BASIC STUDIES ON THE MONGOLIAN GERBIL AS A SUSCEPTIBLE HOST TO FILARIAL INFECTION: COMPARATIVE STUDIES ON SERUM BIOCHEMICAL VALUES BETWEEN THE WILD-COLORED GERBIL AND THE COAT COLOR MUTANTS

KAZUHIRO SHICHINOHE^{1,2}, MASUMI SHIMIZU^{1,2}, KATSUYA HAGA²,

TETSUO IEDOKORO² AND KOICHIRO FUJITA²

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Abstract: We made a comparative study on serum biochemical values of mature Mongolian gerbils between the wild-colored (agouti) and the coat color mutants of both sexes by autoanalyzers. The coat colors of the mutants were white spotted-agouti, albino, black and white spotted-black. All of serum biochemical values we measured of different coat color gerbils were not significantly different each other. We did not find any lipemic sera and any hyperglycemia in all coat color gerbils. Compare to the other common laboratory rodents, patterns and values of serum protein fractions of the gerbils were different. Namely, A/G ratios and rates of γ -globulin fraction of gerbils were much higher than those of mice and rats and were the same as those of human beings.

INTRODUCTION

We made a comparative study on serum biochemical values of Mongolian gerbils between the wild-colored (called agouti) gerbils and the coat color mutants of both sexes in this report.

The Mongolian gerbil (*Meriones unguiculatus*) can be kept and handled so easily in laboratories and is so readily infected with some filarias (Ash and Riley, 1970a,b; Dalesandro and Klei, 1976; Matsuda *et al.*, 1976) that it has been used as an experimental host of filariasis. Biological characteristics of this animal were studied in 1960s when it was started to be used as a laboratory animal (Schwentker, 1963; Ruhren, 1965; Mays, 1969). However after that, it has been raised just only as a material, that means as a host only for providing parasites used for research, and its own characteristics have not been studied systematically.

Genetic influences on host reactions against filarial infections could not be studied because those gerbils commonly used were only agouti type which was not controlled genetically at all. But we have raised and

kept the closed colonies of the coat color mutants of Mongolian gerbils which have white-spotted agouti, albino, black or white-spotted black coat color and those coat color mutants are expected as a model to study influences of genetic background on response against filarial infections. We have studied to characterize the coat color mutants of Mongolian gerbils biologically and hematologically (Shimizu *et al.*, 1990, 1991) and we also need data on serum biochemical values.

MATERIALS AND METHODS

Mongolian gerbils were fed a commercial pellet for small rodents (MF; Oriental Yeast Inc., Tokyo, Japan) and water ad libitum under a conventional condition and housed 5 animals of the same sex in one cage. The room temperature and the humidity were maintained at $24 \pm 2^\circ\text{C}$ and $60 \pm 5\%$, respectively. The coat color mutants of the gerbils maintained in our laboratory were agouti, white-spotted agouti, albino, black and white spotted-black and origins of these gerbils were described in our

1 Department of Laboratory Animal Science, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113, Japan

2 Department of Medical Zoology, Faculty of Medicine, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113, Japan

Table 1 Serum biochemical values of the agouti type and the coat color mutants of Mongolian gerbils (1)

Coat color	Sex	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)
Agouti	Male	89±10	66±10	55± 3*
	Female	89±18	76± 9	37±10
White spotted-agouti	Male	77±14	77± 7	81±17* *
	Female	90±18	91±12	45± 2
Albino	Male	91±18	74±15	41± 9
	Female	102±19	81±16	39±10
Black	Male	88±14	65± 8	57±18
	Female	87±16	76±23	44± 8
White spotted-black	Male	87±14	62±18	77±26
	Female	89±18	63± 9	70±18

The values are derived from 14 to 15-week-old gerbils and represent means ± standard deviations for 10 gerbils of each group. *, ** The difference between sexes is significant by Student's t-test (*p<0.05, **p<0.01). There are no significant coat color differences in those values.

Table 2 Serum biochemical values of the agouti type and the coat color mutants of Mongolian gerbils (2)

Coat color	Sex	GOT (IU/l)	GPT (IU/l)	LDH (IU/l)	ALP (IU/l)
Agouti	Male	388±152	50±16	950±452	164±47
	Female	363±188	61±23	728±476	172±79
White spotted-agouti	Male	355±152	41±22	910±393	99±21
	Female	408±120	29±16	887±433	88±22
Albino	Male	436± 97	43±22	1131±547	145±53
	Female	276±103	43±26	711±459	151±25
Black	Male	422± 55	54±30	1128±197	121±43
	Female	403±139	42±23	1012±559	129±29
White spotted-agouti	Male	342±143	37±14	900±205	82±20
	Female	389± 81	34±11	950±470	110±27

The values are derived from 14 to 15-week old gerbils and represent means ± standard deviations for 10 gerbils of each group. Glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, lactate dehydrogenase and alkaline phosphatase are abbreviated to GOT, GPT, LDH and ALP, respectively. There are no sex differences and no coat color differences in those values significantly by Student's t-test.

Table 3 Serum biochemical values of the agouti type and the coat color mutants of Mongolian gerbils (3)

Coat color	Sex	Uric acid (mg/dl)	Urea nitrogen (mg/dl)	Creatinine (mg/dl)	Sodium (mEq/l)	Potassium (mEq/l)	Chloride (mEq/l)	Calcium (mg/dl)
Agouti	Male	1.2±0.9	23± 8	0.4±0.1	157±2	7.3±1.5	121± 3	8.8±0.3
	Female	0.7±0.2	31± 5	0.5±0.1	157±6	7.0±0.9	123± 6	8.8±0.7
White spotted-agouti	Male	0.5±0.3	26± 6	0.5±0.1	156±2	6.5±2.1	122± 2	7.7±0.8
	Female	0.8±0.5	25± 2	0.5±0.1	155±2	7.8±1.1	121± 1	8.1±0.6
Albino	Male	0.9±0.3	31± 2	0.5±0.1	149±3	10.7±1.2	119± 3	9.0±0.8
	Female	1.2±1.1	21±13	0.5±0.1	147±9	9.4±2.3	113±10	9.4±0.6
Black	Male	0.8±0.2	18± 3	0.3±0.1	151±2	9.5±0.7	118± 1	9.0±0.7
	Female	1.4±0.8	20± 4	0.4±0.1	149±3	10.4±1.4	115± 3	9.2±0.4
White spotted-black	Male	0.7±0.4	18± 3	0.3±0.1	149±3	8.7±0.8	115± 4	9.1±0.9
	Female	1.0±0.7	25± 6	0.4±0.1	147±1	8.8±0.7	113± 2	9.4±0.7

The values are derived from 14 to 15-week old gerbils and represent means ± standard deviations for 10 gerbils of each group. There are no sex differences and no coat color differences in those values significantly by Student's t-test.

Table 4 Serum biochemical values of the agouti type and the coat color mutants of Mongolian gerbils (4)

Coat color	Sex	Total protein (g/dl)	Albumin (g/dl)	A/G ratio
Agouti	Male	5.98±0.30	3.61±0.16	1.56±0.29
	Female	6.24±0.34	3.68±0.22	1.44±0.09
White spotted-agouti	Male	5.92±0.15	3.61±0.13	1.57±0.18
	Female	6.32±0.25	3.65±0.18	1.39±0.25
Albino	Male	5.86±0.21	3.49±0.08	1.47±0.11
	Female	5.80±0.32	3.60±0.13	1.68±0.37
Black	Male	5.62±0.15	3.39±0.10	1.53±0.20
	Female	5.94±0.21	3.56±0.14	1.52±0.27
White spotted-black	Male	5.84±0.39	3.50±0.13	1.51±0.20
	Female	5.76±0.27	3.45±0.08	1.51±0.23

The values are derived from 14 to 15-week-old gerbils and represent means $\pm$ standard deviations for 10 gerbils of each group. There are no sex differences and no coat color differences in those values significantly by Student's *t*-test.

previous report (Shimizu *et al.*, 1990). Ten animals of each coat color of both sexes aged 14 to 15 weeks old were used in the experiment.

Samples of blood were collected from the hearts of animals anesthetized with ether in the morning after overnight fasting. They were centrifugalized and the sera were stored at -80°C until assays.

The autoanalyzer, Hitachi 736 (Hitachi co. Ltd. Japan) or Spotchem (Kyoto Daiichi Kagaku co. Ltd. Japan) was used to measure the followings: glucose (GOD-POD), glutamate oxaloacetate transaminase (GOT) (OAC-POP-POD), glutamate pyruvate transaminase (GPT) (POP-POD), lactate dehydrogenase (LDH) (lactate pyruvate method), alkaline phosphatase (ALP) (p-nitrophenylphosphate), cholesterol (CE-COD-POD), triglyceride (LPL-GYOD-POD), uric acid (uricase-POD), urea nitrogen (urease method), creatine (Jaffe reaction), sodium, potassium and chloride (electrode), calcium (OCPC), total protein (BCG). Protein fractions were measured by using electrophoresis. Those values were evaluated statistically by Student's *t*-test.

RESULTS

Table 1 to 4 show serum biochemical values of Mongolian gerbils including the coat color mutants after overnight fasting. Means of some biochemical values were as follows: glucose 77-102 mg/dl, cholesterol 62-81 mg/dl, triglyceride 37-81 mg/dl (Table 1), GOT 276-436 IU/l, LDH 712-1131 IU/l (Table 2), uric acid 0.5-1.4 mg/dl in males and 0.7-1.4 mg/dl in females (no sex difference) (Table 3), total protein 5.62-6.32 g/dl, A/G ratio 1.39-1.68 (Table 4).

Figure 1 shows patterns of serum protein fractions of

Mongolian gerbils. Mean values of all coat color gerbils of each fraction were as follows: Albumin 57.8-62.3 %, α_1 -globulin 1.9-2.5 %, α_2 -globulin 8.8-11.1 %, β -globulin 4.4-9.2 %, γ -globulin 18.1-23.6 %.

All the values we measured were not significantly different between the agouti gerbil and the coat color mutants.

DISCUSSION

The present paper is the first report of a comparative study on serum biochemical values between the agouti type and the coat color mutants of Mongolian gerbils. In this experiment, significant differences between the agouti gerbil and the coat color mutants were not detected in serum biochemical values. But some values of the agouti gerbils were different from those reported by earlier workers.

Mays (1969) reported that uric acid value had male dominance in Mongolian gerbils, but we could not find such sex difference in that of each coat color gerbil (Table 3). Activity of γ -glutamyltranspeptidase of gerbils (data not shown) was under measurable level similar to that of the other small rodents (Tanimoto, 1988). GOT and LDH of gerbils were extremely higher than those of rats and mice (Table 2). As those samples were not hemolysis and those data were re-confirmed, we thought that is one of particular characteristics of gerbils. The more precise experiments in future are needed to determine it. All the sera from total 100 gerbils we tested were not lipemic at all, although earlier workers pointed out that 30 % of the sera were lipemic (Ruhren, 1965; Rich, 1968; Mays, 1969). This difference might be caused by that earlier workers did not establish the suitable way of feeding for gerbils

Figure 1 Serum protein fractions of the agouti type and the coat color mutants of male (a) and female (b) Mongolian gerbils. The values are derived from 14 to 15-week-old gerbils and represent means $\pm$ standard deviations for 10 gerbils of each group. The coat colors of agouti, white spotted-agouti, albino, black and white spotted-black are abbreviated to Ag, Sp-Ag, Al, BI and Sp-BI, respectively. Each bar represents one of protein fractions as follows; $\square$ albumin, $\blacksquare$ α_1 -globulin, $\square$ α_2 -globulin, $\square$ β -globulin and $\square$ γ -globulin. There are no sex differences and no coat color differences in those values significantly by Student's t-test.

which were newly used as a laboratory animal in those days. Now they are raised in certain condition with nutritionally well-balanced commercial pellets. In fact, values of total cholesterol and triglyceride of gerbils were not so high and similar to those of the other common laboratory rodents (Wolford *et al.*, 1986). Nakama (1977) reported that the Mongolian gerbil was hereditary spontaneously diabetic animal. Bonquist (1972) said that there were a few animals which showed diabetes-like symptoms in a stock of old obese gerbils. But glucose values we tested were within a normal range like mice, rats (Wolford *et al.*, 1986) and humans (Ishii *et al.*, 1981) and glucosuria has not been observed at all either (data not shown). Moreover, food and water intake of gerbils has been stable (Shimizu *et al.*, 1990). These facts reveal that the Mongolian gerbil is not a hereditary spontaneously diabetic animal in general.

The patterns of serum protein fractions of gerbils were very different from those of rats and mice. Namely, A/G ratios (Table 4) and rates of γ -globulin fraction (Figure 1) of the Mongolian gerbils were much higher than those of rats and mice and were the same as

those of human beings (Ishii *et al.*, 1981; Tanimoto, 1989). As globulin value changes according to environmental conditions, the breeding conditions of gerbils might bring the same level of globulin as human beings. Namely, gerbils in our laboratory have been kept under bacteriologically unsterilized condition called conventional condition and that condition was bacteriologically the same as where human beings live. We think that is one of advantages of the gerbil as an experimental model of human filariasis as well as their peculiar sensitivity because the breeding condition of animals would effect on sensitivity to parasites (Shichinohe *et al.*, 1990). The Mongolian gerbil has been known to be the most successful host for experimental filariasis such as *Brugia* spp. (Ash and Riley, 1970a,b), *Dipetalonema vitae* (Dalensandro and Klei, 1976) and *Litomosoides carinii* (Matsuda *et al.*, 1976). This character of the gerbil is really valuable because it is very expensive and difficult for studies of filariasis to use their own natural hosts in laboratories. For example, natural mammalian hosts of *Brugia pahangi* are monkeys, cats and dogs (Edeson, 1959; Edeson and Wilson, 1964) and numerous attempts to introduce this worm into commonly avail-

able laboratory rodents such as rats and mice have been failed (Laing *et al.*, 1961; Ahmed, 1967; Ash and Riley, 1970b; Sucharit and MacDonald, 1973). However, the reason why gerbils are readily infected with them has not been fully understood. The earlier studies on experimental filarial infections in Mongolian gerbils suggested that the sensitivity of gerbils to filarial worms were brought by less responsiveness of cellular immunity against parasites (Lammie and Katz, 1983; Klei *et al.*, 1990). On humoral immunity of gerbils, they had IgG antibody (Tomisato *et al.*, 1983; Farrar *et al.*, 1991), IgE-like antibody in their intact sera, produced it against worm antigen derived from *Dirofilaria immitis* (Shichinohe *et al.*, 1992) and much higher γ -globulin level than mice and rats in the present experiment. These facts that gerbils had enough components of humoral immunity were thought to be interesting in contrast with their peculiar sensitivity to parasites. They might indirectly support the statement of earlier workers that their sensitivity was caused by less cellular immunoreactivity.

Mongolian gerbils commonly used were only agouti type and their genetic background have not been controlled at all in contrast with rats and mice which have been controlled genetically to establish many inbred strains. We have studied characteristics of the coat color mutants of gerbils as compared with the agouti gerbil and until now, we have not found any significant differences between the agouti gerbil and the coat color mutants in the serum biochemical values as well as in biological and hematological values (Shimizu *et al.*, 1990, 1991). Then, if the different coat color gerbils will respond differently each other against filarial infection, it is thought that genetic background of gerbils effects on response against filarial infection. Consequently, present study on serum biochemical values of the Mongolian gerbil would be useful as a basic data to analyze mechanisms of host-parasite relationship using gerbils and the coat color mutants of Mongolian gerbils would be expected an useful model to study an influence of genetic background on reactions against filarial infection.

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