INVITED REVIEW

Clinical Pathology Reference Ranges of Laboratory Animals

Toshiaki MATSUZAWA[1)], Mamoru NOMURA[2)], and Takashi UNNO[3)]

Working Group II, Nonclinical Safety Evaluation Subcommittee of the Japan Pharmaceutical Manufacturers Association, 3–4–1, Nihonbashi-honcho, Chuo-ku, Tokyo 103, [1)]Safety Research Laboratories, Yamanouchi Pharmaceutical Co., Ltd., 1–8, Azusawa 1-chome, Itabashi-ku, Tokyo 174, [2)]Drug Safety Research Center, Daiichi Pharmaceutical Co., Ltd., 16–13, Kita-Kasai 1-chome, Edogawa-ku, Tokyo 134, and [3)]Toxicology Laboratory, Kanebo, Ltd., 5–90, Tomobuchi-cho 1-chome, Miyakojima-ku, Osaka 534, Japan

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ABSTRACT. We analyzed historical control data of clinical pathology testings provided by sixty-seven member companies of the Japan Pharmaceutical Manufacturers Association covering study populations of approximately 7,000 rats/sex, 5,000 dogs/sex, and 700 monkeys/sex. This paper assesses the relationship between conditions of sample collection, methods of measurement, etc. and potential factors contributing to variations in reference data, based on weighted means and standard deviations thereof derived from data for rats, dogs and monkeys for those parameters measured using methods most common to the participating facilities. Parameters included erythrocyte count (RBC), hematocrit (Ht), hemoglobin concentration(Hb), reticulocyte count (Rt), platelet count, total leukocyte count (WBC), differential leukocyte count (%WBC), coagulation time (activated partial thromboplastin time: APTT, prothrombin time: PT), and serum/plasma levels of GOT, GPT, ALP, LDH, glucose, cholesterol, triglycerides (TG), total protein, albumin, urea nitrogen (UN), creatinine, sodium (Na), potassium (K), calcium (Ca), chloride (Cl), inorganic phosphorus (Ip), and CPK. Analyses of the data revealed species differences in RBC, Ht, Rt, platelet count, WBC, %WBC, ALP, LDH, glucose, cholesterol, TG, total protein, UN, creatinine, Ca, Ip, and CPK. There were strain differences in rats in platelet count, WBC, GOT, ALP, UN, creatinine, and CPK. Sex differences were noted for Hb, Ht, WBC, ALP, glucose, cholesterol, TG, total protein, A/G ratio, UN, and Ip. Age differences were observed with RBC, Hb, Ht, Rt, %WBC, GOT, GPT, ALP, LDH, cholesterol, TG, total protein, Ip, and CPK. APTT, PT, ALP, glucose, TG and UN were found to be subject to the influence of fasting/feeding. In rats, Ht, WBC, CPK and K showed differences by the site of bleeding. Observed values for LDH and CPK varied with specimen type, plasma or serum; serum assay values showed greater variation than plasma values.—KEY WORDS: animal, clinical chemistry, hematology, survey data, urinalysis.

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INTRODUCTION

Relatively few studies reporting veterinary clinical pathology reference data for domestic animals such as the cow, horse, sheep and chicken have appeared in recent years [9, 37, 72, 73]. With the general increase in the number of toxicity studies, on the other hand, clinical pathology reference data for laboratory animals have received wide attention, both in Europe and the United States [11, 12, 20, 22, 31, 33, 55, 74, 75, 80] as well as in Japan [26–28, 35, 59, 60, 76–79]. Nearly all these reports, however, deal with historical control data at individual research facilities. Against this background, it is desirable that clinical pathology background data at respective facilities be kept current in pace with the technical changes taking place in testing methods, animal care and feeding, assay procedures, and change of breeders, etc.

To this end, the Japan Pharmaceutical Manufacturers Association (JPMA) in collaboration with the JPMA Nonclinical Safety Evaluation Subcommittee conducted a control survey and issued in 1992 an internal report on clinical pathology reference data covering the recent five-year (1986–1990) period. From the data so collected, the present status of laboratory animal care conditions including age and strain of animals, mode of feeding, diet, drinking water, as well as anesthesia at collection of blood, bleeding site, anticoagulants, and urine collection has been reported elsewhere [38].

The present communication deals with the computation of reference values for clinical pathology parameters and assessments of conditions of sample collection, assay methods, etc. for their relationship as potential factors of variation.

SURVEY PROCEDURE

A survey of historical control data of clinical pathology testings in toxicity studies using laboratory animals was conducted via questionnaires sent to eighty member companies of JPMA.

Species surveyed included the rat, dog, monkey, rabbit, mouse, and other wherever possible, even where little data was available. The survey also examined relevant conditions at individual facilities such as age, strain, mode of feeding, conditions of sample collection (e.g. diet, drinking water, anesthesia, bleeding site, anticoagulants), urine collection and assay conditions (e.g. procedure, instruments, reagents) with which observed values

for clinical pathology parameters usually vary. The historical reference data included data from animals dosed with carriers/vehicles/solvents that are commonly used as negative controls, as well as data from untreated animals.

SURVEY RESULTS

Historical control data of clinical pathology testings obtained from sixty-seven member companies of JPMA were assessed. The approximate total population of animals was 7,000 rats/sex, 5,000 dogs/sex, and 700 monkeys/sex, etc. From data for at least 30 rats/sex, 10 dogs/sex or 5 monkeys/sex per facility obtained by assay methods most common to the participant facilities, the weighted mean and standard deviation of the mean were derived. Observed values exceeding 2 × SD were considered as abnormal values and excluded from analysis. Data from rats at 10 - 20 weeks of age, dogs at 6–9 months

Table 1. Clinical pathology reference ranges in Sprague Dawley rats – 10 to 20 weeks old

Parameters	Units	Analytical methods	Plasma (P) or Serum (S)	Feeding Fed(F) or Deprived (D)	No. of male rats used	Male's mean	Values S.D.	No. of female rats used	Female's mean	Values S.D.
RBC count	$\times 104/mm^3$	ECC		F,D	2816	890.7	75.3	2637	816.9	62.5
Hemoglobin	g/dl	CMH/OMH		F,D	2839	15.9	1.0	2659	15.5	1.0
Hematocrit	%	ECC		F,D	2770	47.0	3.1	2601	45.1	2.8
Reticulocyte	%	NMB+Microscopy		F,D	2235	1.9	0.9	2174	1.8	0.8
Platelet count	$\times 103/mm^3$	ECC		F,D	2606	969.9	201.5	2281	984.1	189.2
WBC count	$\times 102/mm^3$	ECC		F,D	839	94.6	30.9	756	61.4	26.7
Prothrombin time	sec	Conductivity	P	F	558	13.6	2.7	543	12.8	2.3
			P	D	709	17.2	5.6	737	12.7	2.3
APTT	sec	Conductivity	P	F	446	22.4	2.4	434	21.2	2.7
			P	D	409	30.7	5.2	411	22.9	4.0
GOT	IU/l	UV	P,S	F,D	2459	105.9	54.3	2362	104.7	62.8
GPT	IU/l	UV	P,S	F,D	2459	38.2	25.2	2361	40.4	34.2
Total protein	g/dl	Buiret	P,S	F,D	2695	6.2	0.5	2581	6.5	0.6
Albumin	g/dl	BCG	P,S	F,D	2429	3.5	0.7	2247	3.7	0.6
Glucose	mg/dl	GOD/HK	P,S	F	541	189.9	41.1	552	175.7	34.1
			P,S	D	1896	156.7	42.9	1781	140.5	40.2
Total cholesterol	mg/dl	COD	P,S	F	508	60.3	13.1	552	74.2	18.7
			P,S	D	1892	56.7	15.9	1793	71.2	19.6
Triglyceride	mg/dl	GK·GPO·POD	P,S	F	374	150.8	61.3	385	102.8	61.1
			P,S	D	623	74.0	43.5	671	52.5	29.6
Urea nitrogen	mg/dl	Urease·IDP/UV	P,S	F	541	23.3	3.8	552	22.9	5.3
			P,S	D	1861	16.9	3.1	1783	18.6	4.3
Creatinine	mg/dl	Jaffé	P,S	F,D	1824	0.6	0.1	1774	0.6	0.1
Alkaline phospha.	IU/l	PNPP-substrate	P,S	F	692	395.5	162.3	698	252.1	114.3
			P,S	D	1557	164.3	79.2	1539	103.5	65.6
Sodium	mEq/l	Flame photometry	P,S	F,D	2409	143.5	4.3	2295	143.1	3.7
Potassium	mEq/l	Flame photometry	P,S	F,D	2379	4.6	0.6	2306	4.3	0.7
Chloride	mEq/l	Coulometric tit.	P,S	F,D	1746	104.5	3.5	1808	104.7	10.4
Calcium	mg/dl	OCPC	P,S	F,D	2133	9.9	0.7	2032	9.9	0.7
I. phosphorus	mg/dl	Fiske-Subbarow	P,S	F,D	2077	7.1	1.1	1977	6.1	1.3
LDH	IU/l	Pyruvic acid·UV	S	F,D	247	1126.8	778.5	247	1125.6	901.1
			P	F,D	612	186.2	172.9	637	167.6	137.3
CPK	IU/l	NADPH reduced form	S	F,D	319	334.0	228.9	319	291.6	233.7
			P	F,D	233	249.0	247.6	244	204.9	217.7
Urinary sodium	mEq/24 hr	Flame photometry		F	491	1.6	0.6	487	1.2	0.4
				D	204	1.0	0.8	210	0.7	0.7
	mEq/l			F	469	54.0	50.1	486	50.9	48.8
				D	89	71.5	33.7	137	55.8	35.8
Urinary potassium	mEq/24 hr	Flame photometry		F	491	3.5	1.1	488	2.6	0.9
				D	204	2.4	1.4	209	1.5	1.4
	mEq/l			F	469	120.6	118.0	486	115.0	119.5
				D	139	165.3	81.2	137	143.1	85.1
Urinary chloride	mEq/24 hr	Flame photometry		F	480	1.8	0.5	488	1.4	0.4
				D	168	1.4	1.1	163	1.0	1.0
	mEq/l			F	332	57.9	68.4	338	54.6	65.5
				D	133	92.0	52.7	131	83.0	58.2

ECC: Electronic cell counter, CMH/OMH: Cyanomethemoglobin/oxyhemoglobin, NMB:New methylene blue staining, UV: Ultra violet, BCG: Bromo cresol green, GOD/HK: Glucoseoxidase/Hexokinase, COD: Cholesterol oxidase, GK·GPO·POD: Glycelol kinase, glycelol-3-phosphate oxidase, peroxidase, IDP:Indophenol, PNPP/PP: *p*-nitro-phenylphosphate/phenylphosphate, OCPC: *o*-cresolphthalein complexone, NADPH: Nicotinamide adenine dinucleotide phosphate

and monkeys between 2 and 5 years are summarized in Tables 1, 2 and 3, respectively, with stratification according to feeding/fasting and plasma/serum for each parameter.

Hematology

Erythrocyte count, RBC: Measured in most laboratories by electronic particle counting (aperture impedance or laser light scatter method) in an automated cell counter. Values in rats and dogs were higher than that of monkeys. In the rat, RBC increased sharply during the first 10 weeks of age, then steadily until 18 weeks, and gradually declined from about 58 weeks onwards. This trend was more conspicuous among males, and hence consistent with the report of Wolford *et al.* [75]. The count was prone to increase gradually with age in male dogs, whereas no such influence of aging was observed in female. RBC in the rat varied appreciably according to the bleeding site such as the abdominal aorta, retro-orbital sinus, femoral artery, femoral vein and jugular vein. RBC is an important parameter in the detection of anemia and hematopoiesis. Anemia is rarely seen among animals receiving antibiotics or other drugs [7, 8]. An excessive increase in RBC, or polycythemia, is unlikely seen in animals in chronic toxicity studies.

Table 2. Clinical pathology reference ranges in Beagle dogs – 6 to 9 months old

Parameters	Units	Analytical methods	Plasma (P) or Serum (S)	Feeding Fed(F) or Deprived (D)	No. of male dogs used	Male's mean	Values S.D.	No. of female dogs used	Female's mean	Values S.D.
RBC count	$\times 104/mm^3$	ECC		F,D	1863	678.1	72.8	1882	703.1	74.2
Hemoglobin	g/dl	CMH/OMH		D	1863	14.8	1.4	1882	15.5	1.5
Hematocrit	%	ECC		D	1843	44.2	4.6	1864	46.0	4.9
Reticulocyte	%	NMB+Microscopy		D	1243	0.6	0.4	1195	0.6	0.3
Platelet count	$\times 103/mm^3$	ECC		D	1799	296.9	92.1	1763	296.1	89.4
WBC count	$\times 102/mm^3$	ECC		D	1829	114.1	25.9	1859	112.8	25.5
Prothrombin time	sec	Conductivity	P	F	934	7.1	2.1	1001	7.0	2.1
			P	D	584	8.4	2.1	542	8.4	1.9
APTT	sec	Conductivity	P	F	809	15.9	4.4	806	16.2	4.4
			P	D	449	15.4	4.0	413	16.2	3.9
GOT	IU/l	UV	P,S	F,D	1829	27.3	6.8	1744	29.1	8.7
GPT	IU/l	UV	P,S	F,D	1831	32.8	13.8	1744	32.7	13.7
Total protein	g/dl	Buiret	P,S	F,D	1874	5.6	0.4	1799	5.6	0.5
Albumin	g/dl	BCG	P,S	F,D	1561	3.1	0.3	1506	3.2	0.3
Glucose	mg/dl	GOD/HK	P,S	F	1047	104.7	9.9	1025	104.0	10.3
			P,S	D	814	101.7	10.1	750	100.7	10.7
Total cholesterol	mg/dl	COD	P,S	F	1083	154.5	28.1	1045	147.5	28.5
			P,S	D	811	151.3	32.7	754	144.5	30.8
Triglyceride	mg/dl	GK·GPO·POD	P,S	F	419	45.3	14.2	421	50.6	20.4
			P,S	D	421	44.3	13.0	406	42.4	11.6
Urea nitrogen	mg/dl	Urease·IDP/UV	P,S	F	1083	14.7	3.5	1045	16.1	4.0
			P,S	D	814	13.6	3.3	757	14.5	3.8
Creatinine	mg/dl	Jaffé	P,S	F,D	1819	0.7	0.1	1756	0.7	0.1
Alkaline phospha.	IU/l	PNPP-substrate	P,S	F	1011	159.8	68.5	1009	160.9	73.6
			P,S	D	615	159.2	71.6	559	158.5	78.4
Sodium	mEq/l	Flame photometry	P,S	F,D	1783	147.3	3.4	1751	147.5	3.2
Potassium	mEq/l	Flame photometry	P,S	F,D	1783	4.7	0.3	1836	4.6	0.4
Chloride	mEq/l	Coulometric tit.	P,S	F,D	1052	111.7	4.6	1063	111.0	4.2
Calcium	mg/dl	OCPC	P,S	F,D	1573	10.7	0.6	1481	10.7	0.6
I. phosphorus	mg/dl	Fiske-Subbarow	P,S	F,D	1879	6.0	0.9	1786	5.8	1.0
LDH	IU/l	Pyruvic acid·UV	S	F,D	1146	118.6	76.3	1105	114.0	71.8
			P	F,D	617	73.1	31.1	594	74.7	31.3
CPK	IU/l	NADPH reduced form	S	F,D	289	185.5	73.2	432	182.4	73.8
			P	F,D	295	153.8	87.4	290	150.0	67.9
Urinary sodium	mEq/24 hr	Flame photometry		F	379	17.3	8.7	422	17.9	9.8
	mEq/l			F	186	45.7	36.1	168	57.9	43.4
				D	119	70.3	44.8	116	57.8	54.7
Urinary potassium	mEq/24 hr	Flame photometry		F	379	36.1	11.4	422	36.1	12.3
	mEq/l			F	186	151.3	69.7	168	150.9	74.1
				D	119	87.0	46.8	116	89.8	47.6
Urinary chloride	mEq/24 hr	Flame photometry		F	379	27.9	9.2	422	27.3	9.3
	mEq/l			F	96	77.8	44.7	78	78.8	47.6

ECC: Electronic cell counter, CMH/OMH: Cyanomethemoglobin/oxyhemoglobin, NMB: New methylene blue staining, UV: Ultra violet, BCG: Bromo cresol green, GOD/HK: Glucoseoxidase/Hexokinase, COD: Cholesterol oxidase, GK·GPO·POD: Glycelol kinase, glycelol-3-phosphate oxidase, peroxidase, IDP: Indophenol, PNPP/PP: *p*-nitro-phenylphosphate/phenylphosphate, OCPC: *o*-cresolphthalein complexone, NADPH: Nicotinamide adenine dinucleotide phosphate

Hemoglobin concentration, Hb: Most frequently measured using the cyanmethemoglobin method followed by the oxyhemoglobin method. Values did not notably differ between dogs and rats, while monkeys showed somewhat lower values. In rats, there was no difference among strains or between sexes. Hb values in dogs tended to be greater in females than males,whereas values in monkeys showed the reverse tendency. Rat Hb increased progressively with age up to about 10 weeks and was prone to decline slightly starting at 58 weeks of age. In dogs, Hb tended to increase with age till about 10 months of age.

Hematocrit, Ht: Almost all facilities used automated instruments based on the principle of electrical resistance. The microhematocrit method was used in a few facilities. Higher Ht values were observed in rats and dogs compared to the monkeys. No strain difference was noted in rats. Ht values did not differ between sexes in rats, whereas they were greater in female dogs than in males. In rats, values were low during the first 6 weeks of age, remained virtually constant thereafter till 58 weeks, then showed a subsequent slight decline. Ht in dogs tended to increase with age up to about 10 months, while that in monkeys showed greater values in males than in females at 4 years and upward. Ht in rats varied with the site of bleeding; it was somewhat lower for blood drawn from the abdominal aorta than for that from the retro-orbital sinus. Caution should be exercised in handling dogs for blood collection since this parameter shows variation due to stress/excitation [48].

Reticulocytes, Rt: The method most frequently used was the new methylene blue staining technique. Lower Rt values were observed in dogs and monkeys than in rats. Values did not differ between strains of rat. In rats, high Rt values exceeding 10% were recorded at 4–5 weeks of age, followed by a sharp decrease and subsequent near constant levels from 10 weeks onwards. The rat Rt value tended to increase with age after 57 weeks.

Platelet count: Most measurements were made with

Table 3. Clinical pathology reference ranges in cynomolgus monkeys—Young adult to adult, Wild-caught

Parameters	Units	Analytical methods	Plasma (P) or Serum (S)	Feeding Fed(F) or Deprived (D)	No. of male monkeys used	Male's mean	Values S.D.	No. of female monkeys used	Female's mean	Values S.D.
RBC count	$\times 104/mm^3$	ECC		F,D	536	647.7	69.6	500	619.2	65.0
Hemoglobin	g/dl	CMH/OMH		F,D	536	12.1	1.0	500	11.5	1.0
Hematocrit	%	ECC		F,D	547	41.5	4.0	502	40.1	4.1
Reticulocyte	%	NMB+Microscopy		F,D	333	0.4	0.4	387	4.5	4.0
Platelet count	$\times 103/mm^3$	ECC		F,D	476	394.2	118.7	436	403.7	128.3
WBC count	$\times 10^2/mm^3$	ECC		F,D	544	110.0	75.1	500	97.5	34.6
Prothrombin time	sec	Conductivity	P	F	401	10.7	4.6	454	10.6	4.3
			P	D	200	10.6	2.6	206	10.3	2.9
APTT	sec	Conductivity	P	F	401	23.4	5.7	404	23.3	5.1
			P	D	167	27.3	4.2	167	26.1	3.4
GOT	IU/l	UV	P,S	F,D	659	25.3	9.7	625	25.0	11.1
GPT	IU/l	UV	P,S	F,D	641	33.8	23.2	625	38.5	28.0
Total protein	g/dl	Buiret	P,S	F,D	617	7.9	0.6	603	7.8	0.6
Albumin	g/dl	BCG	P,S	F,D	544	4.4	0.4	598	4.2	0.5
Glucose	mg/dl	GOD/HK	P,S	F	269	101.1	32.7	320	96.8	37.0
			P,S	D	330	83.1	18.2	305	79.0	18.5
Total cholesterol	mg/dl	COD	P,S	F	269	128.3	27.5	320	144.7	30.7
			P,S	D	342	141.2	28.4	305	148.3	28.0
Triglyceride	mg/dl	GK·GPO·POD	P,S	F	20	40.6	10.2	101	48.7	22.8
			P,S	D	233	37.3	13.7	203	43.1	17.3
Urea nitrogen	mg/dl	Urease·IDP/UV	P,S	F	269	20.0	4.1	241	19.2	4.2
			P,S	D	330	19.5	3.9	305	19.7	4.1
Creatinine	mg/dl	Jaffé	P,S	F,D	555	1.0	0.2	600	0.9	0.2
Alkaline phospha.	IU/l	PNPP-substrate	P,S	F	238	352.9	172.7	219	250.4	143.3
			P,S	D	174	799.3	350.3	144	375.0	159.0
Sodium	mEq/l	Flame photometry	P,S	F,D	567	150.2	4.6	612	149.2	5.7
Potassium	mEq/l	Flame photometry	P,S	F,D	567	4.4	0.9	612	4.2	0.8
Chloride	mEq/l	Coulometric tit	P,S	F,D	309	107.5	3.5	376	107.9	5.7
Calcium	mg/dl	OCPC	P,S	F,D	502	9.8	0.8	541	9.7	1.0
I. phosphorus	mg/dl	Fiske-Subbarow	P,S	F,D	524	5.3	1.3	535	4.6	1.4
LDH	IU/l	Pyruvic acid·UV	S	F,D	212	526.1	228.0	229	507.1	300.0
			P	F,D	277	327.1	178.3	272	294.9	159.3
CPK	IU/l	NADPH reduced form	S	F,D	85	274.3	307.6	59	279.1	298.5

ECC: Electronic cell counter, CMH/OMH: Cyanomethemoglobin/oxyhemoglobin, NMB: New methylene blue staining, UV: Ultra violet, BCG: Bromo cresol green, GOD/HK: Glucoseoxidase/Hexokinase, COD: Cholesterol oxidase, GK·GPO·POD: Glycelol kinase, glycelol-3-phosphate oxidase, peroxidase, IDP: Indophenol, PNPP/PP: *p*-nitro-phenylphosphate/phenylphosphate, OCPC: *o*-cresolphthalein complexone, NADPH: Nicotinamide adenine dinucleotide phosphate

automated blood cell counting devices which electrically or optically detect platelet particles. The count was greatest in rats, followed in order by monkeys, and dogs. Values in miniature pigs and rabbits were closely similar to those reported by other workers [47]. Sprague-Dawley (SD) rats displayed the highest value, followed by Wistar then F344 rats in this order. No age differences were seen in rats and dogs, in which species there was a gradual decline with age. Most facilities used ethylenediamine tetraacetic acid (EDTA) as an anticoagulant; sodium citrate and heparin were rarely used for this purpose, and measurements using them gave values approximately 30% lower than those from EDTA-treated specimens.

Total leukocyte count, WBC: At nearly all facilities counting was performed using the aperture-impedance system or the flow cytometric system. Values in dogs were greater than in rats and virtually the same as those in monkeys. Male rats showed higher values than female rats. There was no sex differences in dogs and monkeys. WBC tended to be lower in F344 rats than SD and Wistar rats. In rats, WBC of blood drawn from the retro-orbital sinus was approximately twice as high as that of blood drawn from the abdominal vena cava, suggesting differences according to sampling site. It is advisable to draw blood samples from rats at the same time of day since WBC in this species is known to increase in light and decrease in darkness [34].

Differential leukocyte count, %WBC: Hematometric visual counting remains the most frequently used procedure. A noticeable proportion of data, nevertheless, was obtained with automated instruments which included the aperture-impedance system and flow cytometric system. The data did not appreciably differ between the direct visual and automated counting systems. Automated systems tended to give greater neutrophil counts and lower lymphocyte counts than the visual counting method. Unlike rats, dogs exhibited higher percentages of neutrophils. In rats, neutrophils increased and lymphocytes decreased with advancing age.

Blood Coagulation System

Activated partial thromboplastin time, APTT: This was most frequently measured by automated measurement of electrical conductivity, followed by the laser light scatter system. APTTs of rats and monkeys were somewhat longer values in dogs. APTT of rats shows circadian rhythms (diurnal variations), tending to be shorter in the afternoon and longer at night [14]. In rats there was no strain difference, nor any obvious sex or age differences. In male rats, APTT tended to be prolonged on fasting and to recover after feeding. APTT of monkeys was also prolonged on fasting.

Prothrombin time, PT: The electrical conductivity method was most frequently used, followed by the laser light scatter system. Rats showed the longest PT, followed in order by monkeys and dogs. As with APTT, fasting led to prolongation of PT in male rats and in monkeys, and recovery occurred on feeding. As with APTT, circadian rhythms for PT have been reported in rats [14]. No strain differences in PT were observed in rats, nor were significant sex or age differences seen. Activation of factor XII may be influenced by the quality of the material of the test tube in which the sample is drawn and cause a change in PT. Careful checking of the quality of test tube material is therefore important [61]. PT and APTT were tended to be prolonged on fasting [61].

Blood Chemistry

Glutamic oxaloacetic transaminase, GOT/Aspartate aminotransferase, AST: Measurement by ultraviolet spectrophotometry using the Henry or other standard methods were frequently used. Colorimetric determination using the POP or Reitman-Frankel method were also often used. Rats showed higher values for GOT than dogs and monkeys. No obvious sex differences were observed in rats, dogs, or monkeys. In rats, the pattern of variation in this parameter varied to some extent with the facility, but, as a general trend, GOT levels increased with age. Waner *et al.* [69] have reported that a control group of rats in a 104-week toxicity study exhibited the lowest GOT value at 17 weeks of age, the highest value between 30 and 56 weeks and wide individual variations thereafter. GOT levels were somewhat higher in F344 rats than SD rats. There were no appreciable age differences in GOT levels in dogs. Individuals with extremely high levels of GOT are occasionally encountered among monkeys over 3 years of age [76]. In rats, no significant difference in GOT level was seen among blood sampling site, namely the femoral artery, femoral vein, jugular vein and abdominal aorta. Elevation of blood GOT parallels that of blood GPT, but does not necessarily reflect the presence of hepatic disorder because this enzyme occurs at high concentrations in other tissues as well, particularly skeletal muscles. Measurement of this enzyme may not be suitable as a parameter of liver function in monkeys and rats not only because fear of venepuncture tends to cause markedly elevated values, but also because of the considerable variation among individuals [50].

Glutamic pyruvic transaminase, GPT/Alanine aminotransferase, ALT: Most laboratories used ultraviolet spectrophotometry with the Henry or other standard techniques; colorimetric measurement with the POP or Reitman-Frankel method was also used. There were no differences among species or between sexes in blood GPT level. In rats, the pattern of variation varied more or less with the facility but, as a general trend, GOT levels increased with age. In the 104-week toxicity study of Waner *et al.* [69], GPT level of the control group of rats was lowest at 17 weeks of age and highest between 30 and 56 weeks, but significant individuals variation was seen. F344 rats tended to show higher levels than SD rats.No age differences in GPT level were observed in dogs. Extremely high levels have been reported in monkeys over 4 years of age [76]. Rat GPT levels did not differ with bleeding site among the femoral artery, femoral vein, jugular vein and abdominal aorta. The present survey shows little or no difference in GPT activity between serum and plasma levels.

Alkaline phosphatase, ALP: There are two basic methods for the assay of ALP: The first uses *p*-nitrophenylphosphate substrate which the second uses phenylphosphate substrate. The former was more frequently used. ALP levels in rats and monkeys were higher than in dogs. In rats, ALP was highest in the F344 strain, followed in order by the SD and Wistar strains. In both rats and cynomolgus monkeys, males showed higher values than females. There was no difference between sexes in the dog. ALP levels are known to decrease with age in rats, dogs and monkeys [2, 57, 66, 67, 76]. This has been attributed in part to a decline in bone isoenzyme activity [67]. Fasting led to a fall in blood ALP activity in rats,whereas levels rose in monkeys. Blood ALP level was not influenced by food deprivation in dogs. Since circulating ALP activity is largely of small-intestinal/duodenal origin in rats, levels fall with decreased food intake. ALP is widely distributed in various organs, with high tissue levels in the liver, kidneys, small intestine, and bones. ALP fractionation for isoenzymes is therefore essential to determine the tissue origins of circulating ALP [2].

Lactate dehydrogenase, LDH: LDH levels were most frequently by ultraviolet spectrophotometry using pyruvate as a substrate; spectrophotometry using lactate was also used. Blood LDH levels were highest in monkeys, followed in order by rats and dogs. In rats, no strain differences were seen but enzyme levels increased with age. No age differences were noted in dogs or monkeys, excepting rhesus monkeys, in which levels were high at up to 3 years and lowest between 3.5 and 4.5 years, increasing with age thereafter. Fasting had little or no influence on circulating ALP levels. Five LDH isoenzymes are known to exist, because LDH concentration is greater in erythrocytes, leukocytes and platelets than serum, serum levels increase as a result of hemolysis at sampling or when drawn blood is allowed to stand [11]. The present survey disclosed a significant difference between serum and plasma LDH levels in rats and dogs. Serum levels were 6- to 7-fold greater in rats and 1.5- to 1.7-fold greater in dogs and monkeys. Toxicologic assessment based on serum LDH data is difficult, particularly in rats.

Glucose: About half of the facilities used the glucoseoxidase method while the other half used the hexokinase method. Thus, use of the former has decreased and the latter increased since the previous survey [29]. This suggests a current shift to the more precise assay method. Rats and mice showed higher values than the other species in this survey. In dogs, little inter-facility variation was seen. No differences were observed in rats. Male rats exhibited higher glucose levels than female rats at a significant proportion of facilities. Rat serum or plasma LDH levels did not differ between specimens obtained from the retro-orbital sinus and those from the abdominal aorta. Monkeys and rats showed lower values on fasting than feeding, whereas no such influence was seen in dogs. Blood glucose level did not vary noticeably with assay method,but caution is advised because the bleeding site and diurnal variations associated with feeding behavior have both been reported to influence levels [6, 57].

Cholesterol: The cholesterol oxidase method is used. Blood cholesterol levels in dogs and monkeys were approximately twice as high as in rats. Levels were higher in female rats and monkeys and tended to be higher in male dogs. In rats, cholesterol tended to increase with age up to 20 weeks. There was practically no difference between rat serum/plasma cholesterol levels on fasting and those on feeding in the present survey, despite reports describing higher levels in blood obtained on feeding in this species [30, 39, 52, 56]. In female dogs, serum/plasma cholesterol is gradually elevated under the influence of progesterone following estrus, reaching a peak level in about one month [65]. There was no obvious change with age in dogs, or rhesus monkeys. Free and esterified cholesterols in plasma have been reported to increase with age [42, 52].

Triglycerides, TG: The majority of facilities employed the glycerol kinase/glycerol-3-phosphate oxidase/peroxidase (GK-GPO-POD) method. Free glycerol (FG) has been reported to interfere with assay results; found values with the GK-GPO-POD method in which FG has been eliminated, comprising the greatest number of data reported, are presented in Tables. More facilities are now employing this assay method without FG elimination. Higher TG levels were noted in rats than in dogs and monkeys, levels in the latter two of which were comparable. In rats, the serum/plasmaTG level tended to increase with age; moreover, levels were roughly twice as high in blood drawn on feeding than in fasting blood. Diet had no influence in dogs or monkeys, and no strain differences were seen in rats. The TG level was somewhat higher in male rats than in females. Dogs and monkeys showed no difference between sexes. The TG level in rat serum/plasma did not differ with sampling site among the abdominal aorta, jugular vein, femoral artery and femoral vein.

Total protein, TP: Total plasma/serum protein was determined by the biuret method at the majority of facilities. Plasma/serum TP was highest in monkeys, followed in order by rats and dogs. There were no differences among strains of rat. TP increased progressively with age up to 71 weeks in rats and up to 32 months in dogs. Male rats exhibited higher values than female rats, while no difference was observed between sexes of dogs or monkeys. No difference was seen in any species between TP levels in serum and plasma, nor was any obvious difference seen between values on fasting and on feeding.

A/G ratio: Albumin to globulin ratio was calculated from colorimetric quantitation of albumin at about 80% of the facilities; the remainder used densitometric measurement after electrophoresis. The ratio did not differ among strains of rat. In rats and dogs, the ratio tended to be higher in females than in males. It tended to decline with age in rats but no such age difference was seen in dogs. Colorimetric determination of albumin was by the bromcresol green dye (BCG) method. Values with this method

vary with the type, concentration and pH of the buffer solution, and with the type and concentration of surfactant used. It should therefore be noted that the use of reagent products from different manufacturers is directly reflected in assay results.

Urea nitrogen, UN: The urease-indophenol and the urease-UV assay techniques were employed. The latter has recently received widespread application. Rats showed the highest serum UN levels, followed in order by monkeys and dogs. Serum UN declined on fasting; this trend was conspicuous in rats and dogs but practically nonexistent in monkeys. In both rats and dogs, somewhat higher levels were observed in females than in males although data from a few facilities showed no sex differences. There was no sex difference in monkeys. Rat serum UN levels showed no differences by strain or age. Serum UN in dogs increased with age from 6 to 12 months after birth. It is widely recognized that serum UN level is affected by dietary protein content and dietary intake of protein while hemolysis, anticoagulants and preservation of specimens have no significant influence [26].

Creatinine: Most facilities used the alkaline picric acid method utilizing the Jaffé reaction, while some institutions employed the enzymatic method. Values with the latter technique tended to be slightly lower. There was no difference between SD and Wistar rats, while F344 rats showed somewhat lower values than these two. No sex differences were noted in rats, dogs or monkeys. The creatinine level may vary depending not only on assay conditions but also because of hemolysis, the degree of anesthesia for blood collection or reaction with drugs administered [15, 32, 49].

Sodium, Na: Virtually all facilities used flame photometry. The ion specific electrode method and atomic absorption spectrometry were only rarely used. Serum/plasma Na levels did not vary with the analytical method used. Slightly higher levels were reported in monkeys and slightly lower levels in rats, but no species differences were noted. There was no strain difference in rats, and no differences sex or age differences were seen in rats, dogs or monkeys. The species studied showed little or no variation with site or conditions of blood sampling, assay conditions, or feeding conditions.

Potassium, K: The most frequently used method was flame photometry, followed by the use of an ion specific electrode and atomic absorption spectrometry. Measurement was made at the same time as that of Na. There were no significant species differences among rats, dogs and monkeys, and no strain differences in rats. Serum/plasma K levels in rats, dogs and monkeys did not differ between sexes or by age. It is advisable to use plasma to measure this parameter because when serum is used, leakage of K from erythrocytes on standing leads to higher values.

Chloride, Cl: Most laboratories used coulometric titration. Somewhat higher values were observed in dogs than in rats and monkeys. No strain differences were noted in rats. There were no sex or age differences, or differences by feeding conditions.

Calcium, Ca: Nearly all facilities used the *o*-cresolphthalein complexion (OCPC) method, but a few used the alizarin sulfonate method. Both methods are based on a chelation reaction, and therefore yield lower values in assays on specimens sampled with EDTA. This anticoagulant was used at a few facilities. Dogs exhibited slightly higher values, than those for rats and monkeys, which were the same in rats, there were no differences among strains or between sexes. Little or no difference by age, feeding conditions or method of sample collection were seen in any species.

Inorganic phosphorus, Ip: Most of the data were obtained by the Fiske-Subbarow method or the phosphomelybdate method. Serum/plasma levels of Ip were highest in rats and marginally lower in dogs and monkeys in this order. Rats showed no strain differences. In rats, dogs and monkeys, somewhat higher values were observed in males than in females. In rats and dogs, the serum/plasma Ip level decreased with age but did not significantly vary with sampling site or feeding conditions.

Creatinine phosphokinase, CPK: Measurement of CPK activity was by the NADPH (reduced form) method at the majority of facilities. No significant differences in serum/plasma CPK level were observed among rats, dogs, and monkeys. SD rats showed higher values than Wistar and F344 rats. There were no sex differences in rats, dogs or monkeys, and no age differences in rats. In dogs serum/plasma CPK tended to decrease slightly with age. In rats, CPK values are subject to the influence of serum or plasma separation and storage conditions [63]. Serum CPK levels are sharply increased when serum is separated after allowing whole blood to stand at room temperature, but no change occurs in the case of plasma [25]. The present data showed facility differences in serum/plasma CPK level, with a maximum 20-fold difference. There were clearly greater variations in serum than plasma CPK level. The serum CPK values shown in Table 1 exclude abnormal values, which accounted for approximately 60% of data reported. Assays on samples drawn from the retro-orbital sinus of rats showed higher CPK levels with greater variations. In rabbits, repeated blood sampling for serial measurements leads to elevation of serum/plasma CPK [24].

Urinalysis

Urinary volume: 24-hour urine collected from SD rats with *ad libitum* access to food and water ranged from 4 to 24 m*l* per animal. In dogs, 24-hour urinary output under non-fasting conditions with free access to water ranged from 140 to 420 m*l*. When animals were deprived of water, urinary volume decreased markedly to nearly half of that under *ad libitum* supply. Urinary volume was measured using a graduated cylinder in nearly all laboratories. Monkeys showed wide individual variations, and collection conditions varied with facility; nevertheless, urinary output was 50 to 200 m*l* per animal, comparable with data reported by other workers [62].

Urinary protein: Almost all facilities use reagent strip tests forsemi-quantitation of urinary protein. Pretreat-

ment of specimens by boiling or dialysis against distilled water was required in cases where the test gave a false positive or false negative result due to reaction of the reagent with a drug or its metabolite excreted in urine. Specific gravity and pH of urine were also influenced. The reagent strip test data revealed no age difference in rats, dogs or monkeys, significant strain difference in rats, and no sex difference in rats or dogs. Proteins excreted in urine vary with species [23, 25]. Strain differences in urinary protein excretion have been reported in rats [1]; furthermore, the urinary protein fraction pattern in rats also varies with age [70]. The present data suggest that urinary proteins are likely to increase with age, more frequently among male rats than females. Dogs showed a similar trend in agreement with other reports [44, 45]. Urinary albumin is said to increase and urinary globulin decrease in rats with chronic progressive nephrosis [70].

Urinary electrolytes: Flame photometry was the preferred method for determining Na and K in urine, while coulometric titration was preferred for urinary chloride. For rats, 16- to 18-hour urine collection was most common. Urinary electrolyte excretion is usually evaluated in terms of total excretion or concentration in urine. The former was used at 65% of facilities. No sex differences were observed in urinary electrolyte concentration in rats or dogs. There were no appreciable changes with age. Values were higher in samples collected from fasting than feeding animals.

PREANALYTICAL VARIATION

Age-related changes: Rt count, ALP and Ip tended to decrease with advancing age. Conversely, erythrocyte count and serum/plasma protein (globulin) tended to increase with age. A decrease in ALP with age is seen not only in mammals but also in fowls [37]. Regarding WBC, neutrophils have been reported to increase and lymphocytes to decrease with age.The present survey data also showed this tendency. The range of normal values for these clinical laboratory parameters increases expands with age. In rats, so-called chronic progressive nephrosis begins to develop at 10 months of age, and is clinically manifest in some 20% to 30% of animals at the age at which terminal necropsy is conducted in a 12-month dosing toxicity study. This leads to wide variations in the range of normal control values in clinical pathology testings [35, 43, 69]. In dogs, cholesterol and protein are elevated and Ip, ALP, glucose, GOT and CPK are decreased with age [31, 46, 66]. One report describes that cholesterol, glucose and GOT levels in dogs show no significant change between ages 6 to 12 months [26].

Strain differences: These differences are known to exist in rats and monkeys [68]. It is therefore important to use the same strain of animal in any particular toxicity study.

Sex differences: Parameters for which sex differences were seen included Hb, Ht, WBC, ALP, glucose, cholesterol, TG, total protein, A/G ratio, UN and Ip. Sex differences were observed in dogs as well as in rats. It has been reported that female dogs exhibited higher values for cholesterol and UN than males, while male dogs showed higher GPT levels than females [31].

Effect of diet: Among factors affecting clinical laboratory tests are the composition and ingredients of the diet and the amount of feed supplied. It is essential to standardize feeding conditions because the influence of feeding or fasting at the time of blood and/or urine sampling is considerable. For rats, formula diets containing 20% to 29% protein are used in this country and in the United States, whereas those with protein contents of 15% to 20% are used in British facilities. Viewed in consideration of animal nutrition physiology, it may be unwise to feed animals with a diet of the same composition without regard to stage of life, i.e.juvenile, sexually mature, or senile.

When fasted, F344 rats show increased values for RBC, Hb, Ht and platelet count [34]. In SD rats, fasting leads to an increase in erythrocyte parameters and a decrease in WBC, glucose, UN, GPT and ALP [4]. There is room for further investigation as to the necessity of fasting in toxicology evaluation.

Animal handling and stress: Depending on handling of the animal, animals become excited or stressed at sampling of blood or other specimens or depending on handling of the animal. These situations have been reported to lead to the release of catecholamines and corticosteroids, which eventually affects normal reference data for clinical laboratory testings. At collection of clinical specimens, normal values for WBC and blood glucose are altered if animals are excited or frightened on restraint or handled without anesthesia [6]. While erythrocyte counts in rats is relatively stable, that in dogs show marked variations under a variety of stressful conditions [20, 57].

Blood collection procedures: Animals should be bled in random sequence to minimize bias. Hemolysis must be avoided; heparinization and storage temperature should be suitable, plasma separation quick and gentle, and contamination with platelets and other blood cells avoided. In collecting blood samples, the bleeding site, sampling method, duration of preceding fasting and time of sampling all influence laboratory test values.

Bleeding intervals: Bleeding at inadequately short intervals or in a large volume results in anemia of the animal. Not only are hematological parameters affected, but also plasma GPT level are elevated. It has also been reported that increased frequency of blood sampling influences plasma/serum levels of GOT, GPT and ALP, as well as causing variations in Ht, RBC and Rt [53, 54]. Careful consideration should be given to the volume of blood sampled and bleeding intervals to avoid transient oligemic anemia. It has been reported that the maximum amount of blood which can be withdrawn without causing physiological unrest is 10% of total systemic blood volume [19]. It is also advisable to collect blood samples at the same time period of day since certain blood chemical

parameters show diurnal variations, including glucose, hormones and rat plasma/serum ALP [57].

Hemolysis: Hemolysis in test specimens affect not only hematological parameters but also blood coagulation and various biochemical parameters [20]. Leakage from erythrocytes results in noticeably increased levels or activity of AST, LDH, Ip, K, CPK, ALP and cholesterol. Generally, blood samples showing evidence of hemolysis should not be used in laboratory examination. Overt hemolysis is rare; however, even if the blood sample is unhemolyzed at drawing, preservation of whole blood results in changes in corpuscular or serum constituents.

Use of anesthesia: In collecting blood samples, it is important, for humanity reasons, to minimize pain to the animal. Such efforts will also facilitate and speed sample collection, thus reducing effects on blood components. Bleeding site and anesthesia have a profound bearing in rats and mice [43, 58].

Bleeding site and blood sampling method: The site from which blood is sampled differs between Japan and Western countries [38]. Bleeding rats or mice via the retro-orbital sinus, a technique preferred in the United States and Europe, often entails the occurrence of artefacts. It causes ocular damage [19] and carries the potential risk of injuring adjoining tissues such as the orbital tissues and Harderian glands [40, 43, 58]. Attempts to obtain control reference data for clinical pathology parameters with samples collected via the tail vein, jugular vein or sublingual vein have recently been reported [3, 4].

Serum levels of ALP, GOT, GPT and LDH activity and serum K concentration were significantly higher in samples obtained from the posterior vena cava than from the retro-orbital sinus [25]. This finding suggests that these parameters, particularly enzyme activity, are affected by contamination with enzymes leaked from injured blood vessels and adjoining tissues. Friedel *et al.* [18] reported that significantly higher values for plasma LDH, GOT, GPT, CPK and ALP activity were observed with rat plasma samples collected via the orbital sinus than those obtained via the jugular vein. It is widely recognized that analytical values for parameters determined with serial rat blood specimens from the orbital sinus show greater coefficients of variation than with those from the posterior vena cava, the former collection site being commonly employed for repeat sample collection. Since clinical laboratory data are largely dependent on proper phlebotomy techniques, constant efforts to improve blood collection skills is a prerequisite for accurate and precise detection of toxicity.

Sample preparation after blood sampling: Because platelets are fairly rich in LDH and CPK and RBC in LDH, GOT and K [63], plasma enzyme levels become elevated unless plasma separation and assays are performed immediately following blood collection. As described under hemolysis, allowing blood samples to stand for an undue length of time results in an elevation in GOT, LDH, K and CPK and a decrease in Na and glucose in serum. Collected samples should therefore be processed quickly. With regard to the interrelation between post-collection time to assay and serum LDH level, Matsumoto *et al.* [36] reported that enzyme activity was increased approximately 10% in human serum, sevenfold in canine serum and nineteenfold in rat serum when measured 3 hours after sample collection, compared to values at 5 minutes post-collection. The findings stress the need for great caution in blood sample handling. The time for which whole blood is allowed to stand between blood collection and serum separation has a critical influence on clinical laboratory parameters in laboratory animals, especially in rodents. The activity of LDH and GOT [10,16,17] and CPK [41] in whole blood rises on standing at room temperature. This elevation is generally thought to be due to the liberation of these enzymes from platelets during the clotting process. This thrombocytic enzyme release varies markedly with the species; it is rather modest in dogs and monkeys and conspicuous in rabbits and rodents [10,16,17].Serum enzymes are not elevated in heparinized blood. Use of plasma instead of serum is advisable for assays because marked elevations usually occur in serum, particularly in LDH and CPK activity.

Anticoagulants: Heparin is preferable to chelating agents such as EDTA as an anticoagulant for the separation of plasma, because chelating agents affect measurements of ALP, LDH and Ca. Ammonium salt of heparin, however, is not suitable for UN assay by the urease-indophenol method as ammonium ion inhibits urease activity [20].

Plasma versus serum samples: Since normal values for certain clinical laboratory parameters differ between serum and plasma [23], the preferred specimen for each given parameter should be determined. Contamination of blood fluid samples with cell components must be avoided during separation as platelets and RBC contain an abundance of CPK and LDH [51], as well as K. Blood samples should not be refrigerated before or after plasma/serum separation to prevent transudation of these constituents. Both serum and plasma are used in clinical biochemistry examinations in toxicity studies. Stevens and Gallo [57] recommend the use of plasma instudies using laboratory animals, particularly rodents, in view of the marked release of intracellular substances from blood corpuscles, platelets in particular, during blood coagulation.

Storage of sample until measurement: Clinical laboratory testing aim for prompt determination to aid diagnostic evaluation of the clinical condition, and are usually carried out on the same day as sample collection. One should be aware, however, that found values vary with the mode of sample storage. Serum K concentration is elevated during storage of blood as a result of K release from blood cells into serum via decreased active transport and via passive diffusion. This phenomenon occurs with serum as well as plasma, but is more pronounced in the former. Blood samples show greater changes in serum/plasma K concentration when stored at 4°C than at room temperature, resulting from depressed active transport

through the erythrocyte membrane [25]. Caution should be taken in handling such clinical specimens and in evaluating observed assay data therefrom.

Urine collection procedures: It is important to use a suitable quantity of preservative for each 16- to 20-hour urine collection specimen, because bacterial contamination and proliferation causes pH to rise, and such alkaline urine gives a false positive reaction for protein and lowered values for glucose and ketones in the reagent strip tests. Besides their antiseptic effects, preservatives have a second advantage in preventing evaporation of the collected urine [13, 20]. Measurement of urine output is generally performed with the animal deprived of food and water, or while fasting with *ad libitum* access to water. The former is subject to legal restrictions under the Animal Welfare and Act while the latter entails the risk of contamination. An appropriate urine collection method should be chosen to meet the purpose of examination, e.g. renal urine concentration or diuresis. Some investigators point out that administration of water by oral gavage to animals kept from access to food and water leads to decreased urinary electrolyte levels. However, this procedure poses no problems insofar as assessment is made with respect to the amount of excretion per day, and is considered to be most suitable for evaluation in toxicity studies since individual variations in urinary volume are minimized by the specified quantity of water loaded per unit body weight. Urine is advisably collected at a specific time of day because of diurnal variations in urine output and electrolyte excretion [5].

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