Change of Body Weight and Hematologic Value with Aging in Hybrid Mice*

— Preliminary Study —

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The mouse is one of the most popular experimental animal which has wide variation of strains, diet, environment, breeding technique, and diurnal cycle. Total 731 mice (male 372, female 359) were used for the standard data of our laboratory. Proper age for experiment were 30±3 days, body weight were 25±2 gm (male), 23±1 gm. (female), minimal diurnal variation showed from 9 A.M. to 12 P.M.

Key Words: Experimental animal, Body weight, Durnal variation, Hematologic changes.

INTRODUCTION

Various experimental animals have been used in the world, and mouse is the most popular animals for various research work. Many strains of inbred mice were systemized, and many literatures demonstrated that not only growth and development of mice, but also physical characteristics were strongly influenced by strains, diet, handling method, environment and diurnal cycle, even in same strain.[1-5] Therefore, selection of experimental animal is very important and may modify all results.

We need our experimental data for standardized and the basic data for further research. Total 731 hybrid white mice were used and following datas were investigated.

1. Weight with aging, hematologic change including Hb. WBC, Differential count were analyzed for selection of proper age, weight and mean hemotological findings.
2. Diurnal changes for 24 hours were studied for selection of proper time with less extensive variation of hemotolic datas.
3. Based on the data 1 and 2, total 312 mice with equal sex distribution were selected and Hb, WBC, Differential count, clotting time, platelets, urinalysis test were performed for basic data for our laboratory.

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MATERIAL AND METHOD

1. Growth and developmental change with aging

10 mother mice with full term pregnancy by "brother-sister" or "offspring-young parent" mating were selected. All mice were feeded with commercially available mixed diet for small animals. Mother mice were placed one for 1 cage with extreme care, all mice except one delivered within 10 days. Total 48 new born mice were gained with 4-11 litters per each mouse. Delivery date were labeled and mother and all litters placed together until weaning days (18-20 days). As soon as weaning and possible sex differentiation, all mice were tagged for identification and separated by sex. Thereafter, weight were scaled every 2 days until 30 days of age, every 3 days until 60 days, then 1 time per 1 week regularly. Urinalysis were done simultaneously. Hb, WBC, Diferential count were performed every Friday by puncture of tail vein with same pipette and chambers for human test using modified Randolph and Standon method.[9] Used tag was "Tag Size 1 (National

Band & Tab Comp. U.S.A)," scale was "Mouse Scale Model Z-40" (Toconic Farms Inc. USA). Unistx (Young Dong Pharm.) was used for urine protein. Second examination was repeated with 51 new born mice by 10 mother mice and same method of 1st examination were used. Of total 99 mice throughout 1st and 2nd examination, male were 43, female were 56.

2. Diurnal variation

Used animals were 80 hybrid white mice, equal sex distributed, 25±2 gm (male), 23±1 gm (female) weighted, which based on previous experiment 1. All animals were left undisturbed as possible as we could for at least 24 hours before experiment. Blood samples were taken at 3 hours intervals for 24 hours.

3. Mean value of weight, hematology and urine

Based on previous experiment 1 and 2, total 312 hybrid mice with equal sex distribution, 25±2 gm (male), 23±1 gm (female) weighted, total 312 mice hybrid white mice were used for Hb, WBC, differential count, platelet and urinalysis. Experimental procedure was performed from 9 AM to 5 PM which showed least difference of diurnal changes by previous experiment.

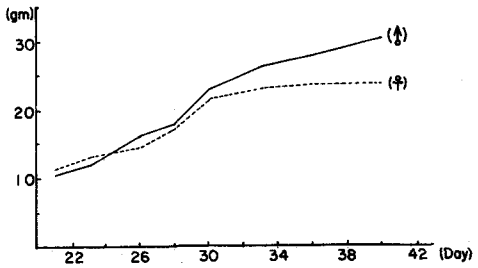

Fig. 1. Body weight change in aging (days)

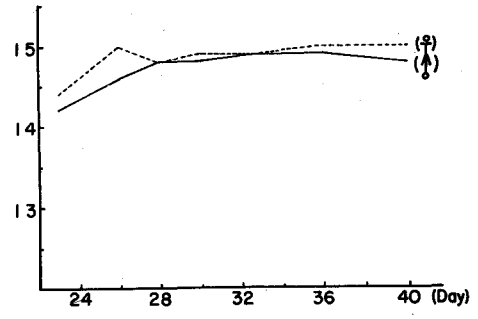

Fig. 2. Hb change in aging (days)

RESULT

1. Growth and developmental changes with aging

WEIGHT: (Table 1, Fig. 1).

Weight of commonly used mice (in my experiment in Mass. General Hospital, USA), 25±2 gm was compatible with 30-36 days of age in male, 30-60 days of age in female. In other words, if we select 25±2 gm weighted male mice, same aged female mice would be 22-24 gm, (23±1 gm). This

Table 1. Body Weight Change in Aging (days)

	Weight (♂)		Weight (♀)	
Age	Mean	S.D.	Mean	S.D.
21	10.5	±0.76	11.6	±0.84
23	11.9	±1.42	13.3	±1.11
26	16.4	±1.82	14.6	±1.31
28	18.0	±1.57	17.4	±1.36
30	23.0	±1.50	21.6	±1.27
33	26.3	±1.59	23.3	±1.38
36	28.0	±1.62	23.6	±1.43
40	30.7	±2.08	24.0	±0.85

Table 2. Hb Change in Aging (days)

	Hb (♂)		Hb (♀)	
Age	Mean	S.D.	Mean	S.D.
23	14.2	±0.58	14.4	±0.59
26	14.6	±0.78	15.0	±0.60
28	14.8	±0.56	14.8	±0.88
30	14.8	±0.52	14.9	±0.51
33	14.9	±0.51	14.9	±0.40
36	14.9	±0.26	15.0	±0.66
40	14.8	±0.42	15.0	±0.35

Table 3. WBC Change in Aging (days)

	WBC ♂ (×10³)		WBC ♀ (×10³)	
Age	Mean	S.D.	Mean	S.D.
23	5.96	±0.53	5.68	±0.55
26	6.56	±0.44	6.31	±0.85
28	6.69	±1.13	6.41	±0.61
30	7.31	±1.08	7.00	±0.55
33	8.50	±0.47	8.06	±0.94
36	8.63	±0.82	8.11	±0.73
40	8.78	±0.57	8.64	±0.77

weight difference showed increasing with aging in our experiment. (30.7 gm in male,.24 gm in female of 40 days of age).

Table 4. Differential Count Change in Aging (days)

Age	Lym, Neu (♂) Mean	S.D.	Lym, Neu (♀) Mean	S.D.
23	65.26	±6.2 ±5.6	65.28	±5.0 ±5.4
26	68.24	±6.0 ±6.1	68.25	±5.4 ±5.1
28	70.22	±3.8 ±4.9	71.23	±5.6 5.1
30	72.21	±3.6 ±3.2	71.22	±2.9 ±2.4
33	73.19	±2.4 ±3.1	74.19	±2.9 ±4.0
36	74.20	±3.5 ±3.2	73.21	±2.2 ±2.4
40	73.20	±2.4 ±3.0	74.19	±3.0 ±3.0

HEMOGLOBIN (Table 2 Fig. 2)

At 23 days of age, Hb. value was 14.2 (male), 14.4 (female), the value was increased gradually with aging. No significant difference whoed after 28 days of age. (14.8 in male, 15 in female at 28 days of age).

WBC (Table 3, Fig. 3).

At 23 days of age, the value was 5960 in male, 5680 in female, this value showed gradual increasing with aging. At 40 days of age, WBC mean value was 8780 in males and 8640 in females, showing 140-500 difference between male and female.

DIFFERENTIAL COUNT. (Table 4, Fig. 4).

At 23 days of age, proportion of lymphocyte was 65% in male and female, segmented neutrophil was 26% in male, 28% in female. Lymphocyte proportion was progressively increased with aging, the mean lymphocyte value was 73% in male, 74% in female, segmented neutrophil was 20% in male, 19% in female at 40 days of age which showed least difference between male and female.

URINALYSIS.

Urine protein tests showed + − +++, variable value which showed no significant correlation with age, sex, even in same mice.

Table 5. Diurnal Variation

	WBC (♂)	Diff. count (%)				WBC (♀)	Diff. count (%)			
	×10³	Lym	Neu	Eos	Deg	×10³	Lym	Neu	Eos	Deg
6	0.07±0.54	72±2.7	23±2.5	1.0	4.7	9.50±0.80	74±5.7	20±3.7	1.0	4.3
9	9.34±0.55	70±2.8	24±1.0	0.3	6.5	9.80±0.30	69±1.0	23±2.0	1.5	7.5
12	11.10±0.64	72±2.0	22±2.0	0.6	5.0	10.80±0.69	73±3.7	19±4.0	0.7	7.0
15	12.67±1.31	70±2.0	25±2.0	1.5	3.5	13.20±1.02	71±2.2	22±4.0	1.3	3.7
18	10.98±1.05	69±1.8	25±4.3	1.3	4.3	11.10±0.87	69±2.3	25±2.0	0.5	3.5
∠1	10.00±1.05	69±3.1	26±4.0	1.0	5.0	10.83±1.60	69±1.4	26±2.1	1.0	4.5
24	8.75±0.54	67±2.6	25±4.0	1.5	6.5	8.67±0.90	68±3.1	25±4.6	1.3	5.5
3	9.28±0.87	70±1.5	24±3.8	1.7	4.0	9.53±0.40	71±2.6	24±1.4	1.3	5.3

Table 6. Mean value & standard deviation

		♂♂ Mean	♂♂ S.D.	♀♀ Mean	♀♀ S.D.
Hb		15.1	±1.72	15.5	±0.93
WBC		9160	±2620	8440	±2220
Diff.	Lym	71	±7.0	73	±5.2
	Neu	22	±6.3	19	±3.8
	Eos	0.9		1.4	
	Deg	6.3	±2.3	6.7	±2.7
Platelet		1.233×10^6	$\pm0.229\times10^6$	1.214×10^6	0.215×10^6
Clotting time (sec)		40	±5	40	±5
Urinalysis		+∼+++		+∼+++	

2. Diurnal variation.

Throughout 4 times repeated experiments, peak value showed at 3 PM in male and female, Nadir was 12 AM which showed some difference from H, Brown's experiment[3] which showed nadir at 9 PM. Cycle variation was a little more irregular in females than males.

3. Mean value

Mean Hb. value was 15.1 in male, 15.5 in female, mean WBC was 9160 in male, 8440 in female, mean lymphocyte proportion was 71% (male), 73% (female), segmented neutrophil was 22% (male), 19% (female) Urine protein was + − +++, no significant difference between male and female. WBC value was slight higher than diurnal experiment, which is suppose due to continued stimuli and different experimental time.

DISCUSSION

Small animal, especially mice has been used for various research as the most popular experimental animal. Many strains, over 200, of mice has nomenclatured and tremendous study of the strain characteristics, systemic breeding, husbandry, nutrition and physiology have reported.[1-3,5,10-12] Also many authors demonstrated by their experiment that physical characteristics were strongly influenced by strain, sex, environment, diet, stress, handling technique and anesthesia.[6-8,13-17,19] Therefore there is no doubt that basic data should be established for each laboratory even though they used same strain since those data can laboratory even though they use same strain since those data can modify any result for any experimental subjects. In my experience, I used C3Hf/sed. mice in Mass. General Hospital which were 8-12 week old, 20-28 gm weighting, but those standard were compatible with 30-36 days old for our hybrid mice of male in Yeung Nam breeding room. Female mice should have some difference in weight if we want same aged mice and same condition.

Hematologic change is strongly influenced by strains, sex[3,10] but the most variable change showed in diurnal cycle experiment, the difference was over 50% between peak and nadir which suggested that selection of time is very important factor for in vivo experiment. E.S Russel (10: 354P) quoted from Budds' report that anesthesia decreased segmented neutrophil in male, lymphocyte in both male and female, which suggested that anesthesia may modify the hematologic responses. In our experiment, we

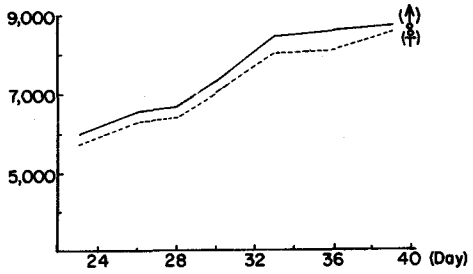

Fig. 3. WBC change in aging (days)

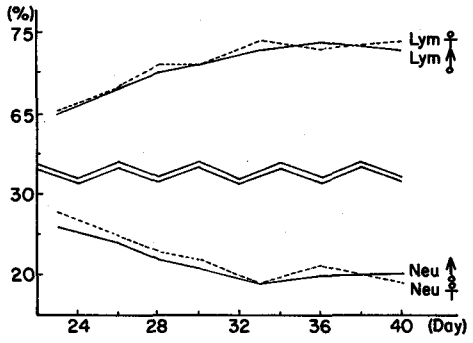

Fig. 4. Differential count change in aging (days)

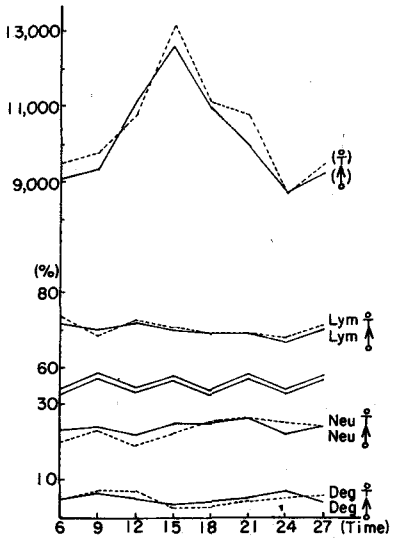

Fig. 5. Diurnal variation

used hybrid mice; not pure strain, which breeded by "brother-sister" or "offspring-young parent" mating and raised in conventional colony, not germ free colony. Unfortunately, we could not excluded the possibility that there may be some difference between our datas and pure inbred strains which handled in strict germ free colonies. It is encouraging, however, that those our data can be helpful for our further experiment as a standard preliminary data, even though these are not perpect.

CONCLUSION

1. Mean weight of 30±3 days old hybrid mice of our laboratory was 25±2 gm in male, 23±1 gm in female, the difference in male and female was increased by aging.
2. Mean Hb. value was 14.2-14.8 in male, 14.4-15.0 in female without significant difference by aging.
3. WBC count showed gradual increasing by aging, mean was 8780 in male, 8640 in female at 30±3 days of age. Differencial count showed that lymphocyte proportion was increased gradually by aging, while segmented neutrophil showed gradual decreasing tendency.
4. Diurnal variation of WBC showed meal pm 3 PM, nadir on 12AM, but no significant variation was observed in differential count.
5. Urinalysis were variable, + − +++, with no correlation with sex, aging and time.
6. In experiment of total 312 hybrid mice, mean Hb. was 15.1 in male, 15.5 in female, WBC was 9160 in male, 8440 in female.
7. Clotting time was approximately 40 sec. in both, male and female, and mean platelet count was 1.23×10^6 in male, 1.21×10^6 in female.

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=국문초록=

한국산 잡종쥐의 성장에 따른 체중 및 혈액상의 변화에 대한 연구

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이성헌 · 조길호 · 신세원 · 김명세

각 실험실마다 사용되는 동물의 종류는 매우 다양하며 특히 소동물의 경우 사용되는 동물의 선택 기준은 실험의 내용은 물론 추후검사의 성적을 수식할 수 있는 요소가 될 수 있으므로 매우 중요하다. 특히 쥐의 경우는 세계각국의 여러 실험실에서 다양한 종류에 대한 실험성적이 많이 보고되어 거의 체계화 되어 있다고는 하나 같은 종류라 하더라도 사료의 종류, 환경, 사육방법등에 따라 발육상태 및 반응이 매우 다르므로 각 실험실의 기본 정상치의 확립은 필수적인 것이다.

이에 본 영남대학병원 치료방사선과교실 소속 방사선 생물학 실험실에서는 잡종백서 731마리를 대상으로 하여

1. 분만후 성장에 이르는 과정을 추적하여 일령에 따른 체중, 혈액상을 분석하여 본 실험실에서 적당하다고 생각되는 일령 및 체중의 기본치를 정하고저 하였으며,

2. 동일한 쥐에서도 하루의 주기적인 변화에 차이가 매우 크므로 이를 분석하여 변화가 비교적 적으며 실험하기에 편리한 시간에서의 기본치를 측정하였고,

3. 위의 성적을 토대로 하여 본 병원 부속 연구소 소속 사육실에서 동일한 조건으로 사육된 암수 동수의 312마리의 쥐를 선택하여 체중, 혈액검사 및 요검사를 시행하여 이 분석결과를 본 실험실에서의 추후 실험을 위한 기본자료로 삼고저 하였다.