Eosinophiles in Passive Anaphylaxis.* (22020)

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It has long been recognized that blood and tissue eosinophilia is a common accompaniment of antigen-antibody activity(1,2,3). The mechanism responsible for the eosinophilia, however, has not been clear. To determine whether a state of active antibody production is a prerequisite for its development the following experiments on passive anaphylaxis were undertaken.

Materials and Methods. Three times crystallized egg albumin (Ea) was used as antigen. The antiserum was from a rabbit ($R194_{4+5}$, ref. 4), which had been injected with a total dose of 40 mg of antigen over a 2 months' period. This antiserum had a precipitable anti-Ea N content at the time of use of 308 μg per ml. Animals for sensitization and for controls were 250 to 325 g guinea pigs. Injections were made into leg veins. Sensitization was accomplished with 23 μg antibody N given intravenously 48 hours prior to the shocking dose of antigen (6 μg Ea N). Blood counts were done on peripheral blood, use being made of ear, leg, and foot veins for this purpose. To prevent coagulation of the blood, it was necessary to wipe the cleaned skin surface and puncture needle (#23 hypodermic needle) with a piece of cotton previously dampened with heparin. White cell counts were performed in a Spencer bright-line counting chamber. Bloods for the total leukocyte counts were diluted 20-fold in a white cell pipette with a solution of acetic acid and methylene blue. Eosinophile counts were done on 10-fold diluted blood in a freshly prepared solution of Phloxin in 0.1% propylene glycol. Both sides of the counting chamber were filled in each instance, four large squares on each side being used in enumerating the total white count and the entire ruled area being used in enumerating the absolute eosinophile count.

TABLE I. Absolute Eosinophile Counts after 6 μg Egg Albumin Nitrogen Intravenously in 9 Sensitized and 7 Non-Sensitized Guinea Pigs.

	Time in hr							
	0	¼–½	1	2	4	6	12	24
	Sensitized with 23 μg anti Ea N							
	201		195	294	165	243		
	33	51	198	165		357		
	66	171		120	141	207		
	30		81	360	240	204		204
	33		6	255	90	123		162
	33	15	99	381	174	147	78	
	12	87	315	135	105	126		
	27	21	117	99	33	21		
	24	57	111	174	288	21		
Mean ± S.E.	51 ± 19	67 ± 24	140 ± 34	220 ± 35	155 ± 29	161 ± 36		
	Non-sensitized controls							
	18	3	24	18	36	21		9
	36	30	27	36	45	36		9
	30	21		30	36	39		30
	30	6	174	36	42	72	27	12
	45	15	66	15	21	9	18	9
	30	15	15	27	18	21		
	3	9	18	24	12	24		
Mean ± S.E.	27 ± 5.1	14 ± 3.4	54 ± 25	29 ± 3.0	30 ± 4.9	32 ± 7.7		
P	>.2	.04	.08	<.01	<.01	<.01		

* Supported by a grant from the A. D. Williams Fund of the Medical College of Virginia.

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TABLE II. Total Leukocyte Counts after 6 μg Egg Albumin Nitrogen Intravenously in 9 Sensitized and 7 Non-Sensitized Guinea Pigs.

	Time in hr							
	0	¼–½	1	2	4	6	12	24
				Animals sensitized with 23 μg anti Ea N				
	9400		9150	13400	9400	9020		
	2150	8400	10800	11100		7600		
		18850		9050	9050	9400		
	6100			14400	6200	7900		8800
	9800			9750	5350	10400		11900
	9750	12200	15750	7950	3100	9650	9250	
	9950	11300	11050	10950	8400	6600		
	14400	9550	8300	12400	4650	5050		
	7150	8500	6850	9800	11100	11300		
Avg	8600	11500	10300	11000	7160	8550		
				Non-sensitized controls				
	5200	10200	8000	11300	8600	16200		5750
	12900	13100	9500	10100	10000	13700		11400
	4900	4450		8900	7200	9150		6300
	6150	5200	6700	5250	6000	3950	7600	5900
	16200	8850	8100	9750	5900	5150	10250	11750
	22850	11200	16450	22050	21000	21850		
	7550	4600	15450	5600	850	4600		
Avg	10550	8230	10600	10400	8510	10700		

Results. Table I and Fig. 1 present the eosinophile counts and Table II presents the total white cell counts in the animals studied. It is clear that there was a significant rise of the eosinophiles in sensitized animals injected with 6 μg of Ea N. This eosinophilia occurred independently of any corresponding rise in the total number of white cells and was maintained for 24 hr in 2 animals examined at this interval. In spite of some variability in the serial counts in these animals, probably due to technical difficulties, the eosinophile differences observed at 2, 4 and 6 hours were statistically highly significant.

It was of interest to note in several instances in a preliminary part of this study moderate eosinophile responses in normal, non-sensitized animals after large (160 μg) doses of Ea N (Table III). This effect was not seen in normal animals at the smaller doses of Ea used later in the anaphylaxis experiments (Table I).

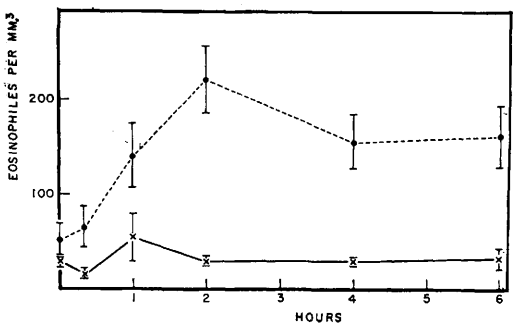

FIG. 1. Mean values and stand. errors of eosinophile responses of sensitized (– – – –) and normal (———) guinea pigs to 6 μg Ea N.

Discussion. That eosinophilia may occur in anaphylaxis independently of active antibody formation is established.‡ This suggests that the stimulus for the eosinophilia is to be found in a product released by the antigen-antibody reaction, or in one of the enzymes activated by the reaction(6). Particularly suggested is histamine itself, which has been reported to produce an eosinophilia in the guinea pig(2,7).

The finding of an eosinophilia in unsensitized guinea pigs after the larger Ea dose recalls the reports by Vaughn(7,8) that eosinophilia may be produced in normal guinea pigs by the injection of ascaris polysaccharide. However, the proper interpreta-

‡ After the conclusion of this study, we became aware of a comment by Campbell (Discussion, reference 5) that he had observed eosinophilia in passive, as well as in active anaphylaxis. The present paper, therefore, confirms and extends his earlier finding.

TABLE III. Blood Eosinophile Response to Injection of Large (160 μg) Doses of Egg Albumin Nitrogen in 4 Normal Guinea Pigs.

Route	Time in hr 0	¼–½	1	2	5	6
I.V.	56	53		175	175	259
"	118	109		68	156	237
I.P.	188	255	350	693	394	160
S.C.	55	33	49	222	316	38

tion of this finding is not clear. The claim (8) that the polysaccharide, independent of immune reaction to it, is primarily toxic and a stimulus to histamine release is not fully supported by the evidence, though this interpretation is certainly possible. Numerous instances have been described both in man and in the experimental animal, wherein antibody to various antigens may be "naturally" acquired. In man, well-documented instances would include the "natural" isoagglutinins and "normal" antidextrans(9). It would be difficult to prove that such "natural" antibodies did not play a role in the above instances. Further, since relatively large doses of antigen have been required for the production of eosinophilia in normal animals the possibility exists that such antibodies might be antibodies to antigen impurity(4). Therefore the experiments in which eosinophilia has been observed in normal animals may need reinterpretation, and particularly is this indicated in those cases(8) in which the animals were noted to die in convulsions, or with dyspnea, after the eosinophilia-producing injections. Such symptoms were not seen in the animals reported in Table III.

Summary and conclusions. It has been shown that eosinophilia in anaphylaxis is not dependent upon active antibody formation by the shocked animal. The development in several instances of a mild eosinophilia following the injection of relatively large doses of Ea into normal guinea pigs was noted.

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Received August 5, 1955. P.S.E.B.M., 1955, v90.

Role of Hyperkalemia in Production of Ventricular Fibrillation Following Hypercapnia.* (22021)

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Rapid return to air breathing following 2 to 4-hour periods of high CO_2 breathing regularly produces hypotension and cardiac arrhythmias in dogs, and may produce ventricular fibrillation and death(1). Recently Sealy, Young, and Harris(2,3) have confirmed these results and have extended them in an attempt to determine the cause of the severe cardiac effect of this rapid fall in CO_2 tension and hydrogen ion concentration. They were impressed, as we were, with the similarity between the electrocardiograms recorded in the immediate post-hypercapnic period and those characteristic of hyperkalemia. These inves-

* This study supported by funds provided under Contract with the USAF School of Aviation Medicine, Randolph Field, Texas, and by Minnesota Heart Assn.