CHANGES IN SERUM IMMUNOGLOBULIN LEVELS AND NEUTROPHILIC PHAGOCYTOSIS AFTER EXERCISE

TAKASHI KUMAE, KAZUO SUGAWARA, KAZUHIKO MACHIDA
and AKIRA SHIMAOKA

Abstract

This paper is intended to give the information we need about personal reactivity to transient loads for the object of estimating human physical fitness. For this purpose, both serum immunoglobulin levels and neutrophilic phagocytosis were assayed as indices to self-defense activities.

To determine the later described reproducibility of experimental results, two independent experiments were made on two different groups—one consisting of twelve and the other of fourteen male voluntary students of our college—exercised in the playground. The serum levels of three immunoglobulins (IgG, IgA and IgM) and two complements (C3 and C4), prior to and immediately after exercise, were compared with each other. They rose without exception after exercise.

Paired T-test revealed that in the group of twelve students, rises in IgG, IgA, C3, α -1-antitrypsin and transferrin were significant at $P < 0.1\%$, 5% , 5% , 5% and 0.1% , and in the group of fourteen rises in IgG, IgA, IgM, C3, C4, α -1-antitrypsin and transferrin were significant at $P < 1\%$, 0.1% , 1% , 0.1% , 0.1% , 0.1% and 0.1% , respectively.

In the meantime, the neutrophilic phagocytosis (%) was significantly decreased immediately after exercise in both groups ($P < 1\%$ and $P < 0.1\%$ respectively).

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key words : Male students ; 1,500 meter running ; nephrometric system

I. Introduction

To carry out investigations into personal resistant activities or protective physical fitness to physical stresses is very important to help live a good life free from disease. How to estimate physical fitness, however, is a difficult problem.

Many reports dealing with the cellular or metabolic effects on muscular work have so far been concerned with lactic acid production during exercise^{1,3)}, free fatty acids and glucose^{3,10)}, or enzyme activity^{2,8,9)}. A few studies of the effect of muscular work on serum immunoglobulin levels and neutrophilic phagocytosis in man were concerned primarily with changes in immunoglobulin levels⁴⁾ or with

changes in neutrophilic phagocytosis in a relatively small number of subjects⁶⁾.

To examine the reproducibility of experimental results, two experiments were made using two different groups (of twelve and fourteen males). The serum levels of immunoglobulins (IgG, IgA and IgM) and complements (C3 and C4) were measured by the nephrometric system¹¹⁾, and phagocytosis against carbon black P32 was measured by a modification of the method described by Hirokawa et al (1960)⁵⁾.

II. Materials and Methods

A. Subjects

The student volunteers were untrained and healthy subjects. In the group of 12 males, the

age ranged from 21 to 30 with the mean at 25.7 years, and in the group of 14 males, 18 to 26, with the mean at 20.0. No subjects had ever taken part in any regular physical activity programs. They were selected at random among our college seniors and freshmen for the 12 and the 14 male group, respectively.

The subjects had been examined for diseases prior to testing ; none had evidence of cardiac, hepatic, metabolic, or muscular diseases. Anthropometric data are shown in Table 1.

Table 1. Anthropometric data of subjects.

Groups	Age (years)	Height (cm)	Weight (kg)
12 males (Seniors)	25.7 $\pm$ 4.0	171.0 $\pm$ 6.5	63.8 $\pm$ 8.7
14males (Freshmen)	20.0 $\pm$ 3.6	169.0 $\pm$ 5.3	62.2 $\pm$ 8.2

B. Exercise

A 1,500 meter run was given as an exercise. According to each one's capacity, the subjects ran 1,500 meters at various speeds in the playground. The mean time record and standard deviations was 419.8 $\pm$ 49.8 sec. for the younger group, and 380.5 $\pm$ 34.0 sec. for the elder.

C. Blood samples

The subjects were kept at rest for at least 30 min. before exercise, and none had taken part in any athletic events on that day. The experiments were made at least 3 hrs. after their last meals. The composition and quantity of the meals were not controlled.

Venous blood samples were obtained prior to and immediately after the 1,500 meter run. Blood (5 ml) was drawn by venipuncture and divided into 2 portions (2 ml and 3 ml). One portion (3 ml) was allowed to clot and centrifuged. The serum was separated and stored at -70°C. A heparin solution (10 μ l, 1,000units/ml) was added to the other portion (2 ml). The anticoagulant blood was immediately used to measure neutrophilic phagocytosis.

D. Measurement of immunoglobulin

The serum levels of immunoglobulins and complements were measured by the nephrometric system (Nephrometer, Kyoto Daiichi Kagaku Co. Ltd). Each collected serum sample and the standard serum (Kallestad Laboratory) were diluted 101 times using a filtered physiological saline that contained 4% (W/V) polyethylene glycol 6,000, mixed with a corresponding filtered antibody (Wako Chemical) saline solution. After allowing to stand 1 hr. at room temperature, the brightness of a scattered light produced by a state of aggregation of an antigen-antibody complex was measured and the amount of the corresponding antigen was determined by the calibration curve method. This measurement is reported to have been applicable to many serum proteins to measure, and the results well agreed with those by the SRID method¹¹⁾. The serum IgG, IgA and IgM were measured by the method proper to immunoglobulins, and C 3 and C 4 by that proper to complements. The other serum proteins, α -1-antitrypsin, and transferrin levels were also measured as indices to the degrees of serum concentration.

E. Method of phagocytosis

The neutrophilic phagocytosis was measured in whole blood by an application of Hirokawa's⁵⁾ method. As the object to be phagocytized, carbon black P32 was chosen and suspended in a Ringer solution containing gum arabic (0.1% W/V). To standardize the particle size, the suspension was repeatedly centrifuged, and the particle size was decided by watching a suspension of 2.5 G to 3.5 G to be formed.

The sediment formed at less than 2.5 G was excluded, and the supernatant at more than 3.5 G was decanted. To keep a constant concentration, the obtained suspension was di-

Fig. 1. Changes in serum immunoglobulin levels due to exercise. Each plotted point represents mean of measurements and vertical bars represent 1 SD. ○, 14 male group and ●, 12 male group.

Fig. 2. Changes in serum complement levels due to exercise. Each plotted point represents mean of measurements and vertical bars represent 1 SD. ○, 14 male group and ●, 12 male group.

luted with a Ringer solution containing gum arabic to an absorption (1.4) at 520 nm using a 1 cm. quartz glass cell.

The thus prepared test suspension (0.1 ml) was added to fresh blood (0.5 ml) made anticoagulant by adding heparin, and the test tube containing thus prepared blood was immersed

Fig. 3. Changes in serum levels of other G-component protein (α -1-antitrypsin and transferrin) due exercise. Each plotted point represents mean of measurements and vertical bars represent 1 SD. ○, 14 male group and ●, 12 male group.

in a water bath heated to 37°C, and the mixture was shaken at intervals of 2 or 3 minutes. After 30 min. one portion of the well shaken

mixture was picked up and smeared over a piece of slide. The slide glass was air-dried and methanol-fixed, and the blood cells were stained with a Giemsa solution (Wako Chemical).

The phagocytosis was measured by observation through a light microscope at 1,000 magnifications with the focus adjusted to the nucleus of each neutrophil. One hundred neutrophils were counted and the results were expressed as

follows: (a) the percentage of cells which ingested carbon black particles; (b) the average number of carbon black particles ingested per leucocyte. Before testing the phagocytosis, the whole white blood cells were counted by the cell counter ACM-503 (Eluma Co. Ltd).

F. Serum laboratory diagnosis

Using the Technicon SMAC (Technicon), serums of all subjects before and after exercise were analyzed to check for any abnormality.

Table 2. Serum immunoglobulin and complement levels before exercise.

Sub. No.	IgG mg/dl	IgA mg/dl	IgM mg/dl	C 3 mg/dl	C 4 mg/dl	At.* mg/dl	Tf.** mg/dl
1	1,057	286	238	230	41.2	241	380
2	961	242	206	101	27.4	267	342
3	1,448	278	227	109	20.3	251	266
4	1,161	164	166	111	40.6	278	287
5	1,681	232	127	118	27.9	266	367
6	883	199	311	160	30.6	284	385
7	1,703	209	183	150	34.3	239	335
8	1,334	250	107	121	27.0	255	320
9	1,465	271	184	133	31.8	206	333
10	1,130	270	263	127	24.6	277	346
11	1,754	306	209	164	31.6	241	351
12	1,798	296	299	209	32.5	285	395
Mean	1,364.6	250.3	210.0	144.4	30.82	257.5	342.3
S. D.	324.5	42.9	62.4	40.6	6.06	23.4	38.4
1	951	242	134	120	24.9	232	328
2	1,339	155	216	127	28.2	256	340
3	1,095	133	189	113	27.0	247	332
4	1,580	416	209	140	21.6	309	387
5	1,005	298	153	116	23.7	231	271
6	1,293	272	178	149	26.5	241	338
7	991	185	251	103	20.6	266	284
8	1,259	272	143	164	37.7	275	313
9	1,236	221	220	146	36.2	261	343
10	1,224	176	204	97	21.0	242	264
11	1,380	359	192	130	18.2	303	330
12	886	147	281	75	16.9	212	250
13	693	125	137	127	20.4	222	308
14	1,415	243	127	132	30.6	274	322
Mean	1,167.6	231.7	188.1	124.2	25.25	255.1	315.0
S. D.	240.5	86.7	46.2	23.0	6.30	28.6	36.8

At.*; α -1-Antitrypsin. Tf.**; Transferrin.

Analytical items were total protein, albumin, blood urea nitrogen, uric acid, creatinine, blood sugar, triglyceride, cholesterol, bilirubin, iron, calcium, inorganic phosphorus, sodium, potassium, chloride, GOT, GPT, LDH, γ -GTP and ALP.

III. Results

A. Serum immunoglobulin and complement levels

Figures 1 and 2 show the immunoglobulin and complement levels in both group prior to and immediately after exercise. These proteins were elevated without exception immediately after exercise. The mean value of IgG at rest was 1365 (mg/dl) in the senior group and 1168 (mg/dl) in the freshman. The levels were upped increased greatly after the 1,500 meter running to 1693(mg/dl)(124% increase $P < 0.1$ %) and 1441(mg/dl)(123% increase $P < 1$ %), re-

Table 3. Serum immunoglobulin and complement levels after exercise.

Sub No	IgG mg/dl	IgA mg/dl	IgM mg/dl	C 3 mg/dl	C 4 mg/dl	At.* mg/dl	Tf.** mg/dl
1	1,390	288	144	163	25.1	249	380
2	1,047	243	205	104	23.3	256	389
3	1,568	296	259	127	26.5	235	301
4	1,435	185	179	130	46.3	281	297
5	2,223	343	212	216	41.9	297	498
6	1,239	246	309	203	32.9	290	476
7	2,089	265	202	182	31.7	275	436
8	1,590	322	120	148	26.9	312	370
9	1,658	251	182	178	28.8	259	358
10	1,238	246	271	124	28.5	270	388
11	2,389	355	243	207	40.0	304	419
12	2,453	404	533	326	53.9	336	540
Mean	1,693.3	287.0	238.3	175.7	33.82	280.3	404.3
S. D.	478.3	60.5	107.1	59.7	9.58	29.0	73.9
1	1,111	297	175	154	27.7	309	403
2	1,446	188	278	160	34.3	309	393
3	1,494	193	268	182	39.9	358	491
4	1,676	422	259	168	22.8	331	443
5	965	287	129	107	22.0	228	277
6	1,510	319	254	174	31.8	288	374
7	1,170	219	321	132	25.6	321	359
8	1,493	343	135	172	40.2	291	339
9	1,463	279	248	187	40.5	355	419
10	1,971	305	358	182	36.5	371	444
11	1,593	426	216	173	23.9	355	422
12	1,668	245	372	133	24.0	335	415
13	1,215	163	171	165	30.8	284	380
14	1,392	246	131	124	37.2	291	314
Mean	1,440.5	280.9	236.8	158.1	31.23	316.1	390.9
S. D.	260.8	80.3	81.1	24.6	6.91	38.6	56.2

At.*; α -1-Antitrypsin. Tf.**; Transferrin.

spectively, in the senior and the freshman groups. The mean increments of IgA and IgM were analogous to the case of IgG; IgA increased to 115% ($P < 5\%$) and 121% ($P < 0.1\%$) and IgM increased to 113% and 126% ($P < 1\%$), respectively. Also the serum levels of complements were higher after exercise nearly at the same rate with the immunoglobulins increments; C3 increased to 122% ($P < 5\%$) and 127% ($P < 0.1\%$) and C4 increased to 110% and 124% ($P < 0.1\%$), respectively. Other proteins also increased (Fig. 3); for example, the increment of α -1-antitrypsin was 109% ($P < 5\%$) in the 12 male group, and 124% ($P < 0.1\%$) in the 14 male group, and transferrin 118% ($P < 0.1\%$) and 124% ($P < 0.1\%$), respectively (see Tables 2 and 3).

B. Neutrophilic phagocytosis

In contrast to the increments of serum pro-

Fig. 4. Initial numbers of neutrophil (Δ , $\blacktriangle$) and leukocyte ($\circ$, $\bullet$); vertical bars represent 1 SD. Δ , $\circ$, 14 male group and $\blacktriangle$, $\bullet$, 12 male group.

teins after work, the number of neutrophils decreased, while the whole number of white blood cells increased (Fig. 4) nevertheless.

Figure 5 shows the phagocytoses in both groups before and after exercise. In this study phagocytic activities were expressed in two terms: the phagocytosis rate and the phagocytosis degree. The phagocytosis rate means the number of neutrophils which phagocytized particles per 100 neutrophils selected at random, and the phagocytosis degree means the average number of particles included in 100 neutrophils. The phagocytosis rate (Fig. 5, upper) significantly decreased both in the 12

Fig. 5. Changes in phagocytic activities due to exercise. Phagocytosis rates ($\circ$, $\bullet$) are described in percent of neutrophils which phagocytized and phagocytosis degree (Δ , $\blacktriangle$) are expressed by the number of average phagocytized particles. Each plotted point represents mean of measurements and vertical bars, 1 SD. $\circ$, Δ , 14 male group and $\bullet$, $\blacktriangle$, 12 male group.

Table 4. Neutrophilic phagocytosis and numbers of white blood cell (WBC) and neutrophil (PMN) before and after exercise.

Sub. No.	Before				After			
	WBC $\times 10^2/\text{mm}^3$	PMN	Phagocytosis ratio	degree	WBC $\times 10^2/\text{mm}^3$	PMN	Phagocytosis ratio	degree
1	71	23	90	8.34	117	21	79	6.31
2	93	55	94	9.17	81	33	86	9.58
3	83	42	86	7.79	81	36	65	6.07
4	94	48	84	7.04	64	24	81	7.87
5	59	28	79	6.98	83	22	79	8.82
6	77	36	93	9.59	105	22	93	10.20
7	82	42	72	4.45	90	27	65	3.72
8	67	29	75	5.52	83	32	54	2.40
9	46	22	77	4.67	82	25	60	4.01
10	80	28	81	8.93	106	35	66	6.10
11	74	33	81	4.74	59	14	53	2.90
12	93	58	84	7.30	60	26	89	5.92
Mean	76.6	37.0	83.0	7.043	84.3	26.4	72.5	6.158
S. D.	14.5	12.1	6.9	1.831	18.3	6.5	13.7	2.584
1	81	45	84	2.92	114	23	71	1.94
2	70	41	72	1.72	95	44	66	2.00
3	59	31	86	3.78	92	26	85	3.03
4	71	24	81	1.98	78	31	73	1.86
5	63	31	78	1.61	83	21	68	2.35
6	75	32	81	1.64	120	34	74	2.18
7	72	53	88	2.11	106	42	75	2.58
8	60	23	75	1.43	71	26	58	1.66
9	73	41	94	3.46	101	35	83	2.94
10	63	21	91	2.47	89	10	82	3.99
11	72	42	83	2.06	94	37	70	1.98
12	57	30	90	2.72	80	30	81	4.07
13	49	21	79	1.58	73	18	72	1.86
14	70	29	93	3.68	74	19	87	3.41
Mean	66.8	33.1	83.9	2.369	90.7	28.3	74.6	2.561
S. D.	8.5	9.8	6.7	0.817	15.4	9.7	8.2	0.805

($P < 1\%$) and the 14 group ($P < 0.1\%$), the mean values changing from 83 (%) at rest to 73 (%) after exercise in the former group and from 84 (%) to 75 (%) in the latter. On the other hand, the phagocytosis degree (Fig. 5, lower) seemed to be inert during the exercise period, though apparently different between the two groups. As for the mean value with standard deviation, it was 7.04 ± 1.83 prior to

exercise in the former and 6.16 ± 2.58 after exercise, and 2.37 ± 0.82 and 2.56 ± 0.81 , respectively, in the latter (see Table 4).

IV. Discussion

The serum levels of immunoglobulins rose without exception after the 1,500 meter running. The mean values of IgG, which usually exist for the most part in serum immunoglo-

Fig. 6. Changes in serum total protein and potassium due to exercise. They were measured by Technicon Autoanalyzer. Each plotted point represents mean of measurements and vertical bars, 1 SD. ○, 14 male group and ●, 12 male group.

bulins were relatively more influenced in form compared with the total serum protein (measured by Technicon Autoanalyzer); an ascent rate of IgG was 124% in the senior group and 123% in the junior, while the total protein showed only 109% increase in both (Fig. 6. left). At present it is difficult to explain how the serum immunoglobulins could increase after exercise, since even their origins have not so far been fully clarified. The serum complements C3 and C4, also increased after exercise, and the former significantly at $P < 5\%$ in the senior group, and both at $P < 0.1\%$ respectively in the junior group. The increments of α -1-antitrypsin and transferrin attained nearly the same rates with immunoglobulins and complements.

These protein increments seemed to be caused by simple water release from serum in the form of sweat; however, not only was there difference between the increments in various proteins but also between individual and total serum protein, which, as above men-

tioned, did not increase at such a high rate. The serum levels of enzymes, minerals, etc. were already measured by Technicon Autoanalyzer (not all the results described here), and the potassium concentration dropped after exercise (Fig. 6. right). The results of the lower ascent rate of total proteins and the descent of potassium suggested that these protein increments were caused not by a concentration of serum, but by an active release of serum from such cells and tissues as well as enzymes.

The suggestions made by many preceding reports dealing with phagocytosis⁷⁾ made us consider that the addition of IgG and/or C3 to test particles would activate neutrophilic phagocytosis, which was therefore expected to be activated by the increased IgG and C3. However, in this experiment, we obtained an unexpected result that the phagocytosis rate decreased after exercise with little changes in the phagocytosis degrees.

The cause of this decrease was not confirmed in the present experimental conditions. The increments of immunoglobulins and complements did not always activate the opsonin; did the neutrophils receive the same damages during the exercise? To inquire into the relationship between immunoglobulins and neutrophilic phagocytoses closely, it will be necessary to measure the serum opsonin activity and the phagocytic activity only in neutrophile itself.

As easily understanding, able for studying the relation between the changes in serum immunoglobulin levels and in neutrophilic phagocytosis, these were expressed as a post-exercise-pre-exercise value/pre-exercise value (Table 5). As shown in table 6, it became clear that the changes in the serum proteins had no relation to the those in phagocytic

Table 5. Changes in serum immunoglobulin and complement levels and neutrophilic phagocytosis due to exercise.

Sub. No.	Immunoglobulins and Complements					Phagocytosis	
	IgG	IgA	IgM	C 3	C 4	rate	degree
1	31.5	0.7	-39.5	-29.1	-39.1	-12.2	-24.3
2	8.9	0.4	-0.5	3.0	-15.0	-8.5	4.5
3	8.3	6.5	14.1	16.5	30.5	-24.4	-22.1
4	23.6	12.8	7.8	17.1	14.0	-3.6	11.8
5	32.2	47.8	66.9	83.1	50.2	0.0	26.4
6	40.3	23.6	-0.6	26.9	7.5	0.0	6.4
7	22.7	26.8	10.4	21.3	-7.6	-9.7	-16.4
8	19.2	28.8	12.1	22.3	-0.4	-28.0	-56.5
9	13.2	-7.4	-1.1	33.8	-9.4	-22.1	-14.1
10	9.6	-8.9	3.0	-2.4	15.9	-18.5	-31.7
11	36.2	16.0	16.3	26.2	26.6	-34.6	-38.8
12	36.4	36.5	78.3	56.0	65.8	6.0	-18.9
Mean	23.5	15.3	13.9	22.9	11.6	-13.0	-14.5
S. D.	11.7	17.9	31.1	28.1	29.1	12.6	23.3
1	16.8	22.7	30.6	28.3	11.2	-15.5	-33.6
2	8.0	21.3	28.7	26.0	21.6	-8.3	16.3
3	36.4	45.1	41.8	61.1	47.8	-1.2	-19.8
4	6.1	1.4	23.9	20.0	5.6	-9.9	-6.1
5	-4.0	-3.7	-15.7	-7.8	-7.2	-12.8	46.0
6	16.8	17.3	42.7	16.8	20.0	-8.6	32.9
7	18.1	18.4	27.9	28.2	24.3	-14.8	22.3
8	18.6	26.1	-5.6	4.9	6.6	-22.7	16.1
9	18.4	26.2	12.7	28.1	11.9	-11.7	-15.0
10	61.0	73.3	75.5	87.6	73.8	-9.9	61.5
11	15.4	18.7	12.5	33.1	31.3	-15.7	-3.9
12	88.3	66.7	32.4	77.3	42.0	-10.0	49.6
13	75.3	30.4	24.8	29.9	51.0	-8.9	17.7
14	-1.6	1.2	3.1	-6.1	21.6	-6.5	-7.3
Mean	26.7	26.1	24.0	30.5	25.8	-11.2	12.6
S. D.	28.4	22.5	22.6	28.0	21.6	5.1	28.3

* These values were calculated using the following equation.

$$(\text{After} - \text{Before}) / \text{Before} \times 100(\%).$$

activity. Table 7 collectively expresses the whole of the above described experiments.

It can be seen herein that the changes of serum immunoglobulin and neutrophilic phagocytic activity that were chosen in this report as indices for the estimation of the protective physical fitness are very interesting factors, because the two had low correlations between

them (they seemed to be nearly independent from each other) and that directions of the changes were reverse.

Concerning whole blood in these experiments, however, there is a possibility that another factor than the above mentioned, inclusive of the exohematic would have brought about the changes. More detailed investiga-

Table 6. Correlations of the changes of serum immunoglobulin, complement levels and neutrophilic phagocytosis in the two groups.

		12 Male Group					
		Immunoglobulins and Complements				Phagocytosis	
		IgG	IgA	IgM	C 3	C 4	rate degree
14 Male Group	IgG		*0.626	0.293	0.352	0.287	0.339 0.173
	IgA	***0.842		**0.740	**0.753	*0.584	0.440 0.234
	IgM	0.528	*0.706		***0.884	***0.913	0.398 0.235
	C 3	**0.774	***0.931	***0.806		**0.767	0.339 0.390
	C 4	**0.770	***0.811	**0.766	***0.822		0.269 0.169
	rate	0.192	0.167	0.430	0.305	0.429	**0.732
	degree	0.412	0.384	0.226	0.281	0.312	-0.054

* ; P<5%. ** ; P<1%. *** ; P<0.1%.

Table 7. Correlations of the changes of serum immunoglobulin, complement levels and neutrophilic phagocytosis.

		Immunoglobulins and Complements				Phagocytosis	
		IgG	IgA	IgM	C 3	C 4	rate degree
IgG			***0.757	*0.394	***0.619	**0.529	0.207 0.337
IgA	—			***0.709	***0.852	***0.703	0.306 *0.406
IgM	—	—	—		***0.841	***0.860	*0.402 0.283
C 3	—	—	—	—		***0.784	0.310 0.349
C 4	—	—	—	—	—		0.314 0.332
rate	—	—	—	—	—	—	0.387
degree	—	—	—	—	—	—	

N=26. * ; P<5%. ** ; P<1%. *** ; P<0.1%.

tions about the changes of serum components and neutrophilic activities under transient loads may be expected to show that these indices could be useful for the estimation of the protective physical fitness.

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References

- 1) Ebashi, H., Shibayama, H., Goto, Y. and Nishijima, N. (1978) : A study on the capability of

the prolonged exercise (3) on the long distance skiing, Bull. Phys. Fit. Res. Inst., **39**, 17-37

- 2) Fowler, W. M. JR., Chowdhury, S. R., Pearson, C. M., Gardner, G. and Bratton, R. (1962) : Changes in serum levels after exercise in trained and untrained subjects, J. Appl. Physiol., **17**, 943-946
- 3) Goto, Y., Kita, N., Tsutsumi, T., Ebashi, H. and Shibayama, H. (1978) : Effect of long-term physical training upon the metabolic and hormonal responses to exercise, Bull. Phys. Fit. Res. Inst., **39**, 38-55

- 4) Hirata, F., Yukawa, K., Ikeda, T., Nishida, H., Saegusa, Y., Fukui, I., Tahara, Y., Sugawara, M., Ishihara, Y., Okazaki, H. and Noguchi, M. (1982) : Correlation between load and immunity during long term training Jpn. J. Phys. Fit. Sports Med., **31**, 460
- 5) Hirokawa, T., Ishikawa, K., Kaihara, M. and Saito, R. (1960) : Examination method of leukocytic phagocytosis, Industrial Medicine, **2**, 1
- 6) Ishihara, Y., Hirata, F., Nakamura, M. and Yukawa, K. (1981) : Physically active life and behavior of leukocyte function, immunity and serum enzymes, Jpn. J. Hyg., **36**, 207
- 7) Nerdrum, H. J. and Berg, K. J. (1964) : Changes on serum glutaminoxaloacetic transaminase and serum lactic dehydrogenase on physical exertion, Scandinv. J. Clin. Lab. Investigation, **16**, 624-629
- 8) Ohno, H., Terayama, K., Hirata, F., Kawarabayashi, T., Watanabe, H., Nishino, M., Takuwa, E. and Taniguchi, N. (1981) : Studies on physical stress. part 1 effect of physical exercise on the activities of various enzymes in human plasma, Jpn. J. Hyg., **36**, 801-810
- 9) Roos, D., Bot, A. A., van Schaik, M. L. J., de Boer, M. and Daha, M. R. (1981) : Interaction between human neutrophils and zymosan particles. The role of opsonins and divalent cations, J. Immunology, **126**, 433-440
- 10) Shibayama, H. and Ebashi, H. (1980) : Characteristics of well-trained athletes in prolonged exercise from the viewpoint of aerobic power, J. Human Ergol., **9**, 55-68
- 11) Tanaka, Y., Matsui, T., Okuda, K., Kawamoto, K. and Yamada, S. (1979) : Quantitative analysis of serum proteins used by the nephelometric system, J. Medical Technology, **23**, 729-732