Brit. J. Pharmacol. (1962), **18**, 101–108.

ANTIHISTAMINE ACTIVITY IN EXTRACTS OF HORSE EOSINOPHILS

BY

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(*Received October* 7, 1961)

Suspensions of horse eosinophils or the watery extracts of such suspensions were injected intraperitoneally into guinea-pigs. The eosinophils were obtained from 9 healthy cross-bred ponies. The number of eosinophils used for each injection varied between 20 and 300 millions. The guinea-pigs were exposed to a 0.6% histamine aerosol 1 to 8 hr later. The eosinophils of 5 ponies produced protection against the aerosol, the eosinophils of the other 4 ponies did not. Protection when produced did not last as long as 23 hr. The active principle in the watery extracts was stable, after freeze drying, for at least a few weeks.

In the preceding paper (Broome, Callow, Feldberg & Kovacs, 1962) it was shown that extracts of plant tumours injected into guinea-pigs protect the animals against a histamine aerosol. One of us (Archer, 1960) has recently developed a method of obtaining suspensions of horse eosinophils relatively free from contamination with other blood cells, and in the present paper it is shown that such suspensions and their watery extracts, injected intraperitoneally into guinea-pigs, also provide protection against a histamine aerosol. The results are in line with previous observations of antihistamine activity shown by suspensions and extracts of leucocytes and of eosinophils.

Our knowledge of the precise physiological function of the eosinophils is still scanty. They increase in the circulating blood during allergic and related disorders in which the symptomatology is thought to be, to some extent at least, due to release of histamine. Eosinophils are not rich in histamine as originally supposed by Code (1937), and there is no evidence that they function as carriers of histamine by removing it from the sites of its release. They respond, however, chemotactically to histamine (Kline, Cohen & Rudolph, 1932; Knott & Pearson, 1934; Riek, 1954; Archer, 1956, 1959, 1960), and they may function in association with histamine release by antagonizing histamine reactions in the tissue by virtue of an antihistamine substance present in these blood constituents.

The first indication for the presence of an antihistamine in eosinophils was given by Kovacs (1950) and by Kovacs & Juhasz (1952), who found that suspensions and extracts of leucocytes injected into guinea-pigs protected the animals against a histamine aerosol and that there was a correlation between the eosinophil content

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of such suspensions and the degree of protection. Vercauteren (1953) showed that extracts prepared from granules of eosinophils inhibited the action of histamine on isolated smooth muscle preparations. More recently, Archer (1960) has shown that watery extracts obtained from suspensions of horse eosinophils injected into the skin of horses antagonize the local oedema formation produced by intradermal histamine.

METHODS

Eosinophils were obtained from 9 healthy cross-bred ponies which had an eosinophil count in their circulating blood of from 700 to 1,000 per mm^3. The eosinophils were injected intraperitoneally into guinea-pigs weighing between 190 and 390 g; (1) as freshly isolated eosinophils suspended in pyrogen-free water; (2) after freezing at $-20°$ C; (3) after freeze-drying; and (4) as watery extracts.

The suspensions of freshly isolated eosinophils were obtained by differential centrifugation. From these suspensions the frozen eosinophils and the watery extracts were prepared. The methods have been described in detail elsewhere (Archer, 1960). The freeze-dried eosinophils were obtained by lyophilization of the frozen cells.

From each suspension of freshly isolated eosinophils counts were made of the eosinophils and of the total leucocytes. From these counts the contamination with the other leucocytes was calculated and the purity expressed as percentage.

For each intraperitoneal injection into guinea-pigs the fresh frozen, or freeze-dried cells, were injected in a volume of 3 to 5 ml. of water or 0.9% sodium chloride solution. The watery extracts were injected in 1 to 1.5 ml. of 0.9% sodium chloride solution. Usually the eosinophils injected into one guinea-pig were derived from a single pony, but in a few experiments each guinea-pig was injected with pooled samples from 2 to 4 ponies. In many experiments the control guinea-pig received an intraperitoneal injection of the same volume of either water or 0.9% sodium chloride solution.

The injected guinea-pig and a control were together exposed to a 0.6% histamine diphosphate aerosol. The details of the histamine aerosol exposure as well as the method adopted for plotting the difference in survival time between each injected animal and its control are described in the preceding paper (Broome, Callow, Feldberg & Kovacs, 1962).

RESULTS

One hundred and three guinea-pigs received intraperitoneal injections of eosinophils. The results are subdivided according to the ponies used for preparing the eosinophils (Fig. 1) and according to the kind of preparation of eosinophils used for injection (Fig. 2; Tables 1 to 4).

Fig. 1 illustrates that the eosinophils of some ponies produced protection of the guinea-pigs against the histamine aerosol whereas the eosinophils of others did not. Protection was produced by the eosinophils of five ponies, and the results obtained in 71 experiments with these eosinophils are shown in Fig. 1A. The numbers 1 to 5 in the squares of this figure refer to these five ponies and no. 6 refers to pooled samples of eosinophils from several ponies in this group. The protection is evident from the fact that in 58 of the 71 experiments the injected guinea-pig survived longer during the histamine aerosol than its control, whereas the control survived longer in eight experiments only. In the remaining five experiments the difference in survival time was either less than 30 sec (4 experiments) or both control and injected animal survived the 20 min exposure (1 experiment). Protection is also evident from a comparison of the number of injected animals with

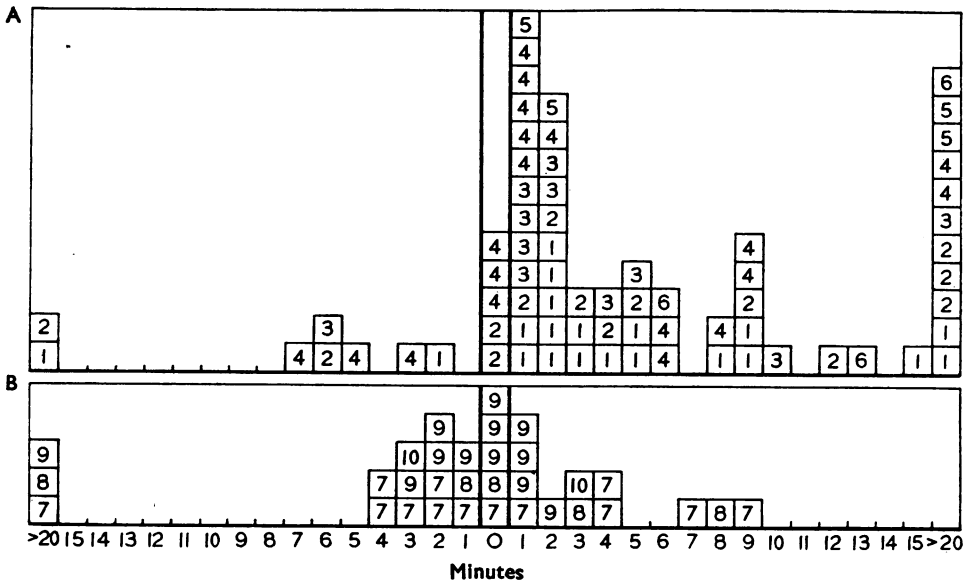

Fig. 1. Differences in survival times between control guinea-pigs and guinea-pigs injected with suspensions or watery extracts of horse eosinophils when exposed to a 0.6% histamine aerosol. The numbers in the squares refer to the ponies from which the eosinophils were obtained, and each square represents the result of one experiment. The abscissa gives the difference in survival time in minutes on the left of 0 if the control, and on the right of 0 if the injected, animal survived longer. The squares given >20 mean that one of the animals survived the 20 min exposure, and the squares above 0 that the difference in survival time was less than 30 sec. (For details of this kind of plotting the results, see Methods of preceding paper.)

that of the controls which stopped breathing during the first 6 or 8 min of exposure and which survived the exposure longer than 11 min or the full 20 min. Of the injected animals 11 (16%) stopped breathing during the first 6 min, and 26 (37%) during the first 8 min, whereas of the controls 31 (44%) stopped breathing during the first 6 min and 51 (72%) during the first 8 min. On the other hand, of the injected animals 29 (41%) survived longer than 11 min, and 12 (17%) survived the 20 min period, whereas of the control animals only 9 (13%) survived longer than 11 min and 3 (4%) survived the 20 min period.

The eosinophils of another group of four ponies, numbered 7 to 10, did not produce protection. The results obtained in 32 experiments with the eosinophils of this group are shown in Fig. 1B. In 15 experiments the control, and in 13 the injected animal survived longer, and in 5 the difference in survival time was less than 30 sec.

In Fig. 2 the same experiments as those shown in Fig. 1 are subdivided into four groups according to whether fresh cells A, frozen cells B, freeze-dried cells C, or watery extracts of the eosinophils D, were used for injection into the guinea-pigs. Tables 1 to 4 give for each experiment the number of eosinophils injected, the purity of the sample, the age of the sample and the interval between injection and exposure to the histamine aerosol.

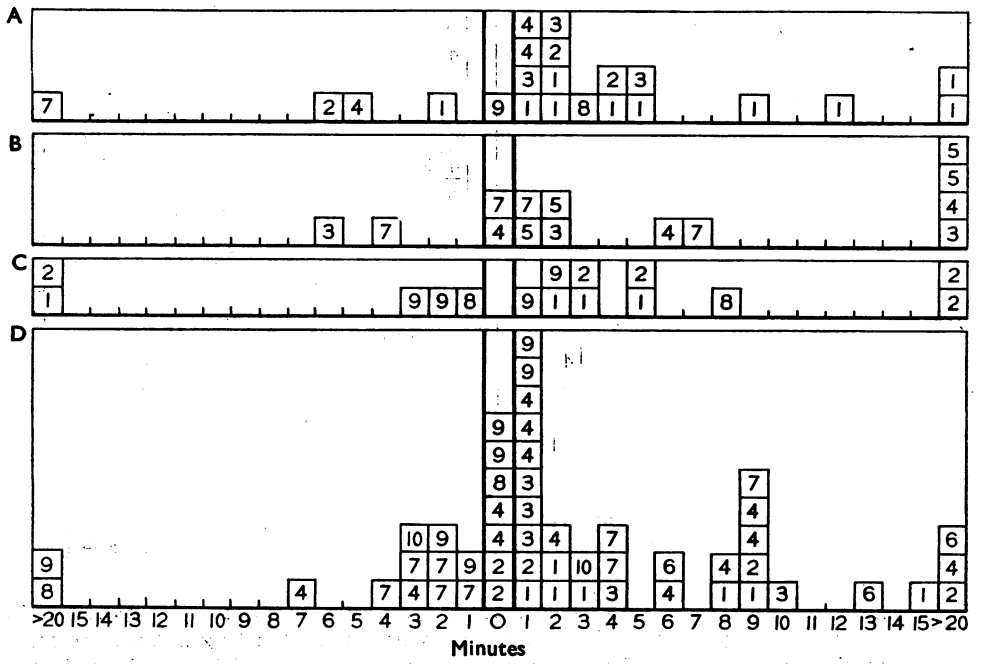

Fig. 2. Differences in survival times between control guinea-pigs and guinea-pigs injected with suspensions of fresh (A), frozen (B), or freeze-dried (C) horse eosinophils or with watery extracts of eosinophils (D) when exposed to a 0.6% histamine aerosol. Same experiments as in Tables 1 to 4. The details of plotting are the same as in Fig. 1.

Twenty-two guinea-pigs were injected with freshly prepared eosinophils 5 to 8 hr before their exposure to the histamine aerosol. Table 1 gives the survival time of each injected animal and of its control, and Fig. 2A shows the difference in survival times between the two. In 19 of these experiments the eosinophils were from ponies nos. 1 to 4, and in 16 of them the injected animal survived longer than its control. Between 20 and 186 millions of eosinophils were injected into each guinea-pig, yet an increase in the number injected did not reveal stronger protection. It is possible that a greater number of experiments would have to be performed in order to obtain evidence for stronger protection with an increase in the number of eosinophils.

Fourteen guinea-pigs were injected with samples of frozen eosinophils. The results are given in Table 2 and Fig. 2B. Eosinophils from ponies nos. 1 to 5 were used for 10 experiments and in 8 of these the injected guinea-pig survived the aerosol longer than its control and in 4 it survived the 20 min period. These experiments show that freezing the eosinophils does not inactivate the active principle and, since some of the samples had been stored for 2 to 5 days, that it was stable in this condition for at least a few days. Since the injected animal survived the 20 min period in 4 experiments, it would appear, as far as one can conclude from the relatively small number of experiments, that suspensions of frozen cells are more active than those of fresh cells. However, two of the guinea-

TABLE 1

EFFECT ON SURVIVAL TIME OF GUINEA-PIGS EXPOSED TO A 0·6% HISTAMINE AEROSOL FOLLOWING INTRAPERITONEAL INJECTION OF A SUSPENSION OF FRESHLY PREPARED EOSINOPHILS

Details of suspension			Exposure of guinea-pigs to aerosol			Difference in survival time in min
			Interval after injection in hr	Survival time		
Pony used	Eosinophils in millions	Purity as %		Injected	Control	
7	159	68	5·5	8′ 15″	>20′	−20
2	22		5·5	9′	15′ 15″	−6
4	173	61	6	7′ 50″	12′ 40″	−5
1	83		5·25	6′ 15″	8′	−2
9	196	60	5·5	6′	6′ 15″	+−
1	20		8	4′ 50″	4′	+1
3	156	59	6	7′ 15″	6′ 10″	+1
4	186	51	5	7′ 5″	5′ 55″	+1
4	172	64	5·75	7′	6′	+1
1	50		5·66	8′ 30″	6′ 25″	+2
1	22		5·66	5′ 50″	4′	+2
2	22		5·5	6′	4′	+2
3	128		6·25	11′ 20″	9′ 15″	+2
8	136	60	5·5	8′	4′ 55″	+3
1	50		5·5	10′	5′ 45″	+4
2	20		6	7′ 40″	4′	+4
1	20		6·5	9′ 45″	4′ 30″	+5
3	173	71	5	11′ 10″	6′ 40″	+5
1	20		6	18′ 50″	10′	+9
2	77		5·5	17′ 10″	4′	+13
1	108	40	5·5	>20′	2′ 30″	+20
1	99	50	5·5	>20′	10′ 15″	+20

pigs were injected with frozen eosinophils from pony no. 5, which was not used for preparing fresh eosinophils, so it may be that the eosinophils obtained from this pony were particularly potent in protecting the guinea-pig against the histamine aerosol.

Some of the guinea-pigs which had been injected with frozen eosinophils, and which had survived the 20 min period or at least had survived longer than their

TABLE 2

EFFECT ON SURVIVAL TIME OF GUINEA-PIGS EXPOSED TO A 0.6% HISTAMINE AEROSOL FOLLOWING INTRAPERITONEAL INJECTION OF A SAMPLE OF FROZEN EOSINOPHILS FROM HORSE BLOOD

Details of sample				Exposure of guinea-pigs to aerosol			Difference in survival time in min
				Interval after injection in hr	Survival time		
Pony used	Eosinophils in millions	Purity as %	Age in days		Injected	Control	
3	183	99	1	5	9′ 25″	15′ 20″	−6
7	194	88	6	5	14′ 25″	18′ 50″	−4
4	206	63	2	5·25	7′	6′ 35″	+−
7	286	91	3	6	10′ 15″	9′ 50″	+−
5	139	60	1	5	7′ 25″	6′ 15″	+1
7	267	74	3	5·5	6′ 15″	5′ 40″	+1
3	155	63	4	5·5	9′ 45″	7′ 40″	+2
5	144	50	2	5	8′ 25″	6′ 15″	+2
4	180	81	2	5·25	13′ 40″	7′ 30″	+6
7	118	53	2	5	13′ 40″	6′ 15″	+7
3	134	59	3	4·75	>20′	5′ 55″	+20
4	144	47	5	5·33	>20′	9′	+20
5	269	92	2	5·25	>20′	11′ 40″	+20
5	135	50	1	5·25	>20′	9′	+20

controls, were re-exposed to the aerosol 1 or 3 days later. At that time there was no longer any sign of protection.

Fifteen guinea-pigs were injected with freeze-dried eosinophils. The results are given in Table 3 and in Fig. 2C. Again the eosinophils obtained from the ponies 1 and 2 produced protection, since seven of the nine guinea-pigs injected with these eosinophils survived longer than their controls. The results show that freeze-drying also does not inactivate the active principle in the eosinophils.

TABLE 3

EFFECT ON SURVIVAL TIME OF GUINEA-PIGS EXPOSED TO A 0·6% HISTAMINE AEROSOL FOLLOWING INTRAPERITONEAL INJECTION OF A SAMPLE OF FREEZE-DRIED EOSINOPHILS FROM HORSE BLOOD

Details of sample				Exposure of guinea-pigs to aerosol			Difference in survival time in min
Pony used	Eosinophils in millions	Purity as %	Age in days	Interval after injection in hr	Survival time: Injected	Survival time: Control	
1	81	50	12	6·75	9′ 40″	>20′	−20
2	195	53	1	6	5′	>20′	−20
9	90	41	7	6	4′ 30″	7′	−3
9	224	83	2	6	4′ 50″	6′ 10″	−1
8	180	74	10	5·5	5′ 30″	6′ 10″	−1
9	90	41	7	5·75	4′ 40″	3′ 40″	+1
1	196	66	8	6·5	6′ 15″	4′ 10″	+2
9	220	73	4	6	11′ 30″	9′ 50″	+2
1	194	71	7	5·75	9′	6′	+3
2	76	50	16	5·75	6′	3′ 20″	+3
1	135	66	3	6	11′ 40″	6′ 35″	+5
2	150	64	2	5·5	15′ 20″	10′ 15″	+5
8	84	55	14	5·75	12′ 20″	4′ 20″	+8
2	90	50	11	6·25	>20′	12′ 5″	+20
2	120	68	3	5·75	>20′	6′ 30″	+20

The active principle can be extracted with water and in this condition remains stable for at least a few weeks. This is evident from Table 4 and Fig. 2D, which give the results from 57 experiments. In 32 of these experiments the guinea-pigs were injected with watery extracts derived from the ponies 1 to 4; no. 6 again refers to pooled samples from these ponies. In 26 of the 32 experiments the injected guinea-pig survived longer than its control; in only two did the control survive longer.

DISCUSSION

The results of the present experiment support the earlier work concerning anti-histamine activity produced by extracts of eosinophils. The finding that such activity was consistently demonstrable with eosinophils from 5 out of 9 ponies suggests great variations in the content of the active substance present in the eosinophils circulating in the blood of individual horses. There is no evident explanation for these variations. All ponies were similarly bred, were housed, fed and run together, and there was no relation to sex or age. They were clinically fit. The only criterion of choice for selection from the herd was a peripheral eosinophil count in the circulating blood of not less than 700/mm³.

From the fact that suspensions of eosinophils and their extracts, injected intra-peritoneally, protect guinea-pigs against the bronchoconstriction of a histamine

TABLE 4

EFFECT ON SURVIVAL TIME OF GUINEA-PIGS EXPOSED TO A 0.6% HISTAMINE AEROSOL FOLLOWING INTRAPERITONEAL INJECTION OF A SAMPLE OF WATERY EXTRACT OF EOSINOPHILS

Details of sample				Exposure of guinea-pigs to aerosol			Difference in survival time in min
Pony used	Eosinophils in millions	Purity as %	Age in days	Interval after injection in hr	Survival time: Injected	Survival time: Control	
8	135	65	9	3·5	5′ 20″	>20′	−20
9	144	63	3	4·75	6′ 25″	>20′	−20
4	417	65	18	3·66	9′ 50″	16′ 30″	−7
7	236	72	31	4	7′ 35″	11′ 55″	−4
4	246	45	11	5·66	6′ 45″	9′ 25″	−3
7	133	54	12	4·75	5′ 20″	8′ 10″	−3
10	135	53	18	5·75	9′ 10″	12′ 20″	−3
7	357	70	22	3·66	6′ 20″	8′ 20″	−2
7	240	68	5	5	6′ 10″	7′ 55″	−2
9	114	55	13	2	4′ 35″	6′ 30″	−2
7	250	52	23	5	4′ 45″	5′ 15″	−1
9	341	70	22	5	6′ 20″	7′ 15″	−1
2	157	67	4	5·75	5′ 35″	5′ 40″	+−
2	233	65	23	5	7′ 40″	7′ 15″	+−
4	183	53	7	4·5	>20′	>20′	+−
4	143	54	8	3·75	5′ 45″	5′ 35″	+−
8	240	100	4	3·5	4′ 55″	4′ 30″	+−
9	141	56	5	5·25	>20′	>20′	+−
9	341	70	22	1	14′ 10″	14′ 10″	+−
1	171	97	24	4	7′ 20″	6′ 10″	+1
2	147	92	8	1	5′ 5″	4′ 30″	+1
3	250	52	23	1	5′ 10″	4′ 10″	+1
3	171	86	35	1·25	5′ 40″	4′ 5″	+1
3	194	55	4	4	8′ 20″	7′ 40″	+1
4	306	100	25	4	6′ 20″	5′ 5″	+1
4	156	57	14	1	4′ 55″	3′ 50″	+1
4	174	50	6	6	7′ 10″	5′ 55″	+1
9	234	68	11	4	5′ 50″	5′ 5″	+1
9	126	50	2	4·33	6′ 42″	6′ 10″	+1
1	205	82	6	4·25	11′ 30″	9′ 50″	+2
1	225	100	2	4·5	8′ 40″	7′ 5″	+2
4	90	67	7	4·75	7′ 15″	5′ 35″	+2
1	114	84	18	2	9′ 10″	5′ 45″	+3
10	188	71	5	3·75	7′	4′ 25″	+3
3	146	63	6	2	10′ 25″	6′ 30″	+4
7	395	65	18	1	18′ 10″	14′ 40″	+4
7	236	72	31	4·25	9′ 10″	4′ 55″	+4
4	345	100	22	1·5	10′ 50″	5′ 10″	+6
6	385	60	19	1	10′ 10″	3′ 55″	+6
1	204	89	7	4	15′ 30″	7′ 15″	+8
4	173	62	20	6	14′ 20″	6′ 40″	+8
1	233	65	23	1·25	16′ 20″	7′ 20″	+9
2	210	45	5	4	16′ 35″	8′	+9
4	378	77	6	1·5	13′ 45″	4′ 45″	+9
4	378	77	6	5·5	17′ 45″	9′ 10″	+9
7	395	65	18	5	15′ 40″	7′ 10″	+9
3	280	83	15	5·5	15′ 40″	5′ 45″	+10
6	385	60	19	4·75	18′ 10″	5′ 5″	+13
1	250	100	6	3·5	19′ 30″	4′ 55″	+15
2	150	96	11	3·5	>20′	9′ 40″	+20
4	253	100	9	1	>20′	8′ 35″	+20
6	216	66	5	5	>20′	5′ 30″	+20

aerosol, no conclusion can be drawn about the specificity or even selectivity of this antihistamine effect. Since similar extracts, however, were shown to antagonize also the local oedema formation produced by histamine in the horse skin, at least two of the three characteristic peripheral histamine effects—contractions of smooth

muscles, increase in permeability of capillaries and glandular secretions—are affected by the active principle of eosinophils. In this connexion it is worth while pointing out that the third of these effects, at any rate the acid gastic secretion, is resistant to synthetic antihistamines.

The only definite fact concerning the properties and nature of the active principle in eosinophils is its water solubility. It is unlikely to be the same substance as that responsible for the antihistamine activity produced by extracts of plant tumours.

Vercauteren (1953) obtained his antihistamine effect with extracts of granules from eosinophils on an isolated smooth muscle preparation, but until his finding is confirmed it is not possible to attribute the antagonistic effect observed *in vivo* against the bronchoconstriction in guinea-pigs and the skin oedema in horses to the same mode of action as that of the synthetic antihistamines.

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