— Note —

Reference Values of Hematological, Serum Biochemical and Hormonal Examinations in Female Cynomolgus Monkeys (*Macaca fascicularis*) of Feral Origin

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Blood samples were collected from clinically healthy female cynomolgus monkeys imported from Indonesia, the Philippines and Malaysia. These animals were maintained under uniform environmental conditions for four to five years. The blood samples were examined for their hematological, serum biochemical and hormonal values. The ranges of the values as well as their arithmetic means and standard deviations have been tabulated with respect to each examination item.

Interest in biomedical use of nonhuman primate species has recently been increasing. We have established a breeding colony of cynomolgus monkey (*Macaca fascicularis*) for the purpose of using this species as a laboratory animal [1, 2]. However, it is currently rather difficult to use the laboratory-bred monkeys for experiments, since their availability is still quite limited. Therefore, the value of wild-imported monkeys is still high, and reference values for their hematological, serum biochemical and endocrinological examinations are needed. Although some reference values of serum chemistry for cynomolgus monkeys have been reported previously [5, 6, 9], useful and practical reference values have remained more or less lacking with regard to clinical biochemistry and endocrinology as the determination of baseline data is often laborious and costly.

At Tsukuba Primate Center for Medical Science (TPC), the National Institute of Health, Japan, a female cynomolgus monkey population of feral origin is being maintained in large under uniform environmental conditions. This population provides a valuable resource for the determination of reference values. Therefore, it was our objective to obtain hematological, serum biochemical and

endocrinological values employing this population.

The cynomolgus monkeys used in this study were imported from Indonesia, the Philippines and Malaysia. They were raised and maintained for four to five years in individual cages (45×60×60cm) set in animal rooms, with a room temperature of 25 ± 2 °C, a relative humidity of 60 ± 5 %, a light and dark cycle of 14L 10D, and fresh air changes of 10 times per hour. Every day the animals were fed with about 100 g of apples and oranges in the morning, and about 70 g of a commercially-prepared monkey diet (Type AS, Oriental Yeast Co., Ltd. Tokyo) in the evening. These animals were estimated to be more than five years old, based on the dental examination at the time of importation and had been reared under uniform environmental conditions at TPC. No animal showed any sign of clinical disorder and any sign of pregnancy at the time of blood sampling. Food was withheld for at least 16 hour prior to each sampling. Blood samples were drawn between 0930 and 1100. For this investigation, 312 animals were used and the mean body weight, standard deviation on the day of sampling were 3.56 ± 0.96 kg in a range of 1.75 to 7.25 kg. The monkeys were sedated

Table 1. Test methods used to establish reference values

Test	Method
Red blood cells	Microcell counter ^a
Hematocrit	Microcell counter ^a
Hemoglobin	Cyanomethemoglobin ^a
Mean cell volume	Microcell counter ^a
White blood cells	Microcell counter ^a
Glutamic oxaloacetic transaminase	NADH-NAD ^b
Glutamic pyruvic transaminase	NADH-NAD ^b
Total protein	Biuret ^b
Albumin	Bromcresol green ^b
Albumin-globulin ratio	Calculated ^b
Blood urea nitrogen	Urease ^b
Glucose	Glucose oxidase ^b
Total cholesterol	Enzymatic ^b
Free cholesterol	Enzymatic ^b
High density lipoprotein cholesterol	Enzymatic ^b
Phospholipids	Enzymatic ^b
Free fatty acids	Enzymatic ^b
Uric acid	Enzymatic ^b
Triglycerides	Enzymatic ^b
Thymol turbidity test	Thymol reaction ^b
Alkaline phosphatase	p-Nitrophenyl phosphate hydrolysis ^b
Alcohol dehydrogenase	Alcohol substrate ^b
γ -Glutamyl transpeptidase	γ -Glutamyl-p-nitroanilide substrate ^b
Lactate dehydrogenase	NADH-NAD ^b
Amylase	Enzymatic ^b
Creatinine	Kinetic Jaffe ^b
Sodium	Flame emission spectrophotometry ^c
Potassium	Flame emission spectrophotometry ^c
Calcium	Flame photometry ^d
Cortisol	Radioimmunoassay ^e
Renin	Radioimmunoassay ^f
Angiotensin-I	Radioimmunoassay ^f
Insulin	Enzymeimmunoassay ^g

a : CC-108, 110, MH-301, Toa Medical Electronics Co., Ltd. b : 706, Hitachi Ltd.
c : AA275, Varian-Techtron Co., Ltd. d : Flame-30 C, Nippon Bunkoh Medical Co.,
Ltd. e : Spac Cortisol kit II, Daiichi Radioisotope Labs., Ltd. f : Renin Riabead,
Dainabot Co., Ltd. g : Insulin EIA kit, Kainos Labs. Inc.

with intramuscular injection of ketamine hydrochloride (Ketalar 50, Park Davis-Sankyo Co., Ltd. Tokyo) with the dosage of approximately 5 mg/kg body weight. Samples were taken by femoral vein puncture. Two ml of blood were collected in a vacuum blood collection tube containig EDTA-2 Na as anti-coagulant for hematological measurement that were performed on the day of blood withdrawal. In additino, 5 ml were collected in a tube without anti-coagulant for serum biochemical and endocrinological measurements. Serum samples were separated by centrifugation and divided into two aliquots, one of which was stored at 4°C for serum biochemical measurements performed on the day after the blood

sampling, and the other aliquot was stored at -80°C until use for hormone assays.

The measurement items and analytical methods are summarized in Table 1. Prior to measurement of the present blood samples, both the accuracy and precision of each measuring procedure were examined. The coefficients of variations of hematological and serum biochemical tests ranged from 0.8% to 3.8% between assays (Cellcheck-400, Toa Medical Electronics Co., Ltd. Kobe, Beckman Decision Multilevel Comprehensive Chemistry Control Serum, Beckman Instruments. Tokyo). The coefficients of variation of the radioimmunoassays (RIAs) and of the enzymeimmunoassay (EIA) were less than 5 % wihin assay and

Table 2. Blood and serum values of adult female cynomolgus monkeys of feral origin, showing sample size (n), Mean (x), Standard deviation (SD) and observed range (O. R.) for each value

Test	Unit	n	x	SD	Min.	O. R. -	Max.
Red blood cells	$\times 10^4$ cells/mm ³	300	608	63	418	-	776
Hematocrit	percent	309	40.8	4.5	30.2	-	53.3
Hemoglobin	g/dℓ	301	11.3	1.3	8.1	-	14.7
Mean cell volume	fl	306	67	5	56	-	88
White blood cells	$\times 10^3$ cells/mm ³	302	9.7	2.8	4.5	-	19.4
Glutamic oxaloacetic transaminase	Karmen units	297	29.5	7.8	11.0	-	75.0
Glutamic pyruvic transaminase	Karmen units	298	31.7	15.7	6.0	-	95.0
Total protein	g/dℓ	307	8.25	0.69	6.71	-	9.95
Albumin	g/dℓ	291	4.05	0.20	3.02	-	4.70
Albumin-globulin ratio		310	0.97	0.15	0.69	-	1.33
Blood urea nitrogen	mg/dℓ	308	15.1	3.8	7.2	-	23.4
Glucose	mg/dℓ	278	44	12	14	-	96
Total cholesterol	mg/dℓ	301	120	27	62	-	201
Free cholesterol	mg/dℓ	293	21	6	6	-	50
High density lipoprotein cholesterol	mg/dℓ	30	56	27	19	-	145
Phospholipids	mg/dℓ	30	181	42	111	-	300
Free fatty acids	mEq/ℓ	30	0.89	0.41	0.34	-	2.08
Uric acid	mg/dℓ	30	0.65	0.44	0.4	-	2.3
Triglycerides	mg/dℓ	312	58	22	15	-	116
Thymol turbidity test	Kunkel units	82	0.8	0.6	0	-	1.8
Alkaline phosphatase	King-Armstrong units	258	8.6	2.3	2.8	-	49.6
Alcohol dehydrogenase	IU/ℓ	196	1.39	0.78	0.16	-	3.65
γ -Glutamyltranspeptidase	IU/ℓ	194	65	21	19	-	116
Lactate dehydrogenase	IU/ℓ	198	563	218	95	-	997
Amylase	IU/ℓ	198	713	213	300	-	1170
Creatinine	mg/dℓ	183	1.1	0.2	0.6	-	2.1
Sodium	mEq/ℓ	190	160	6	140	-	177
Potassium	mEq/ℓ	180	5.8	0.7	4.2	-	9.0
Calcium	mEq/ℓ	181	10.1	0.4	8.4	-	11.5
Cortisol	μ g/dℓ	120	6.23	0.12	2.75	-	9.17
Renin	ng/ml/hr	107	7.6	4.5	1	-	24
Angiotensin-I	ng/ml	107	0.27	0.33	0.1	-	1.8
Insulin	μ U/ml	126	41	31	2	-	183

less than 10% between assays.

Mean values for female cynomolgus monkeys are presented in Table 2. No remarkable relation between each value and the phase of menstrual cycle of each monkey was observed.

It has already been reported that human RIAs and EIAs were available for measuring serum concentrations of cynomolgus monkey insulin [8]. Serum concentrations of angiotensin-I in cynomolgus monkey were measured by a human EIA kit. It is well known that an anti-human angiotensin-I usually has crossreactivity to angiotensin-I from many mammalian species, such as equines, dogs, pigs and rats [7]. We employed a human angiotensin-I RIA kit to measure serum levels of angiotensin-I in cynomolgus monkey.

Concerning reference values for serum biochemistry in the cynomolgus monkey, several papers have been published. However, the number of animals used for these studies was not large enough. Even in the largest-scale study in previous papers, only about thirty animals per group were used [3, 4, 9]. In the present study, at least, thirty animals were used for each test even on the smallest scale. Because of the lack of seasonal variation in the feeding conditions for our cynomolgus monkeys, we processed our data collectively without regard to the season of the blood sampling. The maximum number of animals used in the present study reached more than three hundred in some items. This number is seemed to be satisfactory enough to determine

the reference range of the cynomolgus monkey. We already reported that the hematological and serum biochemical values of laboratory-bred monkeys [10]. The laboratory-bred female cynomolgus monkeys aged over 10 years showed the higher values in mean cell volume, albumin, albumin-globulin ratio, Triglycerides and lower values in total protein than those of the wild-imported monkeys examined in this study. The difference of blood and serum properties between laboratory-bred animals and wild-imported animals should be further examined. As conclusion, the present reference values should prove useful to those who wish to use cynomolgus monkeys of feral origin as experimental animals.

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野生由来メス・カニクイザル (*Macaca fascicularis*) の血液学的, 血清生化学的および内分泌学的測定値

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野生由来メス・カニクイザルを用いて, 血液学的 (15項目), 血清生化学的 (24項目), および内分泌学的 (4項目) 測定を実施した。対象とした動物は, 東南アジアより輸入され, 当センターに入荷してから4年ないし5

年間, 個別ケージで飼育されたものである。使用頭数は, 測定項目によって異なるが, 最少30頭, 最大312頭である。本研究によって, 野生由来メス・カニクイザルの参考値を提供することができたものと判断される。