Relation between Peroxidase Granula and Giemsa-stained Granula of Leucocytes after the Administration of Morphine Hydrochloride.

118th Report of the Peroxidase Reaction.

By

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Introduction.

In a preceding experiment¹⁾, we were able to produce a very peculiar blood picture in rabbits into which morphine had been injected in a considerable amount.

The picture was very similar to that produced in rabbits with cerebral hemispheres removed. All or most myeloid leucocytes in the streaming blood showed a very strong peroxidase reaction (of Sato and Sekiya)²⁾, while the oxidase reaction (of Winkler-Schultze)³⁾ did not show any perceptible change. We gave the name "Anencephalic Blood Picture" meaning "Atelencephalic Blood Picture", or "Contra-striatal Blood Picture" meaning the counter picture of the "Striatal Blood Picture (Sato 1924)."⁴⁾

Now in the case of rabbits with morphine administered, the Giemsa-stained films showed an increase of the Giemsa-stained granules in pseudoeosinophiles, so that the picture would induce one to think of the possibility that a relation might exist between the peroxidase granules and the Giemsa-stained granules of pseudoeosinophiles. The present paper treats of the problem.

1) A. Sato and S. Kuribayashi, Tohoku J. Exp. Med., 1939, **35**, 522.

2) A. Sato and S. Sekiya, Tohoku Igaku Zasshi, 1923, **6**, 534.

3) W. Schultze, Münch. med. Wehschr., 1909, **56**, 167; 1910, **57**, 2171.

4) A. Sato, Tohoku Igaku Zasshi, 1924, **8**, 79.

Method of Experiment.

1. The same animals were used (Nos. 4, 5, 6 and 7 in Tables 4, 5, 6; 7 and 8 in our preceding paper¹⁾).

2. Blood examination.

Blood was taken from an ear vessel before the injection of morphine hydrochloride, and 3, 6 and 24 hours after it. And then blood was examined every 24 hours till the 5th day of experiment in two cases (Nos. 4 and 5 in Table 1), and in the other two cases (Nos. 6 and 7 in Table 1) till the 10th day of experiment.

3. Method of blood examination.

Blood films were stained by use of the Tohoku Pediatric Method⁵⁾, with a slight modification: Solution B was applied for five minutes instead of 1 minute in the Tohoku Pediatric Method. Control films were stained with the Giemsa-stain.

Result of Experiment.

The percentage of the cells with strongly peroxidase-positive granules among 100 pseudoeosinophile leucocytes increased remarkably in number and reached the maximum 2 days after the first injection of morphine hydrochloride in all the cases (Nos. 4, 5, 6 and 7 in Table 1). It showed a gradual decrease and approached the initial on the 5th day of experiment in all the cases.

The percentage of the cells with Giemsa-stained granules among 100 pseudoeosinophile leucocytes increased in number 2 days after the first injection of morphine hydrochloride and then showed a gradual increase. It reached the maximum in 3 cases (Nos. 4, 5 and 7 in Table 1) on the 5th day of experiment, in the other one case (Nos. 6 in Table 1) on the 6th day. Then it showed a gradual decrease and approached the initial on the 10th day of experiment. As to the Giemsa picture Cf. Figure of the 28th Hemotological Paper* (Giemsa stain before and after morphine administration).

Comment.

As has been shown above, the percentage of the pseudoeosinophiles with strong peroxidase reaction, and that of those with the Giemsa-stained granules on blood films from the same rabbits were quite

* A. Sato and S. Kuribayashi, Tohoku J. Exp. Med., 1939, 35, 535.

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

TABLE 1.

Relation between the peroxidase granula and the giemsa-stained granula of leucocytes on subcutaneous of 1% solution of morphine hydrochloride.

Sex and class of rabbits	Body weight (in kilos)	Date of experiment	Time of blood count	Amount of 1% solution of morphine hydrochloride subcutaneously injected	Cells with strong peroxidase reaction among 100 pseudoeosinophile leucocytes	Cells with Giemsa-stained granules among 100 pseudoeosinophile leucocytes
♀ Rb (No. 4)	2.43	25. XII. 1937	9:20 (Before injection)	45 c.c.	3%	4%
		25. "	9:30 a.m.		7 "	6 "
		25. "	12:30 A.M. (3 hours after ")		17 "	6 "
	2.49	26. "	3:30 P.M. (6 hours after)	20 c.c.	33 "	17 "
		26. "	9:30 A.M. (1 day after)		52 "	28 "
	2.40	27. "	9:30 A.M. (2 days ")		18 "	30 "
	2.50	28. "	9:30 A.M. (3 " " ")		9 "	27 "
	2.50	29. "	9:30 A.M. (4 " " ")		4 "	35 "
	2.50	30. "	9:30 A.M. (4 " " ")			
	2.50	30. "	9:30 A.M. (4 " " ")			
♀ Rb (No. 5)	3.04	10. I. 1938	9:20 A.M. (Before injection)	50 c.c.	2 "	3 "
		10. "	9:30 A.M.		8 "	6 "
		10. "	12:30 A.M. (3 hours after)		16 "	12 "
	2.80	11. "	3:30 P.M. (6 " " ")	37 c.c.	27 "	18 "
		11. "	9:30 A.M. (1 day after)		70 "	20 "
	2.80	12. "	9:30 A.M. (2 days ")		18 "	32 "
	2.70	13. "	9:30 A.M. (3 " " ")		12 "	35 "
	2.73	14. "	9:30 A.M. (4 " " ")		3 "	40 "
	3.00	15. "	9:30 A.M. (5 " " ")			
	3.00	15. "	9:30 A.M. (5 " " ")			
♂ Rb (No. 6)	2.97	17. "	9:50 A.M. (Before injection)	50 c.c.	2 "	5 "
		17. "	10 A.M.		7 "	6 "
		17. "	1 P.M. (3 hours after)		9 "	7 "
	2.86	18. "	4 P.M. (6 " " ")	30 c.c.	20 "	21 "
		18. "	10 A.M. (1 day after)			

TABLE 1. Continued.

Sex and class of rabbits	Body weight (in kilos)	Date of experiment	Time of blood count	Amount of 1% solution of morphine hydrachloride subcutaneously injection	Cells with strong peroxidase reaction among 100 pseudoeosinophilic leucocytes	Cells with Giemsa-stained granules among 100 pseudoeosinophilic leucocytes
♂ Rb (No. 6)	2.88	19. I. 1938	10 A.M. (2 days after)		55%	38%
	2.84	20. "	10 A.M. (3 " ")		19 "	45 "
	2.85	21. "	10 A.M. (4 " ")		6 "	48 "
	2.87	22. "	10 A.M. (5 " ")		2 "	65 "
	2.77	23. "	10 A.M. (6 " ")		3 "	70 "
	2.85	24. "	10 A.M. (7 " ")		3 "	43 "
	2.93	25. "	10 A.M. (8 " ")		2 "	34 "
	2.90	26. "	10 A.M. (9 " ")		3 "	17 "
	2.90	27. "	10 A.M. (9 " ")		2 "	5 "
♂ Rb (No. 7)	2.67	25. "	9:20 A.M. (Before injection) 9:30 A.M.	40 c.c.	2 "	10 "
		25. "	12:30 P.M. (3 hours after)		5 "	13 "
		25. "	3:30 (6 " ")		9 "	16 "
	2.66	26. "	3:30 A.M. (1 day after)	30 c.c.	24 "	23 "
	2.66	27. "	9:30 A.M. (2 days ")		62 "	42 "
	2.76	28. "	9:30 A.M. (3 " ")		17 "	42 "
	2.74	29. "	9:30 A.M. (4 " ")		9 "	43 "
	2.74	30. "	9:30 A.M. (5 " ")		3 "	52 "
	2.70	31. "	9:30 A.M. (6 " ")		4 "	30 "
	2.75	1 II.	9:30 A.M. (7 " ")		2 "	28 "
	2.70	2. "	9:30 A.M. (8 " ")		2 "	17 "
	2.72	3. "	9:30 A.M. (9 " ")		2 "	11 "
	2.70	4. "	9:30 A.M. (10 " ")		0 "	11 "

TABLE 2.

Relation between the Giemsa-stained granula and the pathological granules on subcutaneous injection of 1% solution of morphine hydrochloride.

Sex and class of rabbits	Body weight (in kilos)	Date of experiment	Time of blood count	Amount of 1% solution of morphine hydrochloride subcutaneously injected	Pathological granules among 100 pseudo-eosinophiles leucocytes	Giemsa-stained granules among 100 pseudo-eosinophiles leucocytes
♂ Rb (No. 6)	2.97	17. I.	9:50 A.M. (Before injection)	50 c.c.	4%	5%
		17. "	10 a.m. 1 P.M. (3 hours after)		6 "	6 "
		17. "	4 P.M. (6 " ")		6 "	7 "
	2.86	18. "	10 A.M. (1 day after)	30 c.c.	14 "	21 "
	2.88	19. "	10 A.M. (2 days ")		25 "	38 "
	2.84	20. "	10 A.M. (3 " ")		39 "	45 "
	2.85	21. "	10 A.M. (4 " ")		45 "	48 "
	2.87	22. "	10 A.M. (5 " ")		50 "	65 "
	2.77	23. "	10 A.M. (6 " ")		60 "	70 "
	2.85	24. "	10 A.M. (7 " ")		50 "	43 "
	2.93	25. "	10 A.M. (8 " ")		40 "	34 "
	2.90	26. "	10 A.M. (9 " ")		16 "	17 "
	2.90	27. "	10 A.M. (9 " ")		4 "	5 "
♂ Rb (No. 7)	2.67	25. I.	9:20 A.M. (Before injection)	40 c.c.	5 "	10 "
		25. "	9:30 A.M. 12:30 P.M. (3 hours after)		8 "	13 "
		25. "	3:30 P.M. (6 " ")		10 "	16 "
	2.66	26. "	9:30 A.M. (1 day after)	30 c.c.	18 "	23 "
	2.66	27. "	9:30 A.M. (2 days ")		38 "	42 "
	2.76	28. "	9:30 A.M. (3 " ")		38 "	42 "
	2.74	29. "	9:30 A.M. (4 " ")		42 "	43 "
	2.74	30. "	9:30 A.M. (5 " ")		50 "	52 "

TABLE 2.* Continued.

Sex and class of rabbits	Body weight (in kilos)	Date of experiment	Time of blood count	Amount of 1% solution of morphine hydrochloride subcutaneously injected	Pathological granules among 100 pseudoeosinophiles leucocytes	Giemsa-stained granules among 100 pseudoeosinophiles leucocytes
♂ Rb (No. 7)	2.70	31. I.	9:30 A.M. (6 days after)		30%	30%
	2.75	1. II.	9:30 A.M. (7 " ")		26 "	28 "
	2.70	2. "	9:30 A.M. " " (8 " ")		15 "	17 "
	2.72	3. "	9:30 A.M. " " (9 " ")		14 "	11 "
	2.70	4. "	9:30 A.M. " " (10 " ")		10 "	11 "

different. On the 5th or 6th day of the morphine injection, on which the changed peroxidase picture regained the normal again, the Giemsa-stained granules showed the maximum, attaining the normal picture again on the 10th day of experiment only.

From this difference of the percentage and of the course of the change, it will be understood easily that the peroxidase-granules were quite different from the Giemsa-stained granules. But why did the latter increase? It is probable that, on the administration of morphine in such a large amount, pathological granules might show a considerable increase. So we stained the films according to the prescription of Mommsen⁶⁾, using the buffer solution. And the result is as shown in Table 2.

The percentage of the pseudoeosinophiles with Giemsa-stained granula and that of those with the pathological granula were about equal (Cf. Table 2). Besides, the red cell count was remarkably reduced on the 5th or 6th day of experiment when the cells with the Giemsa-stained granules showed the maximum percentage, and nucleated red cells occurred in a considerable number, presenting thus a toxic anemia. It is highly probable that the increase of the Giemsa-stained granules was that of pathological granules due to an administration of morphine in a large amount.

6) H. Mommsen, *Jahrb. f. Kinderheilk.*, 1927, **116**, 293.

Summary.

The percentage of cells with strongly peroxidase positive granula among pseudoeosinophile leucocytes increased remarkably and reached the maximum 2 days after the first administration of morphine. Then it showed a decrease and approached the initial on the 5th day of experiment. The percentage of the cells with Giemsa-stained granula among pseudoeosinophile leucocytes increased in number and reached the maximum 5-6 days after the first injection of morphine hydrochloride. It decreased and approached the initial on the 10th day of experiment.

Conclusion.

After an administration of morphine in a considerable amount, the myeloid leucocytes in the streaming blood will show a very strong peroxidase reaction (of Sato and Sekiya)²⁾ quantitatively as well as qualitatively, while the oxidase reaction (of Winkler-Schultze)³⁾ will remain as normally strong as ever. The increase of peroxidase granules, however, have nothing to do with the increase of the Giemsa-stained granules. The former is the result of effect of morphine on cerebral hemispheres, while the latter is the manifestation of effect of the alkaloid on the organism.