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

76th Report.

Effect of Yakriton* upon Tuberculin from the
Hematological Point of View.

By

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It may be easily conceived that, if tuberculin is injected in normal rabbits, a definite blood picture will result. However, according to the contention of some authors, namely Tschistowitsch,¹⁾ Rieder,²⁾ Fauconnet³⁾ and Arneth,⁴⁾ a definite picture will not result. Now I tried to see if a definite change of normal blood picture, due to Koch's old tuberculin would occur or not, and what kind of influence yakriton would,—if a definite change in question were to be obtained at all,—exert upon the change.

Method of Experiment.

1. Animals for experiment. Normal rabbits were put to Sato and Sakurada's⁵⁾ liver function test (three times) and only *b*-classed (with low liver ability for ammonia-detoxicating) animals were used. The experiment was begun one week later than the last liver function test.

2. Koch's old tuberculin (Kitasato Institute For Infectious

* Throughout the work, emulsified yakriton was used.

1) N. Tschistowitsch, Berl. klin. Woch., 1891, **28**, 835.

2) H. Rieder, Beiträge zur Kenntnis der Leukocytose., Leipzig 1892, 153.

3) Ch. J. Fauconnet, Deutsch. Arch. f. klin. Med., 1905, **82**, 167.

4) J. Arneth, Die qualitative Blutlehre, Vol. I., Leipzig 1920, 321.

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

Diseases). This was subcutaneously used in the amount of 0.1 c.c. per kilo of body weight.

3. The administration of yakriton. Regarding the use of yakriton, it was subcutaneously injected in the amount of $\frac{1}{5}$ R.A.U. per kilo of body weight, 30 minutes prior to use of Koch's old tuberculin. The preparation contained 1. R.A.U. in 1 c.c.

4. Blood taking. Blood was taken before feeding, and the blood picture was followed in the 5th hour after the tuberculin injection.

5. Blood count method. Blood count was made by the use of Sato and Shoji's⁶⁾ counting chamber peroxidase method. Using this method, not only the total leucocyte count, but the relation between the lymphatic and the myeloid cells can be readily and simply found.

For the purpose of studying nuclear shift the Tohoku Pediatric Method⁷⁾ was used. Each time, 300 pseudoeosinophiles were counted and the different types of cells determined.

Result of Experiment.

A. In the case of Koch's old tuberculin injection (Cf. Table I).

1. Total white cell count. Up to the 1st or the 2nd hour after the injection, it showed a leucopenia. Then leucocytes increased up to the 5th hour, reaching the maximum in number in the 3rd or the 4th hour.

TABLE I.

Blood picture of b-classed rabbits on the subcutaneous injection of Koch's old tuberculin in the amount of 0.1 c.c. per kilo of body weight.

Nos. of rabbits, Body weight (in kilos)	Time of blood count	White cells*		Lymphatic cells*		Myeloid cells*		Nuclear shift (in percentage)				
		Total	(%)	%	(%)	%	(%)	I	II	III	IV	V
No. 1 (No. 4520) 2.47	Before Old tuberculin injection	8500	(85)	66	(56)	34	(29)	4.0	37.7	44.3	13.3	0.7
	After 1 hour	6300	(63)	57	(36)	43	(27)	5.0	41.0	42.3	10.7	1.0
	" 2 hours	7200	(72)	37	(27)	63	(45)	6.3	43.3	40.7	9.7	0.7
	" 3 "	9800	(98)	36	(36)	64	(62)	16.7	46.7	31.3	5.0	0.3
	" 4 "	10300*	(103)	35	(36)	65	(67)	16.3	49.3	31.7	2.3	0.3
	" 5 "	9200	(92)	24	(22)	76	(70)	13.0	53.0	30.7	3.3	0

6) A. Sato and Shoji, Journal of Laboratory and Clin. Med., 1928, 13, 1058.

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

Nos. of rabbits, Body weight (in kilos)	Time of blood count	White cells*		Lym-phatic cells*		Myeloid cells*		Nuclear shift (in percentage)				
		Total	(%)	%	(%)	%	(%)	I	II	III	IV	V
No. 2 (No. 4521) 2.20	Before Old tuberculin injection	7500	(75)	70	(53)	30	(22)	5.3	37.3	42.3	13.7	1.3
	After 1 hour	6500	(65)	50	(33)	50	(32)	7.7	50.3	31.3	10.0	0.7
	" 2 hours	6300	(63)	39	(24)	61	(39)	13.7	57.7	23.7	5.0	0
	" 3 "	10800	(108)	30	(33)	70	(75)	16.3	54.7	25.3	3.7	0
	" 4 "	9400	(94)	24	(23)	76	(71)	23.7	60.7	14.0	1.7	0
	" 5 "	9700	(97)	28	(28)	72	(69)	20.7	61.7	17.0	0.7	0
No. 3 (No. 4522) 2.62	Before Old tuberculin injection	7400	(74)	56	(42)	44	(32)	4.7	38.0	42.3	13.3	1.7
	After 1 hour	6500	(63)	43	(28)	57	(37)	9.7	50.0	32.0	9.0	0
	" 2 hours	10500	(105)	26	(26)	74	(79)	10.0	45.7	34.0	10.3	0
	" 3 "	9900	(99)	27	(27)	73	(72)	13.0	46.7	32.0	8.3	0
	" 4 "	9100	(91)	25	(23)	75	(68)	15.3	58.3	23.7	2.7	0
	" 5 "	11000	(110)	22	(24)	78	(86)	18.3	60.7	21.0	0	0
No. 4 (No. 4523) 1.85	Before Old tuberculin injection	12800	(128)	57	(73)	43	(55)	9.3	36.7	42.3	11.0	0.7
	After 1 hour	11200	(112)	40	(45)	60	(67)	19.7	55.7	20.7	4.0	0
	" 2 hours	14100	(141)	30	(43)	70	(98)	28.3	57.0	13.3	1.3	0
	" 3 "	17100	(171)	32	(55)	68	(116)	35.3	51.0	13.7	0	0
	" 4 "	19600	(196)	27	(53)	73	(143)	37.3	53.0	9.7	0	0
	" 5 "	13500	(135)	22	(30)	78	(105)	37.7	52.0	11.7	0.7	0
No. 5 (No. 4524) 2.00	Before Old tuberculin injection	8700	(87)	68	(59)	32	(28)	7.0	39.0	40.7	12.0	1.3
	After 1 hour	7300	(73)	52	(38)	48	(35)	8.7	46.7	34.7	9.7	0.3
	" 2 hours	7500	(75)	43	(33)	57	(42)	12.7	51.7	27.3	8.3	0
	" 3 "	10700	(107)	33	(35)	67	(72)	18.3	55.0	21.7	4.7	0.3
	" 4 "	11300	(113)	24	(28)	76	(85)	21.3	56.7	18.0	4.0	0
	" 5 "	11000	(110)	25	(28)	75	(82)	28.3	59.3	9.7	2.7	0

* Explanation to the Table.

White cells Total (%)		Lymphatic cells % (%)		Myeloid cells % (%)	
8500	(85)	66	(56)	34	(29)
8500 is the white cell count per cmm, and (85) or 85% means that a normal rabbit is arbitrarily assumed to have 10000 white cells in cmm.		66 or 66% means 66% out of 8500 cells. (56) or (56)% means $66 \times \frac{85}{100}\%$.		34 or 34% means 34% out of 8500 cells. (29) or 24% means $34 \times \frac{85}{100}\%$.	

2. Lymphatic cells. These elements showed a gradual decrease in number after the injection, and, if observed relatively to the myeloid elements, the decrease was especially marked, the maximum of the

Fig. 1. (a) No. 3. Blood picture in *b*-classed rabbit on tuberculin injection.

Fig. 1. (b) No. 2. Blood picture in *b*-classed rabbit on tuberculin injection.

the tuberculin injection, which was temporarily marked in the 2nd or the 3rd hour. Afterwards it became normal again, and then showed a slight leucocytosis in the 5th hour. In No. 8 rabbit (in Table II) no change occurred during the 5th hour.

2. Lymphatic cells. They showed gradually a lymphopenia after the tuberculin injection, reaching the maximum in number in the 5th hour. This decrease was not so remarkable as in the case of the use of tuberculin alone, with the result that in the cases of Nos. 7, 8 and 10 (in Table II) they showed almost no change or, if any

decrease falling on the 4th or the 5th hour.

3. Myeloid cells. They showed a remarkable increase in number, absolutely as well as relatively, after the injection, and attained the maximum in the 4th or the 5th hour.

4. Nuclear shift. The nuclear shift was very marked, the cells of Class I increasing in percentage about four or five times, in the 4th or the 5th hour after the injection. This shift was due to an increase of cells with rod-shaped nucleus, and of those of Type II, and, of course, to a decrease of those of Types III, IV and V.

B. In the case of yakriton (and tuberculin) administration (Cf. Table II.)

1. Total white cell count. It showed a slight leucocytosis after

change at all, only a slight increase in number in the 1st or the 2nd hour after the tuberculin injection.

3. Myeloid cells. They showed a myelocytosis, absolutely as

TABLE II.

*Blood picture of b-classed rabbits in case of yakriton administration
30 minutes before Koch's old tuberculin injection. Yakriton
used in the amount of $\frac{1}{3}$ R.A.U. per kilo of body weight.*

Nos. of rabbits, Body weight (in kilos)	Time of blood count	White cells* Total (%)	Lym-phatic cells* % (%)	Myeloid cells* % (%)	Nuclear shift (in percentage)				
					I	II	III	IV	V
No. 6 (No. 4525) 1.94	Before 30 minutes Yakriton injection	6400 (64)	77 (49)	23 (15)	6.7	36.7	44.3	10.0	2.3
	Before Old tuberculin injection	6000 (60)	75 (45)	25 (15)	8.0	40.7	41.0	8.7	1.7
	After 1 hour	6000 (60)	61 (33)	39 (27)	7.3	38.3	40.0	13.0	1.3
	" 2 hours	7700 (77)	52 (40)	48 (37)	7.3	46.7	36.7	8.3	1.0
	" 3 "	6300 (63)	42 (27)	58 (36)	8.0	43.3	36.0	11.7	1.0
	" 4 "	5900 (59)	33 (19)	67 (40)	12.0	50.0	33.0	5.0	0
	" 5 "	5800 (58)	36 (21)	64 (37)	10.0	46.7	36.0	6.7	0.7
No. 7 (No. 4526) 2.00	Before 30 minutes Yakriton injection	6800 (68)	76 (52)	24 (16)	5.3	40.0	44.7	10.0	0
	Before Old tuberculin injection	6900 (69)	69 (48)	31 (21)	9.7	46.3	37.7	6.7	0
	After 1 hour	8100 (81)	55 (44)	45 (37)	6.7	41.7	41.3	8.7	0.7
	" 2 hours	10700 (107)	53 (58)	47 (49)	11.3	48.3	30.3	10.0	0
	" 3 "	6800 (68)	54 (37)	46 (31)	9.3	40.0	39.7	9.0	2.0
	" 4 "	7300 (73)	40 (29)	60 (44)	8.3	36.3	36.7	15.3	1.3
	" 5 "	8400 (84)	38 (32)	62 (52)	6.7	39.0	41.7	11.0	1.7
No. 8 (No. 4527) 2.40	Before 30 minutes Yakriton injection	7900 (79)	65 (43)	35 (36)	9.3	40.7	38.3	10.7	1.0
	Before Old tuberculin injection	8000 (80)	65 (53)	33 (27)	11.7	43.3	34.7	9.7	0.7
	After 1 hour	8100 (81)	56 (45)	44 (36)	8.3	41.3	38.7	10.3	1.3
	" 2 hours	8000 (80)	59 (48)	41 (32)	9.0	39.7	39.0	10.0	2.3
	" 3 "	8500 (83)	40 (33)	60 (50)	5.7	35.7	42.3	13.3	3.0
	" 4 "	8300 (83)	38 (32)	62 (51)	9.3	39.0	41.3	8.3	2.0
	" 5 "	9600 (96)	36 (34)	64 (62)	8.3	45.3	37.7	8.3	0.3
No. 9 (No. 4528) 1.60	Before 30 minutes Yakriton injection	7100 (71)	76 (54)	24 (17)	7.0	41.3	39.0	11.7	1.0
	Before Old tuberculin injection	7800 (78)	72 (56)	28 (22)	10.0	46.0	36.0	7.3	0.7
	After 1 hour	8000 (80)	60 (48)	40 (32)	6.7	36.7	45.3	10.0	1.3
	" 2 hours	8200 (82)	60 (49)	40 (33)	6.7	38.3	38.3	14.3	2.3
	" 3 "	10400 (104)	52 (54)	48 (50)	9.3	38.7	40.7	10.7	0.7
	" 4 "	8500 (85)	50 (43)	50 (42)	8.0	42.7	41.0	8.3	0
	" 5 "	8000 (80)	45 (36)	55 (44)	10.0	41.7	39.0	9.3	0

Nos. of rabbits, Body weight (in kilos)	Time of blood count	White cells*		Lym-phatic cells*		Myeloid cells*		Nuclear shift (in percentage)				
		Total	(%)	%	(%)	%	(%)	I	II	III	IV	V
No. 10 (No. 4529) 1.67	Before 30 minutes	8300	(83)	78	(65)	22	(17)	11.7	43.0	38.7	6.7	0
	Yakriton injection											
	Before	9000	(90)	72	(65)	28	(25)	13.7	45.7	36.3	4.3	0
	Old tuberculin injection											
	After 1 hour	9300	(93)	69	(64)	31	(29)	13.0	45.0	36.7	5.3	0
	" 2 hours	9100	(91)	64	(58)	36	(33)	17.7	48.3	29.7	4.0	0.3
	" 3 "	12000	(120)	64	(78)	36	(42)	12.3	56.0	26.7	5.0	0
	" 4 "	8100	(81)	63	(51)	37	(30)	12.7	52.0	28.7	6.7	0
	" 5 "	11300	(113)	48	(54)	52	(59)	13.3	49.3	30.0	7.3	0

* Cf. Footnote to Table I.

well as relatively, after the tuberculin injection, reaching the maximum in the 5th hour, but this relation was not so remarkable as in the case of tuberculin injection alone.

4. Nuclear shift. Cells of Class I reached approximately twice the percentage in the 3rd or the 4th hour after the tuberculin injection. As to Nos. 8 and 9 rabbits (in Table II) cells of Class I did not increase, but decreased, and cells with Types IV and V increased in the 1st or the 2nd hour after the injection.

Summary.

A. If Koch's old tuberculin is subcutaneously injected in the amount of 0.1 c.c. per kilo of body weight into normal *b*-classed rabbits, a definite picture will be produced. The blood picture is described below:

1. Total white cell count. Up to the 1st or the 2nd hour a leucopenia; then a marked leucocytosis up to the 5th hour.

2. Lymphatic cells. Up to the 5th hour a marked lymphopenia, absolutely as well as relatively, which becomes marked still more as time passes on.

3. Myeloid cells. Up to the 5th hour a very marked myelocytosis, absolutely as well as relatively, which becomes marked still more with the passing of time.

4. Nuclear shift. Cells of Class I reach approximately four or five times the percentage in the 4th or the 5th hour after the injection.

B. If yakriton is subcutaneously injected in the amount of $\frac{1}{2}$ R.A.U. per kilo of body weight into normal *b*-classed rabbits, 30 min-

Fig. 2. (a) No. 9. Blood picture in *b*-classed rabbit after use of yakriton on tuberculin injection.

Fig. 2. (b) No. 8. Blood picture in *b*-classed rabbit after use of yakriton on tuberculin injection.

utes before Koch's old tuberculin injection (0.1 c.c. per kilo of body weight), the blood picture will be changed as below:

1. Total white cell count. Up to the 1st or the 2nd hour, normal or a slight leucocytosis. A temporarily marked leucocytosis in the 2nd or the 3rd hour, then up to the 5th hour normal, or, a slight leucocytosis again.

2. Lymphatic cells. Gradually a lymphopenia, reaching the maximum in the 5th hour. This decrease is not so pronounced as in the case of tuberculin injection alone. Up to the 1st or the 2nd hour no change, or, a slight increase in the absolute number.

3. Myeloid cells. Gradually a myelocytosis, absolutely as well as relatively, reaching the maximum in the 5th hour. This increase is not so marked as in the case of tuberculin injection alone.

4. Nuclear shift. Cells of Class I reached about twice the percentage in the 3rd or the 4th hour. Up to the 1st or the 2nd hour, cells of Class I decrease and those of Types IV and V increase.

Contrary to what the above mentioned authors state,^{1,2,3,4} I have obtained a definite change of blood picture resulting from the injection of old tuberculin. The reason is, I believe, very simple. I have used selectively only *b*-classed animals. It would have been interesting to

do the same experiment in *f*-classed ones too, but unfortunately I was unable to obtain a sufficient number of such rabbits during the present work.

Remarks.

The result above described (Tables I and II, Figs. 1 and 2) can best be shown diagrammatically in Fig. 3 (Cf. Fig. 3).

Fig. 3. Diagram showing the blood change after tuberculin (in continual line) and that after yakriton and tuberculin (in dotted line).

As to the question whether the nuclear shift is of a regenerative or a degenerative nature, it is of the combined form both in the case of tuberculin and in the case of yakriton and tuberculin. Only in the former case a more degenerative picture is seen.

Conclusions.

1. If Koch's old tuberculin is subcutaneously injected into *b*-classed rabbits in the amount of 0.1 c.c. per kilo of body weight, a definite blood picture is obtained.

2. If yakriton, the detoxicating hormone of the liver, is subcutaneously used into *b*-classed rabbits in the amount of $\frac{1}{2}$ R.A.U. per kilo of body weight, 30 minutes before Koch's old tuberculin injection, yakriton will counteract tuberculin, and endeavour to minimize the blood change which would occur if tuberculin were used alone.