Albumin-Deficient Rat Mutant

Abstract. *An analbuminemic colony was established from Sprague-Dawley rats. Analbuminemia was inherited as an autosomal recessive trait. The rates of growth and reproduction of the mutant rats were no different from those of normal rats. Biochemically, the mutant was characterized by an extraordinarily low serum albumin content and a hyperlipidemia. Total serum protein in the mutant rat was similar to that of control Sprague-Dawley rats, with increased globulin. Serum cholesterol was inversely correlated with a decrease in albumin; the correlation coefficient for ablumin was −.92. These mutant rats may serve as a model of human familial analbuminemia and may also be useful in elucidating the functional roles of albumin.*

In 1977, we found an analbuminemic rat among hypercholesterolemic Sprague-Dawley rats (*1*) and, through breeding experiments (*2*), we established a rat strain with analbuminemia (NAR, Nagase analbuminemia rat).

The electrophoretic and immunoelectrophoretic patterns of serum proteins were determined in the NAR strain (Fig. 1). Upon electrophoresis of the proteins albumin was barely detectable in the NAR serum (Fig. 1A). Immunoelectrophoresis of the proteins revealed a faint precipitin line corresponding to albumin (Fig. 1B); this was in sharp contrast to the distinct precipitin line given by the serum of normal rats.

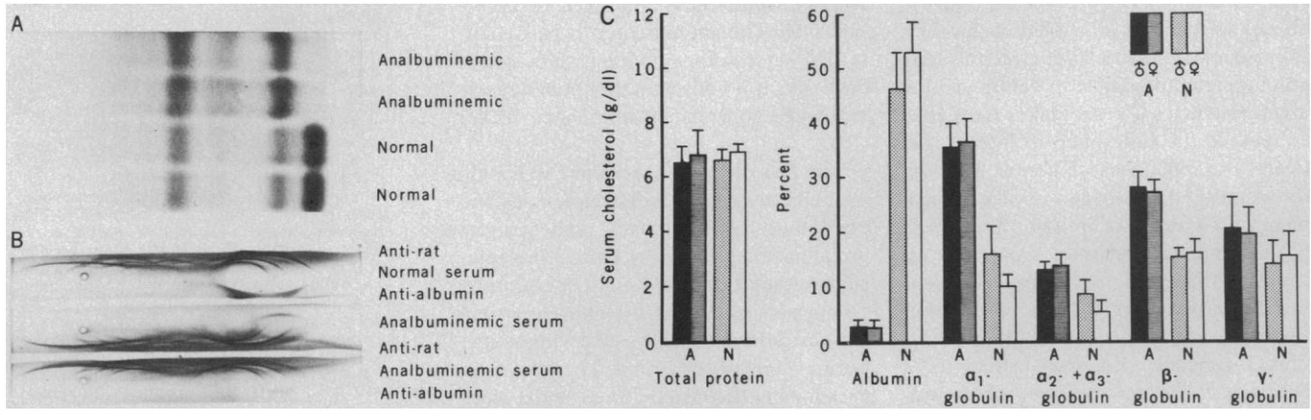
Rats of the NAR strain develop not only analbuminemia but also a hyperlipidemia, which greatly increases their serum cholesterol and triglyceride concentrations. The correlation between the albumin fraction value and cholesterol concentration was high ($r = -.92$) (Fig. 2).

The reproductive ability of the NAR strain during inbreeding—defined by fertility rate, litter size, and rearing rate—was compared with the reproductive ability of normal rats. The values were as follows: the fertility rate was 50 to 100 percent for NAR and 30.8 to 100 percent for normal rats; the NAR strain produced 6.4 to 13 young per litter and the normal rats, 5.8 to 11; the rearing rate was 50 to 100 percent for the NAR strain and 63 to 93 percent for the normal rats. These results, indicating that there was practically no difference between the two groups, suggest that analbuminemia has little effect on reproductive ability. In external appearance, the rats of the NAR strain do not differ from normal rats except that the former have a slightly smaller body size.

The mode of inheritance of analbuminemia is displayed in Table 1. The F_1 generation did not show analbuminemia, and the mean frequency of analbumi-

Table 1. Frequency and segregation of analbuminemia in F_1, and F_2, and backcross progeny. Abbreviations: A, analbuminemia; N, normal. The value for χ^2 (1, 0.05) = 3.84.

Cross	Sex	*N*	Analbuminemic	Normal	Frequency of analbuminemia (%)	χ^2
A × N	♂	26	0	26	0	0
A × N	♀	15	0	15	0	0
AN × AN	♂	16	3	13	18.8	0.333
AN × AN	♀	25	6	19	24.0	0.013
AN × A	♂	28	18	10	64.0	2.286
AN × A	♀	27	15	12	55.6	0.333
AN × N	♂	9	0	9	0	0
AN × N	♀	10	0	10	0	0



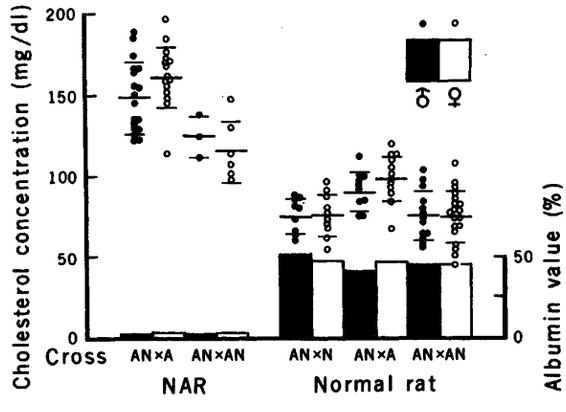


Fig. 1 (top). Analysis of serum proteins. (A) Cellulose acetate membrane electrophoresis of 0.3 μl of serum was carried out on Titan III strips (Helena Laboratories) with a constant current of 0.8 mA/cm for 80 minutes at 10°C in 0.15*M* tris-Veronal buffer (*p*H 8.8). After electrophoresis, the membrane was stained with Ponceau S, destained in 5 percent acetic acid, dehydrated in methanol, and cleared in a 20 percent acetic acid in methanol solution. The strip was then subjected to densitometry. (B) The serum of an NAR or normal rat was analyzed by immunoelectrophoresis either with rabbit antiserum against whole rat serum (*anti-rat*) or with specific antiserum against rat albumin (*anti-albumin*) (*9*). (C) Distribution pattern of serum proteins from normal rats (*N*) and analbuminemic rats (*A*). The fractional values were obtained by cellulose acetate membrane electrophoresis. Fig. 2 (bottom). Correlation between cholesterol concentration and the fractional value of albumin in normal rats (*N*) and analbuminemic rats (*A*). Serum cholesterol was measured by the modified method of Liebermann-Burchard (*10*). Solid circles indicate male rats and open circles, females. Each circle represents the value for one animal. A long bar indicates mean value; two small bars indicate standard deviation. Columns (hatched, males; open, females) indicate mean percentage values for albumin obtained by electrophoresis of serum on cellulose acetate membrane.

0036-8075/79/0810-0590$00.50/0

nemia in the F_2 progeny was 22 percent. In the animals backcrossed to NAR, the frequency of the mutant trait was around 50 percent, whereas in the backcross to normal rats, no analbuminemia was found. These data indicate that analbuminemia is inherited as an autosomal recessive trait.

The first case of human analbuminemia was reported by Benhold *et al.* (*3*); since then, others have studied the etiology and metabolic aspects of the human disease (*4*-*7*).

A decrease of albumin accompanied by an increase of globulins occurs in the serum of analbuminemic patients, and high cholesterol level has been reported (*5*). The analbuminemic rats should serve as a model for understanding the human disease, and may also be suitable for studying the function of albumin.

SUMI NAGASE
KANE SHIMAMUNE
Department of Chemistry, Sasaki Institute, Tokyo, Japan 101
SEIGO SHUMIYA
Central Institute for Experimental Animals, Kawasaki, Japan 221

References and Notes

1. In 1974 and 1976, Nagase first recognized the existence of analbuminemia in dead Sprague-Dawley rats but was unable to find living analbuminemic rats in the colony. Hattori *et al.* (*8*) succeeded in breeding a strain of rat that developed a high degree of hypercholesterolemia after feeding them a high (2 percent) cholesterol diet for 3 days. In these experiments, Sprague-Dawley rats of both sexes were fed a high cholesterol diet; these rats were divided into high and low responders according to their serum cholesterol levels. By repeated matings between siblings that were high responders, progeny of both sexes became progressively more susceptible to dietary hypercholesterolemia; some of them developed spontaneous hypercholesterolemia on a cholesterol diet. These rats were delivered to us in the F_4 generation, and further mating between siblings was performed at our institute.
2. Two lines of NAR and normal Sprague-Dawley rats were bred. The rats were kept on a diet of CE-2 (Clea Japan, Tokyo) and had free access to water. Room temperature was maintained at 23° ± 2°C throughout the experiments.
3. H. Benhold, H. Peters, E. Roth, *Verh. Dtsch. Ges. Inn. Med.* **60**, 630 (1954).
4. H. Keller, A. Morell, G. Noseda, R. Riva, *Schweiz. Med. Wochenschr.* **102**, 33 (1972).
5. E. J. Cormode, D. M. Lyster, S. Israels, *J. Pediatr.* **86**, 862 (1975).
6. H. Boman, M. Hermodson, C. A. Hammond, A. G. Motulsky, *Clin. Genet.* **9**, 513 (1976).
7. J. P. Goule *et al.*, *Ann. Biol. Clin. (Paris)* **34**, 403 (1976).
8. Y. Hattori, N. Numata, K. Shibata, *Jpn. J. Pharmacol.* **27**, 96 (1977).
9. P. Graber and C.A. Williams, *Biochim. Biophys. Acta* **10**, 193 (1953).
10. T. C. Huang, C. P. Chen, V. Wefler, A. Rafetery, *Anal. Chem.* **33**, 1405 (1961).

3 January 1979; revised 27 March 1979

Toxaphene, a Complex Mixture of Polychloroterpenes and a Major Insecticide, Is Mutagenic

Abstract. *Toxaphene, the most widely used chlorinated insecticide, is mutagenic in the* Salmonella *test without requiring liver homogenate for activity. This insecticide is a complex mixture (more than 177 polychloroterpenes) with carcinogenic activity in rodents. Some but not all of the mutagenic components are easily separated from the insecticidal ingredients.*

More than 10^9 pounds (0.5×10^9 kg) of the insecticide toxaphene (*1*) have been manufactured since 1947. The yearly production now totals about 40×10^6 pounds (*2*). The insecticide, which is used on cotton (86 percent of the total amount) and food crops (*3*), is a complex mixture of at least 177 polychlorinated compounds (overall composition, $C_{10}H_{10}Cl_8$) (*4*). It is produced by the extensive chlorination of technical-grade camphene obtained by isomerization of α-pinene, a by-product of turpentine distillation. Only ten components have been identified, including the most toxic ingredient, and they account for less than one-fourth of the mass of the mixture (*5*). Eighteen or more components are used in amounts individually exceeding 10^6 pounds per year (*5*, *6*).

The carcinogenic and mutagenic potential of toxaphene is of interest because of its long history of use, continuing importance, and complex composition. Toxaphene is a carcinogen in rodents (*7*). Toxicological problems have also been encountered with two related polychlorocamphene insecticides. Strobane, produced in the United States (*2*), causes liver hepatomas in male mice (*8*). Another polychlorocamphene insecticide, produced in the U.S.S.R. and of unknown composition relative to toxaphene, is associated with chromosomal and other abnormalities in humans at high occupational concentrations (*9*). In pregnant female rats this insecticide is transported to the fetus, and in male rodents it causes testicular degeneration and endocrine changes (*10*).

We used mutagenesis assays as a first step in defining the potential carcinogenic components of toxaphene (*1*). When tested in histidine-requiring strains of *Salmonella typhimurium* (*11*), toxaphene was mutagenic in strains TA98 and TA100, giving 325 revertants per milligram in the latter case (Fig. 1). All further discussion in this report is based on findings with the more sensitive TA100 strain. Mutagenic activity was reduced by 50 percent in the presence of rat or mouse liver homogenate [Fig. 1, toxaphene (+S9)]; this finding suggests possible nonspecific interactions with glutathione or macromolecules (for example, RNA).

Because of the chemical complexity of toxaphene, we fractionated the mixture to determine whether the mutagenic and toxic activities are separable. The direct mutagenic activity observed did not correspond exactly to the major toxic components, as suggested by the following evidence.

1) The most easily isolated major toxic component, heptachlorobornane-I (*5*), did not have mutagenic activity, either with or without liver S9, in any of the standard tester strains (TA1535, TA1537, TA1538, TA98, and TA100) (Fig. 1).

2) The direct mutagenic activity was not lost upon treatment of toxaphene with ethanolic potassium hydroxide under conditions (molar ratios of 1:1 or 1:10, 24 hours, 25°C) that dehydrochlorinate the major identified *gem*-dichloro toxic components.

3) The mutagenic activity of a recrystallized toxaphene fraction (from isopropanol) was less than that of the more polar mother liquor fraction (Fig. 1). Some of the direct mutagenic activity was in the polar fractions from chromatography of toxaphene on a silicic acid column rather than the nonpolar fractions most acutely toxic to animals and usually analyzed by gas chromatography (GC) (*12*) (Fig. 1, methanol and hexane fractions).

4) A series of toxaphene samples produced in different years that were similar in toxicity and retention pattern on open-tubular-column GC had about a fourfold range of direct mutagenic activity (*13*).

We attempted to remove the direct mutagens from toxaphene but were unsuccessful. The mutagenic activity was not reduced when a solution of toxaphene in carbon tetrachloride was washed with water or aqueous methanol (up to 20 percent). A portion of the activity survived passage in hexane solution through a concentrated sulfuric acid column (*14*), as would chlorobornanes and other chloroalkanes. A potent but minor (0.3 percent) mutagenic fraction was retained on a Celite column on chromatography as a hexane solution but was then eluted with methanol; however, most of the direct mutagenic activity remained with the bulk of the material that eluted in the hexane.

Heptachlorobornane-I and possibly other major toxic components of tox-

0036-8075/79/0810-0591$00.50/0