Studies on the Detoxicating Hormone of the Liver (Yakriton).

97th Report.

The Difference of Urinary Elimination of Phenolsulphonphthalein
(Injected Intravenously) in Rabbits with Different Liver
Power; and Influence of Yakriton upon It. Part 2.

By

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Introduction.

In Part 1¹⁾ (96th Report on Yakriton) of the present work, I reported of the urinary elimination of phenolsulphonphthalein, injected intravenously, in rabbits as follows:—

The most part of the phenolsulphonphthalein, injected intravenously, in a rabbit will be excreted during the 1st hour of dye injection.

1. But there is a difference of the rate of dye elimination according to the strength of ammonia-detoxicating liver power. In *f*-classified rabbits (= with strong liver), it is always accelerated.

2. But if yakriton is injected to *b*-classified rabbits (= with weak liver), in the amount of 1/2 R.A.U.* per kilo of body weight, the rate becomes remarkably accelerated, as in the case of *f*-classified rabbits with no use of yakriton.

However, in the series of the experiments in the preceding paper,¹⁾ I always used yakriton in the amount of 1/2 R.A.U. per kilo of body weight.

Prof. Sato²⁾ is of opinion that yakriton will exert detoxicating

* R.A.U. means Rabbit-Ammonia-Unit., Tohoku J. Exp. Med., 1927, 8, 351.

1) F. Ohta, Tohoku J. Exp. Med., 1940, 39, 1.

2) A. Sato, Tohoku J. Exp. Med., 1929, 13, 502.

action on different poisons with different numbers of units or with different phases of "yakritisation." So it is considered that the effect of yakriton upon accelerating the rate of elimination of phenolsulphonphthalein will be different according to the differences in the numbers of units of yakriton used. And I performed further experiments for the purpose of investigating the effect of yakriton used in different units upon the rate of elimination of the dye.

The present paper is Part 2 of the present work.

Method of Experiment.

1. Animals used for the experiment.

Healthy adult male rabbits were used. They were put to Sato and Sakurada's³⁾ liver function test by means of ammonium chloride 3 times (once a day) every other day and only *b*-classed (low-classed) rabbits were used. They were put to the experiment proper after a lapse of more than a week after the last liver function test.

2. Method of the phenolsulphonphthalein test.

It was the same as is described in the preceding paper.¹⁾

3. General schedule of experiments.

The rabbits were divided into 4 groups, according to the differences of "yakritisation."

1. The 1st group.

The rabbits in this group were put to phenolsulphonphthalein test with no use of yakriton, and then just a week later the test was repeated with use of yakriton in the amount of 1/4 R.A.U. per kilo of body weight 30 minutes prior to phthalein injection, as is shown in Fig. 1.

2. The 2nd group.

The experiment of this group differed from that of the 1st group only in the amount of yakriton used. 1 R.A.U. was used instead of 1/4 R.A.U., as is shown in Fig. 1.

3. The 3rd group.

The experiment of this group differed from that of the 1st group only in the amount of yakriton used. 2 R.A.U. were used instead of 1/4 R.A.U., as is shown Fig. 1.

4. The 4th group.

The experiment of this group differed from those of the preceding

3) A. Sato and H. Sakurada, Tohoku J. Exp. Med., 1927, 8, 347.

Fig. 1. Schedule of experiment.

3 groups in the number of injections of yakriton and the amount of it. In this experiment it was injected in the amount of $\frac{1}{2}$ R.A.U. per kilo of body weight at 9:00 a.m. repeatedly for 4 consecutive days and 30 minutes after the last yakriton injection the test was repeated, as is shown in Fig. 1.

Result of Experiment.

The results of my experiments are shown in Tables 1, 2, 3 and 4. They will be observed conveniently in the following 4 items:—

1. The 1st group.

As is shown in Table 1, yakriton caused no change of the total amount of dye excreted in the urine during the whole time. Compare 82.2% without yakriton and 82.0% with it on an average (Cf. Table 1). But the rate of the dye elimination was accelerated in the rabbits Nos. 1, 3 and 4 by use of yakriton. And the effect was especially remarkable in the rabbits Nos. 3 and 4, while it was slightly delayed

TABLE 1.
Result of the 1st group

When yakriton was used, it was injected in the amount of $\frac{1}{4}$ R. A. U. per kilo of body weight 30 minutes prior to the phenolsulphonphthalein injection.

No. of rabbits	Date of experiment	Use of yakriton	Room temperature	Body weight (in kilos)	Amount of dye excreted in the urine (% to the whole amount injected)							Total	Change of the rate of dye elimination by use of yakriton
					Lapse of time after dye injection								
					0°-½°	½°-1°	1°-1½°	1½°-2°	2°-3°	3°-4°			
No. 1 (6500)	28. IX 5. X	No use With use	19°C 21°C	2.15 2.21	35.5 48.6	24.0 18.1	17.5 8.1	2.5 6.7	2.0 2.3	+*	81.5 83.8	Accelerated	
No. 2 (6501)	28. IX 5. X	No use With use	19°C 21°C	1.77 1.97	38.0 36.0	24.0 29.0	12.4 8.0	5.0 4.0	2.2 5.0	+	81.6 82.0	Slightly delayed	
No. 3 (6502)	29. XI 6. X	No use With use	20°C 21°C	1.77 1.80	38.8 50.2	18.5 12.5	10.3 8.5	9.5 5.0	4.5 3.0	+	81.6 79.2	Remarkably accelerated	
No. 4 (6503)	29. XI 6. X	No use With use	20°C 21°C	1.97 1.90	27.5 47.5	37.5 20.5	8.0 6.0	6.0 4.0	5.0 3.5	+	84.0 81.5	Remarkably accelerated	
No. 5 (6504)	30. XI 7. X	No use With use	21°C 18°C	1.94 1.97	35.0 32.5	27.0 28.0	17.0 15.0	5.0 7.0	2.5 2.0	+	86.5 84.5	Slightly delayed	
No. 6 (6505)	30. XI 7. X	No use With use	21°C 18°C	2.24 2.21	54.5 52.3	9.0 10.0	8.0 11.3	4.0 4.1	2.8 3.3	+	78.3 81.0	Slightly delayed	
Average	No use of yakriton				38.2	23.3	12.2	5.3	3.2	+	82.2	Slightly accelerated	
					61.5		17.5						
					79.0								
	With use of yakriton				44.5	19.7	9.5	5.1	3.2	+	82.0		
				64.2		14.6							
				78.8									

* Dye was identified by means of amyllic or butylic alcohol in an amount as small as negligible.

in the rabbits Nos. 2, 5 and 6, in spite of the use of yakriton. So the amount of dye excreted during the 1st $\frac{1}{2}$ hour, for instance, was increased by 16.5% on an average, as is shown in Table 5.

2. The 2nd group.

As is shown in Table 2, yakriton caused no change of the total amount of dye excreted during the whole time. Compare 74.9% without yakriton and 76.6% with it on an average (Cf. Table 2). But the rate of the dye elimination was remarkably accelerated in all the rabbits, except for No. 12, in which almost no influence was seen. So the amount of dye excreted during the 1st $\frac{1}{2}$ hour, for instance, was increased by 36.4% on an average (Cf. Table 5).

3. The 3rd group.

TABLE 2.
Result of the 2nd group.

When yakriton was used, it was used in the amount of 1 R. A. U. per kilo of body weight 30 minutes prior to the phenolsulphonphthalein injection.

No. of rabbits	Date of experiment	Use of yakriton	Room temperature	Body weight (in kilos)	Amount of dye excreted in the urine (% to the whole amount injected)							Total	Change of the rate of dye elimination by use of yakriton
					Lapse of time after dye injection								
					0°-½°	½°-1°	1°-1½°	1½°-2°	2°-3°	3°-4°			
No. 7 (6506)	1. X	No use	21°C	2.20	37.5	27.2	12.5	3.0	2.0	+	82.2	Remarkably accelerated	
	8. "	With use	18°C	2.24	58.6	12.5	4.1	3.2	2.3	+	80.7		
No. 8 (6507)	1. "	No use	21°C	1.95	30.0	39.4	8.2	4.4	3.3	+	85.4	Remarkably accelerated	
	8. "	With use	18°C	1.85	55.7	20.0	4.6	3.2	2.7	+	86.2		
No. 9 (6508)	12. "	No use	17°C	2.23	46.7	19.5	3.5	2.5	1.7	+	73.9	Remarkably accelerated	
	19. "	With use	16°C	2.26	60.5	8.5	2.5	2.0	1.0	+	74.5		
No. 10 (6509)	12. "	No use	17°C	2.34	43.2	25.0	5.0	3.4	1.6	+	78.2	Remarkably accelerated	
	17. "	With use	16°C	2.30	60.0	10.9	4.3	2.6	1.8	+	79.6		
No. 11 (6510)	13. "	No use	17°C	2.25	40.9	20.5	7.3	5.9	2.7	+	77.3	Remarkably accelerated	
	20. "	With use	12°C	2.31	57.8	10.8	2.5	2.2	1.8	+	75.1		
No. 12 (6511)	13. "	No use	17°C	2.15	45.7	24.3	6.9	2.9	3.3	+	83.1	not changed	
	20. "	With use	12°C	2.15	46.2	30.4	5.0	2.0	1.0	+	84.1		
Average	No use of yakriton				40.7	20.9	7.2	3.7	2.4	+	74.9	Remarkably accelerated	
					61.6 10.9								
					72.6								
Average	With use of yakriton				55.6	12.2	3.9	2.5	1.8	+	76.0	Remarkably accelerated	
					67.8 6.4								
					74.2								

* Cf. Foot-note to Table 1.

As is shown in Table 3, yakriton caused no change of the total amount of dye excreted during the whole time. Compare 73.7% without yakriton and 78.6% with it (Cf. Table 3). But the rate of the dye elimination was accelerated by use of yakriton in all the rabbits, except for Nos. 13 and 18, in which it was remarkably delayed, in spite of the use of yakriton. The effect of yakriton was especially remarkable in rabbit No. 14. So the amount of dye excreted during the 1st 1/2 hour, for instance, was increased by 13.0% on an average (Cf. Table 5).

4. The 4th group.

As is shown in Table 4, yakriton caused no change of the amount of dye excreted during the whole time. Compare 73.3% without yakriton and 74.0% with it on an average (Cf. Table 4). But the rate

TABLE 3.
Result of the 3rd group.

When yakriton was used, it was injected in the amount of 2 R. A. U. per kilo of body weight 30 minutes prior to the phenolsulphonphthalein injection.

No. of rabbits	Date of experiment	Use of yakriton	Room temperature.	Body weight (in kilos)	Amount of dye excreted in the urine (% to the whole amount injected)							Total	Change of the rate of dye elimination by use of yakriton
					Lapse of time after dye injection								
					0°-½°	½°-1°	1°-1½°	1½°-2°	2°-3°	3°-4°			
No. 13 (6512)	14. X	No use	17°C	2.27	41.3	14.8	8.7	3.5	2.6	+	70.9	Remarkably decreased	
	21. "	With use	14°C	2.30	25.5	31.4	5.5	4.8	3.8	2.0	73.0		
No. 14 (6513)	31. X	No use	17°C	2.25	35.4	26.3	8.2	3.2	1.6	+	74.7	Remarkably accelerated	
	7. XI	With use	13°C	2.23	55.4	8.2	5.4	2.3	3.0	+	74.4		
No. 15 (6514)	31. X	No use	17°C	2.03	45.0	20.5	9.0	4.0	2.0	+	80.5	Remarkably accelerated	
	7. XI	With use	13°C	2.10	65.0	10.0	5.2	3.2	+	+	83.4		
No. 16 (6515)	1. "	No use	15°C	2.07	37.5	27.6	8.0	5.0	1.5	+	79.6	Slightly accelerated	
	8. "	With use	14°C	2.28	46.1	20.0	8.4	1.8	2.6	+	78.9		
No. 17 (6516)	1. "	No use	15°C	2.00	35.0	22.0	3.5	2.5	2.4	+	65.4	Remarkably accelerated	
	8. "	With use	14°C	2.18	50.5	11.8	3.6	2.6	4.0	+	72.5		
No. 18 (6517)	2. "	No use	14°C	2.51	40.0	16.8	5.8	2.9	4.0	+	69.5	Remarkably delayed	
	9. "	With use	14°C	2.45	22.0	33.5	7.6	9.2	5.2	2.1	79.6		
Average					39.0	21.6	7.2	3.5	2.4	+	73.7	Slightly accelerated	
					No use of yakriton								
					60.6 10.7								
					71.3								
					44.1	19.1	5.9	6.0	2.9	0.6	78.6		
With use of yakriton													
63.2 11.9					75.1								

* Cf. Foot note to Table 1.

of the dye elimination was accelerated in the rabbits Nos. 19 and 21 by use of yakriton, while it was not influenced in the rabbit: No. 22, and slightly or remarkably delayed in the rabbits Nos. 20, 23 and 24 in spite of the use of yakriton. So the amount of dye excreted during the 1st 1/2 hour, for instance, was decreased by 7.4% on an average (Cf. Table 5).

Discussion.

From these results, it is apparent that there is a difference of the effect of yakriton upon accelerating the rate of the dye elimination according to the differences of "yakritisation." In the series of experiments reported in this paper, the effect of yakriton displayed it-

TABLE 4.

Result of the 4th group.

When yakriton was used, it was injected in the amount of $\frac{1}{2}$ R. A. U. per kilo of body weight once a day for 4 consecutive days, and 30 minutes after the last yakriton injection the 2nd phenolsulphonphthalein test was commenced.

No. of rabbits	Date of experiment	Use of yakriton	Room temperature	Body weight (in kilos)	Amount of dye excreted in the urine (% to the whole amount injected)							Total	Change of the rate of dye elimination by use of yakriton	
					Lapse of time after dye injection									
					0°-½°	½°-1°	1°-1½°	1½°-2°	2°-3°	3°-4°				
No. 19 (6519)	2. XI 9.	No use	14°C	2.81	45.0	22.5	4.0	3.6	1.0	+	76.1	Remarkably accelerated		
		With use	14°C	2.82	55.6	16.5	3.0	3.0	2.5	+	80.6			
No. 20 (6520)	4. " 11.	No use	14°C	2.72	42.9	18.5	6.0	3.2	2.3	+	72.9	Slightly delayed		
		With use	15°C	2.80	35.5	30.2	5.4	3.0	2.0	+	76.1			
No. 21 (6521)	4. " 11.	No use	14°C	2.03	40.0	17.5	4.0	3.6	3.5	+	67.6	Remarkably accelerated		
		With use	15°C	2.05	50.0	10.0	5.0	3.5	3.0	+	71.5			
No. 22 (6522)	8. " 15.	No use	16°C	2.32	47.6	13.0	2.6	1.8	2.3	+	67.5	Not changed		
		With use	15°C	2.34	50.8	7.0	5.0	3.6	2.5	+	68.9			
No. 23 (6523)	8. " 15.	No use	16°C	2.72	48.1	14.8	7.4	3.0	2.6	+	75.9	Remarkably delayed		
		With use	15°C	2.65	34.6	17.3	6.1	4.6	6.1	+	68.7			
No. 24 (6524)	9. " 16.	No use	14°C	2.02	45.0	22.5	7.5	3.0	2.0	+	80.0	Remarkably delayed		
		With use	12°C	2.06	22.5	36.0	10.5	4.0	3.0	2.5	78.5			
Average					44.8	18.1	5.3	3.0	2.1	+	73.3	Not changed		
					No use of yakriton									
					62.9		8.3							
					71.2									
					41.5	19.5	5.8	3.6	3.2	0.4	74.0			
With use of yakriton														
61.0		9.4												
70.4														

* Cf. Foot note to Table 1.

TABLE 5.

Difference of the rate of the dye elimination in each group between in cases of no use of yakriton and in case of use of it.

	No. of groups	Lapse of time after dye injection			
		$\frac{1}{2}$ °	1°	2°	4°
In case of use of yakriton	1	+ 16.5%	+ 4.3%	- 2.5%	- 2.4%
— larger by —	2	+ 36.4 "	+ 10.0 "	+ 2.2 "	+ 1.4 "
that in case of no use of it	3	+ 13.0 "	+ 4.3 "	+ 5.0 "	+ 6.6 "
	4	- 7.4 "	- 3.0 "	- 1.1 "	+ 0.9 "

self most remarkably in the 2nd group (Cf. Tables 1, 2, 3, 4 and 5). But even in the 2nd group it was not so remarkable as in the experi-

ment in *b*-classed rabbits, reported in the preceding paper,¹⁾ in which yakriton was always used in the amount of 1/2 R.A.U. per kilo of body weight, and the amount of dye excreted during the 1st 1/2 hour after dye injection, for instance, was increased by 53.2% on an average by use of it (Cf. Tables 2 and 5 of the preceding paper,¹⁾ and, Tables 2 and 5 of this paper). As has been shown in a number of reports* published from this Laboratory, yakriton is the most effectual if used in an appropriate amount (R.A.U.). It does not follow that a larger amount is better than a small amount. There are instances, in which 1/5 R.A.U. is much more efficacious than 1 R.A.U. In my own experiments the amount of yakriton of 1/2 R.A.U. per kilo of body weight (used in the experiment reported in the preceding paper¹⁾) seems the most suitable for the purpose of accelerating the rate of dye elimination.

However it is very interesting to me that, though the most favourable result was obtained by a single use of yakriton in the amount of 1/2 R.A.U. per kilo of body weight, a favourable result could not be obtained by that amount, if it was used for 4 consecutive days repeatedly—as in the 4th group.

Remarks.

Kawakatsu⁴⁾⁵⁾ reported that in both cold-blooded and warm-blooded animals the elimination of dyestuffs, which were excreted principally through the kidneys, was influenced by the 4 seasons—the rate of dye elimination was always accelerated in summer. In my own series of experiments, the cooler the weather became, the slower the rate of dye elimination became, and the smaller the amount of dye

* For instance,—

1. K. Asakura, Tohoku J. Exp. Med., 1930, 15, 476.

He reported, in his study on the Effect of Yakriton against Acute Phosphorus Poisoning (23rd Report), that yakriton used in the amount of 1/4 R.A.U. was more effective than that used in the amount of 3/4, 1, 5 or 50 R.A.U.

2. T. Sato, Tohoku J. Exp. Med., 1933, 20, 408.

He reported, in his study on the Effect of Yakriton on Uranyl Nephritis (29th Report), that yakriton used in the amount of 1/2 R.A.U. was more effective than that used in the amount of 1 R.A.U.

3. M. Hasegawa, Tohoku J. Exp. Med., 1938, 32, 163.

He reported, in his study on the Effect of Yakriton upon Chronic Nephritis (85th Report), that yakriton used in the amount of 1/2 R.A.U. was more effective than that used in the amount of R.A.U.

- 4) Ry. Kawakatsu, Jikken Shokwakibyō Gaku, 1930, 5, 1801.

- 5) Ry. Kawakatsu, Jikken Shokwakibyō Gaku, 1931, 6, 526.

excreted through the kidneys became (Cf. Table 2 of the preceding paper,¹⁾ and Tables 1, 2, 3 and 4 of this paper). However, in my experiment, the influence of the weather upon the rate of dye elimination need not be taken into consideration, because in my 2 phenolsulphonphthalein tests—one without use of yakriton and the other with it—were performed always within a short interval, a week, during which—for the duration of the performance of experiment at least—almost no change of room temperature occurred, as has been shown in Tables 2 and 3 of the preceding paper,¹⁾ and Tables 1, 2, 3, and 4 of this paper.

Further, in the series of 6 experiments of mine, there was a fairly great individual fluctuation in the rate of dye elimination, not only between *f*-classed and *b*-classed rabbits, but also among *b*-classed ones themselves (Cf. Tables 2 and 3 of the preceding paper,¹⁾ and Tables 1, 2, 3 and 4 of this paper). However, my 2 phenolsulphonphthalein tests—one without yakriton and the other with use of it—were performed always in the same rabbits, and so the individual fluctuation need not be taken into consideration, upon observing the effect of yakriton.

On the other hand, it must be remarked that in the series of my experiments the rabbits had been fixed on a rack in a dorsal position for about 4 1/2 hours in hunger. Could not have been the liver power of the rabbits impaired by being kept in that state for such a long time? And if the liver power of them was impaired by being kept so during the 1st test, could it have completely recovered from it by the time the 2nd test was performed? In order to solve this problem, I performed the following experiment:—

Four *f*-classed rabbits were used for the purpose. Since the evening of the day before the experiment, they had been in hunger, and on that day they were in the morning injected with 40 c.c. of physiologic saline solution intraperitoneally and had been fixed on a rack in a dorsal position for about 4 1/2 hours. And after about 4 1/2 hours, they were put to Sato and Sakurada's³⁾ liver function test. In consequence of it, 3 of them were reduced to *c*-class and the other one to *d*-class, as is shown in Table 6. But they attained *f*-class standard again after a lapse of one week, as was shown by Sato and Sakurada's³⁾ liver function test (Cf. Table 6). So it was known that the liver power of rabbits kept in such a state as described above for a long time, was impaired, but it completely recovered after a lapse of one week. Therefore, the result of the awkward experiments need not be

taken into account on position and state imposed on rabbits during phenolsulphonphthalein test.

TABLE 6.
Result of control experiment.

Liver power for ammonium chloride						
Date of experiment		Before experiment			Immediately after being fixed in a hunger state for about 4½ hours	After further a week
		22. XI	24. "	26. "	3. XII	10. "
Number of rabbits and their body weight (in kilos)	No. 1 (6564)	<i>f</i> 2.30	<i>f</i> 2.25	<i>f</i> 2.32	<i>c</i> 2.20	<i>f</i> 2.32
	No. 2 (6565)	<i>f</i> 2.65	<i>d</i> 2.50	<i>f</i> 2.55	<i>c</i> 2.50	<i>f</i> 2.70
	No. 3 (6566)	<i>f</i> 2.80	<i>f</i> 2.81	<i>f</i> 2.76	<i>d</i> 2.72	<i>f</i> 2.83
	No. 4 (6567)	<i>f</i> 2.41	<i>f</i> 2.44	<i>f</i> 2.46	<i>c</i> 2.40	<i>f</i> 2.50

It is to be remarked that if the present work had been done after rabbits were fed early in the morning, the effect of yakriton might have been somewhat different, if the study of Prof. Sato⁶⁾ in an early paper of yakriton is taken into consideration.

Summary.

Yakriton exerts, as has been published in Part 1¹⁾ of the present paper, an accelerating effect upon the rate of urinary elimination of phenolsulphonphthalein injected intravenously.

1. Now when yakriton was used 30 minutes prior to dye injection, it was not so effective in the amount of 1/4 R.A.U. or 2 R.A.U. (per kilo of body weight) as in the amount of 1 R.A.U. (per kilo) (Cf. Tables 1, 2, 3 and 5).

2. When yakriton was used 30 minutes prior to dye injection, it was not so effective in the amount of 1 R.A.U. (per kilo of body weight) as in the amount of 1/2 R.A.U. (per kilo) (Cf. Tables 2 and 5, of the preceding paper,¹⁾ and Tables 2 and 5 of this paper).

3. When yakriton was injected in the amount of 1/2 R.A.U. per kilo of body weight for 4 consecutive days, and dye was injected 30 minutes after the last injection of it, the rate of the dye elimination was not accelerated (Cf. Tables 4 and 5).

6) A. Sato, Tohoku J. Exp. Med., 1928, 11, 265.

Conclusion.

In the effect of yakriton upon accelerating the rate of urinary elimination of phenolsulphonphthalein, injected intravenously, there are differences according to differences of "yakritisation" as in cases of detoxication of poisons.