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METABOLISM OF GLUCURONIC ACID IN FATIGUE DUE TO PHYSICAL EXERCISE

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Concerning the cause of fatigue, there have been many assumptions on the basis of energy accumulation of substances inducing fatigue. There have been a lot of studies (1, 2) on piling up of the so-called fatigue substances. In the book edited by Katsunuma and Asahina (3), it was reported that the so-called fatigue substances were found by Ueda, Weichart, Pieron, Knipping, Denisenko, Asahina, Ozawa, Vorobew, Pravdic and Neminsky. They assumed that these substances were the metabolites from degraded protein.

As mentioned above, there are many reports concerning the cause of fatigue, but we cannot but conceive some questions for their chemical nature.

In our investigation on the appearance of fatigue due to physical exercise, the fact that a certain kind of metabolic substance was produced and accumulated from the energy source consumed by the physical exercise.

Our investigation was begun to study the relationship between the change of this metabolic product and the change of glucuronic acid metabolism due to physical exercise in the body.

The first experiment was done by Tamura in participation in Abe's studies (4, 5) on agents relieving fatigue and increasing efficiency, in 1942. The summary of this study is as follows: the rat was made to run as long as possible on the rotating belt and the time being almost impossible to run was adopted as running time. The running was repeated three times after a short rest.

This running test revealed that the central stimulants such as caffeine and amphetamine prolonged only the 1st running time, while glucose and glucuronic acid prolonged all of the 1st, the 2nd and the 3rd running time. In other words, the reducing agents

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such as glucose and glucuronic acid much more prevented the appearance of fatigue than the central stimulants.

This finding led us to the next deduction: in a living body, although toxic metabolites produced as a consequence of physical exercise are naturally detoxicated as protective response and consequently the appearance of fatigue is suppressed, fatigue may appear in the shape of autointoxication due to the toxic metabolites when the detoxicating ability in the body was inhibited because of the excess production of the toxic metabolites. The judgement of the grade of fatigue is mostly done by measuring the substances produced or increased due to physical exercise and other labours.

The method of measuring fatigue by using urine is divided into two types in view of the reactive substances to be measured: one is the method of measuring protein metabolism, including urinary microprotein method, urinary protein differential method, Donaggio reaction and Masuyama method (6-8) etc, and the other is the method of measuring reducing substance, namely differential method of urinary reducing substance. From pharmacological standpoint of the effects of these reactive substances upon a living body, it may be thought of that the change of the toxic substance can be examined with the protein method and that the defense mechanism of a living body against the toxic substance, in other words, the change of antidote, can be examined with the reducing substance method. If fatigue appears as a result of the accumlation of the protein metabolic product and the decrease of antidote against this product, the former may increase and the latter on the contrary may decrease as physical exercise is prolonged. As a result of examination on the method of measuring fatigue by means of urine of school children (9), it was found that the reactive substances made from protein metabolism increased with light outdoor exercise and that the reducing substances. particularly glucuronic acid, temporarily increased at first and then decreased. Basing upon the above experiment, it may be implied that this reaction is positive, even in light exercise, before the appearance of fatigue. Our experiments, therefore, were begun with the examination of the reactive substance in urine above noted.

EXPERIMENTS AND RESULTS

1. Toxic metabolic products of protein nature increasing in urine and serum (10, 11)

a) The reactive substances in the sediment precipitated by addition of acetone in urine and serum

The reactive substances of Donaggio reaction and Masuyama filter paper hanging reaction in urine or dialyzed serum were precipitated by addition of acetone. This sediment was called AP. This acetone-insoluble substance (AP) was extracted with water in pH 7.0. Then this extract was precipitated again by addition of saturated lead acetate solution. This sediment was removed deprived of the lead and was filtered and then was called APP_3 and showed a toxicity against the function of organs. The clear extract after removing the above precipitated sediment was alkalized with ammonia solution into pH 8.0 and then was precipitated by addition of saturated lead subacetate solution. This sediment, after being deprived of the lead, was dried up. This substance included

glucuronide and showed no toxicity, and was called APP_4. The analytic method is represented in Table 1.

From the point of pharmacological view, it was proved that the toxic substance (APP_3) stopped the frog heart beating and inhibited the activities of various enzymes and the ability of physical exercise, while the substance including glucuronide had not such effects (Table 2). But we found, when glucuronic acid contained in the substance including glucuronide was destroyed by hydrolysis, this substance showed the various toxic actions (Table 2). This substance was detected also in serum. The swimming test of rats shown in the Table 2-A was carried out as follows: water trank, which was

TABLE 1. Analysis of acetone-insoluble substance in urine and serum (AP)

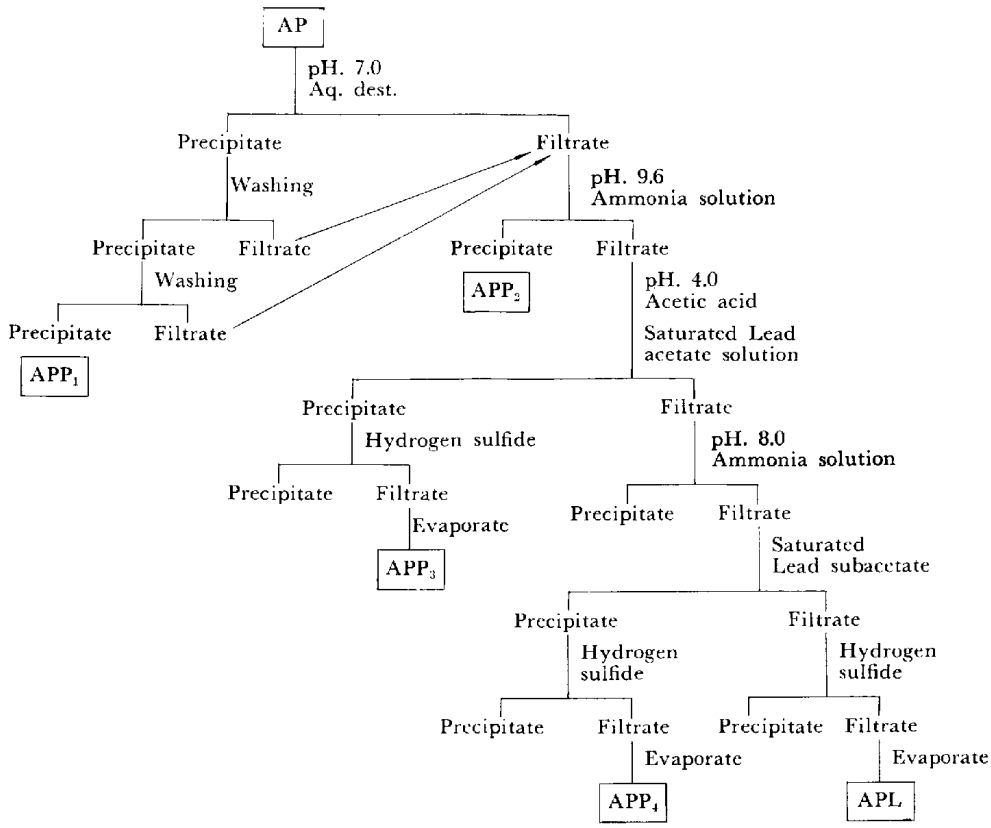

TABLE 2. Comparison of toxicity of the acetone-insoluble substance.

A) Toxicity against frog heart beating*

APP_1, APP_2, APP_4, APL	APP_3	APP_4 which is hydrolyzed
over 50 mg/5ml shows no toxicity	5 mg/5ml impedes the heart beating	10 mg/5ml stops the heart beating

* In experiments, Yagi's reflux methodwas applied and *Rana nigromaculata* was used.

The reflux fluid was 5 ml.

B) Toxicity against acetylcholinesterase*

	AP	APP_1	APP_2	APP_3	APP_4	APL
10 mg/3ml	10	18	23	35	15	3
25 mg/3ml	21	23	54	82	22	3
100 mg/3ml	40	59	65	95	29	16

* Acetylcholinesterase activity was measured by Ammon's method (12). The numeral indicates the percent of inhibition of this activity.

C) Toxicity against swimming record of rat (average of 10 rats)

	1st swimming—30 min—2nd swimming rest	30 min—3rd swimming rest
	min	min
No-treatment	31.0±5.2	22.3±5.3	17.5±4.0
APP_3 10 mg/100g inj. 3 times	22.7±9.4	9.3±3.1	6.9±2.6

Swimming was repeated 3 times.

partitioned into 5 parts (volume of each part is $33\times20\times19$ cm^3) and an electric thermoregulator was set in the middle part, and 19°C was kept by continuous stirring. A rat tired with prolonged swimming floated vertically in rotating movement and then sank into the bottom of trank. The rat was made to swim till it sank again; the time taken for it was adopted as the swimming time. In control test, a rat was administrated 5 ml/kg of physiological saline (intraperitoneally), 30 minutes before the 1st swimming, and immediately after the 1st and 2nd swimming.

In the experiment to examine the influence of the toxic substance in urine upon the ability of physical exercise, a rat was injected with 100 mg/kg APP_3 only once before the 1st swimming in above-mentioned manner.

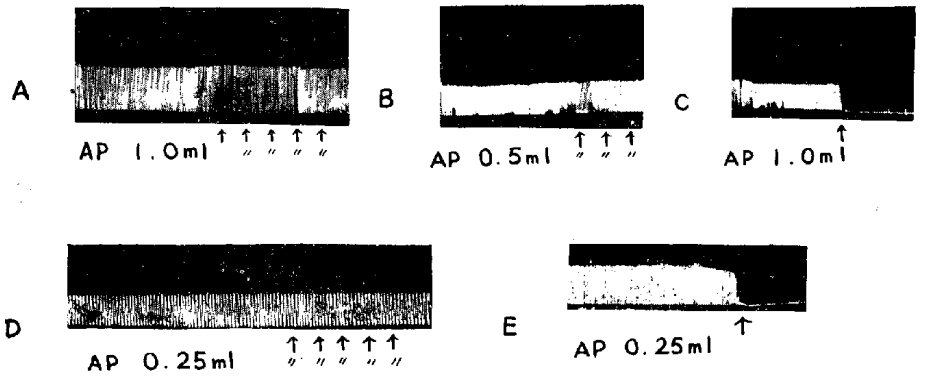

FIG. 1. Effect of acetone-insoluble substance on the frog heart beating.
AP in A, B and C was in urine and AP in D and E was in serum.
A : before the boat-training, B : after the boat-training, appearing no fatigue, C : after the boat-training, appearing severe fatigue, D : before the boat-training, E : after the boat-training, appearing severe fatigue.

b) Toxicity in this sediment strengthened after physical exercise

In this experiment, we adopted the boat-racing as physical exercise.

The method of collecting urine in the boat-racing test was as follows. The 1st urine was collected for one hour in the rest condition, before exercise; the 2nd urine, at the beginning of light exercise when they felt no fatigue; the 3rd urine, at the end of heavy exercise in the morning when they felt severe fatigue. The method of collecting urine in the afternoon was the same as in the morning.

The toxicity of these substances increased after physical exercise (Fig. 1).

The amount of APP_3 was about 0.3 g per liter in normal urine, but about 1.0 g per liter in urine after physical exercise.

c) Chemical analysis of the toxic substance

On the paper chromatography, this substance consisted of 5 spots. Under ultra-violet light, the 1st and the 2nd spots had the fluorescence, the 3rd was large, but the 4th and the 5th were dark spots not so large (Fig. 2).

According to Staudinger's analytic method (13), chemical composition of this substance in urine are F_{II}, SF_{II} and SF_{III}. Consequently, this substance may be a compound formed from amine, amino acid, peptides and the other elements.

The 3rd spot including 80 percent of the toxic substance should have most powerful toxicity, because a small amount of it stopped the frog heart beating completely and the other spots, on the contrary, could not have such an effect unless they were used in much greater amount. According to these experiments, we recognized that the representative of the toxic substances was the 3rd spot, and then we decided to examine the 3rd spot of the toxic substance (APP_3) in detail. Consequently, it was proved that

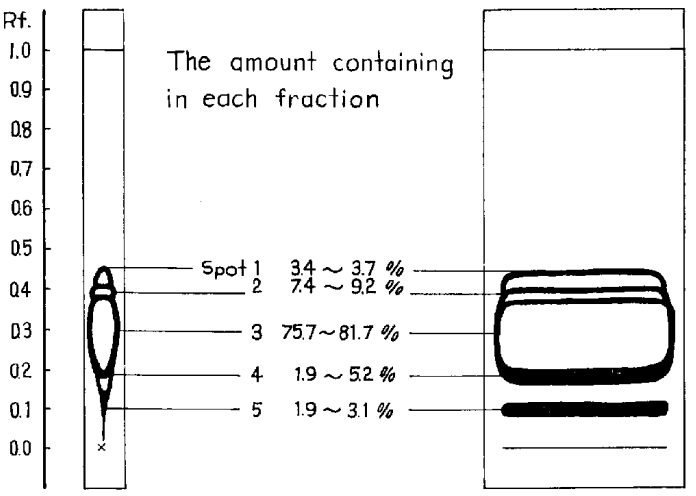

FIG. 2. Paper chromatography of the toxic substance (APP_3) (trace). Paper chromatography was performed by one-dimensional ascending method. Toyo filter paper No. 51 was used as filter paper and the mixture of n-Butanol 40%, Acetic acid 10% and Water 50%, used as solvent.

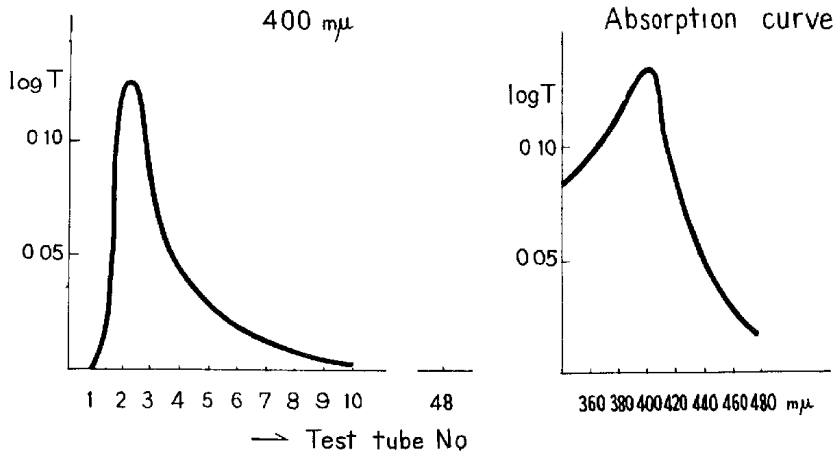

FIG 3 Sephadex G-25 chromatography of the toxic substance (APP_3)
APP_3 (0 5 g) were dissolved in 5 ml 0 9% NaCl solution and applied to the column (1 × 20 cm Sephadex G 25 16 ml) Elution was carried out with water Fraction (5 ml) were collected on an automatic fraction collector (test tube No 1–48) The total effluent was 240 ml Each fraction was added to colour reagent which consists of Ethylcellosolve 50 ml, Ninhydrin 500 mg, Hydrindantin 40 mg and McIlvaine buffer and then examined by spectrophotometer

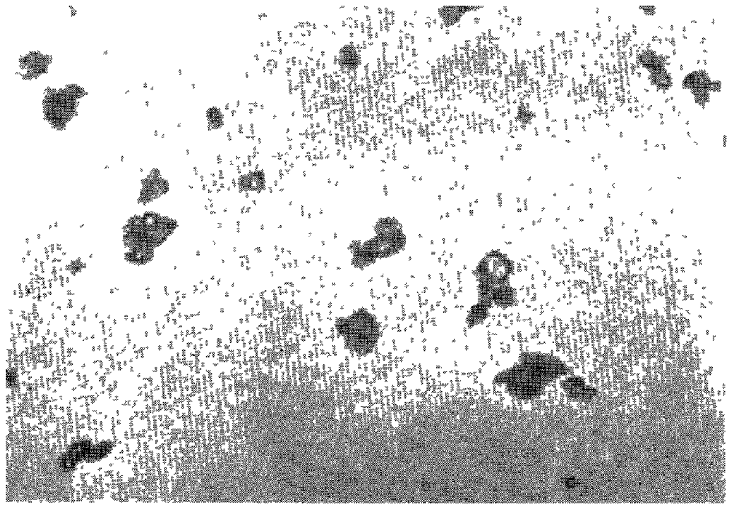

FIG 4 Crystallines of the toxic substance (APP_3) (× 150)

the 3rd spot showed a characteristic absorption curve through Sephadex G-25 (Fig. 3) and caused a reaction of primary amine These crystallines were shown in Fig. 4. We are now identificating it, to what kind of amine it belongs

2. *Changes of glucuronic acid content induced as a consequence of fatigue due to physical exercise in a living body (14–16)*

Method of examining glucuronic acid content was as follows The glucuronic acid

content in urine was measured by Fishman and Green's method (17). The glucuronic acid content in blood and serum was also measured by Fishman and Green's method (17).

a) Effect of the physical exercise on glucuronic acid content in human urine or serum

The cycling and the boat-racing were adopted as the physical exercise. In both

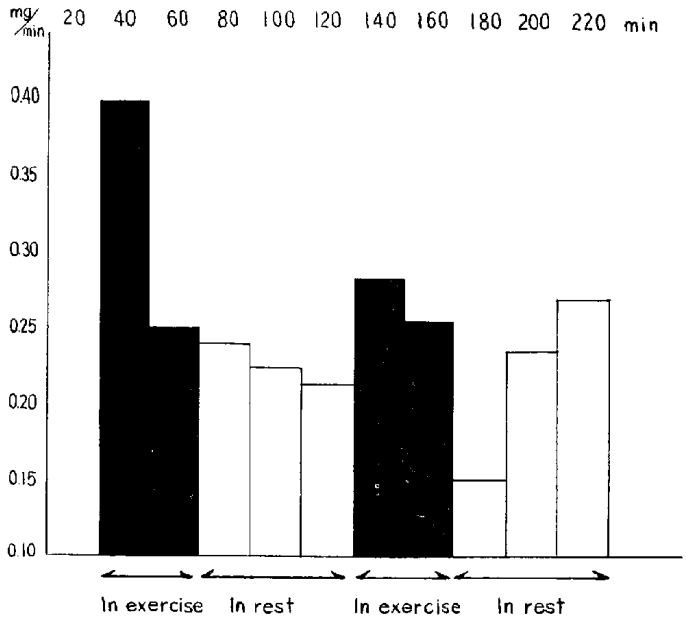

FIG. 5. Effect of the cycling on glucuronic acid content in urine (average of 5 men).

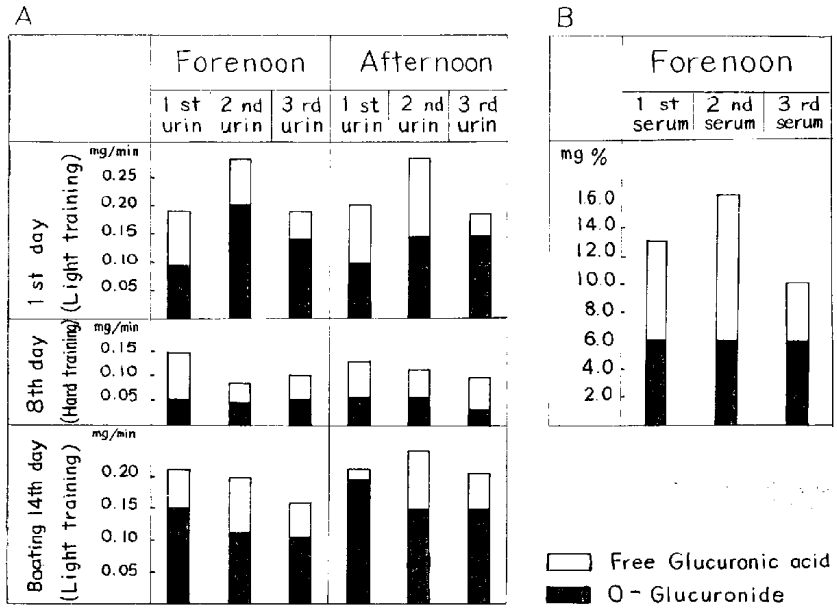

FIG. 6. Effect of the boating on glucuronic acid content.
A : in urine, B : in serum (average of 5 men).

exercises, glucuronic acid content in urine increased at the beginning of the exercise, but decreased at the end of the prolonged exercise (Figs. 5 and 6). In case of severe fatigue, decreasing of it was very remarkable. This tendency was the same in the serum (Fig. 6).

Though, in the boat-racing, on the 14th day of the training (light training) glucuronic acid in urine returned to normal content, on the 8th day of the training (heavy training) there was only a small amount of glucuronic acid in the urine at the beginning or the end of the exercise (Fig. 6).

b) Effect of the swimming on glucuronic acid content in liver, kidney and serum and on hepatic glycogen content in rat

Method of measuring the glucuronic acid content in organs was as follows. As soon as experimental animals were sacrificed by rapid decapitation, their organs were weighed immediately. Each organ was added with water 4 times as much as its weight, and then homogenized for 3 minutes. Glucuronic acid in 2 ml of the clear supernatant fluid after centrifuging (10,000 r.p.m., 15 minutes) was measured the same as in the blood.

Hepatic glycogen was measured by Fujii's method (18), immediately after extripation of the organ. Though glucuronic acid content in the liver or serum increased slightly after the 1st or 2nd swimming, it decreased markedly after the 3rd swimming

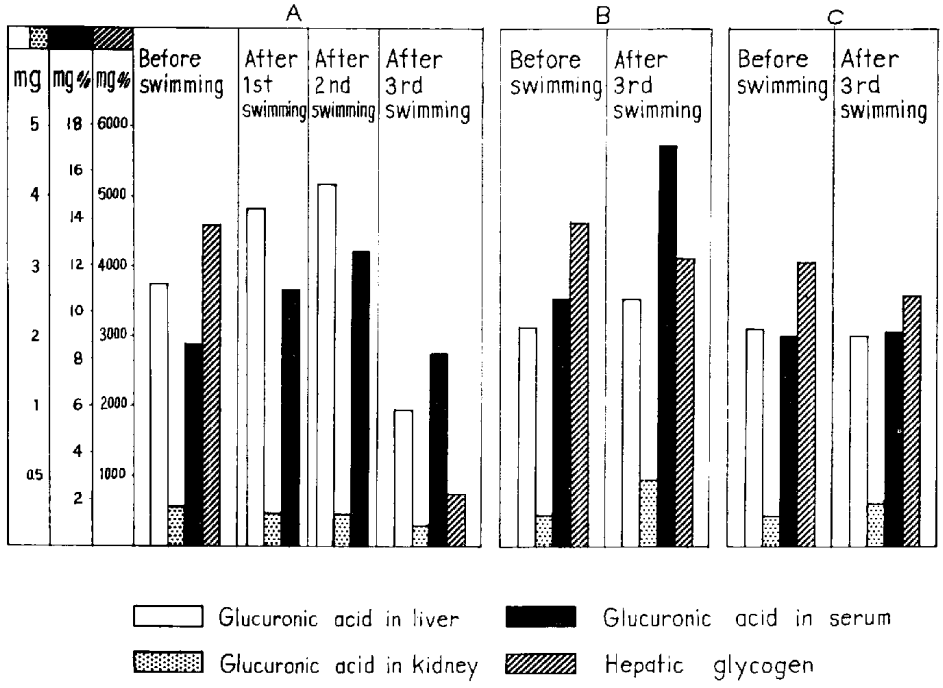

FIG. 7. Effect of the swimming on glucuronic acid content in liver, kidney and serum and hepatic glycogen content.

A : no-treatment, B : intraperitoneal injection of 100 mg/kg glucuronolactone at 30 minutes before swimming, C : intraperitoneal injection of 100 mg/kg glucose at 30 minutes before swimming (average of 10 rats).

(Fig. 7-A). Glucuronic acid content in the kidney and hepatic glycogen content decreased gradually as the swimming continued (Fig. 7-A).

c) Effect of the physical exercise on glucuronic acid content in glucuronide fraction in the sediment precipitated by addition of acetone in human urine or serum

Method of measuring the glucuronic acid content in glucuronide fraction in the sediment precipitated by addition of acetone was as follows: The precipitate which was formed by addition of 4 times as much volume of acetone to urine, was resolved in 50 times as much volume of 90 percent acetic acid. After the clear supernatant fluid was removed, the precipitate was washed several times with alcohol and at last with ether. The glucuronic acid in this precipitate dissolved in a small amount of water was measured the same as in the urine.

The result was shown in Fig. 8. The change of this glucuronic acid content was similar to that of glucuronic acid content in the urine or serum.

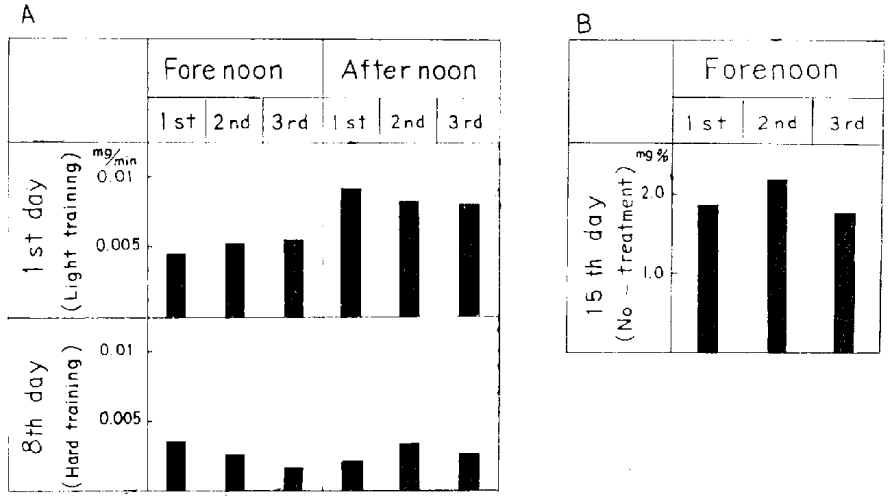

FIG. 8. Effect of the boating on glucuronic acid content in glucuronide fraction in precipitated sediment by addition of acetone.
A : in urine, B : in serum (average of 5 men).

3. Effect of the administration of glucuronolactone and glucose on the appearance of fatigue due to physical exercise (15, 16)

In the boat training, the boatman was administrated orally 6 g glucuronolactone twice a day before boat training. In animal test, each rat was injected intraperitoneally with 100 mg/kg glucuronolactone or 100 mg/kg glucose which was dissolved in 5 ml/kg of water instead of physiological saline. The swimming method and others were already described in Chaper 1.

a) Effect of the administration of glucuronolactone on glucuronic acid content in human urine and serum

By the administration of glucuronolactone before boat training, glucuronic acid content in human urine and serum, and glucuronide fraction in the sediment precipitated

by addition of acetone in human urine or serum apparently increased and did not decrease at the end of boat training. These results were more remarkable on the 4th day (20 day after the beginning of training) of administration of glucuronolactone (Fig. 9).

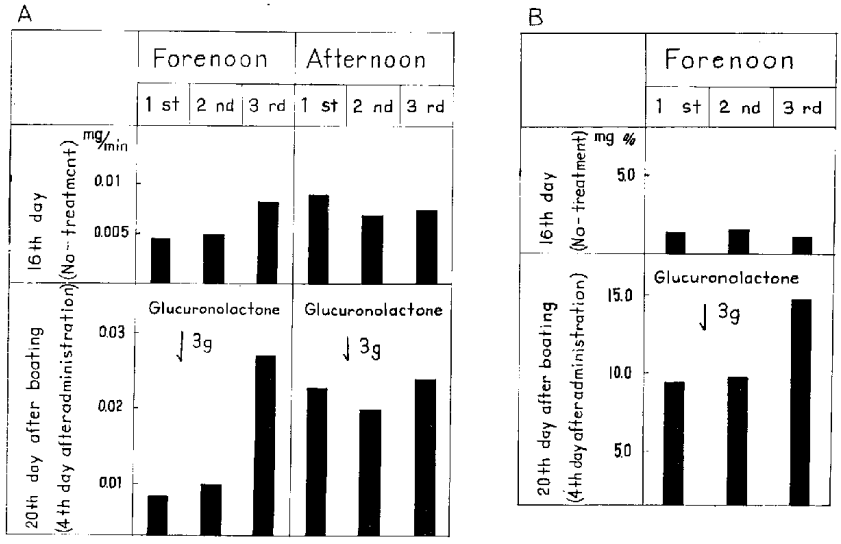

FIG. 9. Effect of administration of glucuronolactone on glucuronic acid content.
A : in urine, B : in serum (average of 5 men).
The boatmen were administrated orally with glucuronolactone 6 g twice a day for 4 days.

b) Effect of the administration of glucuronolactone on glucuronic acid content in liver, kidney and serum and on hepatic glycogen content in rat

By the administration of glucuronolactone before swimming, the swimming record of rat apparently prolonged. After the 3rd swimming, glucuronic acid content in liver, kidney and serum increased, and hepatic glycogen content did not decrease at all (Fig. 7-B).

c) Effect of the administration of glucose on glucuronic acid contentin liver, kidney and serum and on hepatic glycogen content in rat

By the administration of glucose before swimming, the swimming record of rat prolonged, too. After the 3rd swimming, glucuronic acid content in liver, kidney and hepatic glycogen content did not decrease so much, but their content was less than that obtained in case of the administration of glucuronolactone (Fg. 7-C).

4. Effect of the administration of glucuronolactone and glucose on o-aminophenylglucuronide synthesis (19)

The activity of *o*-aminophenylglucuronide synthesis of liver was measured by Levvy and Storey's method (20).

a) Effect of the swimming on o-aminophenylglucuronide synthesis of liver in rat

Though the activity of *o*-aminophenylglucuronide synthesis of liver was promoted at the end of the 1st swimming when glucuronic acid content increased, it was inhibited definitely at the end of the 3rd swimming when glucuronic acid content decreased (Table 3).

b) Effect of the toxic substance (APP_3) in urine and serum on o-aminophenylglucuronide synthesis

In vitro, the activity of *o*-aminophenylglucuronide synthesis was increased slightly by addition of a very small amount of the toxic substance (APP_3), but was decreased undoubtedly by addition of 0.005 percent the toxic substance (APP_3) or more (Table 4).

TABLE 3. Effect of the swimming on *o*-aminophenylglucuronide synthesis of liver *in vivo*.

	Control	After 1st swimming	After 3rd swimming
Average	503.9* ±21.1	559.1 ±40.9	301.4 ±17.1
Inhibition of glucuronide synthesis (%)	0	−11.0	40.2

* μg *o*-aminophenylglucuronide/g dry weight liver/ 1.5 hour (average of 10 rats).

TABLE 4. Effect of the toxic substance (APP_3) on *o*-aminophenylglucuronide synthesis of liver *in vitro*.

	No-treatment	Amount of APP_3 (%)							
		5×10^{-4}	25×10^{-4}	5×10^{-3}	25×10^{-3}	5×10^{-2}	1×10^{-1}	2.5×10^{-1}	5×10^{-1}
Average	503.9* ±21.1	589.2 ±20.5	572.2 ±23.8	471.9 ±18.4	291.9 ±29.1	251.3 ±15.1	193.1 ±14.4	148.8 ±6.8	105.5 ±14.3
Inhibition of glucuronide synthesis (%)	0	−16.9	−13.6	6.2	42.1	50.1	61.7	70.5	79.1

* μg *o*-aminophenylglucuronide/g dry weight liver/ 1.5 hour.

c) Effect of glucuronolactone on the decrease of o-aminophenylglucuronide synthesis of liver due to the toxic substance (APP_3)

At first we examined the effect of glucuronolactone on the activity of *o*-aminophenylglucuronide synthesis of liver *in vitro* (Table 5).

This synthesis was accelerated by addition of 10^{-5} mol or 10^{-4} mol glucuronolactone, but inhibited by addition of 10^{-3} mol glucuronolactone or more *in vitro*.

TABLE 5. Effect of glucuronolactone on *o*-aminophenylglucuronide synthesis of liver *in vitro*.

Glucurono-lactone (mol)	10^{-5}	5×10^{-5}	10^{-4}	5×10^{-4}	10^{-3}	5×10^{-3}	10^{-2}	2×10^{-2}
Average	504.0* ±22.3	502.8 ±14.4	520.7 ±38.0	508.4 ±17.7	368.0 ±20.2	169.7 ±23.3	96.3 ±13.1	50.3 ±7.6
Inhibition of glucuronide synthesis (%)	0	0	−3.3	0.9	27.0	66.3	80.9	90.0

* μg *o*-aminophenylglucuronide/g dry weight liver/ 1.5 hour.

TABLE 6. Effect of glucuronolactone and APP_3 on *o*-aminophenylglucuronide synthesis of liver *in vitro*.

Glucuronolactone (mol)	—	10^{-4}	10^{-3}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
APP_3 (%)	0.025	—	—	0.025	0.025	0.025	0.025
Average	297.3* ±27.0	513.5 ±29.2	355.9 ±20.0	472.8 ±14.9	498.5 ±17.4	202.4 ±29.2	1.7 ±1.6
Inhibition of glucuronide synthesis (%)	41.0	−1.9	29.5	6.2	1.1	59.8	96.6

* μg *o*-aminophenylglucuronide/g dry weight liver/1.5 hour.

The inhibition of *o*-aminophenylglucuronide synthesis induced by addition of 0.025 percent the toxic substance (APP_3) was counteracted by addition of a small amount of glucuronolactone which had no suppressive effect on this synthesis (Table 6).

d) Effect of glucose on the decrease of o-aminophenylglucuronide synthesis due to the toxic substance (APP_3)

This synthesis was not affected by addition of 10^{-4} mol glucose, but was inhibited by addition of 10^{-3} mol glucose or more *in vitro*. The inhibition of *o*-aminophenylglucuronide synthesis induced by addition of 0.025 percent toxic substance (APP_3) was not affected by addition of 10^{-4} mol glucose (Table 7). This result was varied with that of addition of glucuronolactone as described above.

TABLE 7. Effect of glucose and APP_3 on *o*-aminophenylglucuronide synthesis of liver *in vitro*.

Glucose (mol)	—	10^{-4}	10^{-3}	10^{-2}	10^{-4}	10^{-3}	10^{-2}
APP_3 (%)	0.025	—	—	—	0.025	0.025	0.025
Average	346.1* ±31.8	478.1 ±33.0	430.5 ±41.5	389.6 ±40.5	362.0 ±31.1	347.2 ±19.1	293.0 +26.3
Inhibition of glucuronide synthesis (%)	31.3	6.5	15.8	23.8	28.2	36.3	41.8

* μg *o*-aminophenylglucuronide/g dry weight liver/ 1.5 hour.

e) Effect of the injection of glucuronolactone and glucose on the decrease of o-aminophenylglucuronide synthesis of liver due to the swimming in rat

Injecting intraperitoneally glucuronolactone and glucose to the rat at rest inhibited slightly the activity of *o*-aminophenylglucuronide synthesis of liver. Though the activity of *o*-aminophenylglucuronide synthesis of liver was inhibited remarkably after the 3rd swimming, it was slightly less inhibited by glucose injection than in case of no treatment, but more than by glucuronolactone (Table 8). The activity of this synthesis was apparently inhibited by the toxic substance (APP_3) both *in vitro* and *in vivo*.

Though, injecting intraperitoneally the toxic substance (APP_3) exceedingly inhibited the activity of this synthesis of rat after the 3rd swimming, by addition of glucurono-

TABLE 8. Effect of glucuronolactone and glucose on *o*-aminophenylglucuronide synthesis of rat's liver *in vitro* (average of 10 rats).

A) Rest

No-treatment	525.8*±57.4
Glucuronolactone	491.8 ±52.2
Glucose	440.7 ±61.5

B) After 3rd swimming

No-treatment	285.6 ±51.2
Glucuronolactone	501.7 ±68.2
Glucose	322.7 ±88.3

* μg *o*-aminophenylglucuronide/ g dry weight liver/ 1.5 hour.

Rats were injected 3 times intraperitoneally with 100 mg/kg of glucuronolactone and glucose.

TABLE 9. Effect of APP_3 and glucuronolactone or glucose on *o*-aminophenylglucuronide synthesis of rat's liver *in vitro* (average of 10 rats).

APP_3	236.1*±21.3
APP_3+glucuronolactone	376.5 +47.5
APP_3+glucose	295.0 ±52.7

* μg *o*-aminophenylglucuronide/g dry weight liver/ 1.5 hour.

Rats were injected 3 times intraperitoneally with 100 mg/kg of glucuronolactone, glucose and APP_3 and sacrificed after 3rd swimming.

lactone with APP_3 it was inhibited less than in case of no addition (Table 9). Such an effect was not brought about with glucose (Table 9).

5. Effect of glucuronolactone and glucose in disorder of the beating of an isolated frog heart induced by the toxic substance (APP_3) in urine or serum

As mentioned above, we examined the effect of some agents on the isolated heart of *Rana nigromaculata* by Yagi's reflux method (21).

Paper chromatography was performed by one-dimensional ascending method. Toyo filter paper No. 51 was used as filterpaper and the mixture of *n*-butanol 40, acetic acid 10 and water 50 as solvent. In order to differentiate glucuronide from glucuronolactone, the solvent of mixture of *n*-butanol 6, pyridine 4 and water 3, or *n*-butanol 2, pyridine 1 and water 1 was used.

Fluorescence was determined by Ultra-violet light (Wave length: 2536 Å cycle).

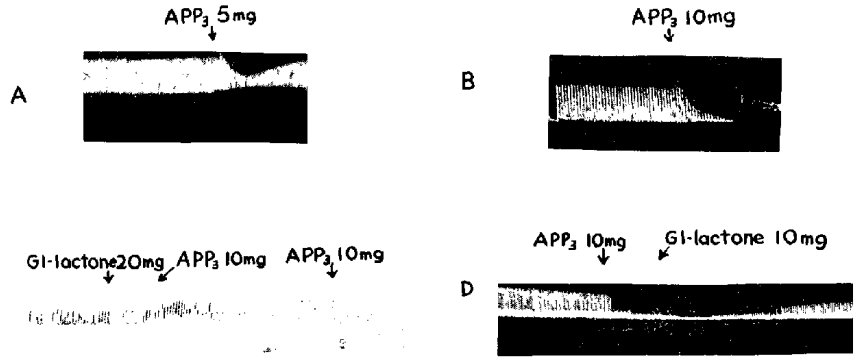

FIG. 10. Effect of glucuronolactone on disorder of the beating of an isolated frog heart due to the toxic substance (APP_3).

A : heart beating did not stop by 10 mg/5 ml APP_3, B : heart beating stopped by 10 mg/5 ml APP_3, C : 20 mg/5 ml glucuronolactone prevented the stopping of heart beating due to 20 mg/5 ml APP_3, D : stopped heart beating beated again with 10 mg/5 ml glucuronolactone.

a) Effect of glucuronolactone on disorder of the beating of an isolated frog heart due to the toxic substance (APP_3) in urine and serum

With administration of glucuronolactone, an isolated frog heart which had stopped beating by the toxic substance (APP_3) began to beat again (Fig. 10-C). Moreover, administration of glucuronolactone prevented the stopping of heart beating due to the toxic substance (APP_3) (Fig. 10-D). Therefore, the harmful effect of the toxic substance (APP_3) was detoxicated prophylactically and therapeutically by administration of glucuronolactone.

b) Effect of glucose on the disorder of the beating of an isolated frog heart due to the toxic substance (APP_3) in urine and serum

With administration of glucose, slight disorder of the beating of an isolated frog heart due to the toxic substance (APP_3) was restored to normal condition but beating in stopping was not recovered (Fig. 11-A, B).

FIG. 11. Effect of glucose on disorder of the beating of an isolated frog heart due to the toxic substance (APP_3).
A : slight disorder of the heart beating due to 5 mg/5 ml APP_3 was recovered, B : stopped heart beating due to 10 mg/5 ml APP_3 did not beat againt with glucose.

c) Paper chromatography of the amine-like substance combined with glucuronolactone in an isolated frog heart

The toxic substance (APP_3) was conjugated into a new glucuronide by addition of glucuronolactone to an isolated frog heart. This glucuronide is hydrolyzed by dilute acid, but not by β-glucuronidase (Fig. 12).

Basing upon the above described experiment, we could conclude that this toxic substance was detoxicated by glucuronolactone. That is to say, a harmful action to the body of this amine-like substance in the urine could be suppressed by use of glucuronolactone. This phenomenon can not be understood in view of our present knowledge concerning the glucuronic acid metabolism. In case of administration of glucuronolactone, residual glucuronolactone after decomposition turns glucaric acid or gulonic acid, entering into the glucose pathway. In the plant an enzyme which makes UDP-glucuronic acid from glucuronic acid has been discovered, while such an enzyme has not been found in the animal at present. Consequently, it should be considered that this compound is a form different from UDP-glucuronic acid. From this view-point it was examined whether the combination of amine and glucuronolactone was *o*-glucuronide or not.

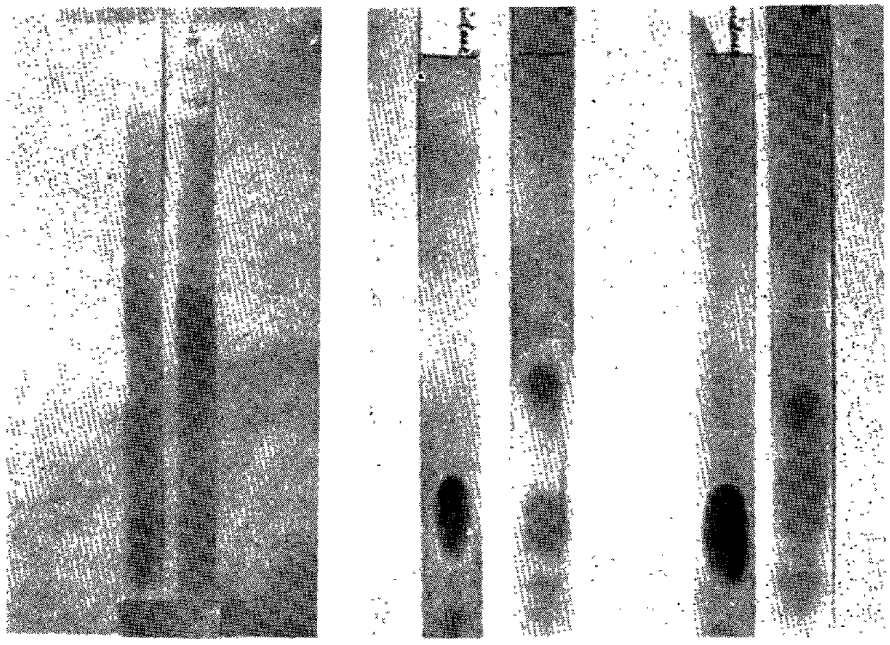

FIG. 12. Paperchromatography of the toxic substance (APP_3, Amine) combined with glucuronolactone in an isolated frog heart.

(a) : spot of glucuronolactone, (b) : spot of APP_3 added with glucuronolactone in an isolated frog heart, (c) : spot of APP_3 added with glucuronolactone in an isolated frog heart was unable to separate with considerably high unit of β-glucuronidase in 48 hours, (d) : this spot was able to separate with N-HCl at 50°C in 48 hours, (e) : spot of APP_4 including glucuronic acid was unable to separate with β-glucuronidase, (f) : this spot was able to separate with N-HCl at 100°C in 15 minutes.

At first we examined the reflux fluid, by paper chromatography, on condition that with addition of the toxic substance (APP_3) to reflux fluid of a frog heart the beating of the heart stopped, and we did the same experiment on condition that with immediate addition of glucuronolactone the beating began again.

The latter chromatograph different from the former's, was consisted of two spots, namely glucuronide and glucuronolactone. The spot of this glucuronide, being the same as the spot of glucuronide in urine, was not to be separated with considerably high unit of β-glucuronidase in 48 hours, but was separated with N-HCl at 50°C in 15 minutes (Fig. 12).

As a result of the experiment using an isolated frog heart, we may conclude that this amine-like substance was combined with glucuronolactone into a form unlike o-glucuronide. On the other hand, because glucuronide contained in acetone-precipitated sediment excreted into the urine, was not be separated with considerably high unit of β-glucuronidase in 48 hours, but was separated with N-HCl at 100°C in 15 minutes, it appears that glucuronide in urine partly consists of glucuronide.

6. The activity of β-glucuronidase in organs

We determined the activity of β-glucuronidase of rat's organs, using two groups, one at rest and the other after the 3rd swimming. The activity of β-glucuronidase was measured by Tsukamoto's method (27).

The result was shown in Table 10. The activity of β-glucuronidase in liver with no treatment was only slightly developed by the swimming, while with glucuronolactone or glucose the activity in each organ was mostly promoted by the swimming (Table 3).

TABLE 10. The activity of β-glucuronidase in organes.

	A			B	
In liver	Control	28,649* ±3,778	In liver	Control	32,458* ±7,171
	Glucuronolactone	28,646 ±4,667		Glucuronolactone	36,540 ±5,669
	Glucose	30,579 ±4,377		Glucose	34,940 ±7,576
In kidney	Control	6,201 ±1,404	In kidney	Control	5,552 ±1,174
	Glucuronolactone	5,278 ±1,106		Glucuronolactone	7,541 ±1,201
	Glucose	6,099 ±1,348		Glucose	6,216 ±1,116
In serum	Control	5,289** ± 656	In serum	Control	5,120** ±1,035
	Glucuronolactone	8,215 ±1,570		Glucuronolactone	8,760 ±1,208
	Glucose	5,342 ±1,315		Glucose	8,510 ±1,403

* unit/g/hr. ** unit/dl/hr.

Rats were injected 3 times intraperitoneally with 100 mg/kg of glucuronolactone and glucose. A indicates the activity in rest and B indicates the activity after 3rd swimming.

DISCUSSION

It has been demonstrated from the above described experiment that the toxic substance (amine-like substance) in urine and serum increased and glucuronic acid in a living body decreased due to physical exercise. Furthermore, as is previously said, the toxic substance in urine is present also in normal urine, increasing in the amount in the course of disease as well as physical exercise. On the occasion of severe disease, glucuronic acid in human urine decreases likewise.

With the administration of glucuronolactone or glucose before physical exercise, in any case, the time for running and swimming was prolonged. The prolongation of running and swimming time caused by the administration of glucuronic acid and glucose may suggest that they will exert a detoxicating action in a living body. On the basis of the above experiment, it may be said that glucuronolactone exercises a detoxicating action, as was noted in an isolated frog heart, over amine-like substances which cause a disharmonious state in a body by suppressing the enzyme being produced and increased due to physical exercise and which stop the heart beating.

In this condition the toxic substance (APP_3) increasing in urine and serum after physical exercise is turned into a new glucuronide. Although it is not clear whether this substance is *n*-glucuronide or not, it does not seem to be *o*-glucuronide. At any rate the presence of UDP-glucuronic acid may be unnecessary for the conjugation of this

amine-like substance.

Whether this phenomenon takes place in our body or not, when fatigue due to physical exercise appears, is a question to be settled. It seems that the administration of glucuronolactone shows good result in suppressing the appearance of fatigue due to physical exercise. There has been demonstrated a relationship between the administration of glucuronic acid and the activity of β-glucuronidase. It has been also said that nature glucuronic acid in each organ does not decompose and the good effect is developed as a consequence of the inhibition of β-glucuronidase activity by the administration of glucuronolactone. So we examined the activity of β-glucuronidase in each organ. What changes in β-glucuronidase activity are brought due to physical exercise? In a few cases of our studies, however, the activity of β-glucuronidase was developed in swimming; even with the administration of glucuronolactone and glucose, its activity was not decreased.

Judging from these results, it does not seem that this phenomenon which has been influenced by addition of glucuronolactone is due to β-glucuronidase.

Also, prolongation of the running and swimming time is caused by the administration of glucose. Glucose is to be turned into glucuronolactone in the glucose pathway; moreover UDP-glucose is the precursor of UDP-glucuronic acid. If glucuronolactone is given, it was largely decomposed, entering in the body. In any animal body an enzyme which makes UDP-glucuronic acid from glucuronic acid has not been discovered. Though the administration of glucose has theoretically more advantages to form glucuronide than that of glucuronolactone, we found more glucuronic acid content, in the animal urine or serum in case of glucuronolactone administration than in case of glucose. From the result of these experiments, it is assumed that the detoxication in a living body is stronger with glucuronolactone administration than with glucose.

In any case, it seems that there is an intimate connection between glucuronic acid in our body and the appearance of fatigue due to physical exercise.

It is an interesting problem that the toxic substance (amine-like substance) increasing in blood and serum due to physical exercise and severe disease was turned into harmless substance (glucuronide) in an isolated frog heart, by addition of glucuronolactone, as shown in paper chromatography experiment. As a result of our studies, this glucuronide was not formed through the Dutton's glucuronide pathway. From Dutton's speech at the 10th glucuronic acid symposium (23), we also could ascertain an important matter of the utilization of exogenous glucuronic acid. He said "UDP-glucuronic acid is not an only form with regard to utilization of exogenous glucuronolactone. I merely found one of the utilization pathways. I think, another pathway is probably to be found. We must keep in our mind to find the new pathway."

We can hardly refrain from entertaining many doubts about this problem, but will study further into the conjugation pathway of this amine-like substance and glucuronolactone.

SUMMARY

The results of our study concerning the relationship between the appearance of fatigue and glucuronic acid metabolism are as follows:

1. We analysed the substance which increased in urine or serum of a living body as fatigue due to physical exercise appeared. The acetone-insoluble substance precipitated by addition of lead acetate (APP_3) which contained in above mentioned substance had various toxic actions. According to Staudinger's analytic method, this substance may be a compound formed from amine, acid, peptide and the other elements. On the paper chromatography, the 3rd spot including 80 percent of the toxic substance should have most powerful toxicity. Moreover, this spot showed a characteristic absorption curve through Sephadex G-25 and caused a reaction of primary amine.

2. Glucuronic acid content in urine, serum, liver or kidney and hepatic glycogen content of a living body decreased after hard physical exercise, but did not decrease with the administration of glucuronolactone.

3. With the administration of glucuronolactone the swimming record of rat prolonged and the appearance of fatigue in human lessened, too.

4. In accordance with decrease of glucuronic acid content in urine or serum caused by hard swimming, the activity of *o*-aminophenylglucuronide synthesis of rat's liver was inhibited, but the administration of glucuronolactone prevented this inhibition. *In vitro*, the activity of *o*-aminophenylglucuronide synthesis of rat's liver was inhibited by addition of the toxic substance (APP_3), but the addition of glucuronolactone prevented this inhibition. Such an effect was not brought about with addition of glucose.

5. Disorder of an isolated frog heart due to the toxic substance (APP_3) was checked both prophylactically and therapeutically with the administration of glucuronolactone. On the paper chromatography, the spot of the toxic substance (APP_3) added with glucuronolactone in an isolated frog heart was not to be separated with considerably high unit of β-glucuronidase in 48 hours, but it was separated with N-HCl at 50°C in 15 minutes. For this reason, it may be assumed that such a toxic effect of the toxic substance (APP_3) was perhaps detoxicated as a result of synthesizing, with addition of glucuronolactone, a certain kind of new glucuronide unlike *o*-glucuronide.

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