Blood Histamine, Leukocytes and Platelets in Experimental Serum Disease in Rabbits. (17461)

CAROLYN FORMAN, ELIZABETH MERTENS, MILTON GRAUB AND WILLIAM EHRICH

From the Philadelphia General Hospital and the Graduate School of Medicine of the University of Pennsylvania, Philadelphia, Pa.

The role of histamine in allergic diseases is poorly understood. During anaphylactic shock the blood histamine level has been found to be markedly elevated in dogs and guinea pigs immediately following the reinjection of antigen.[1-5] It is believed that this elevation can account for practically all dominant symptoms of this condition except the incoagulability of the blood which is explained by outpouring of heparin.[5] However, in rabbits, horses, and calves the blood histamine level has been shown to be depressed.[5,6] In rabbits this deviation can plausibly be explained by liberation of histamine from the buffy coat which in these animals contains 200 γ of histamine per 100 cc of blood as compared with 2 to 4 γ in dogs and 12 to 25 γ in guinea pigs.[5] The depression of the blood histamine in horses and calves is difficult to explain on this basis for these animals contain only 2 to 4 γ of histamine per 100 cc of blood.

The role of histamine in serum disease has been studied by several investigators. Dammin and Bukantz[7] were unable to produce the vascular lesions of this disease in normal rabbits by giving them daily intravenous injections of 0.1 mg of histamine base per kg of body weight for periods varying from 1 to 62 days. Injecting histamine into the rabbit's skin seldom caused erythema or whealing, and the site of injection, unlike that of an Arthus phenomenon, generally did not fluoresce following the intravenous injection of fluorescein. Moreover, in rabbits injected with horse serum, neohetramine given subcutaneously in doses of 10 mg per kg twice daily did not affect the appearance of circulating antibodies, the Arthus reaction of the skin or the development of arteritis.[7] Benadryl in doses of 5 mg per kg every 8 hrs was likewise ineffective.[8]

However, the latter drug in doses of 2 or 10 mg twice daily, and drug 1627, another antihistaminic agent, have been reported to impede the development of the myocardial lesions of serum disease,[9] and benadryl in doses of 5 mg per kg every 8 hrs apparently offered some protection against the production of lesions in the valves.[8] These results have not been duplicated.[7,8] Antistine has been found to prevent experimental glomerulonephritis in rabbits produced by anti-kidney serum,[10] but the glomerulitis of experimental serum disease was not affected by antihistaminics.[11]

In view of these various findings it seemed of interest to study the blood histamine level in experimental serum disease, and as the histamine of the blood is known to be contained in the buffy coat, to investigate the leukocytes and platelets as well.

Material and Methods. All experiments were performed in Chinchilla rabbits averaging 1900 g in weight. Twenty-five rabbits were given 15 cc per kg of "normal horse serum" (Squibb) containing 1:10000 sodium ethyl mercuri thiosalicylate plus 0.2% phenol. The remaining 15 rabbits received horse

1 Dragstedt, C. A., and Mead, F. B., *J. Immun.*, 1936, **30**, 319.

2 Dragstedt, C. A., and Mead, F. B., *J. Pharm. and Exp. Therap.*, 1936, **57**, 419.

3 Simon, A., and Staub, A. M., *Compt. rend. Soc. de biol.*, 1937, **125**, 815.

4 Code, C. F., *Am. J. Physiol.*, 1939, **127**, 78.

5 Dragstedt, C. A., *Physiol. Rev.*, 1941, **21**, 563.

6 Code, C. F., and Hester, H. R., *Am. J. Physiol.*, 1939, **127**, 71.

7 Dammin, G. J., and Bukantz, S. C., *J. Am. Med. Assn.*, 1949, **139**, 358.

8 Roberts, R. C., Crockett, K. A., and Laipply, T. C., *Arch. Int. Med.*, 1949, **83**, 48.

9 Kyser, F. A., McCarter, J. C., and Stengle, J., *J. Lab. and Clin. Med.*, 1947, **32**, 379.

10 Reubi, F., *Helvet. Med. Acta*, Suppl. 18, 1946.

11 Janeway, C. A., oral communication, 1949.

serum without preservative (Wyeth).* Seventeen days later the surviving animals were desensitized with 1 cc of serum intravenously and 2 days thereafter they were reinjected with another 15 cc per kg. Twenty-four rabbits received serum only, 16 were given 20 mg of neohetramine in a 4% solution subcutaneously 4 times daily (at 9 AM and at 1, 5 and 9 PM). The injections of the drug were discontinued 3 days after reinjection of the serum, *i.e.*, 4 days before the last 4 rabbits receiving the drug were sacrificed. Histamine was determined according to Code's method.[12] The blood was collected from the heart in quantities of 10 cc. In order to avoid a possible change in the blood histamine level by repeated bleeding, blood was drawn as a rule only once or twice from the same animal except in a last series of 3 rabbits which were bled 5 times each. The dates of collection are indicated in Fig. 1. Leukocyte and platelet counts were first done on certain days only, but in our last series of 3 rabbits 12 determinations were made in each animal. All counts were made shortly before blood was collected for histamine determination. They were made on blood obtained by nicking the marginal vein of the ear with a sharp razor blade. Groups of 4 animals were sacrificed and studied microscopically 3, 4, 5, 7, 14 and 16 days after the first injection of serum, and 2, 6 and 7 days after the second.

Results. The Arthus reaction of the skin elicited by intradermal injection of 0.1 cc of horse serum was, as in the experiments of Dammin and Bukantz,[7] not appreciably changed by neohetramine in 20 rabbits tested. Analysis of the urine at the time of sacrifice showed no significant difference between the rabbits receiving neohetramine and the controls during the later stages of the experiment. However, the proteinuria which occurred during the week following the first injection of

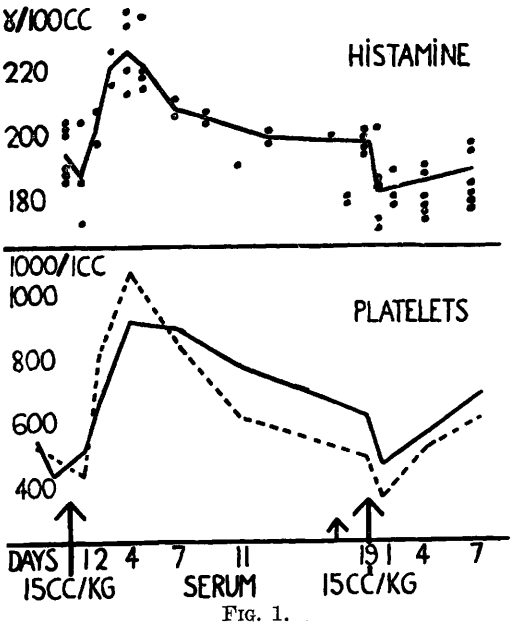

FIG. 1.

Histamine and platelet levels in serum disease. The large arrows indicate the first and second injection of 15 cc of horse serum per kg, the small arrow the injection of the desensitizing dose of 1 cc.

horse serum was abolished by the antihistaminic (Table I). The histamine level of the blood before injection averaged 194 γ per 100 cc in 6 rabbits tested. After the intravenous injection of 15 cc of horse serum per kg the level rose to an average of 226 γ on the 4th day and then gradually returned to normal (Fig. 1). Reinjection of serum on the 19th day was followed by a sharp drop in histamine to an average of 182 γ on the 1st day. There was no elevation of this material during the first 7 days after the second injection.

The rabbits which received neohetramine revealed an average blood histamine level of

TABLE I.

Protein Content of Urine Following the First Injection of Serum.

Days after inj.	Without Neohetramine	With Neohetramine
4	+	0
	+ −+	0
5	+	0
	+	0
7	(+)	(+)
	(+)	0
14	(+)	0
	0	0

* We are indebted to Dr. J. Seifter of the Wyeth Institute of Applied Biochemistry for supplying us with the neohetramine solution, and Dr. B. Scott Fritz and Dr. J. H. Brown of the Wyeth Incorporated Biological Laboratory at Marietta, Pa., for giving us the horse serum.

[12] Code, C. F., *J. Physiol.*, 1937, **89**, 257.

TABLE II.
Leukocyte and Platelet Levels Following the First and Second Injection of Serum in Rabbits No. 1, 2, 3.

Days after inj. Rabbit No.	Neutrophiles			Lymphoid cells			Eosinophiles			Basophiles			Platelets (thousands)		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
						Before inj.									
	1622	2967	2342	4400	3349	4314	31	0	0	187	64	136	543	524	756
	1228	1872	2630	4182	4017	4558	30	30	37	140	121	185	427	499	685
						After first inj.									
1	1408	988	2442	4847	2660	4514	131	95	74	164	57	370	511	428	533
2	4493	1084	1588	7128	3729	4027	60	25	0	300	202	265	660	817	475
4	3640	6837	3220	3100	4596	3187	0	0	0	0	57	133	908	1068	701
7	2784	3559	4232	5193	5703	3976	42	49	0	291	439	342	898	850	936
11	3353	2550	5408	3724	3858	7452	112	0	0	261	302	330	774	610	670
19	5490*	1404	1350	5270*	3591	7850	0	27	0	330	378	800	613	483	1064
						After second inj.									
1	4085*	1564	7709	2074	1717	2399	31	17	0	0	102	102	458	357	727
4	1952	1748	2138	8991	7343	3680	59	50	0	828	849	1192	562	524	669
7	1639	2275	2984	6423	4302	3581	89	71	37	709	462	858	682	609	790

* This elevation is accounted for by a retro-aural abscess which developed in this rabbit.

73 γ per 100 cc (42 to 102) from the 3rd to the 17th day after the first injection, and 72 γ after the second. The blood for these determinations was drawn mostly 4 hrs after the last injection of neohetramine.

The results of the leukocyte and platelet counts in the 3 most completely studied rabbits are given in Table II and Fig. 1. It is apparent that the first injection of serum caused first a rise in eosinophiles (on the 1st day) which was followed by a rise in neutrophiles (between the 2nd and 4th day) and lymphoid cells (on the 4th day) whereas the basophiles were not much changed. While 2 of the rabbits behaved much alike with the exception of a high neutrophile count on the 19th and 20th day in Rabbit 1 (caused by a retro-aural abscess), and while most of our counts in other rabbits fell well within the range of the counts of these 2 animals, Rabbit 3 behaved differently showing a peak in neutrophiles on the 11th day and one in lymphoid cells between the 11th and 19th day. At autopsy this animal revealed the most severe arteritis of all animals of this series; it also showed a marked gomerulitis though this was less severe than that in Rabbit 2.

The reinjection of serum on the 19th day caused a marked drop in lymphoid cells and basophiles one day after injection. This was followed by a marked rise of the 2 with a peak on the 4th day. The average rise in lymphocytes was 178% (161% over the normal control counts), that in basophiles 1300% (590% over the normal control counts). The neutrophiles showed no consistent change.

The platelets were markedly elevated following the first injection of serum with a peak on the 4th day; they showed a marked drop one day after the second injection and thereafter returned to normal (Fig. 1). Rabbit 3 differed again from this course, whereas most of our counts in other rabbits fell well within the range of counts of Rabbits 1 and 2. The course of the platelets closely paralleled that of histamine.

The important morphological changes in our rabbits are given in Table III. It can be seen that the rabbits which were treated with serum containing preservative showed more pulmonary involvement and less myocarditis, glomerulitis and arteritis than those receiving serum without preservative. This difference may have been due to precipitation and therefore increased retention in the pulmonary vessels of the preservative containing serum, for sections of these lungs showed numerous emboli blocking arterioles and capillaries of the lungs, whereas those of animals receiving serum without preservative did not. The chief difference in the reactions toward the

TABLE III.
Frequency (a)* and Intensity (b)† of the Various Lesions Caused by Serum.

No. of inj. of serum	Neohetramine	No. of rabbits	Mesenchymal myocardium a.	Mesenchymal myocardium b.	Reactions, lungs a.	Reactions, lungs b.	Glomerulitis a.	Glomerulitis b.	Valvulitis a.	Valvulitis b.	Arteritis a.	Arteritis b.
			Serum with preservative									
1	with	8	62.5	52.5	100	48.5	0	0	0	0	0	0
	without	8	50	52.5	100	83	12.5	17	0	0	0	0
2	with	3	100	57.5	100	100	33.3	33	66.7	25	0	0
	without	3	100	62.5	100	92	33.3	17	50	100	33.3	50
	" ‡	6	100	75	100	71	66.7	21	40	83	16.7	50
			Serum without preservative									
1	with	3	100	21	66.7	31	0	0	0	0	0	0
	without	4	100	44	100	34.5	25	17	0	0	0	0
2	with	2	100	75	100	50	100	54	100	83	50	50
	without	2	100	31	100	56	50	50	—	—	50	75
	" ‡	6	100	39.5	100	71	83.3	67	40	67	50	67

* The frequency has been expressed in per cent of animals affected in order to facilitate comparison.
† The intensity has been expressed in per cent of the maximum intensity observed. The figures were calculated from the positive cases only.
‡ These animals were members of separate groups not receiving neohetramine.

first and second injection of serum was a greater frequency and intensity particularly of glomerulitis, valvulitis and arteritis following the latter. In fact, valvulitis and arteritis occurred in these experiments only after the second injection. The mesenchymal reactions in lungs and myocardium were more severe, too, though less strikingly so, as may be expected if our contention is correct that these reactions are chiefly primary reactions instrumental in the production of antibody, whereas glomerulitis and arteritis are chiefly allergic in nature, *i.e.*, the result of an antigen-antibody reaction.[14,15]

Neohetramine was found to depress the mesenchymal reactions in lungs and myocardium. This effect was most noticeable in the organs whose mesenchyme reacted most severely to the serum, *i.e.*, in the lungs, in the rabbits treated with serum containing preservative, and in the lungs and myocardium, in the animals which were given serum without preservative. Also the 2 cases of glomerulitis observed after the first injection of serum occurred both in animals without neohetramine. Following the 2nd injection no significant difference was observed between the two series of experiments. However, the antihistaminic drug was discontinued 4 days before the last animals were sacrificed.

Discussion. The first intravenous injection of a large dose of horse serum in our rabbits caused a rise in histamine and platelets, the peak occurring on the 4th day. The reinjection of the same quantity of the same serum 19 days later was followed by a marked drop of the two elements during the first day, and there was no rise above normal during the remaining 6 days of the experiments. The rise in platelets after the first injection resembled that following various infections, the exposure to ultraviolet light, and other injuries,[16] while the drop in histamine after reinjection was in accord with the observed fall of histamine in anaphylactic shock in rabbits.[5] The rise in platelets following the first injection and the absence of a rise after reinjection furnish an explanation for the recent observation of increased blood coagulability during the first week following the first injection of serum and its absence after the second.[17] The close parallel between histamine and platelets in our rabbits (Fig. 1) is in keeping with the demonstration by Minard[18] and others that in rabbits most of the blood histamine is contained

14 Ehrich, W. E., Seifter, J., and Forman, C., *J. Exp. Med.*, 1949, **89**, 23.

15 Forman, C., Seifter, J., and Ehrich, W. E., *J. Allergy*, 1949, **20**, 273.

16 Gunn, F. D., *Arch. Path.*, 1931, **12**, 153.

17 Moore, R. C., and Waugh, D., *J. Exp. Med.*, 1949, **89**, 541.

in the platelets.

It is obvious that the rise in histamine and platelets following the first injection of serum cannot be regarded as an allergic phenomenon; it is apparently part of the primary reaction of the organism against noxious agents in general. During the period of histamine and platelet elevation following the first injection of serum all rabbits tested which received serum only contained protein in the urine, whereas, those which were given neohetramine did not. Though one may be tempted to explain this as a specific antihistaminic effect, there is reason to believe that this was due to a non-specific action of the drug upon the permeability of the capillaries possibly via the hyaluronidase-hyaluronic acid mechanism; for it has been found that antihistaminic drugs decrease the permeability of tissue membranes *in vitro*[19] as well as that of capillaries *in vivo*,[20,21] and it has also been observed that hyaluronidase enhances the leakage of protein through the glomeruli in serum disease in rabbits.[22] However this may be, microscopic examination of the kidneys showed that the proteinuria in our animals was not caused by proliferative glomerulitis, but it was associated with degenerative changes such as swelling of the loops and pyknosis of the nuclei as described and illustrated in a previous paper.[14]

The mesenchymal reactions in the lungs following the first injection of serum were distinctly, and those of the myocardium slightly depressed by neohetramine. The latter observation is in accord with the findings of Kyser and coworkers.[9] The depressing effect of neohetramine, if real, is opposite to that of hyaluronidase[22] and therefore may well be explained by a non-specific action of this drug on the permeability of tissue membranes causing diminished penetration of lungs and myocardium by antigen. The development of arteritis, glomerulitis and valvulitis following reinjection, on the other hand, was not affected. These observations are in keeping with our view that the mesenchymal reactions in lungs and myocardium are direct reactions to the antigen instrumental in the production of antibody, whereas arteritis and glomerulitis are the result of an antigen-antibody reaction.[14,15]

Of the changes of the leucocytes, the marked rise in basophiles following the second injection of serum is of particular interest. As the reinjection is followed by outpouring of heparin,[5] and there is reason to believe that the basophiles are cellular sources of heparin,[23] it suggests itself that the rise in basophiles may be a regenerative phenomenon prompted by the heparin consuming effect of the anaphylactic reaction.

Conclusions. It appears that in rabbits histamine is linked via the histamine containing platelets with blood coagulation, and this is connected via heparin with the heparin producing basophiles. All these elements are involved in serum disease. The first injection of serum is followed by a rise in histamine and platelets, the second by a drop in these elements. The destruction of the white cell layer following reinjection calls forth an outpouring of heparin which in turn is followed by basophilia in the blood. Only the changes following reinjection can be regarded as allergic. Neohetramine was found to effect only the primary reactions following the first administration of serum. It suppressed the rise in blood histamine and the proteinuria observed during the first week following the first injection, and also impeded the primary mesenchymal reactions in lungs and heart which are believed to be instrumental in antibody formation. There are reasons to believe that this was not a specific antihistaminic effect, but that it was due to a non-specific action of the drug upon the permeability of tissue membranes possibly via the hyaluronidase-hyaluronic acid mechanism.

18 Minard, D., *Am. J. Physiol.*, 1941, **132**, 327.

19 Seifter, J., Baeder, D. H., and Dervinis, A., PROC. SOC. EXP. BIOL. AND MED., 1949, **72**, 136.

20 McGavack, T. H., Elias, H., and Boyd, L. J., *Am. J. Med. Sc.*, 1947, **213**, 418.

21 Leger, J., and Masson, G., *ibid.*, 1947, **214**, 305.

22 Seifter, J., and Ehrich, W. E., to be published.

23 Jorpes, J. E., Heparin, Oxford University Press, 1946.

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