included adenocarcinomas, adenomas and mesenchymal tumors. The *in vitro* metabolic activations of dimethylnitrosamine and other carcinogenic nitrosamines, such as diethylnitrosamine, methylamylnitrosamine and BHBN by liver S9 of analbuminemic rats and SD rats were not significantly different.[22]

4) Induction of Gastric Tumors by N-Methyl-N′-nitro-N-nitrosoguanidine (MNNG)

Analbuminemic rats were also more susceptible than control SD rats to induction of gastric tumors by MNNG at a concentration of 67–83 μg/ml given in their drinking water for 32 weeks. The incidences of gastric tumors including adenocarcinoma, adenoma and sarcoma in week 44 were 12/17 (70%) in male analbuminemic rats and 8/21 (38) in male SD

Table I. Summary of Results of Carcinogenesis Experiments in Analbuminemic Rats

| Carcinogen | Target organ | Sex of animals | Incidence of tumors | | Authors | Year | Ref. |
|---|---|---|---|---|---|---|---|
| | | | Analbuminemic rats | Sprague-Dawley rats | | | |
| N-Butyl-N-(4-hydroxybutyl)nitrosamine | Bladder | male | 16/16(100)[a] | 3/18(17)** | Kakizoe *et al.* | 1982 | 12 |
| 3′-Methyl-4-dimethylaminoazobenzene | Liver | male | 25/32(78) | 28/30(93) | | | |
| | | female | 23/35(66) | 16/29(55) | Sato *et al.* | 1980 | 20 |
| N-Dimethylnitrosamine | Kidney | male | 38/50(76) | 23/62(37)** | Nagase *et al.* | 1986 | 21 |
| N-Methyl-N′-nitro-N-nitrosoguanidine | Stomach | male | 12/17(70) | 8/21(38) | Sugiyama *et al.* | 1986 | 23 |
| 7,12-Dimethylbenz[*a*]anthracene | Breast | female | 7/20(35) | 18/25(69)* | Nagase *et al.* | 1984 | 25 |
| Azoxymethane | Intestine | male | 23/23(100) | 21/29(72)** | Nagase *et al.* | 1983 | 27 |
| 3-Methylcholanthrene | Skin | male | 24/24(100) | 19/20(95) | Takahashi *et al.* | 1985 | 29 |
| | Subcutaneous | male | 7/8 (88) | 4/12(33)* | | | 29 |

*a*) Numbers in parenthesis are percentages.
Statistical difference was examined by applying the one-sided Fisher's exact probability test. $^{**}P<0.01$, $^{*}P<0.05$.

rats.[23] MNNG induces stomach tumors in rats by a direct action on gastric epithelial cells that results in methylation of nucleic acids and proteins.[24]

5) Induction of Mammary Tumors by 7,12-Dimethylbenz[*a*]anthracene (DMBA)

When female rats were given a single dose of DMBA at 110 mg/kg body weight dissolved in olive oil by gastric intubation at 8 weeks of age, the incidences and average numbers of mammary tumor 23 weeks later were 7/20 (35.0%) and $1.7 \pm 0.2$ in analbuminemic rats and 18/25 (69.2%) and $2.3 \pm 0.2$ in SD rats, respectively.[25] Thus female analbuminemic rats were less susceptible to carcinogenesis of the mammary gland. They also showed a significantly lower plasma prolactin level ($176 \pm 67$ ng/ml) than controls ($308 \pm 52$ ng/ml) during proestrus at 7–8 weeks of age. The serum level of prolactin is considered to have a marked influence on carcinogen-induced mammary tumorigenesis in rats.[26] Namely, it increases the mitotic activity, thus providing favorable conditions for the action of carcinogens, and promotes progression of neoplastic foci.

6) Induction of Intestinal Tumor by Azoxymethane

Male, analbuminemic rats and SD rats, initially 6 weeks old, were given a subcutaneous injection of 8 mg/kg body weight of azoxymethane once a week for 10 weeks, and were killed in week 27. The incidence of tumors was 23/23 (100%) in analbuminemic rats and 21/29 (72.4%) in SD rats. The tumors in the two groups were histologically similar, but differed in number, location and size. In analbuminemic rats, $7.8 \pm 4.2$ tumors were found in the large intestine, and $22.0 \pm 7.8$ in the small intestine, whereas SD rats had averages of $1.1 \pm 1.4$ in the large intestine and $0.2 \pm 0.6$ in the small intestine. The tumors in analbuminemic rats were also larger and more invasive than those in SD rats.[27] The serum bile acid level was found to be $2.02 \pm 0.51$ $\mu$g/ml ($n=15$) in analbuminemic rats and $20.86 \pm 3.72$ $\mu$g/ml ($n=10$) in SD rats.[28] Cholic acid was the main biliary bile acid in both types of rat and it was mostly present as the taurocholic-conjugate.[28] The level of bile acids excreted in the intestinal tract should be assayed in analbuminemic and SD rats. The higher susceptibility of analbuminemic rats to induction of tumors in the small intestine as well as the large intestine might be due to abnormal metabolism of bile acids.

7) Skin Carcinogenesis by 3-Methylcholanthrene

The skin of the back of male analbuminemic and SD rats, initially 4 weeks old, was painted with 0.3% 3-methylcholanthrene in acetone 3 times a week for a total of 50 times. After 46 weeks, the incidences of skin tumors were 24/24 (100%) in analbuminemic rats and 19/20 (95%) in SD rats.[29] In another experiment, male analbuminemic and SD rats were given two subcutaneous injections of 50 mg/kg body weight of 3-methylcholanthrene dissolved in sesame oil with an interval of 1 week between injections. The incidences of subcutaneous sarcomas 17 weeks later were 7/8 (88%) in analbuminemic and 4/12 (33%) in SD rats.[29]

The results of these carcinogenesis experiments are summarized in Table I.

It was initially thought that the target organs of carcinogens might be different in analbuminemic and normal rats. However, cancers developed in the same organs in the two strains of rats after administration of various carcinogens. This finding implies that the patterns of transport of the carcinogens studied so far might not be influenced by lack of albumin. It was observed that in some organs, the incidence of cancer was different in analbuminemic rats and SD rats. Possible mechanisms for this will be discussed below.

### *Possible Mechanisms of Higher Susceptibility of Analbuminemic Rats to Carcinogenesis in Various Organs*

Analbuminemic rats were found to show increased susceptibility to carcinogenesis in organs such as the urinary bladder, kidney, stomach, and intestine and in subcutaneous tissue. Possible mechanisms for this higher susceptibility are as follows:

1) The higher susceptibility of analbuminemic rats is due to increased cellular DNA adduct formation by the carcinogens in their target organs. Assuming that the amount of carcino-

gen reaching the target organ is the same in analbuminemic and SD rats, this possibility must be ascribed to increased penetration of the carcinogen through the cell membrane. After treatment with BHBN, the total amount of BCPN, a proximate carcinogen of BHBN,[30] excreted in the urine per 24 hours was similar in analbuminemic and SD rats. Recently, Nagase *et al.* studied the reason for the high susceptibility of analbuminemic rats to BHBN and found that the amount of BCPN in bladder tissue, detected by high-pressure liquid chromatography, was significantly higher in analbuminemic rats than in SD rats (personal communication). Thus the high susceptibility of analbuminemic rats to induction of bladder cancer by BHBN might be due to increased transportation of BCPN from the urine into the bladder tissue. This implies that the membrane permeability of bladder epithelial cells to BCPN is changed, probably by alteration in the membrane lipid composition as a result of lack of albumin in the serum or epithelial membrane. Consistent with this possibility, the fragility of red blood cells was found to be increased in analbuminemic rats,[31] resulting in an increased efflux of potassium, which was prevented by addition of rat albumin. These findings suggest that alteration in membrane lipid in erythrocytes as a result of lack of serum albumin may increase the permeability of the erythrocyte membrane to potassium in analbuminemic rats.

2) No difference was found in *in vitro* activations of DMN, diethylnitrosamine, methylamylnitrosamine and BHBN by the S-9 fractions obtained from the livers of analbuminemic and SD rats.[22] As far as BHBN is concerned, any difference of metabolic activation should not be related to the higher incidence of bladder cancer because the excreted amount of BCPN, its metabolite, in the urine was the same in both strains of rats.

3) The higher susceptibility of analbuminemic rats to induction of gastric tumors by MNNG might be due to increased proliferation of cells and DNA methylation of gastric mucosa. This possibility requires study by comparison of analbuminemic and SD rats by counting the number of bromodeoxyuridine-positive cells per pit column in the pyloric glands.[32]

4) The levels of known and as-yet-unknown growth factors may be increased in analbuminemic rats with the general increase in the protein fraction in compensation for lack of albumin. It is possible that increased incidence of some induced cancers in analbuminemic rats could be due to the increased amounts of growth factors, leading to increased proliferation of initiated cells. Since growth factors have been shown to have diverse biological effects on cellular proliferation and differentiation,[33] this possibility requires examination.

5) Increased susceptibility of analbuminemic rats to various carcinogens may be associated with increased tumor promotion. Since the serum tryptophan level in analbuminemic rats is 1/5 to 1/4 that of normal rats[13] and their testosterone level[34] is abnormally low, endogenous promoters may not be relevant at least to increased susceptibility to BHBN in this strain. We have reported the tumor-promoting effect of L-isoleucine and L-leucine on bladder carcinogenesis in F344 rats.[35] However, the levels of these amino acids in the plasma are the same in SD and analbuminemic rats,[13] and those in the urine are not known. Increased amounts of bile acids, especially secondary bile acids such as deoxycholic acid and lithocholic acid in the feces were suggested to be related with large bowel carcinogenesis.[36] Bile acid excretion in the feces in analbuminemic rats has not been examined yet.

Many tumor promoters produce oxygen radicals which damage DNA.[37] Absence of albumin may result in suppression of oxygen radical-scavenging action, usually exerted by albumin. Hyperlipidemia in analbuminemic rats may produce increased amounts of lipid peroxide which evokes an intracellular cascade reaction for tumor promotion.[38]

Thus at present no single mechanism can explain the higher susceptibility of analbuminemic rats to induction of various carcinomas.

*Detection of Albumin-positive Cells in the Liver of Analbuminemic Rats*

It is very interesting to note that the reversion of hepatic cells to albumin synthesis occurred in the liver of analbuminemic rats

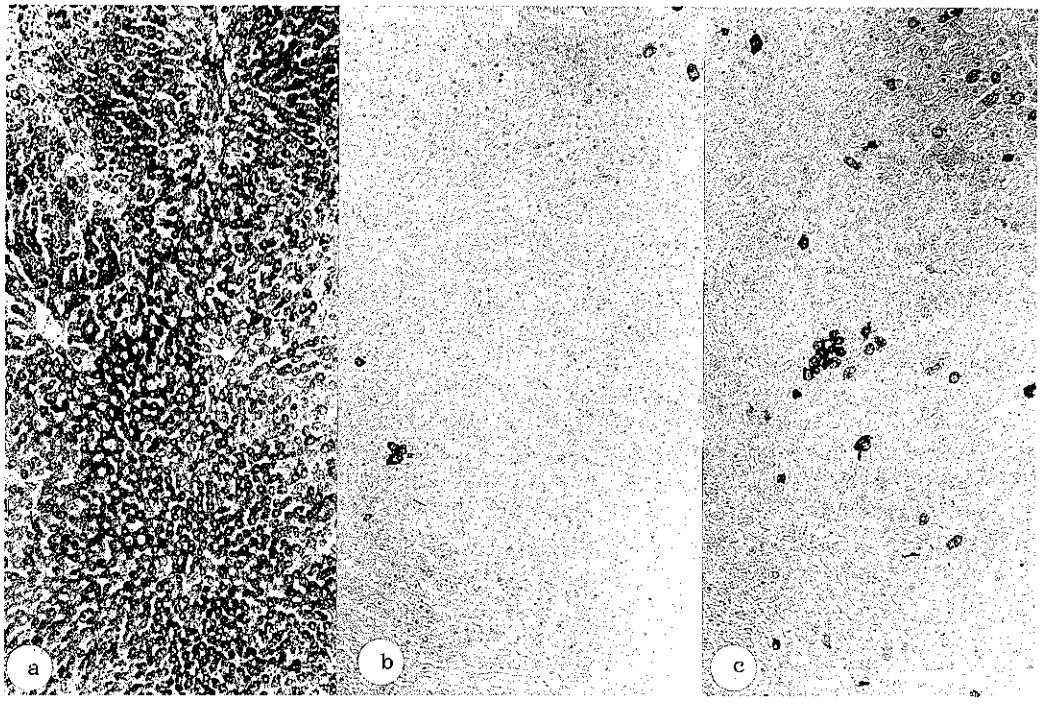


Fig. 3. Albumin-positive cells detected by the peroxidase-antiperoxidase method (×100). a, Liver of normal Sprague-Dawley rat; b, liver of an untreated analbuminemic rat (20 weeks old); c, liver of an analbuminemic rat treated with 3′-methyl-4-dimethylaminoazobenzene. (Cited from *Jpn. J. Cancer Res. (Gann)*, 77, 153–159 (1986) with permission)

after administration of mutagens and/or carcinogens. This is in accordance with the presence of a very small amount of albumin (10 to 20 $\mu$g/ml) in the serum of untreated analbuminemic rats.[39] Production of serum albumin by the revertant hepatic cells can be readily studied immunohistochemically.[40] Most hepatocytes are albumin-positive in normal rat liver. However, the frequency of albumin-positive cells in analbuminemic rats increased with age, being $10^{-5}$ at birth and $6\times10^{-3}$ at 45 weeks of age. After treatment with 3′-methyl-4-dimethylaminoazobenzene and 2-acetylaminofluorene, the number of albumin-positive cells increased greatly, being 7–10 times that of untreated rats at 10–16 weeks of carcinogen treatment (Fig. 3). The number of albumin-positive cells did not decrease after 3′-methyl-4-dimethylaminoazobenzene feeding was discontinued. This appearance of albumin-producing cells may be due to somatic mutation because 1) albumin-positive cells showed a scattered distribution in the liver, 2) their number increased with age and after administration of some hepatocarcinogens, 3) the appearance of albumin-positive cells was irreversible and 4) albumin-positive cells appeared in clusters in regenerating liver after partial hepatectomy, suggesting clonal growth of genetically altered cells. The original mutation in the analbuminemic rat is a deletion of a stretch of seven base pairs. Therefore, a reverse mutation to the wild type by exact insertion of the deleted stretch is highly unlikely. Conceivable alternative mutations are; 1) secondary mutations in the albumin gene itself resulting in splicing at the correct site or in forming a new splicing site. 2) Mutation(s) in the genes for the machinery involved in the splicing, such as splicing enzymes allowing the machinery to tolerate the deletion and thus resulting in correct splicing of the albumin gene product.

## EPILOGUE

Analbuminemic rats are mutants in which albumin mRNA processing in the liver is blocked owing to a seven-base-pair deletion in the 9th intron of the albumin gene. It is a noteworthy finding that rats can live without serum albumin. This animal line shows normal serum osmolality and normal levels of serum electrolytes, but has a variety of abnormalities to compensate for the deficiency of albumin. They are increased serum globulin, lipids, cholesterol and urea nitrogen, and decreased serum tryptophan, prolactin, testosterone and bilirubin. Probably there are many as yet undetected abnormalities in this animal. This strain is highly susceptible to induction of tumors of the bladder, kidney, stomach, intestine and subcutis by various carcinogens. Hyperlipidemia associated with the lack of albumin may change the membrane lipid composition, altering its permeability to a certain carcinogen(s). Analbuminemic rats are useful for short-term screening tests for bladder carcinogens by measurement of concanavalin A agglutination of isolated bladder cells. Separation by flow cytometry of revertant hepatocytes induced by administration of mutagens or carcinogens and analysis of these cells to determine the molecular mechanism of reversion would be very interesting. Such experiments may promote future studies on gene therapy of analbuminemia in rats.

## ACKNOWLEDGMENT

The authors are indebted to Dr. S. Nagase, Sasaki Institute, for helpful discussion during the preparation of this manuscript.

*(Received March 11, 1988/Accepted May 31, 1988)*

## REFERENCES

1) Okamoto, K. and Aoki, K. Development of a strain of spontaneously hypertensive rats. *Jpn. Cir. J.*, **27**, 282–293 (1963).
2) Flanagan, S. P. 'Nude,' a new hairless gene with pleiotropic effects in the mouse. *Genet. Res.*, **8**, 295–309 (1966).
3) Bennhold, H. Über einen Fall von Kompletter Analbuminemie wesentliche klinische Krankheitszeichen. *Verh. Dtsch. Ges. Inn. Med.*, **60**, 630–634 (1954).
4) Russi, E. and Weigand, K. Analbuminemia. *Klin. Wochenschr.*, **61**, 541–545 (1983).
5) Nagase, S., Shimamune, K. and Shumiya, S. Albumin-deficient rat mutant. *Science*, **205**, 590–591 (1979).
6) Szpirer, J., Levan, G., Thorn, M. and Szpirer, C. Gene mapping in the rat by mouse-rat somatic cell hybridization: synteny of the albumin and $\alpha$-fetoprotein genes and assignment to chromosome 14. *Cytogenet. Cell Genet.*, **38**, 142–149 (1984).
7) Esumi, H., Okui, M., Sato, S., Sugimura, T. and Nagase, S. Absence of albumin mRNA in the liver of analbuminemic rats. *Proc. Natl. Acad. Sci. USA*, **77**, 3215–3219 (1980).
8) Esumi, H., Takahashi, Y., Sekiya, T., Sato, S., Nagase, S. and Sugimura, T. Presence of albumin mRNA precursors in nuclei of analbuminemic rat liver lacking cytoplasmic albumin mRNA. *Proc. Natl. Acad. Sci. USA*, **79**, 734–738 (1982).
9) Esumi, H., Takahashi, Y., Sato, S., Nagase, S. and Sugimura, T. A seven-base-pair deletion in an intron of the albumin gene of analbuminemic rats. *Proc. Natl. Acad. Sci. USA*, **80**, 95–99 (1983).
10) Rothshild, M. A., Oratz, M. and Schreiber, S. S. Albumin synthesis. I and II. *N. Engl. J. Med.*, **286**, 748–757, 816–821 (1972).
11) Weisiger, R., Gollan, J. and Ockner, R. Receptor for albumin on the liver cell surface may mediate uptake of fatty acids and other albumin-bound substances. *Science*, **211**, 1048–1050 (1981).
12) Kakizoe, T., Komatsu, H., Honma, Y., Niijima, T., Kawachi, T., Sugimura, T. and

Nagase, S. High susceptibility of analbuminemic rats to induced bladder cancer. *Br. J. Cancer,* **45,** 474–476 (1982).
13) Emori, T., Sugiyama, K. and Nagase, S. Tryptophan metabolism in analbuminemic rats. *J. Biochem.,* **94,** 623–632 (1983).
14) Cohen, S. M., Arai, M., Jacobs, J. B. and Friedell, G. H. Promoting effect of saccharin and DL-tryptophan in urinary bladder carcinogenesis. *Cancer Res.,* **39,** 1207–1217 (1979).
15) Honma, Y., Kakizoe, T., Komatsu, H., Niijima, T., Sugimura, T. and Nagase, S. Agglutination assay of bladder cells by concanavalin A proved the high susceptibility of analbuminemic rats to bladder carcinogens. *Cancer Lett.,* **19,** 7–11 (1983).
16) Ito, N., Arai, M., Sugihara, S., Hirao, K., Makiura, S., Matayoshi, K. and Denda, A. Experimental urinary bladder tumors induced by N-butyl-N-(4-hydroxybutyl)nitrosamine. *Gann Monogr. Cancer Res.,* **17,** 367–381 (1975).
17) Arai, M., Cohen, S. M., Jacob, J. B. and Friedell, G. H. Effect of dose on urinary bladder carcinogenesis induced in F344 rats by N[4-(5-nitro-2-furyl)-2-thiazolyl]formamide. *J. Natl. Cancer Inst.,* **62,** 1013–1016 (1979).
18) Hosaka, Y., Muramatsu, M., Matsushima, T., Niijima, T. and Nagase, S. Low susceptibility of analbuminemic rats to induction of bladder cancer by N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide. *Jpn. J. Cancer Res. (Gann),* **76,** 577–582 (1985).
19) Nagase, S., Shumiya, S., Takahashi, M., Maekawa, A. and Hayashi, Y. Urinary bladder carcinogenesis by N-butyl-N-(4-hydroxybutyl)nitrosamine in an analbuminemic congenic strain of rats with an ACI genetic background. *Jpn. J. Cancer Res. (Gann),* **77,** 1198–1200 (1986).
20) Sato, S., Makino, R., Esumi, H., Takahashi, Y., Sugimura, T., Nagase, S. and Tanaka, H. Studies on carcinogenesis in analbuminemic rats. *Proc. Jpn. Cancer Assoc., 39th Annu. Meet.,* 55 (1980).
21) Nagase, S., Shumiya, S., Emori, T. and Tanaka, H. High incidence of renal tumors induced by N-dimethylnitrosamine in analbuminemic rats. *Gann,* **74,** 317–318 (1983).
22) Wakabayashi, K., Fujita, Y., Nagao, M., Makino, R., Sato, S., Sugimura, T. and Nagase, S. Metabolic activation of carcinogens by S9 fraction of liver from analbuminemic rats. *Proc. Jpn. Cancer Assoc., 41st Annu. Meet.,* 67 (1982) (in Japanese).
23) Sugiyama, K., Nagase, S., Maekawa, A., Onodera, H. and Hayashi, Y. High susceptibility of analbuminemic rats to gastric tumor induction by N-methyl-N′-nitro-N-nitrosoguanidine. *Jpn. J. Cancer Res. (Gann),* **77,** 219–221 (1986).
24) Sugimura, T. and Kawachi, T. Experimental stomach carcinogenesis. *In* "Gastrointestinal Tract Cancer," ed. M. Lipkin and R. A. Good, pp. 327–341 (1978). Plenum Publishing Corp., New York.
25) Nagase, S., Shumiya, S., Tanaka, H. and Nagasawa, H. Mammary tumor induction in analbuminemic rats by 7,12-dimethylbenz[*a*]anthracene. *Eur. J. Cancer Clin. Oncol.,* **20,** 283–286 (1984).
26) Nagasawa, H. Causes of age-dependency of mammary tumor induction by carcinogens in rats. *Biomedicine,* **34,** 9–11 (1981).
27) Nagase, S., Shumiya, S., Emori, T. and Tanaka, H. Induction of intestinal tumors in analbuminemic rats by azoxymethane. *Proc. Jpn. Cancer Assoc., 42nd Annu. Meet.,* 50 (1983) (in Japanese).
28) Takikawa, H., Seyama, Y., Sugiyama, Y. and Nagase, S. Bile acid profiles in analbuminemic rats. *J. Biochem.,* **97,** 199–203 (1985).
29) Takahashi, M., Onodera, H., Maekawa, A., Hayashi, Y. and Nagase, S. Carcinogenicity test of 3-methylcholanthrene in analbuminemic rats' skin. *Proc. Jpn. Cancer Assoc., 44th Annu. Meet.,* 80 (1985) (in Japanese).
30) Hashimoto, Y., Suzuki, E. and Okada, M. Induction of urinary bladder tumors in ACI/N rats by butyl(3-carboxypropyl)nitrosamine, a major urinary metabolite of butyl(4-hydroxybutyl)nitrosamine. *Gann,* **63,** 637–638 (1972).
31) Sugiyama, K., Emori, T., Shumiya, S. and Nagase, S. Anemia and potassium permeability of red blood cells in analbuminemic rats. *Exp. Anim.,* **33,** 307–318 (1984).
32) Ohgaki, H., Sato, S. and Sugimura, T. Differences of cell kinetics in gastric mucosa of susceptible ACI and resistant Buffalo rats after treatment with MNNG. *Proc. Jpn. Cancer Assoc., 46th Annu. Meet.,* 65 (1987).
33) Goustin, A., S., Leof, E. B., Shipley, G. D. and Moses, H. L. Growth factors and cancer. *Cancer Res.,* **46,** 1015–1029 (1986).
34) Takahashi, M., Inano, H., Tamaoki, B. and Nagase, S. Reduced activity of androgen biosynthesis in the testes of rats with analbuminemia. *J. Steroid Biochem.,* **24,** 871–876 (1986).
35) Nishio, Y., Kakizoe, T., Ohtani, M., Sato, S., Sugimura, T. and Fikushima, S. L-Isoleucine and L-leucine: tumor promoters of bladder cancer in rats. *Science,* **231,** 843–845 (1986).

36) Narisawa, T., Magadia, N. F., Weisburger, J. H. and Wynder, E. L. Promoting effect of bile acids on colon carcinogenesis after intrarectal instillation of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in rats. *J. Natl. Cancer Inst.*, **53**, 1093–1097 (1974).
37) Slaga, T. J. Overview of chemical carcinogenesis and anticarcinogenesis. *In* "Radioprotectors and Anticarcinogens," ed. O. F. Nygaard and N. G. Simic, pp. 437–470 (1983). Academic Press, New York.
38) Trosko, J. E., Jone, C. and Chang, C. C. Oncogenes, Inhibited intercellular communication and tumor promotion. *In* "Cellular Interactions by Environmental Tumor Promoters," ed. H. Fujiki, E. Hecker, R. E. Moore, T. Sugimura and I. B. Weinstein, pp. 101–113 (1984). Japan Scientific Societies Press, Tokyo.
39) Makino, R., Esumi, H., Sato, S., Takahashi, Y., Nagase, S. and Sugimura, T. Elevation of serum albumin concentration in analbuminemic rats by administration of 3′-methyl- 4 -dimethylaminoazobenzene. *Biochem. Biophys. Res. Commun.*, **106**, 863–870 (1982).
40) Makino, R., Sato, S., Esumi, H., Negishi, C., Takano, M., Sugimura, T., Nagase, S. and Tanaka, H. Presence of albumin-positive cells in the liver of analbuminemic rats and their increase on treatment with hepatocarcinogens. *Jpn. J. Cancer Res. (Gann)*, **77**, 153–159 (1986).