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HISTAMINE, EOSINOPHILS AND BASOPHILS IN THE BLOOD

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Some evidence has been accumulated which indicates that the eosinophils of the blood at times contain histamine (Code, 1937*b*; Code & MacDonald, 1937; Parrot & Gabe, 1945; Graham, Lowry, Wheelwright, Lenz & Parish, 1955). When such evidence was first presented, it was reported also that the number of eosinophils in the blood may be increased without any increase in the concentration of histamine (Code, 1937*b*; Code & MacDonald, 1937). Although this observation was rather the exception in the initial study (Code, 1937*b*), it became much more the rule in the later investigations. In the blood of humans with eosinophilia, for example, no direct correlation was usually found between the number of eosinophils and the amount of histamine (Randolph & Rackemann, 1941; Went & Rex-Kiss, 1941; Graña, Recarte & Balea, 1943; Valentine, Pearce & Lawrence, 1950). A number of patients in each series, however, had eosinophilia and a concentration of histamine in the blood which was well above the normal range. This observation might be interpreted as showing, in accord with the original suggestion, that eosinophils at times contain histamine. Nevertheless, the results of study of the larger series indicate that if, under conditions of good health, eosinophils contain histamine, then in the presence of eosinophilia when presumably some abnormal circumstances are present, they often contain much less or none at all. The first objective of this study was to determine whether any correlation exists between the number of eosinophils and the concentration of histamine in the blood of animals and healthy humans.

The administration of either adrenocorticotrophin or cortisone to healthy humans or animals reduces the number of eosinophils in the circulating blood (Hills, Forsham & Finch, 1948; Thorn & Forsham, 1949; Sprague, Power, Mason, Albert, Mathieson, Hench, Kendall, Slocumb & Polley, 1950). If the

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eosinophils contain histamine then its concentration in the blood should decrease with the decrease in eosinophils when one of these hormones is given. Herbert, de Vries & Rose (1950) reported testing for this effect by the administration of ACTH to two patients with eosinophilia. Since there was already some indication that the eosinophils of patients with eosinophilia might at times contain little or no histamine, and since a significant change in the number of eosinophils did not occur in the blood of either of the patients studied by Herbert *et al.* (1950) at the time the concentration of histamine in the blood was determined, the second objective of this study became to establish whether or not the concentration of histamine in the blood of animals or healthy humans changes during the eosinopenia induced by the administration of cortisone or ACTH.

While the investigation just outlined was in progress, Riley & West (1953) reported that mast cells of tissue are rich in histamine. Since the basophils of the blood resemble in some features the mast cells of the tissues, we were forced to extend our study from esinophils alone to include consideration of the basophils. An earlier report by Graham and her co-workers published in abstract form (Graham, Wheelwright, Parish, Marks & Lowry, 1952) had indicated that basophils act as carriers of significant quantities of histamine, at least in the blood of patients with myelocytic leukaemia. The support given to this assertion by Riley & West (1953) led to the third objective of our study: to determine whether or not there is a relationship between the number of basophils and the amount of histamine in the blood of animals and healthy humans.

An earlier search for a relationship between the numbers of the various types of white cells and the concentration of histamine in the blood had been abandoned by one of us (Code) because of a lack of precision in the method of enumerating the different white cells of the blood. Differential white cell counts in a smear of the blood coupled with a count of their total numbers in the blood had been employed. In our hands, at least, estimates by this means of the exact numbers of the different white cells, particularly of the rarer types, were dishearteningly variable. Development, by others, of more accurate methods, employing dilution and white-cell-chamber counting procedures for enumeration of both eosinophils (Randolph, 1944) and basophils (Moore & James, 1953), removed this block and was the final stimulus to the investigation.

METHODS

Observations were made on dogs, guinea-pigs and humans. The five dogs studied were adrenalectomized because we had found that, as in humans (Recant, Hume, Forsham & Thorn, 1950), the number of eosinophils in the blood of dogs is increased in the absence of the adrenal glands. Tests were made on the blood of four of these animals over periods of 1–2 years. The fifth was studied only during one test of the effect of cortisone.

Estimates of the condition of the adrenalectomized animals were made from the intake of food, changes in body weight, volume of erythrocytes by haematocrit readings, concentration of blood urea, 24 hr output of urinary chlorides during a fixed intake of chloride, and occasional determinations of sodium and potassium in the serum. The concentration of chloride was determined by the method of Schales & Schales (1941), and that of sodium and potassium by use of a flame photometer. Except where otherwise stated, all of the results presented in this report were obtained while the dogs were maintained in good health. Most of the animals were maintained for periods of a month or more before the tests with deoxycorticosterone alone: a single injection or repeated injections of 10 mg of cortisone or more a day were given on five different occasions to one dog and on one occasion to each of the other four.

It has been reported that in the guinea-pig the number of eosinophils in the circulating blood differs significantly between male and female, and among females between virgin and multipara (Dworetzky, Code & Higgins, 1950; Dews & Code, 1951). Tests were made to check these differences and to determine if they were accompanied by changes in the concentration of histamine in the blood. In addition, cortisone or ACTH, which uniformly lowers the eosinophil counts in the blood of these animals (Dworetzky *et al.* 1950; Dews & Code, 1951), was given intramuscularly in single doses of 50 mg or 50 Armour units respectively, and the concentration of histamine and the number of eosinophils were determined 12–24 hr later. In a final series of tests the basophils as well as the eosinophils were enumerated, and the effect was determined of the administration of cortisone on the numbers of these cells and on the concentration of histamine in the blood.

Finally, three groups of volunteers were studied; each was composed of five men whose ages ranged from 30 to 43 years. All were in good health and none had ever exhibited significant allergy. In the first group, the number of eosinophils and the amount of histamine in the blood were determined before, during and after a 3 day period in which 50 mg of cortisone was taken by mouth every 6 hr (200 mg/day). For the members of the second and third groups the number of basophils in the blood was determined as well as the number of eosinophils and the amount of histamine. Cortisone was given by mouth to each of the members of the second group at the rate of 200 mg /day for 3 days and 400 mg was taken by each member of the third group in two doses of 200 mg each, one taken at midnight and the other at 5 a.m.

The concentration of histamine in the blood was determined by means of a modification (Code, 1937*a*) of the Barsoum & Gaddum (1935) procedure. All values are expressed in terms of histamine base. Duplicate samples of blood were always used and their volume as a rule was 5 ml. In almost all instances, after the trichloroacetic acid filtrates had been boiled with hydrochloric acid and dried twice in the presence of alcohol, the dry material was extracted three times, using 10, 5 and 5 ml. of absolute ethanol. This additional extraction was carried out to reduce the concentration of potassium (Code, Hallenbeck & Gregory, 1947). The alcohol-insoluble material was discarded after separation by centrifugation. The alcohol-soluble material was taken to dryness. Later, when the assay of histamine was to be made, the dried residue was taken up in Tyrode solution and neutralized. Sometimes the extracts were centrifuged before assay but usually this was unnecessary. The Tyrode solution used to prepare the final extract was diluted with one part of distilled water to three parts of Tyrode solution. In most instances the volume of Tyrode solution used was the same as that of the original sample of blood. With dog's blood, however, such small amounts of histamine were often encountered that it was found necessary to double the quantity of blood for satisfactory assay on the ileum of the guinea-pig. This was accomplished by taking 8 or 10 ml. of blood and using 4 or 5 ml. of diluted Tyrode solution in the final volume of the extract.

The eosinophils and basophils were always enumerated in the same sample of blood as was used for the determination of histamine. As a routine the blood was drawn through a siliconed or polished needle into a 10 ml. siliconed syringe. If eosinophils only were to be counted, the syringe was rinsed with a saline solution of heparin just before the blood was taken and the needle was left filled with the solution. If basophils were to be enumerated, no heparin could be used since it combines with the dye in the diluting fluid and thus reduces the staining of the basophils.

Somewhat more than 10 ml. of blood was drawn, the barrel of the syringe was withdrawn and 5 ml. of blood was removed with a siliconed pipette for the determination of histamine. Then samples were drawn into pipettes for enumeration of eosinophils or basophils. The duplicate aliquot for estimation of histamine was next withdrawn and often duplicate samples for eosinophil or basophil enumeration were removed. The blood for the enumeration of eosinophils was diluted with an aqueous propylene glycol phloxine solution as recommended by Randolph (1944), and that for enumeration of basophils with an ethanol-aqueous solution of toluidine blue as devised by Moore & James (1953). Each type of cell was counted directly by use of two Levy counting chambers with double-Fuchs-Rosenthal ruling (four ruled areas) for each pipette.

All samples of blood were taken in the morning, usually between 8 and 9 o'clock. When repeated observations were made on the same individual an effort was made to take the blood during the same hour each day. Venous blood was used for the dogs and humans and no anaesthetic was employed. Blood was taken from the guinea-pigs, however, by cardiac puncture, while the animals were anaesthetized by the inhalation of ether or by the subcutaneous injection of 50 mg pentobarbital sodium/kg body wt.

RESULTS

Adrenalectomized dogs

No truly characteristic basophils were identified in the blood of the dogs we studied. The method which allowed ready recognition and enumeration of basophils in the blood of guinea-pigs and human beings did not reveal basophils in the blood of the dogs. Neither did the more conventional methods of fixation of a blood smear by absolute alcohol or heat followed by staining with toluidine blue or alcoholic thionine (Michels, 1938). Occasionally cells showing faint basophilic characteristics were found, but even these were so few and so atypical that the conclusion has been reached that the blood of the dogs we observed did not contain significant numbers of basophils.

'Spontaneous' changes. Gradual and rather sweeping changes in the number of eosinophils and the amount of histamine sometimes occurred in the blood of the four dogs which were observed for many months. Because these changes could not be related to therapeutic measures or to alterations in the general health of the animals, they were labelled 'spontaneous'. There were eighteen such episodes during the two years of observation, and in two-thirds of them there was a rough parallelism between the shifts in the number of eosinophils and the concentration of histamine (Fig. 1, dog A). Often one component changed, and the other followed in a day or two. In the remaining third of the spontaneous episodes, the concentration of histamine in the blood sometimes remained low and fixed, while the number of eosinophils rose and fell (Fig. 1, dog D), and at other times the eosinophil count remained high while the concentration of histamine fluctuated. It was apparent that more drastic changes than those which appeared spontaneously would have to be produced to expose a relationship, or lack of one, between the number of eosinophils and the concentration of histamine in the blood. Administration of cortisone offered such a prospect.

Effects of cortisone. In the doses used, cortisone always caused a decisive fall in the number of eosinophils and a parallel reduction in the quantity of histamine in the blood (Figs. 2, 3). Soon after cortisone had been given and subsequent to the decisive fall, eosinophils often temporarily accumulated in the blood stream in considerable numbers without a rise in the concentration of histamine (Figs. 2, 3). It was as if some eosinophils, free of histamine, had burst into the circulation.

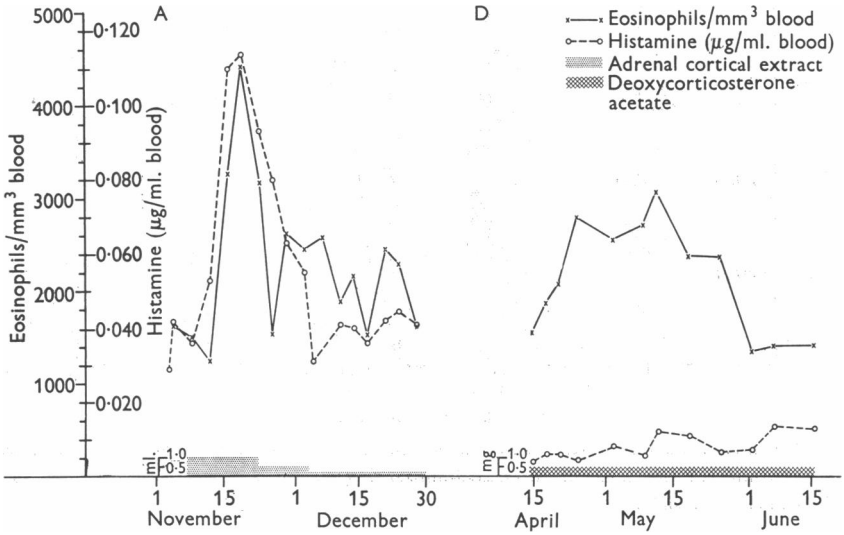

Fig. 1. Spontaneous changes in the number of eosinophils and amount of histamine in the blood of adrenalectomized dogs A and D.

The fifth dog of the series (Fig. 3, dog E), which was studied for only a week, had the highest eosinophil count and the greatest amount of histamine in the blood of any dog ever tested in our laboratory except, of course, under acute conditions such as anaphylactic shock when the histamine is not contained in the cells of the blood but is free in the plasma (Code, 1939). At necropsy a foreign-body abscess was found in the pleural cavity and this may have contributed, in some unknown fashion, to the large number of eosinophils and the excessive amount of histamine found in the blood. The administration of cortisone produced the same parallel decline in the numbers of eosinophils and amounts of histamine in the blood of this animal as it did in the blood of the other dogs (Fig. 3).

Over-all relationship. To show the relationship between histamine and eosinophils in the blood, a graph was constructed on which the concentration of histamine in a sample of blood was plotted against the number of eosinophils in the same sample. Since the values obtained on dog E were few in number and unusual in magnitude, they have not been included on the graph

although they have the same general relationships as those for the other dogs. All other estimates made throughout the study have been included (Fig. 4). Five of the 154 points on the graph represent blood in which the concentration of urea was slightly elevated and the animals had mild adrenal insufficiency.

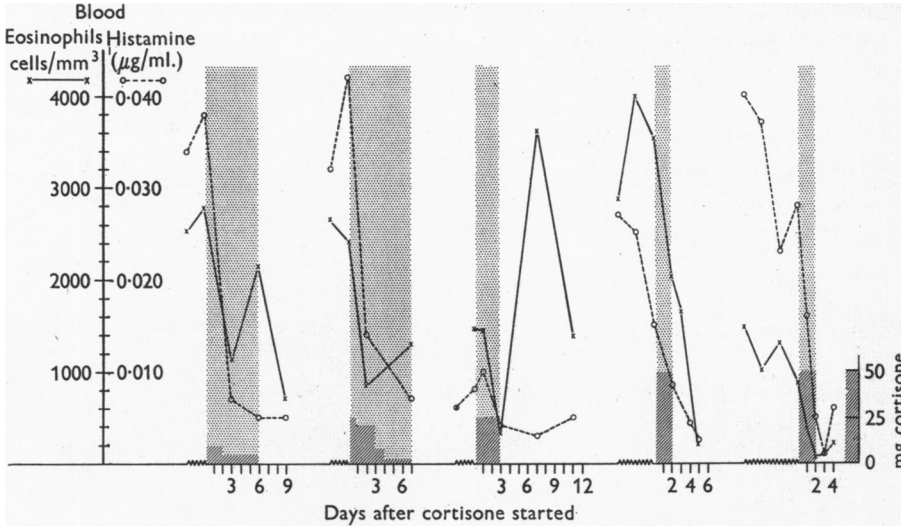

Fig. 2. The effect of various doses of cortisone on the number of eosinophils and the concentration of histamine in the blood of adrenalectomized dog A. Shaded area indicates duration of treatment with cortisone.

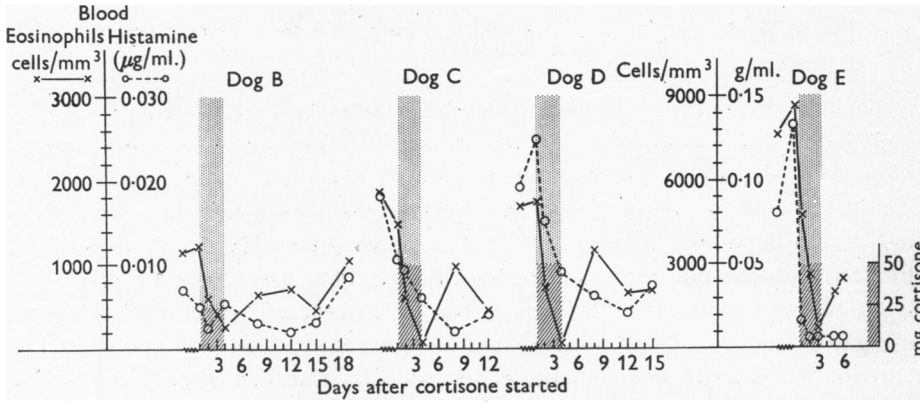

Fig. 3. The effect of the administration of cortisone for 3 days to adrenalectomized dogs B, C and D, and for 2 days to adrenalectomized dog E, on the number of eosinophils and the concentration of histamine in the blood.

All others were derived from blood taken when the animals were in good general condition. These data indicate that a relationship does exist between the histamine and the eosinophils in the blood (Fig. 4). A high concentration of histamine only occurred in the presence of large numbers of eosinophils.

Similarly, if the eosinophil count was low, the concentration of histamine was also always low. High eosinophil counts, however, occurred in blood containing little histamine. Thus, the eosinophils certainly did not always contain histamine. Indeed, at times they were apparently devoid of it. When they contained it, they raised the concentration of histamine in the blood. The 'load' of histamine assumed by each eosinophil must have been variable. The data indicate, however, that there was a maximal load which the eosinophils could or

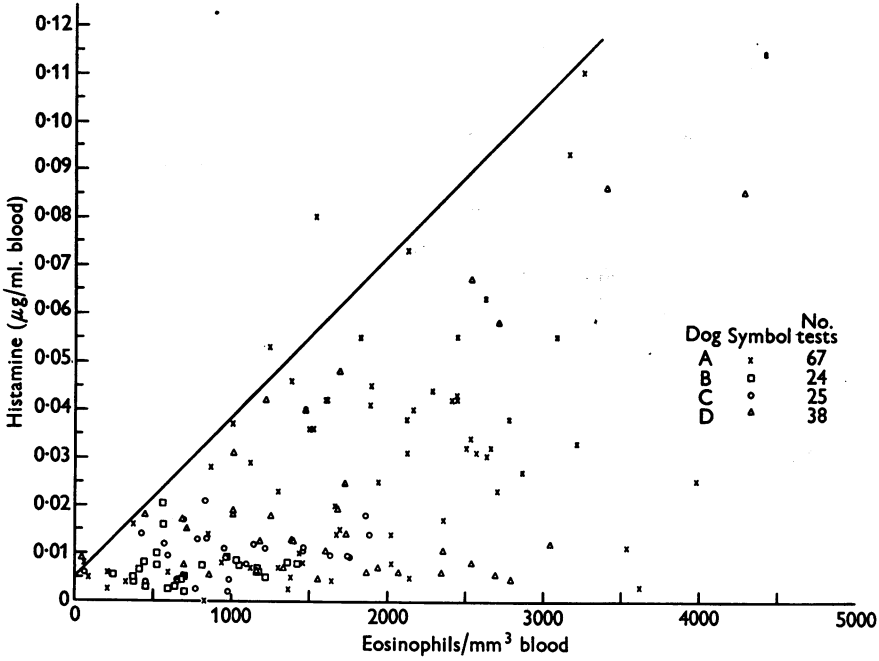

Fig. 4. The concentration of histamine plotted against the number of eosinophils found in the blood of adrenalectomized dogs A, B, C and D.

would assume, because for each range of eosinophil counts a maximal concentration of histamine was attained in the blood. Within the experimental errors of the methods involved, the relationship is represented by a straight line which rests sharply on the upper edge of the plotted points (Fig. 4). Only two points fall significantly above it and none of the values obtained for dog E would rest above it. The coefficient of regression indicates that the maximal load of histamine that the eosinophils of adrenalectomized dogs can assume is 0·03 ng/eosinophil. In dogs, therefore, when the eosinophil count is zero, the concentration of histamine in the blood is negligible. The line along the upper edge of the points intercepts the zero axis of the eosinophil counts at less than 0·01 μg histamine/ml. blood. Thus no other compartment of the blood of these dogs besides that afforded by the eosinophils apparently contained more than a

trace amount of histamine. Finally, within this concept, when the concentration of histamine in the blood is low, sweeping changes may occur in the numbers of eosinophils without changes in the concentration of histamine, precisely as has been reported. When the concentration of histamine in the blood is high, however, large numbers of eosinophils will then be present in the blood, just as they were in the dogs we studied, and under these circumstances if drastic reductions in the numbers of eosinophils occur, the concentration of histamine should also fall, exactly as it did when cortisone was given. These relationships of course would not obtain in such conditions as anaphylactic shock when any excess histamine is free in the plasma (Code, 1939).

TABLE 1. Mean concentration of histamine and number of eosinophils in blood of guinea-pigs

			Untreated group				Treated Group				
					Blood					Blood	
Series	Season	Anaesthesia	No.	Mean wt. (g)	Eosinophils (per mm³)	Histamine (μg/ml.)	No.	Hormone	Mean wt. (g)	Eosinophils (per mm³)	Histamine (μg/ml.)
Males	Spring	Ether	11	882 ±39	49 ±13	0·071 ±0·006	11	Cortisone	832 ±20	2·5 ±0·5	0·052 ±0·007
Females Virgins	Spring	Ether	20	829 ±21	119 ±20	0·086 ±0·018	14	Cortisone	—	30 ±10	0·061 ±0·008
Multiparae	Spring	Ether	12	1055 ±33	129 ±17	0·101 ±0·010	—	—	—	—	—
	Summer	Ether	18	—	185 ±45	0·076 ±0·007	—	—	—	—	—
	Summer	Pentobarbital	14	1017 ±36	104 ±21	0·060 ±0·007	18	ACTH	996 ±31	17 ± 7	0·057 ±0·006

Guinea-pigs

Eosinophils and histamine in blood. In the first group of observations the eosinophils but not the basophils were counted. The studies were made during the spring and summer months and since the results obtained at the two seasons differed somewhat the series were kept separate in the initial analysis of the data. In the spring series the samples of blood were always drawn while the guinea-pigs were under ether anaesthesia. As expected, in the untreated animals the eosinophil counts of the multiparae were in the highest ranges, those of the males in the lowest and the virgins in the intermediate ranges (Table 1). The variability was so great among the females, however, that the differences between the virgins and the multiparae were not significant. Despite this variability the concentration of histamine in the blood of the three groups followed the eosinophil counts in a rough fashion, the mean concentrations of histamine being highest in the multiparae and lowest in the males although again the differences between the means were hardly significant.

Cortisone was given to groups of males and virgins and it decisively lowered the eosinophil counts. The mean concentration of histamine in the blood followed the downward trend of the eosinophils, but the differences between the mean values of the treated and untreated members of the groups of the same sex were not significant (Table 1). The trend toward a parallelism between the number of eosinophils and the amount of histamine in the blood was, however, unmistakable (Fig. 5). The correlation coefficient, for the five groups,

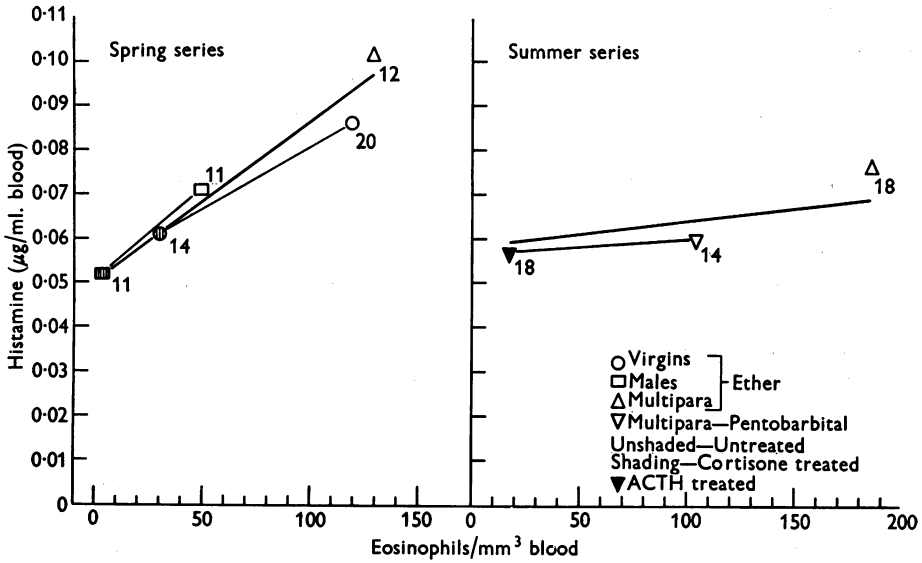

Fig. 5. Mean concentration of histamine and mean number of eosinophils found in the blood of guinea-pigs. Numerals below symbols indicate number of animals in each group.

of 0·98 ($P < 0·01$) and the linear regression indicated a significant relationship between eosinophils and histamine. The mean load of histamine assumed by each eosinophil as estimated by the regression coefficient was 0·34 ng ($P < 0·01$). Extrapolation of the linear regression to zero for the eosinophil count indicated that the amount of histamine in the extra-eosinophil compartment in the blood of these guinea-pigs was large and usually contained between 0·05 and 0·06 μg histamine/ml. blood (Fig. 5).

The experiments done in the summer months were confined to multiparae. There were three groups of animals. One control group was anaesthetized with ether and received no ACTH or cortisone. Another group, anaesthetized with pentobarbital sodium, served as controls for a group of animals treated with ACTH, the blood from which was also drawn under pentobarbital anaesthesia. Although the eosinophil counts of the untreated groups were generally high, particularly of those of the first control group tested under ether (Table 1), and even though ACTH caused a definite decrease in the numbers of eosinophils, the blood histamine in the control series was uniformly

low and this low value was little affected by treatment with ACTH (Fig. 5). The tendency for the concentration of histamine in the blood to be lowest when the eosinophil counts were lowest, and highest when the eosinophil counts were highest (Table 1; Fig. 5), was not significant, for the correlation coefficient of the values comprising the means of the three groups was not significantly different from zero. The data again indicated a sizable extra-eosinophil compartment containing between 0·05 and 0·06 μg histamine/ml. blood. Thus, although the extra-eosinophilic compartment was not expanded in the blood of these animals over that of the spring series, it did contain a much greater proportion of histamine, in fact most of the histamine in the blood. It is concluded that the eosinophils in the blood of both the control and treated animals of the summer series were carrying little if any histamine. It seems clear that when the concentration of histamine in the whole blood of guinea-pigs approaches that of the extra-eosinophilic portion, then large numbers of eosinophils may be present and decisive reductions in their numbers may occur without significant changes in the concentration of histamine in the blood.

Eosinophils, basophils and histamine. Up to this point in the study the most consistent finding in regard to guinea-pigs was that a considerable proportion of the histamine of the blood was outside the eosinophils. The discovery by Riley & West (1953) that the mast cells of the tissues contain large quantities of histamine suggested immediately that the extra-eosinophilic histamine of the blood of guinea-pigs might reside in the basophils. Therefore, simultaneous determinations of the numbers of eosinophils and basophils and the concentration of histamine in the blood were made in a series of fifty-one guinea-pigs. The series was made up of virgin females and non-gravid multiparae. Cortisone was given to nine of the guinea-pigs.

The administration of cortisone reduced the numbers of both eosinophils and basophils below those customarily found under control conditions. When it became clear that the hormone was not having a clear-cut differential effect on the two types of cells, the results of the tests with cortisone and the control tests were combined and separate correlations were sought between numbers of eosinophils and basophils and the amount of histamine in the blood (Fig. 6).

Depletion of both eosinophils and basophils in the blood of guinea-pigs did not lower the concentration of histamine in the blood below that previously assigned to the extra-eosinophilic compartment. Thus, in the guinea-pigs we studied, the extra-eosinophilic histamine of the blood was not contained in ths basophils. The correlation between the number of basophils and the amount of histamine in the blood was poorer than the correlation between the amount of histamine and the number of eosinophils (Fig. 6). Neither correlation was good (Fig. 6), owing apparently to the large and somewhat variable proportion of the total histamine lying in the blood outside both these types of cells.

Over-all relationship of eosinophils and histamine. The tests in which the basophils were included indicated that in the blood of guinea-pigs the relationship between numbers of eosinophils and amounts of histamine was somewhat closer than that between numbers of basophils and amounts of histamine. It should be emphasized, however, that much more data have been collected on the eosinophils than on the basophils and that with more extensive observations on basophils better definition of the possible relationship of these cells to histamine may emerge.

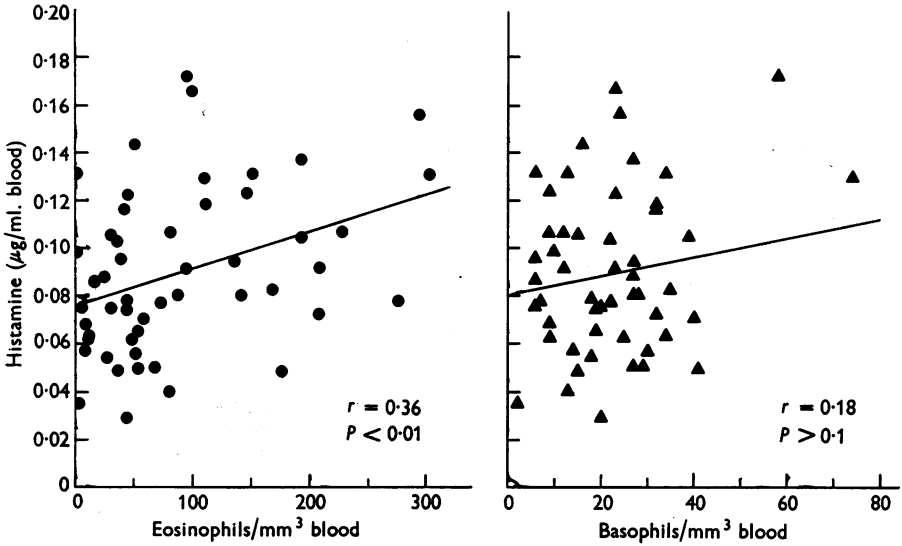

Fig. 6. Search for relationships in the concentration of histamine in the blood and the number of eosinophils (left panel) and the number of basophils (right panel) in the blood of fifty-one guinea-pigs. In each instance the concentration of histamine and the numbers of both types of cells were determined in the same samples of blood.

Since ample data on eosinophils were available, a final attempt to clarify the relationship of eosinophils and histamine was made by use of the estimates collected in the study. The 150 samples of blood tested in the first series and the 51 in the second were included in the final search for a relationship. These 201 samples of blood were grouped according to the eosinophil counts. Samples with counts of 0–50/mm³ blood were placed in the first group, those with counts of 51–100 in the second, those with 101–150 in a third and those with counts of more than 151 were placed in a last group. The mean number of eosinophils and the corresponding mean concentration of histamine found in each group of blood samples were calculated and plotted (Fig. 7). When the concentration of histamine was 0·06 or more μg/ml. and the eosinophils numbered 0 to 150, a direct relationship is indicated between numbers of

eosinophils and amounts of histamine in the blood (Fig. 7). The correlation coefficient of all the estimates comprising the three points was 0·44 ($P < 0·001$). With eosinophil counts of more than 150 there was a break in the relationship, and eosinophils containing much less histamine were present in the blood. Although not shown in Fig. 7, this was particularly true of the summer series which involved many multiparae with rather high counts and some with counts sufficiently elevated to be regarded as representing eosinophilia.

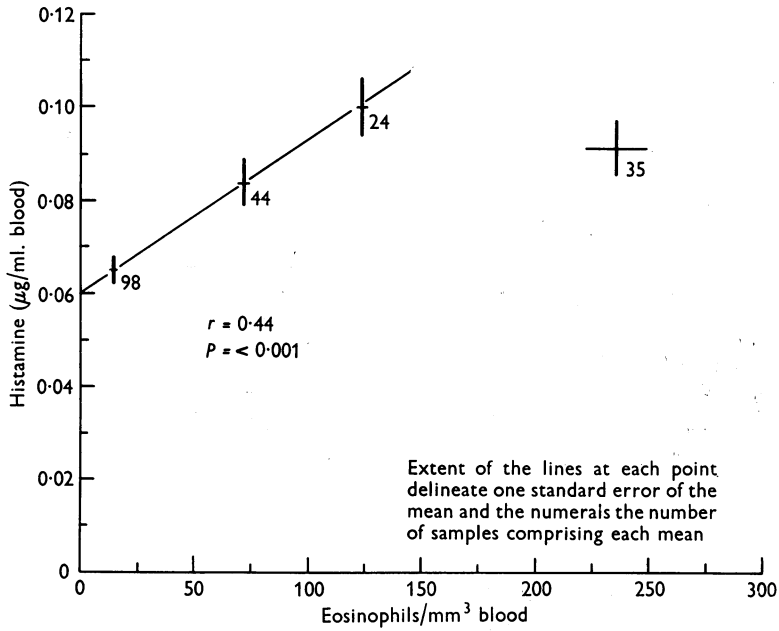

Fig. 7. Mean concentration of histamine and mean number of eosinophils in blood of guinea-pigs. Samples of blood were grouped according to eosinophil counts of 0–50, 51–100, 101–150, and >150/mm³ blood; vertical line and number at each point indicate S.E.M., and number of samples.

Extrapolation to zero of the line of linear regression for the counts up to 150 indicated again that in guinea-pigs an extra-eosinophilic compartment of the blood usually contains about 0·06 μg histamine/ml. The regression coefficient for these points indicated a mean load of 0·33 ng histamine per eosinophil for counts up to about 150. With counts of more than 150 the mean concentration of histamine per eosinophil must have declined steadily.

The following conclusions have been drawn from the data collected from all of the guinea-pigs we studied. From 50 to nearly 100% of the histamine extractable from the blood of guinea-pigs is outside both the eosinophils and basophils. In different groups of guinea-pigs the mean concentration of histamine in this compartment is usually 0·05–0·06 μg histamine/ml. blood, although the differences between individual animals may far exceed this range.

The eosinophils and basophils of the blood sometimes contain histamine but their association with it is by no means fixed. Although the data available for the comparison are somewhat meagre, they do indicate that of the two types of cells in the blood of guinea-pigs the eosinophils more often carry histamine. When the concentration of histamine in the blood rises above that usually found in the extra-eosinophilic, extra-basophilic compartment, the eosinophil numbers are also customarily elevated and a rough correlation may then be present between numbers of eosinophils and concentration of histamine in the blood. Nevertheless, since large numbers of eosinophils may be present without increase in the histamine, the load of histamine assumed by each eosinophil is variable and at times negligible or absent.

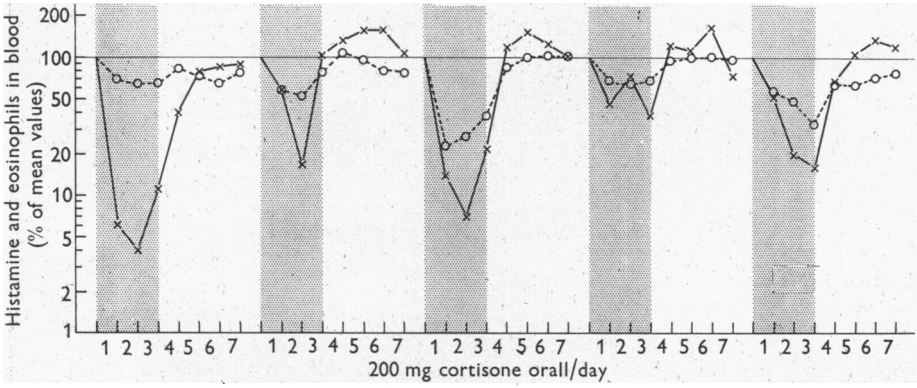

Fig. 8. Changes in the number of eosinophils (×) and the concentration of histamine (○) in the blood of five healthy men during and after ingestion of 200 mg of cortisone per day for 3 days. Values obtained on at least 3 pre-cortisone days constituted controls; shaded areas delineate periods of administration of cortisone.

Humans

Eosinophils and histamine. The ingestion of 200 mg cortisone/day for 3 days caused prompt and pronounced reduction in the numbers of eosinophils in the blood of each of the five persons who took the hormone, and the eosinopenia was always accompanied by a decisive decline in the concentration of histamine (Fig. 8). When administration of cortisone was discontinued, the eosinophils and histamine both returned toward the control values (Fig. 8). The parallelism, however, was most definite during the decline, for when administration of cortisone was discontinued, as in the dogs, the eosinophil counts sometimes rose temporarily above the control value without a corresponding rise in the values for histamine (Fig. 8).

The determinations of histamine in the blood drawn from a subject before, during and after administration of cortisone were always made on the same strip of guinea-pig ileum. When the quantitative assessments had been

completed, the contractions of the intestine to fixed doses of the standard solution of histamine were compared with those given by a fixed volume of each of the extracts. The tests clearly showed that the changes in histamine were in ranges which could be readily estimated (Fig. 9).

Plotting the concentration of histamine against the number of eosinophils found in each sample of blood from the five individuals indicated that within rather wide limits there was a relationship between the amount of histamine and the number of eosinophils (Fig. 10). The correlation coefficient of the values was 0·67 ($P < 0·001$). The mean amount of histamine carried per eosinophil, as estimated from the regression coefficient, was 0·16 ng ($P < 0·001$).

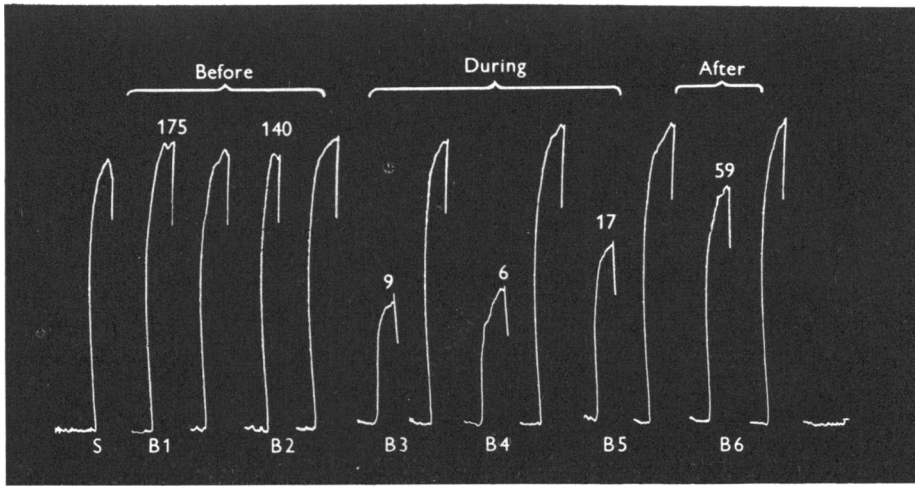

Fig. 9. Record of consecutive contractions of a strip of guinea-pig ileum in response to alternating additions of fixed volumes of a standard solution of histamine, S, and extracts of consecutive daily samples of blood B1–B6 taken from a healthy man before, during and after the administration of cortisone. The fixed dose of the standard was 0·15 ml. of a 1×10^{-7} strength solution of histamine and the fixed dose of the extract of blood was 0·3 ml. Numerals above contractions of ileum are numbers of eosinophils per mm^3 blood.

Extrapolation of the line of linear regression, which was calculated from the eosinophil and histamine values (Fig. 10), suggests that between 0·01 and 0·02 μg histamine/ml. blood would usually still be present even if all the eosinophils were absent. Thus, in the blood of normal persons, as well as in the blood of dogs and guinea-pigs, histamine may reside outside the eosinophils. The data, viewed as a whole, however, indicated a relationship between the amount of histamine and the number of eosinophils in the blood of the healthy persons we studied and that in these individuals the eosinophils were carrying some histamine.

Eosinophils, basophils and histamine. When the work of others indicated that basophils may contain histamine it was realized that the interpretation

just presented of the results obtained with eosinophils alone would become complicated if the administration of cortisone lowered the numbers of basophils as well as of eosinophils in the circulating blood. The first tests established beyond any doubt that cortisone did indeed reduce the numbers of basophils in a way similar to that in which it reduced the numbers of eosinophils (Code, Mitchell & Kennedy, 1954). When the second group of subjects took 200 mg cortisone/day by mouth for 3 days, the eosinopenic response was similar to that seen in the first series of tests (compare Figs. 8 and 11), the basophils decreased in every instance, but were less affected than the eosinophils and

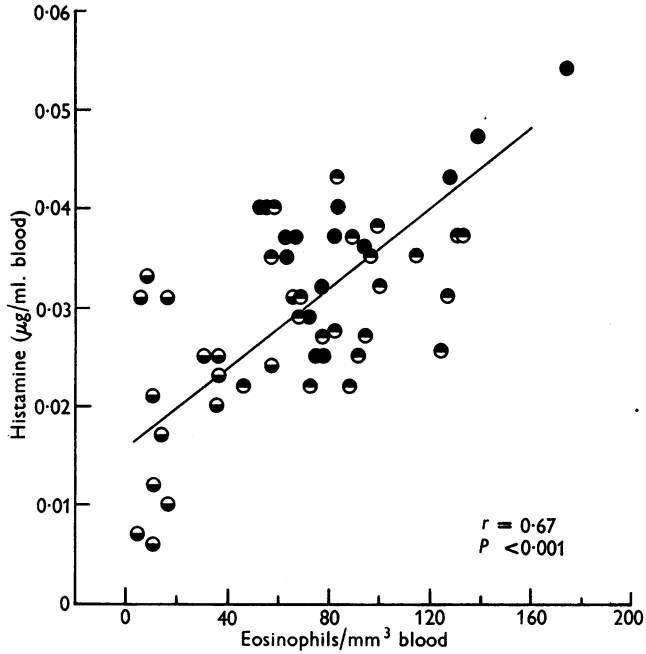

Fig. 10. Relation of the concentration of histamine and the number of eosinophils in the blood of five healthy men before (●), during (◒) and after (◓) the administration of cortisone.

the concentration of histamine always declined (Fig. 11). When the changes were expressed as percentage of control values, the parallelism was closer between histamine and basophils than between histamine and eosinophils (Fig. 11).

In an attempt to shift the relative degree of the decline in the numbers of the two types of cells and if possible to produce more drastic reductions in their numbers, 400 mg cortisone was taken by mouth by the five subjects in the third group. The numbers of both types of cells declined in a similar fashion but to a greater degree than when the smaller doses had been taken. The concentration of histamine was also more decisively reduced (Fig. 12).

The results of the last two series of tests, therefore, were combined and the number of eosinophils and the number of basophils occurring in each sample of blood were plotted separately against the concentration of histamine found in the sample (Fig. 13). A somewhat closer relationship was then noticed between the basophils and histamine than between the eosinophils and histamine. The

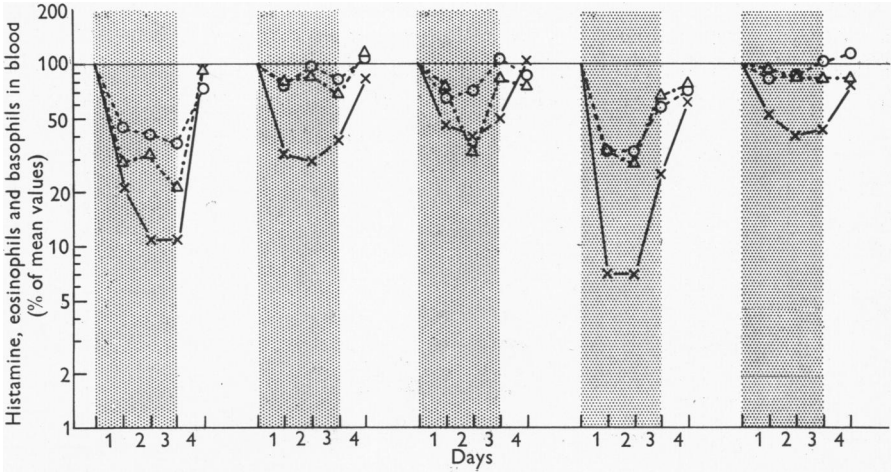

Fig. 11. Changes in the numbers of eosinophils (×) and basophils (△) and in the concentration of histamine (○) in the blood of five healthy men during and following the daily ingestion 200 mg of cortisone for 3 days. Determinations made on 2 or more days before the administration of cortisone were used for calculation of means of control values; periods of administration of cortisone are delineated by shaded areas.

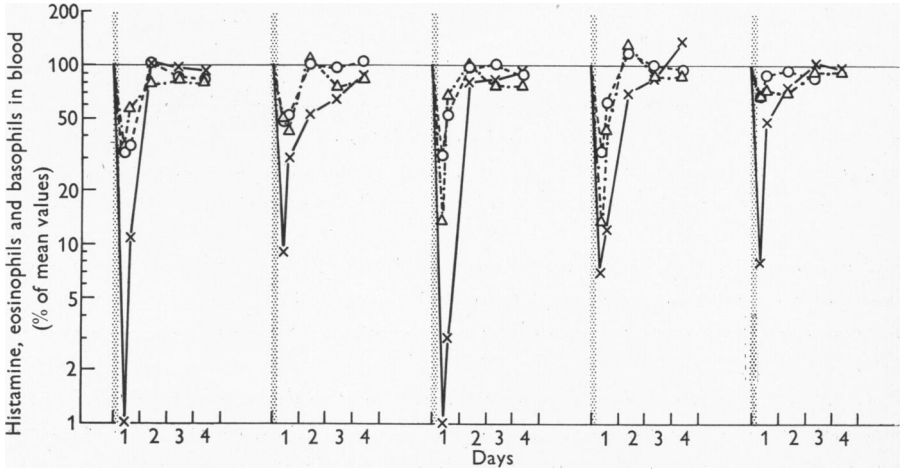

Fig. 12. Changes in the numbers of eosinophils (×) and basophils (△) and in the concentration of histamine (○) in the blood of five healthy men after the ingestion of 400 mg of cortisone; the shaded strip indicates time of taking cortisone. Mean control values were based on estimates made on at least 2 days before administration of cortisone.

correlation coefficients for both sets of data were, however, similar, and both were significant (Fig. 13). Extrapolation of the linear regression of the basophil-histamine relationship to zero basophils gave zero or nearly zero blood histamine (Fig. 13). But under the conditions of these tests no eosinophils would then have been present in the blood. Extrapolation of the linear regression of the eosinophil-histamine relationship to zero eosinophils did not indicate that the concentration of histamine in the blood would then be zero, but under the conditions of these tests some basophils would still be present in the blood.

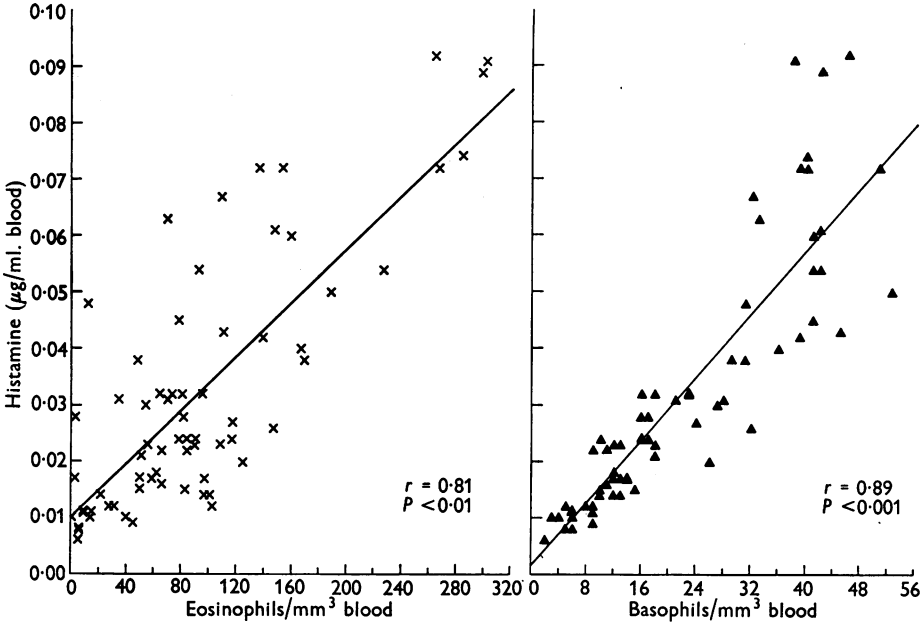

Fig. 13. Relationship between the concentration of histamine and the numbers of eosinophils (left panel) and the numbers of basophils (right panel) in the blood of eight healthy men before, during and after the ingestion of cortisone. The eosinophils and basophils were counted in the samples of blood which were used for the determination of histamine.

Because cortisone affected both eosinophils and basophils similarly, the precise partition of histamine between them could not be determined. Our data, however, support three main contentions concerning the blood of healthy humans: (1) that histamine is carried by basophils and that it can be and usually is also carried by eosinophils; (2) that the amount of histamine carried per eosinophil is less, and much more variable than that carried per basophil; indeed, the eosinophils may sometimes contain little or no histamine; and (3) that if both these types of cells are eliminated from the circulating blood by the action of cortisone, little, if any, histamine remains in the blood.

DISCUSSION

The eosinophil is apparently the main carrier of histamine in the blood of the dog, but the situation in the blood of guinea-pigs is much more complex. Our data indicate that much of the histamine in the blood of guinea-pigs is not associated with either the eosinophils or the basophils, although both these types of cells, and more especially the eosinophils, at times contain histamine. The load per cell seems to be variable. The physical boundaries of the other histamine-carrying component or components of the blood have not been clarified by our results.

The results obtained in healthy humans following the administration of cortisone support the general thesis that in man the eosinophils and basophils account for all or nearly all the histamine in the blood and that both types of cells may carry histamine. This conclusion is in direct agreement with the results reported recently by Graham *et al.* (1955).

When large numbers of eosinophils are present in the blood of dogs, guinea-pigs and humans, a break in the relationship to histamine often occurs. Under these circumstances the eosinophils may contain very little or no histamine. The administration of cortisone or ACTH under such conditions while reducing the numbers of eosinophils in the blood will have little or at times no effect on the concentration of histamine in the blood. This set of circumstances is most likely to occur in the presence of eosinophilia and probably pertained in the patients studied by Herbert *et al.* (1950) and Rose (1950).

Administration of cortisone offers an effective means of clearing the blood, of dogs and humans, of most of its histamine. Following such treatment eosinophils may return to the blood in rather large numbers but with little histamine in them. Cortisone has apparently 'unloaded' them or brought into the circulation 'unloaded' cells. Further study of these cells would be greatly aided by discovery of a means by which they could be forced to assume their maximal load of histamine. In humans the load of the basophils seems much more fixed than that of the eosinophils. In myelogenous leukaemia both types of cells, in their adult and immature forms, may apparently become maximally loaded with histamine (Code & MacDonald, 1937). Unfortunately the controlling factors and the mechanisms producing this circumstance are not known.

SUMMARY

1. A search has been made for possible relationships between the concentration of histamine and the numbers of eosinophils and basophils in the blood of adrenalectomized dogs, intact guinea-pigs and healthy humans. Cortisone was given to each of the species.

2. The administration of cortisone always reduced the number of eosinophils and basophils in the blood. In dogs and humans the concentration of histamine

in the blood also diminished. In guinea-pigs the effect on the histamine in the blood was less decisive.

3. The relationship between the concentration of histamine and the numbers of eosinophils and basophils in the blood was different in the three species. Evaluation of the results obtained in each led to the following conclusions:

(*a*) All or nearly all the histamine in the blood of dogs is carried by eosinophils but the amount associated with each eosinophil is variable and sometimes negligible.

(*b*) Some of the histamine in the blood of guinea-pigs is associated with the eosinophils and some may also reside with the basophils but the proportion of the total amount in the blood which is tied to these cells is variable. As a rule most of the histamine of the blood of guinea-pigs is not contained in either the eosinophils or the basophils.

(*c*) Most of the histamine in the blood of healthy humans is associated with the basophils and eosinophils. The amount carried by each eosinophil is less and much more variable than that associated with each basophil. Sometimes the eosinophils of humans contain no histamine.

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