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

80th Report.

Effect of Yakriton* on Phenylhydrazin Anemia.

By

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It is well known that phenylhydrazin causes a very severe anemia. Besides, it has been pointed out by Domarus,¹⁾ Jo,²⁾ Takamori,³⁾ and Nishitani⁴⁾ that the type of anemia produced by the poison is very similar to pernicious anemia in human pathology. And, as it is well known again that liver or its preparations are very effectual^{5,6)} in human pernicious anemia, a number of investigators have tried liver preparations for the treatment of phenylhydrazin anemia in animal experimentation. While Jastrowitz and Neidhard,⁷⁾ Friedländer and Steinitz,⁸⁾ Susanna⁹⁾ and Takimoto and Kawagoi¹⁰⁾ hold the liver therapy to be efficacious, Adlersberg and Gottsegen,¹¹⁾ Gottsegen,¹²⁾ and Strisower¹³⁾ consider it as quite inefficacious.

* Throughout the work emulsified yakriton was used.

1) A. Domarus, Arch. f. exp. Pharm. u. Path., 1908, **58**, 319.

2) S. Jo, Nihon Byôri Gakkai Zasshi, 1916, **6**, 489.

3) T. Takamori, Kyôto Igakkai Zasshi, 1921, **18**, 1407.

4) M. Nishitani, Nihon Biseibutsu Gakkai Zasshi, 1926, **20**, 989.

5) G. H. Whipple, F. S. Robscheit and G. W. Hooper, Amer. Jour. Physiol., 1920, **53**, 236.

6) G. R. Minot and W. P. Murphy, Bost. Med. and Surg. Jour., 1926, **195**, 410.

7) H. Jastrowitz and K. Neidhard, Zeitsch. f. g. exp. Med., 1931, **75**, 526.

8) E. Friedländer and E. Steinitz, Dtsch. med. Wsch., 1933, 135.

9) V. Susanna, Rev. d. Pharm. et Thé. exp., 1931, **2**, 113.

10) S. Takimoto and H. Kawagoi, Nihon Shôkakibyô Gakkai Zasshi, 1931, **30**, 395.

11) D. Adlersberg and G. Gottsegen, Arch. f. exp. Path. u. Pharm., 1929, **142**, 323.

12) G. Gottsegen, Wien. Klin. Wschr., 1934, 462.

13) R. Strisower, Wien. Med. Wschr., 1928, 1371.

In the present paper, I desire to report on a tentative experiment on the effect of yakriton for experimental phenylhydrazin anemia. Yakriton is, as will be seen from Prof. Sato and Yoshimatsu's paper,¹⁴⁾ most probably a chemical entity and is free from amino acids, being thus different from that liver principle which is said to be effective in human pernicious anemia. So much more interesting must be the results presented in this present paper of mine.

Method of Experiment.

1. Class of animals. Normal healthy rabbits which were of high-classed liver power were used. In other words, healthy rabbits were put to Sato-Sakurada's liver function test and only *f*-classed¹⁵⁾ were used for the present work. As to the results with *b*-classed rabbits I shall be able to report in the near future.

These *f*-classed animals were fed on *tofukara*.**

2. The poison used. 0.015 grms. of phenylhydrazin hydrochloride (Kahlbaum) were injected subcutaneously per kilo of body weight into the back of a rabbit. The injection of the poison was performed every day during the experiment.

3. Yakriton. The preparation used contained $\frac{1}{2}$ R.A.U.*** in 1 c.c. It was used subcutaneously injected in the amount of $\frac{1}{10}$ R.A.U. per kilo of body weight, every day during the experiment. The injection of yakriton was made into the back of a rabbit 30 minutes prior to that of the phenylhydrazin, which was injected always on the side of the body opposite to the place of the administration of the yakriton.

4. Blood examination. Blood was taken in each case before the injection.

Red cells were examined by use of Hayem's solution and white cells by use of Türk's solution. For the differentiation of white cells the Tohoku Pediatric Method,¹⁶⁾ a modification of Sato-Sekiya's copper peroxidase reaction, was used.

Pathological erythrocytes were examined either by that method or by Manson's method.

14) A. Sato and S. Yoshimatsu, Tohoku J. Exp. Med., 1926, 8, 234.

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

** Tōfu is a food made from soy beans, and is thus a kind of bean curd, and Kara is the refuse left over from the beans; this is swollen in water, then ground and pressed.

*** R.A.U. = Rabbit-Ammonia-Units.

16) A. Sato, T. Suzuki and R. Shibata, Tohoku J. Exp., Med. 1934, 24, 195.

Reticulocytes were examined by use of 1% brillanteresil blue solution. Hemoglobin was measured with Sahli's hemometer.

5. General schedule of the experiment. Always a couple of rabbits were used in one series of experiment. The injection of phenylhydrazin was begun on the same day on the control animal as on the yakriton animal in which the hormone was used 30 minutes earlier, while in the control no yakriton was used.

When the control happened to die, then the injection of the poison was also suspended in the yakriton animal, and from then on only yakriton was injected daily, till the normal or almost normal state with the almost normal blood picture presented itself again.

TABLE I a.

Control animal (No. 1.....No. 5051) with phenylhydrazin—control to No. 2.

Day of experiment		Before the injection		After the injection		
				1	2	3
Date		30. I	31. I	1. II	2. II	
Body weight (kilo)		2.700	2.700	2.600	2.650	
Phenylhydrazin (per kilo grm.)			0.015	0.015	0.015	
Erythrocytes per cmm. (Millions)		7.3	7.4	5.6	3.8	
Hemoglobin (Sahli)		80	80	60	45	
Reticulocytes %		8	2	12	90	
Leucocytes per cmm.		10,000	9,000	14,700	21,200	
Percentage of white cells	Lymphocytes	50.0	46.0	43.0	40.0	
	Polymorphonuclears	46.5	52.0	54.0	56.0	
	Eosinophiles	1.0	1.0	0.5	0.5	
	Mast cells	2.0	0.5	1.0	2.0	
	Monocytes	0.5	0.5	1.5	1.5	
	Leucocyte-index	0.9	1.0	1.2	1.4	
Schilling-Arneth's nuclear shift	M	0	0	0	0	
	J	0	0	0	0	
	St	6.0	7.0	20.0	24.0	
	II	30.0	32.0	40.5	40.5	
	III	45.0	43.0	32.0	27.0	
	IV	16.0	15.0	5.5	8.0	
	V	3.0	3.0	2.0	0.5	
Index		0.1	0.1	0.3	0.3	
Aniso-and poikilocytosis		—	—	+	++	
Polychromasia		+	+	++	+++	
Punctata basophilia		—	—	—	++	
Normoblasts per cmm.		—	—	100	3,300	

Result of Experiment.

As stated above, the present experiment was performed on *f*-class-ed rabbits alone.

1. Total red cell count. Before starting the experiment total red cell count was 6.5–7.8 millions per cmm. in each rabbit.

a. The control group. The total count was after 2 consecutive injections of the poison reduced to half the preexperimental number 3.8–4.0 millions. And each animal died after three consecutive injections.

b. The yakriton group. After 2 consecutive injections of the

TABLE
Yakriton animal (No. 2.....No. 5052)

Day of experiment		Before the injection						
				1	2	3	4	5
Date		30. I	31. I	1. II	2. II	3. II	4. II	5. II
Body weight (kilo)		3.000	3.200	3.050	3.020	3.300	3.150	3.000
Yakriton per kilo (R.A.U.)				$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$
Phenylhydrazin (per kilo grm.)				0.015	0.015	0.015		
Erythrocytes per cmm. (Millions)		7.0	7.4	5.9	4.5	3.8	1.3	1.1
Hemoglobin (Sahli)		80	80	65	55	35	25	25
Reticulocytes %		2	2	10	75	100	100	100
Leucocytes per ccm.		9,000	10,000	15,600	22,600	29,400	26,700	32,600
Percentage of white cells	Lymphocytes	52.0	54.0	32.0	50.0	37.0	30.0	31.0
	Polymorphonuclears	44.0	41.0	66.5	47.5	59.5	69.5	68.0
	Eosinophiles	1.0	0.5	0	1.0	0.0	+	0
	Mast cells	2.0	3.0	1.0	1.0	0.5	0	0
	Monocytes	1.0	1.5	0.5	0.5	2.0	0.5	1.0
	Leucocyte-index	0.9	0.8	2.0	0.9	1.6	2.3	2.2
Shilling-Arneth's nuclear shift	M	0	0	0	0	0	0	0
	J	0	0	0	0	0.5	1.0	2.0
	St	7.0	8.0	16.0	20.0	32.0	17.0	24.0
	II	32.0	33.0	40.5	47.5	47.0	53.0	41.5
	III	42.0	39.0	37.0	28.0	7.5	24.0	27.5
	IV	15.0	16.0	6.0	4.0	3.0	5.0	5.0
	V	4.0	4.0	0.5	0.5	0	0	0
	Index	0.1	0.1	0.2	0.3	0.5	0.2	0.4
Aniso-and poikilocytosis		—	—	+	++	+++	+++	+++
Polychromasia		+	+	+	++	+++	+++	+++
Punctata basophilia		—	—	+	++	++	++	++
Normoblasts per cmm.		—	—	+	5,200	4,600	3,900	5,700

poison, the total count was reduced to 4.5–5.4 millions instead of 3.8–4.0, and to 3.5–4.0 millions only after 3 consecutive injections. Anemia occurred later than in the control group. And if the 4th injection of the poison was not performed in the yakriton animal as the consequence of the death of the respective control animal following the 3rd injection, then red cells in young form began to appear and though anemia attained the highest degree numbering only 1.1–1.9 millions of red cells, no death would occur in the yakriton group. The restoration to the normal or almost normal state and blood picture came about on the 19th, 20th or 25th day after the first injection of the poison.

I b.

with phenylhydrazin and yakriton.

After the injection										
6	7	8	10	12	14	16	19	21	23	25
6. II	7. II	8. II	10. II	12. II	14. II	16. II	19. II	21. II	23. II	25. II
3,020	3,150	3,150	3,200	3,200	3,250	3,250	3,300	3,300	3,300	3,350
$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$
1.5	1.6	2.0	3.2	4.1	4.5	4.6	5.4	5.8	6.3	7.6
25	30	35	45	55	60	65	70	75	80	80
95	75	50	39	20	5	4	3	2	2	2
19,100	15,900	13,800	9,400	7,000	5,400	6,600	14,000	14,000	11,000	10,000
40	35.0	45.0	43.0	63.0	60.0	68.0	50.0	52.0	47.0	55.0
45.5	63.0	49.0	50.5	31.5	36.0	28.5	48.5	45.0	50.5	43.0
0.5	0.5	0.5	0.5	0.5	+	0.5	0.5	0.5	0.5	0.5
2.0	0.5	3.5	4.0	2.5	2.5	1.0	0.5	1.0	1.5	1.0
2.0	1.0	2.0	2.0	2.5	1.5	2.0	0.5	1.5	0.5	0.5
1.1	1.8	1.1	1.1	0.5	0.6	0.4	0.9	0.9	1.1	0.8
0	0	0	0	0	0	0	0	0	0	0
1.0	0	0.5	0	0	0	0	0	0	0	0
28.0	15.0	17.5	21.0	16.0	13.5	20.0	11.5	7.0	5.0	7.0
44.5	35.0	35.5	40.0	45.0	39.0	39.0	39.0	39.0	30.0	32.0
23.5	36.5	32.0	32.0	33.0	37.0	29.0	36.0	45.0	49.0	42.0
2.0	10.5	12.0	5.5	4.0	8.5	11.5	11.5	7.0	14.0	16.0
1.0	3.0	2.5	1.5	2.0	2.0	0.5	2.0	2.0	2.0	3.0
0.4	0.2	0.2	0.3	0.2	0.2	0.3	0.1	0.1	0.1	0.1
++	++	++	+	+	+	+	—	—	—	—
+++	+++	+++	+++	++	++	++	+	+	+	+
++	++	++	+	—	—	—	—	—	—	—
11,500	20,300	10,500	2,200	400	50	60	—	—	—	—

TABLE II a.

Control animal (No. 3.....No. 5053) with phenylhydrazin—control to No. 4.

Day of experiment		Before the injection		After the injection		
				1	2	3
Date		18. II	19. II	20. II	21. II	
Body weight (kilo)		2.500	2.560	2.500	2.550	
Phenylhydrazin (per kilo grm.)			0.015	0.015	0.015	
Erythrocytes per cmm. (Millions)		7.1	7.0	5.5	3.8	
Hemoglobin (Sahli)		80	80	60	45	
Reticulocytes %		2	2	13	80	
Leucocytes per cmm.		11,000	12,000	15,000	17,000	
Percentage of white cells	Lymphocytes	60.0	62.0	45.0	40.0	
	Polymorphonuclears	37.5	35.0	41.0	58.0	
	Eosinophiles	0.5	0.5	0.5	+	
	Mast cells	1.5	1.5	2.0	1.0	
	Monocytes	0.5	1.0	1.5	1.0	
	Leucocyte-Index	0.6	0.6	0.9	1.4	
Schilling-Arneth's nuclear shift	M	0	0	0	0	
	J	0	0	0	0	
	St	7.0	7.0	20.0	24.0	
	II	36.0	35.0	40.0	40.0	
	III	41.0	42.0	31.5	28.5	
	IV	14.0	13.0	6.5	7.5	
	V	2.0	3.0	2.0	0	
	Index	0.1	0.1	0.3	0.3	
Aniso-and poikilocytosis		—	—	+	++	
Polychromasia		+	+	++	+++	
Punctata basophilia		—	—	—	+	
Normoblasts per cmm.		—	—	100	350	

2. Hemoglobin. This was of 75–80 hemeteric value or reading (Sahli).

a. The control group. The value was 40–45 after 2 consecutive injections of the poison.

b. The yakriton group. The hemometeric value was 40–55 after 2 consecutive injections of the poison and 35–40 after 3 consecutive injections.

The smallest value occurred 2 or 3 days after suspending the in-

TABLE II b.
Yakriton animal (No. 4 No. 5054) with phenylhydrazin and yakriton.

Day of experiment	After the injection																		
	Before the injection	1	2	3	4	5	6	7	8	9	11	13	15	17	19				
Date	18. II	19. II	20. II	21. II	22. II	23. II	24. II	25. II	26. II	27. II	28. II	2. III	4. III	6. III	8. III	10. III			
Body weight (kilo)	2.450	2.500	2.550	2.570	2.500	2.500	2.500	2.440	2.350	2.340	2.300	2.340	2.300	2.350	2.350	2.400			
Yakriton per kilo (R.A.U.)			$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$				
Phenylhydrazin (per kilo grm.)			0.015	0.015	0.015														
Erythrocytes per cmm.	7.5	7.8	6.5	5.0	3.5	1.9	2.3	2.5	2.9	3.8	4.7	5.1	5.9	6.2	6.7	7.2			
Hemoglobin (S a h i)	80	80	60	50	40	25	35	45	50	55	60	65	70	75	80	80			
Reticulocytes %	2	2	13	25	100	100	100	80	70	36	20	10	5	5	2	2			
Leucocytes per cmm.	13,000	11,000	13,800	17,000	29,700	25,600	25,900	21,100	6,800	6,700	7,200	6,300	6,400	5,400	6,700	8,500			
Percentage of White cells	Lymphocytes	70.0	65.0	36.0	33.0	31.0	33.0	40.0	55.0	51.0	50.0	55.0	58.0	64.0	56.0	50.0			
	Polymorphonuclears	24.5	30.5	60.0	62.5	64.5	65.0	55.0	40.0	48.0	45.0	39.5	39.5	31.0	39.0	47.0			
	Eosinophiles	0.5	0.5	0	0.5	0.5	0.5	0.5	1.5	0	+	0.5	0.5	0.5	1.0	0.5			
	Mast cells	4.0	2.0	2.5	3.5	3.0	0	3.5	3.0	0	3.5	2.5	1.5	3.5	3.5	2.0			
	Monocytes	1.0	2.0	1.0	0.5	1.0	1.5	1.0	0.5	1.0	0.5	0.5	0.5	1.0	0.5	0.5			
Leucocyte-Index		0.4	0.5	1.7	1.9	2.1	1.9	1.4	0.7	0.9	0.9	0.8	0.7	0.5	0.7	0.9			
Abnormalities nuclear shift	M	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0			
	J	0	0	0	0	0	0	2.5	1.0	0	0	0	0	0	0	0			
	St	6.0	7.0	14.0	20.0	19.0	25.0	34.5	16.0	0	17.0	12.5	10.5	9.0	8.0	7.0			
	II	30.0	33.0	50.0	49.0	45.0	47.5	48.0	42.0	41.0	35.0	45.5	49.0	34.0	36.5	38.5			
	III	45.0	47.0	49.5	26.0	30.0	23.0	14.0	38.0	37.5	39.0	36.0	35.0	45.0	44.5	42.0			
Sclerobillings	IV	16.0	12.0	5.5	4.0	4.5	3.5	1.0	3.0	4.0	4.0	6.0	6.0	11.0	9.0	9.5			
	V	3.0	2.0	1.0	1.0	1.0	1.0	0	0.5	0	0.5	1.5	2.0	1.0	2.0	3.0			
	Index	0.1	0.1	0.2	0.3	0.2	0.3	0.7	0.3	0.2	0.2	0.1	0.1	0.1	0.1	0.1			
Aniso-and poikilocytosis	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
Polychromasia	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
Punctata basophilia	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
Normoblasts per cmm.	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+			

jection of the poison and was 20-25. The restoration to the normal value came about on the 17th or 23rd day of the first injection of the poison.

3. Reticulocytes. Before commencing the experiment the percentage of the cells was 2-3% of all the red cells in each animal.

a. The control group. The percentage was increased to 70-90% after 2 consecutive injections of the poison.

b. The yakriton group. The percentage was increased to 20, 25 or 75% respectively after 2 consecutive injections of the poison and became 100% after 3 consecutive injections. This percentage continued for three days and then was reduced to the normal percentage on the 15th, 17th, or 21st day of the first injection of the poison.

TABLE III a.

Control animal (No. 5.....No. 5055) with phenylhydrazin—control to No. 6.

Day of experiment		Before the injection		After the injection		
				1	2	3
Date		27. III	28. III	29. III	30. III	
Body weight (kilo)		2.500	2.570	2.500	2.250	
Phenylhydrazin (per kilo grm.)			0.015	0.015	0.015	
Erythrocytes per cmm. (Millions)		7.0	6.5	5.3	4.0	
Hemoglobin (Sahli)		80	80	60	40	
Reticulocytes %		2	2	25	70	
Leucocytes per cmm.		12,000	10,000	17,800	18,000	
Percentage of white cells	Lymphocytes	50.0	60.0	48.0	32.0	
	Polymorphonuclears	46.0	35.0	47.5	60.5	
	Eosinophiles	0.5	0.5	0	0.5	
	Mast cells	1.5	3.0	4.0	5.5	
	Monocytes	2.0	1.5	0.5	1.5	
	Leucocyte-Index	0.9	0.6	0.9	1.9	
Schilling-Arneth's nuclear shift	M	0	0	0	0	
	J	0	0	0	0	
	St	9.0	7.0	20.0	34.0	
	II	33.5	38.0	38.0	36.5	
	III	42.5	42.5	35.0	23.5	
	IV	13.0	10.5	5.0	5.0	
	V	2.0	2.0	2.0	1.0	
Index		0.1	0.1	0.3	0.6	
Aniso-and poikilocytosis		—	—	++	###	
Polychromasia		+	+	++	###	
Punctata basophilia		—	—	—	—	
Normoblasts per cmm.		—	—	—	200	

Of the three rabbits of this group the most weakened was the one that showed an increase up to 75%, while the other two, far less weakened, were of 20 or 25% of reticulocytes. From this fact a rapid, intense increase of reticulocytes may not be a good sign.

4. Normoblasts. No normoblasts were met with before the experiment.

a. The control group. After 2 consecutive injections of the poison, normoblasts appeared to the number of 3,300, 350 or 200 per cmm. After the first injection the cells occurred only in one of the three.

b. The yakriton group. After 2 consecutive injections of the poison normoblasts appeared to the number of 5,200, 400 or 80 in the yakriton group, and after 3 consecutive injections, the number was 4,600, 1,900 or 300 respectively. And the largest number of the cells was in each case 20,300, 10,300, or 6,500 respectively on the 7th or 6th day of the first injection of the poison.

The disappearance of the cells took place 11, 15 or 19 days after the first injection of the poison. And in this experiment the animal with a very early appearance of normoblasts in a large number was much more weakened than the other two were. Also the restoration to the normal state took place much later in this animal.

5. Polychromasia and basophilic punctate. In the control group these phenomena were more or less striking than in the yakriton group, and in the latter group they were at their highest when anemia attained the severest grade.

6. Anisocytosis, poikilocytosis and wrecked red cells. They appeared earlier and more intensely in the control group than in the yakriton group.

7. The total white cell count. It was 9,000–13,000 before the experiment.

a. The control group. The count was 17,000–21,200 after 2 consecutive injections of the poison.

b. The yakriton group.

Fig. 1. No. 3. Rabbit (Cf. Table II a)

P = phenylhydrazin

The count was 17,300–22,600 per cmm. after 2 consecutive injections of the poison, and 19,800–27,700 per cmm. after 3 consecutive injections. The most intense leucocytosis was seen on the 3rd or 5th day of the experiment and was of the count 19,800, 29,700 or 32,600 respectively. Thus before the 3rd day no difference of white cell pictures between the two groups was seen. The percentage distribution of different white cells did not show any difference between the two groups either.

8. Nuclear shift. This was slightly more striking in the control group than in the yakriton group, and in the latter group the shift was the most pronounced when anemia attained the highest degree.

Summary.

The present work was undertaken to test whether or not yakriton, —the detoxicating hormone of the liver, which does not contain the liver principle effectual in human pernicious anemia,—was effectual for experimental phenylhydrazin anemia in rabbits. Only *f*-classed animals were used in the present experiment.

The result was that the control group which received a daily subcutaneous injection of 1% phenylhydrazin in the amount of 1.5 c.c. per kilo of body weight succumbed on the third injection, while the yakriton group which received, besides phenylhydrazin, a daily injection of yakriton in the amount of $\frac{1}{10}$ R.A.U. per kilo of body

weight, was able to outlive the third injection of the poison and the ensuing severe anemia.

It has thus been shown, that yakriton, the detoxcating hormone of the liver, is effectual in phenylhydrazin poisoning or in the severe anemia caused by it.

Conclusion.

In *f*-classed rabbits, yakriton,—the detoxicating hormone of the liver, which does not contain that principle which is said to be effectual in human pernicious anemia—is effectual in rescuing animals from severe anemia due to phenylhydrazin poisoning.