THE NATURE, SIGNIFICANCE AND MECHANISM OF EOSINOPENIA CAUSED BY ADRENOCORTICAL STIMULATION.

BY.

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INTRODUCTION.

Eosinophils.

Eosinophils form only a small proportion of the white blood cells. They have, however, aroused the curiosity of many investigators during the past hundred years. According to Rud (1947), an Englishman, Wharton Jones, described coarsely granulated cells in the blood in 1846; Max Schultzc first described the eosinophile cell as a morphological entity, while Ehrlich in 1878 carried out staining procedures to demonstrate their acidophile characterstic and named them eosinophils.

Apart from their staining properties, eosinophils differ from neutrophil polymorphs in three distinct ways. Firstly, their nuclei are more regular in shape with, in the majority of cases, only two well-formed lobes; secondly, their granules are distinctly coarser than that of the neutrophil; thirdly, the average size of the cells is slightly greater.A picture of an Eosinophil demonstrating the first two points of distinction is shown in Plate I.

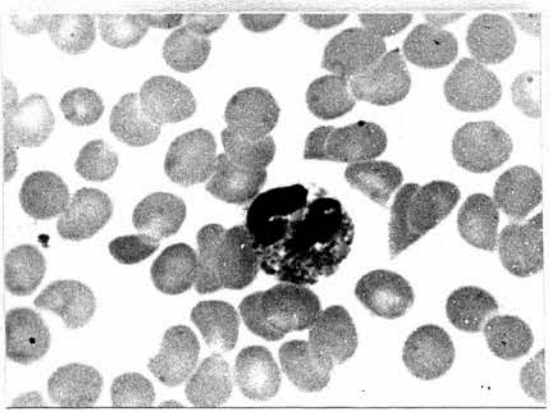


PLATE I.

Examples of a staff and three-lobed cells are shown in Plates II and III. These are less common and are found mainly in cases of eosinophilia.

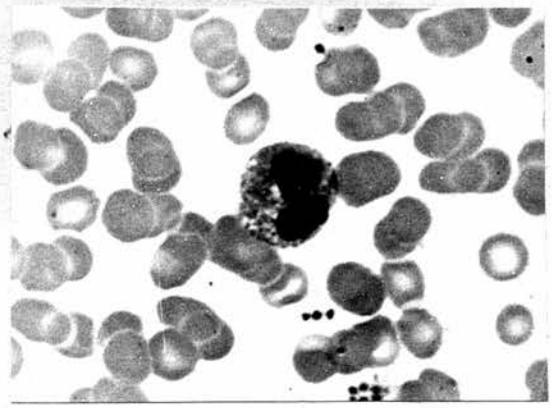


PLATE II.

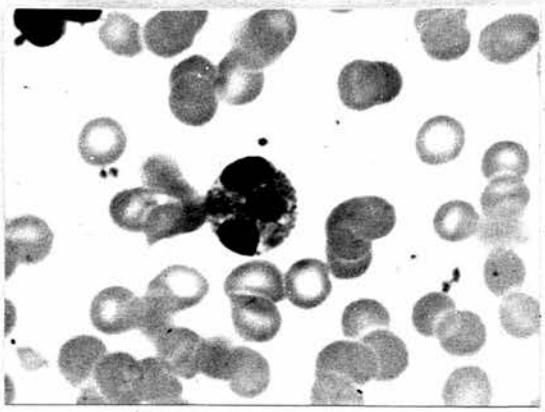


PLATE III.

The fact that eosinophils are quite distinct entities of the blood, and not merely products of artificial staining methods, is confirmed by information on their chemical constituents. At one time, Code (1937) considered that the cells were rich in histamine, but this view has been fairly conclusively proved incorrect by Valentine, Pearce & Lawrence (1950). One of the major constituents is iron, and there is also a high proportion of lipoid in the granules. The cells are more resistant

to lysis than their brother leucocytes, and according to Osgood (1937) their expectation of life is greater than neutrophils.

In a brief review (Cape 1952) an attempt was made to assess present knowledge of eosinophils. This knowledge has been obtained mainly by a study of eosinophilia from both a clinical and experimental point of view. It points to the influence of "foreign protein" as the main stimulus to an increased level of eosinophils in the blood. Consideration of the many varied causes of eosinophilia shows that, perhaps without exception, this element of "foreign protein" is present: such cases range from asthma to skin diseases, drug intoxications to parasitic infestations, and certain ill-defined groups of disorder such as polyarteritis nodosa, Loeffler's syndrome and Tropical eosinophilia. On the other hand, a disappearance of eosinophils from the blood stream has been noted in a variety of conditions. Rud (1947), quotes Naegeli as setting down five groups of pathological conditions which produce this effect. These are firstly, acute infectious diseases, with some exceptions, followed by postinfectious eosinophilia; secondly all intoxications in their early stages; thirdly, all large operative incisions; fourthly, pernicious anaemia and finally, certain internal secretory disturbances. Emil Schwarz (1914) noted the occurrence of eosinopenia and linked this with the endocrine system.

The concept of the Adaptation Syndrome introduced by Hans Selye was, however, the first clear account of the

cause of this eosinopenia; Dalton and Selye (1939) included the reaction as a constant finding during a condition of stress. During the past fifteen years this "eosinopenic" effect of stress has been shown by many authors to be due to stimulation of the suprarenal cortex by corticotrophin from the anterior pituitary.

Link between Eosinophils and the Endocrine Glands.

The work of Selye followed by that of Thorn, Forsham, Prunty and Hills (1948) has clearly established that the administration of corticotrophin has a specific effect on the eosinophils. The nature and mechanism of this effect is the subject of the studies which are described below. Before considering them, some further general comments on links between eosinophils and the endocrine system provide a background to the experimental work.

Rud (1947) has shown that there is a persistent diurnal variation in the levels of eosinophils. This observation has been confirmed by Swanson et al. (1952), Bonner (1952) and others. It seems that the level of eosinophils rises and falls with the delicate rhythm of the homeostatic mechanisms of the body. There is a morning increase in urinary excretion of lipoid soluble reducing steroids which exceeds the morning increase in glomerular filtration rate, (Goldman & Bassett, 1952). This suggests increased suprarenal cortical activity at that time, perhaps due to the "shock" of getting out of bed! Such an increased activity may be linked with the falling numbers of eosinophils found in the blood during the forenoon period.

The current belief is that the hypothalamus exerts a controlling influence on homeostasis. Hume (1949) has demonstrated by skilful experiments that this area plays an essential part in the mechanism by which stresses produce adrenocortical stimulation and eosinopenia. Fig. I is a

digrammatic representation of his results. It should be noted that, while the pathway from injured area to the hypothalamus is a nervous one, he postulates a humoral connection between the hypothalamus and the anterior pituitary.

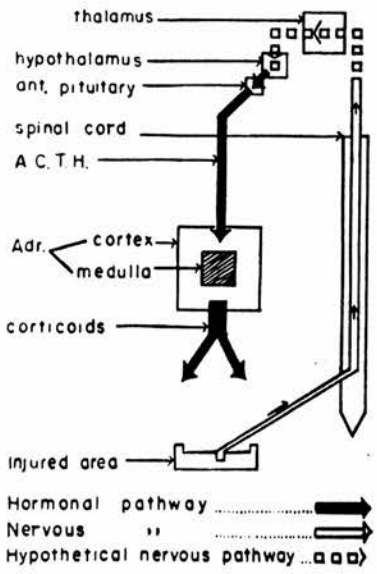


FIG. I.

Earlier writers, of whom Rud quotes Eppinger, Hess Schilling and others, suggested that the eosinophils were under the control of the autonomic nervous system. They described a vagotonic blood picture characterised by lymphocytosis and eosinophilia and a sympathicotonic picture in which the numbers of lymphocytes and, more particularly, eosinophils fell. More recent work has confirmed the accuracy of these observations but has shed new light on the mechanisms involved. It would now seem likely that the controlling influence on the levels of eosinophils, and most probably of all the white cells, is a humoral one.

Aims of the Present Studies.

In the series of studies which are described below, it is intended to describe firstly, the effect on the eosinophils of the blood of giving corticotrophin, and, secondly, an attempt to discover the mechanism of this effect. The studies are, therefore, divided into two groups.

Methods.

One essential was to obtain a satisfactory stimulation of the adrenal cortex. This was acheived by giving ACTH in an intravenous infusion. McIntosh (1951) has shown that intramuscular ACTH may be destroyed at the site of injection before it has an opportunity to be absorbed. It was considered that a slow, even intravenous drip would give a consistent stimulating action on the supratenal cortex. In all cases a fresh vial of ACTH (Armour) was dissolved in sterile water and added to 500 c.c. of 5% dextrose in water immediately before administration. This ensured that the ACTH was fresh and active.

The method used for counting eosinophils was a modification of the method first used by Dunger (1910). This method has been criticised by Henneman, Wexler and Westenhaver (1949). They claim a large and rapid decrease in cell count with passage of time after dilution of blood in the white cell pipette; a similar decrease was noted when repeated counts were performed on oxalated blood samples kept for 24 or more hours.

These findings have not been confirmed by my personal experience. While there is no doubt that if the diluted blood is left for periods of 1 hour or more, a decrease in numbers of eosinophils will occur, no significant drop occurs for periods of less than that time. One feels that too much reliance cannot be placed on Henneman's results, as our "in vitro" study of the effect of adrenal steroids on eosinophils has shown that the reduction in numbers of eosinophils in a sample of oxalated blood is not significant after 18 hours incubation at 37 C. For experimental work, when it is possible to perform the eosinophil counts shortly after taking the blood, the Dunger method, gives a clearer definition of the cells for counting purposes, and is preferable to that of Randolph (1944). All counts were carried out 5 - 15 minutes after the specimen of blood was diluted with the cosin-acetone mixture. This mixture was kept below 4 C in a refrigerator to keep it fresh, and was renewed frequently. I have personally performed well over a thousand eosinophil counts using this method and have felt satisfied with it.

Details of the various methods used are given in the appendix.

THE EFFECT OF THE ADMINISTRATION OF ACTH ON EOSINOPHILS.

a. Qualitative.

This effect is now well known, and is the basis of the Thorn Test, (Thorn et al.1948) of adrenocortical function. Fig.2 illustrates the effect of giving a patient an intravenous infusion of 500 c.c. 5% dextrose in water containing 10 mgm. of ACTH over a period of 8 hours. The numbers of eosinophils present falls, at first slowly, then during the third to fifth hours more rapidly, and finally levels off as it approaches zero. In patients with a normal suprarenal cortex, the drop in numbers of eosinophils is such that after 4 hours of stimulation there will be less than 50% of the original eosinophils remaining in the blood stream.

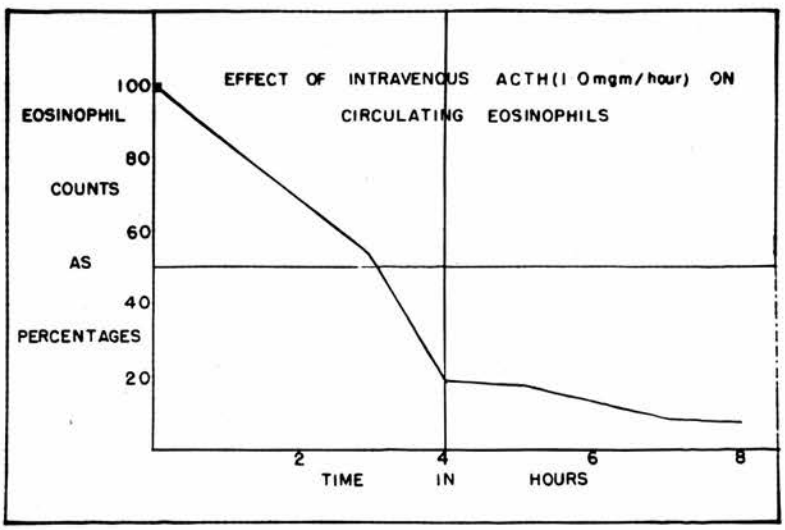


FIG. 2.

b.Quantitative.

Further study of the quantitative aspects of this reaction has been undertaken (Cape, Carruthers, Robinson, Palmer & McIntosh 1952). The technique used was to vary the amounts of ACTH in the infusion and compare the results obtained by plotting them on a graph. Five individuals were studied. All received a series of infusions, with an interval of 3-5 days between each to exclude the possible occurrence of progressive adrenocortical hyperplasia. The total amounts of ACTH in the infusions varied from 0.4 mgm to 16.0 mgm. The rate of the intravenous drip was regulated to allow the 500 c.c. infusion to run in over a period of 8 hours. The dose given was then expressed in mgm/hour.

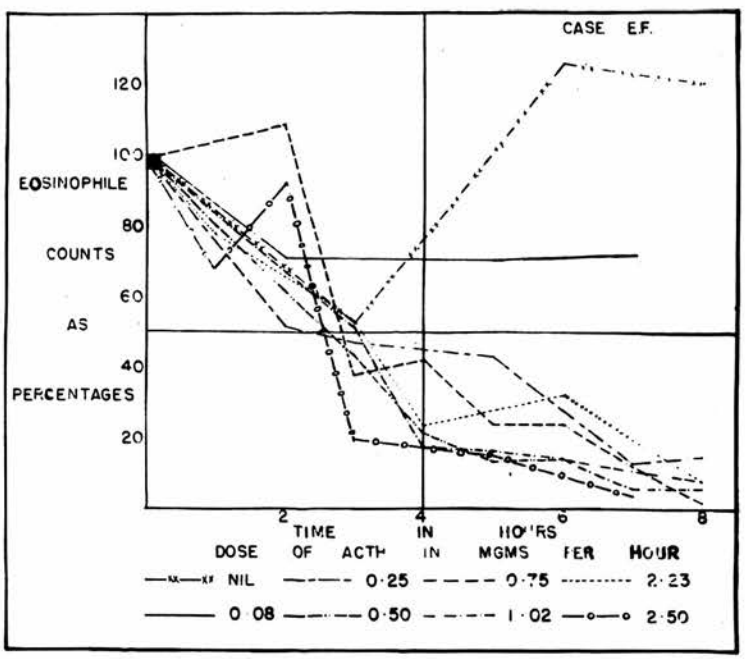


FIG. 3.

Fig. 3 shows the results in the first case. It will be seen that the graphs fall into two main groups. The

first group representing nil and 0.05 mgm per hour shows no progressive fall in the number of eosinophils. The second is that in which a fall of 50% or more has occurred within 4 hours; it includes all doses of 0.10 mgm per hour and greater.

The second case (Fig.4) shows a very similar picture.

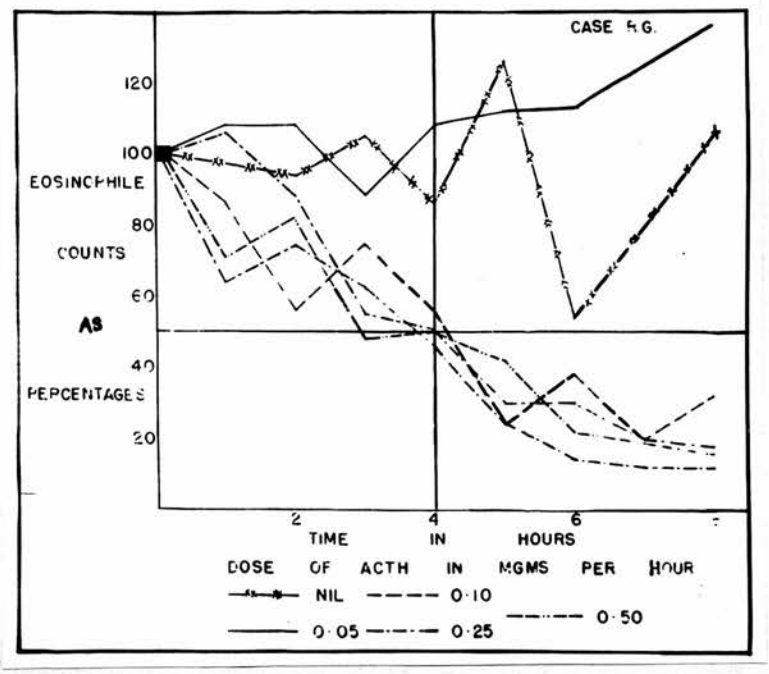


FIG. 4.

The two cases illustrate that, once a fully effective dose is given, further increases do not effect the rate and amount of fall; for example, in the first case 0.25 mgm per hour produces as rapid a fall as 2.5 mgm per hour. Minor differences in the curves are not considered significant, being due to inaccuracies inherent in counting eosinophils.

These two patients were studied personally. Full details of the individual eosinophil counts are given in

The other three cases showed similar results. In two of these an intermediate group is seen between those showing no response and those showing a full response. This intermediate group was present over a small range of dosage, varying from 0.075 mgm per hour to 0.175 mgm per hour. (Figs.5 & 6) The final case is illustrated in Fig.7.

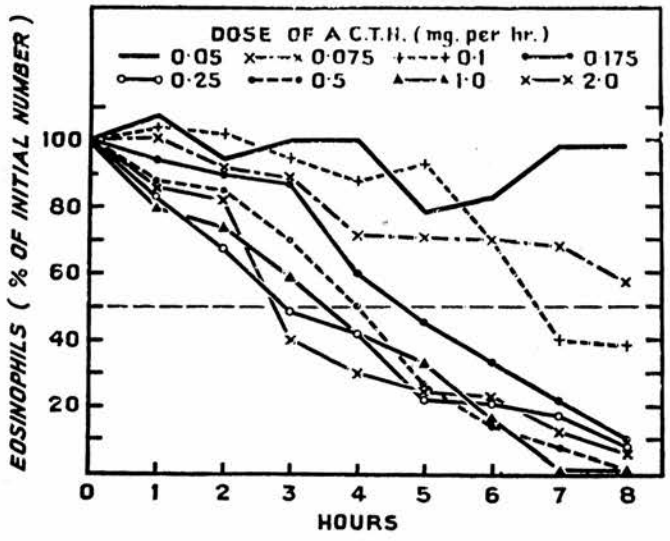


FIG. 5.

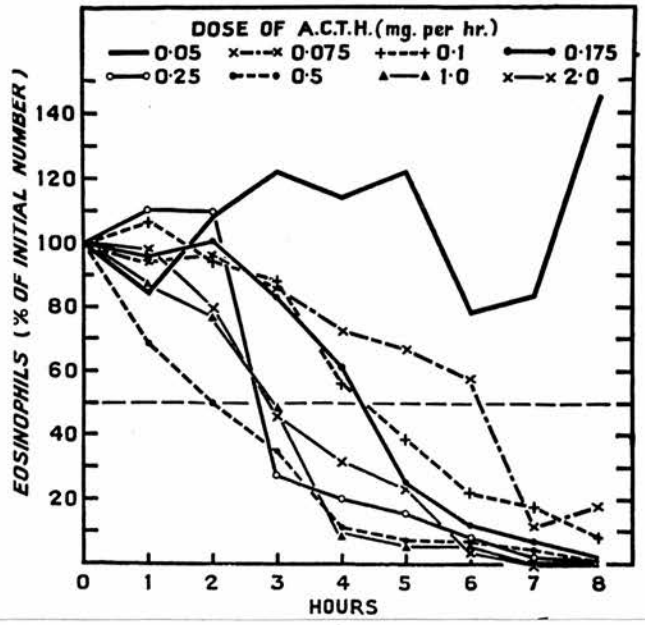


FIG. 6

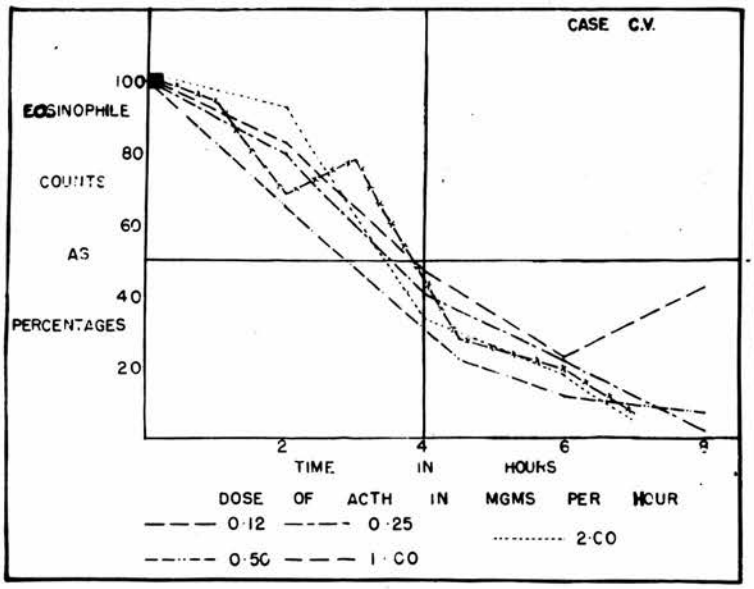


FIG. 7.

The results obtained show that the minimum effective rate of administering intravenous ACTH to produce a maximum fall in the level of circulating eosinophils in the minimum period of time lies between 0.1 mgm per hour and 0.25 mgm per hour in the five patients studied. As a corollary to this it would seem logical to suggest that the amount of ACTH produced by the pituitary in response to stress, over and above the normal amount secreted, must be at least 0.1 to 0.25 mgm per hour. While the minimum effective doses found in the five cases were all of the same order, there were minor variations and it is likely that each individual has his own critical level at which a full eosinopenic response occurs. One should also state that it was not possible to use the same batch of ACTH in all cases, although the same batch was used in all administrations to the same patient. This may account for some of the differences

from individual to individual. From a quantitative point of view there was only a very small difference between a completely non-effective dose and a fully effective one.

The Effect of the Administration of intravenous ACTH to a Splenectomised Patient.

The same technique as that described above was employed to study one patient who had a splenectomy performed about six months previously. She was a young girl of 21 with severe crippling rheumatoid arthritis present since childhood. This had been accompanied latterly by a low total white count and palpable spleen. The latter was removed in the hope of improving her condition, without much success. Following the operation she developed an eosinophilia. The results obtained in her case are shown in Fig. 8.

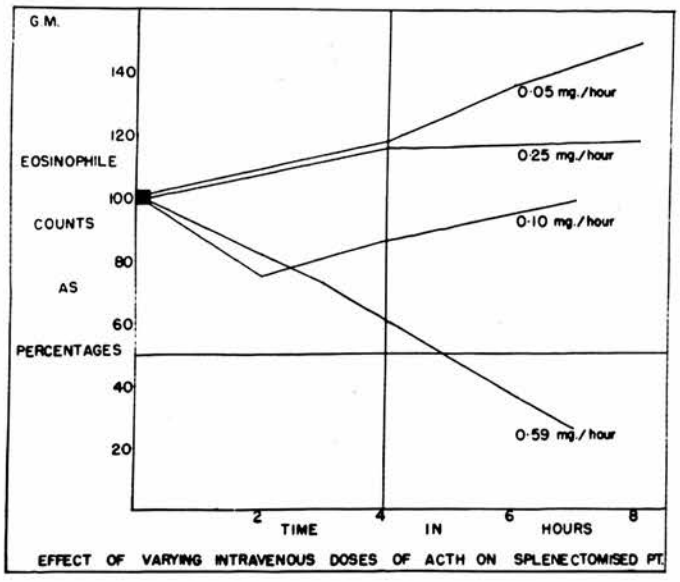


FIG.8.

Unfortunately it was not so detailed as in the other cases, because counting of eosinophils was made technically more difficult by massive rouleaux formation of red cells and increased resistance on their part to lysis under the i influence of the staining fluid. Both Dunger's and Randolph's methods were tried with the same results. Finally 1000 cell differential counts were carried out on peripheral blood, and total white cell counts, from which the numbers of eosinophils were calculated. Bonner (1952) found this method satisfactory, provided 800 cells were counted in the differential. It is interesting to note that the minimum effective dose of ACTH to produce eosinopenia in this case is appreciably greater than in the other cases. Again the differences in batch of ACTH must be remembered.

Effect of ACTH on Eosinophils in a case of Addison's Disease.

One patient with Addison's Disease was given an infusion containing 16 mgm ACTH over 8 hours. Eosinophil counts were performed before and 2, 6 and 8 hours after the beginning of the infusion. No eosinopenic effect occurred. The results are shown in Table I. This observation confirms many earlier reports and illustrates that the eosinopenic effect occurrs only in the presence of a normal adrenal cortex.

Hours after beginning ACTH Infusion.(2mgm/hr)	Eosinophil Counts.
0	409
2	429
6	507
8	443

Table I. The effect of ACTH on Eosinophils in Addison's Disease.

Effect of ACTH administration on the eosinophils of patients with an eosinophilia.

In my experience the presence of eosinophilia of slight or marked degree does not alter the reaction to intravenously administered ACTH to any extent. This is true whatever the cause of eosinophilia. The only difference lies in the fact that the 50% level may not be reached in 4 hours in some cases. Three examples of this are shown in Table II. The most dramatic effect was seen in the patient No.2 who suffered from a severe iododerma with a marked eosinophilia (Cape 1954). The first case was considered to have a Loeffler's Syndrome, and the third was a young boy with a severe reticulosis.

Patient Number.	Cause of Eosinophilia.	Time after commencement of ACTH.	Eosinophil count.
1.	Loeffler's Syndrome.	0 hrs.	1,069
		4 hrs.	676
		8 hrs.	149
2.	Iododerma.	0 hrs.	8,866
		4 hrs.	5,316
		8 hrs.	1,772
3.	Reticulosis.	0 hrs.	1,331
		4 hrs.	912
		8 hrs.	440

Table II. Effect of intravenous ACTH on Eosinophilia.

The Significance of Eosinopenia.

From the above studies it is fair to suggest that the eosinopenic effect of corticotrophin forms a sensitive test of adrenocortical function. Expressed in another way, a significant fall of eosinophils in the peripheral blood is an indication that the adrenal cortex has been stimulated and is putting out an increased secretion. It does not, however, indicate the degree of stimulation.

McIntosh and Holmes (1951) have shown this conclusively. They gave two patients varying amounts of ACTH in a continuous intravenous infusion over 24 hours. The amounts varied from none to 160mgm. A full eosinopenic response was obtained with 5mgm in 24 hours (approximately 0.21 mgm per hour cf. above). They also measured the urinary output of 17-Ketosteroids as a measure of suprarenal cortical activity. This excretion increased steadily with doses of 2.5, 5, 10 and 20 mgm per 24 hours, but 40, 80 and 160 mgm doses

showed proportionately much less marked increases. Similar results were obtained by Renold, Forsham, Maisterrena and Thorn (1951). They gave 20 mgm ACTH intravenously over an 8 hour period on five consecutive days. Eosinophil counts were performed before and 10 hours after the beginning of each infusion. 17-Ketosteroid excretion was measured over each 24 hours period. The eosinophil count fell to zero on the first day and subsequent days, but the 17-Ketosteroid excretion increased progressively till the third day, after which the level remained the same. These results show that the adrenal cortex was stimulated to a greater degree than that required to produce eosinopenia.

More direct evidence was obtained by Nelson, Samuels, Willardson and Tayler (1951). They describe a method of estimating 17 - hydroxycorticosteroids in the blood, these substances presumably emanating from the suprarenal cortex. The administration of 15 mgm ACTH in a continuous drip over 24 hours raised the level of these substances to 40 microgrammes per 100 ml; while 5 mgm, administered in the same way over the same period raised the level to 30 microgrammes per 100 ml. Information about eosinophil counts is not given, but from the studies described above, one can assume both these amounts would produce a full eosinopenic response.

Summary of the effect of the administration of ACTH on Eosinophils.

The above studies show :-

1. Administration of ACTH to a person with a normal suprarenal cortex results in a

progressive fall in the level of circulating eosinophils of 50% or more in 4 hours, while after 8 hours continuous intravenous ACTH the level is only about 5-20% of the original one.

2. The minimum rate of intravenous ACTH required to produce this effect was from 0.1 - 0.25 mgm per hour in the five cases studied.

3. Administration of increased rates of intravenous ACTH do not alter the eosinopenic effect. They will, however, result in an increase in adrenocortical activity, measured by the urinary excretion of 17-Ketosteroids, and blood levels of 17-hydroxycorticosteroids.

4. A fall in circulating eosinophils is thus a sensitive indicator of adrenocortical stimulation, but it does not imply a maximum or "all-out" effort on the part of the gland.

THE MECHANISM OF THE EOSINOPENIA.

It is established that a hormonal substance, or substances, is produced by the adrenal cortex after stimulation of this gland by corticotrophin, which substances cause a marked drop in the numbers of circulating eosinophils. Three hypotheses may be put forward to explain how this occurs. These are :-

1. There may be a peripheral intravascular lysis of the cells;
2. There may be a sequestration of the cells from the blood stream into a tissue or tissues, temporarily or permanently;
3. There may be inhibition of production or release of the cells in the bone marrow.

The latter possibility might be associated with, or combined with, either or both of the first two.

'In Vitro' Studies.

If a peripheral intravascular lysis of eosinophils occurs it would seem likely that a similar action on the cells would take place 'in vitro', if they were exposed to the same hormonal substance. In collaboration with Dr. Thomas and Dr.Palmer, the author has investigated this possibility (Cape, Thomas, Palmer, 1952). Two series of observations were made. The first was a study of the effects of plasma taken from patients with an ACTH-induced eosinopenia on the eosinophils of blood from a second individual. Such

specimens of plasma will be referred to as 'eosinopenic plasma' for the sake of clarity. The second was a study of the direct effects of adrenal steroids on eosinophils.

1. The Effect of 'Eosinopenic Plasma' on Eosinophils.

In this series of experiments, 'eosinopenic plasma' was obtained in ten instances from patients who had been receiving intravenous ACTH for at least six hours, and whose eosinophil counts had dropped to very low levels. It was presumed that at this time the plasma from such patients must contain potent eosinopenic material derived from the stimulated adrenal cortex. Samples of blood were obtained from a selection of donors. Eosinophil counts were performed on each sample, after which they were divided into two portions. To one was added 'eosinopenic plasma', the second was used as a control. All samples were incubated at 37°C in an air oven for from 4 - 18 hours. Further eosinophil counts were carried out on all samples after 4 hours, on three samples after 8 hours, and on one sample after 18 hours.

The first incubation was carried out using one part of plasma to one of donor blood. It was considered, however, that dilution of the humoral substances present might be sufficient to inhibit a lytic action, and all subsequent incubations were performed using nine parts of plasma to one of donor blood.

From the first six incubations and the accompanying six control specimens, blood films were prepared and stained by Wright's stain.

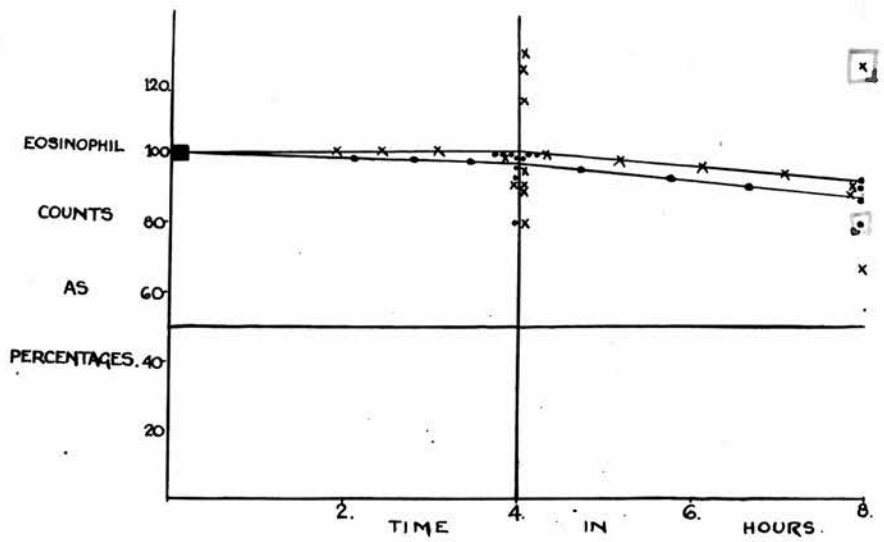
Duration of incubation (hrs.)	Eosinophil count/c.mm. Before incubation	After incubation. Control blood.	After incubation. Blood + Plasma.
4	3,275	3,220	3,244
4	2,600	2,697	2,664
4	66	66	66
4	660	650	770
4	55	55	44
4	111	111	100
4	455	422	555
8		422	333
4	122	122	111
8		111	111
4	166	133	222
8		144	111
4	333	333	300
18		266	422

Table III. Effect of Plasma from Patient with ACTH-induced Eosinopenia on Eosinophils of Donor Blood.

The detailed results are shown in Table III, and are graphically represented in Fig.9. It will be seen that no alterations occurred in the eosinophil counts after the incubations. Careful study of the blood films revealed no morphological changes between the eosinophils of control or diluted blood after the incubations. The mean lines shown in Fig.9 of the control & experimental counts, both show a slight decrease at the end of 8 hours. This is presumably due to destruction of a small proportion of cells caused by repeated handling and shaking of the blood samples. There was also a reduced degree of accuracy in the counts on

the diluted blood, which the results illustrate. In all cases other than the first two, where the count was high, four or more Fuchs-Rosenthal chambers were counted.

X Represents counts on blood + plasma, dots the control counts. Crossed is mean of former, dotted line of latter.



□ . Counts after 18 hours.

FIG. 9.

Thus using the technique described, no evidence of a destructive effect on eosinophils by 'eosinopenic plasma' was demonstrated.

2. The Effect of ACTH, Cortisone and Compound F on Eosinophils in vitro.

In this series of observations a suitable amount (see Appendix) of one of the three hormones, ACTH, cortisone and Compound F, was added to 5cc of blood, and to one series of specimens a mixture of ACTH and cortisone. The same technique of incubation and repeated eosinophil counts was

followed as in the previous study.

Hormone used.	Concentration hormone mgm/100 c.c. blood.	Eosinophils/c.c. after incubation 0 hours.	4-6 hours.	8-10 hours
Cortisone	0.68	122	111	
"	"	341	260	272
"	"	198	189	161
"	"	212	237	198
"	"	126	147	111
ACTH	0.06	198	183	172
"	"	212	191	186
"	"	126	118	111
"	"	182	194	161
"	"	255	399	
ACTH and cortisone.				
"	0.68	198	215	180
"	"	212	169	175
"	"	126	95	100
"	"	205	205	177
"	"	55	44	
Compound F.	0.68	293	250	260
"	"	127	115	122
"	"	146	138	132
"	"	135	151	121
"	"	106	93	105
Cortisone.	20.0	143	111	122
"	"	105	111	110
"	"	176	194	183
"	"	110	73	100
"	"	321	366	312
Compound F.	20.0	143	122	153
"	"	67	57	72
"	"	270	267	300
"	"	161	158	139
"	"	237	222	219

Table IV. Effect of Hormones on Eosinophils after Incubation.

The results obtained are shown in Table IV, and are represented graphically in Fig.10. The tests were repeated

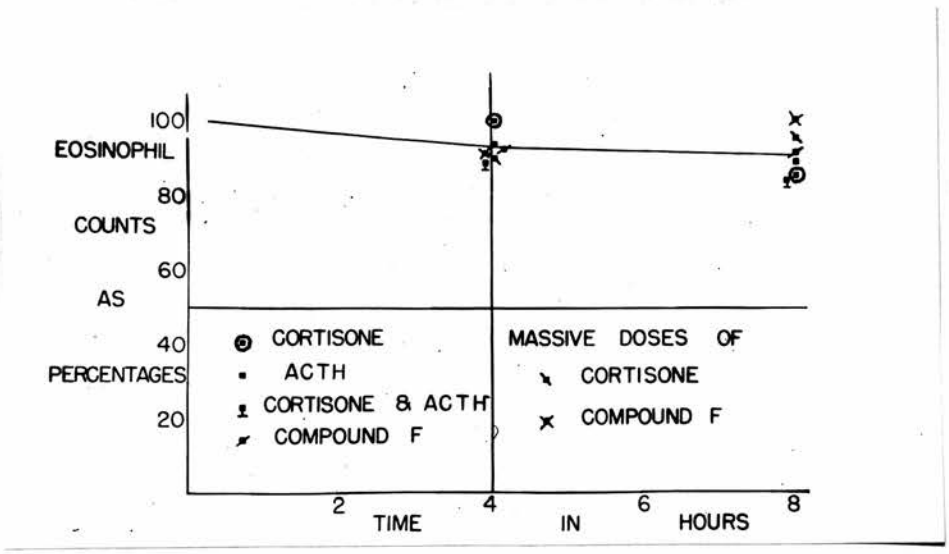

100
EOSINOPHIL
80
COUNTS
60
AS
40
PERCENTAGES
20
CORTISONE
ACTH
CORTISONE & ACTH
COMPOUND F
MASSIVE DOSES OF
CORTISONE
COMPOUND F
2
4
6
8
TIME IN HOURS

FIG. 10.

twice with cortisone and Compound F as it was felt that the amounts of the hormones used in the first series might be insufficient.

It can be seen that in no instances was there a significant alteration in the numbers of eosinophils present. As in the previous study no significant destruction of eosinophils was found.

3. The Role of Anticoagulants.

Anticoagulants were of necessity used in both these studies. In most cases this was a mixture of potassium & ammonium oxalate (Heller & Paul 1934). While this could theoretically interfere with the hormones' activity, there is no evidence that it does. In the remaining incubations herapin was used. Godlowski (1951) has shown that full heparinisation will prevent the eosinopenic response to ACTH occurring. His contention was that heparin brings eosinophils stored in the tissues, into the blood stream, thus counteracting the eosinopenic effect of adrenal steroids. This action could not occur in the test-tube studies described. Further reference will be made to this later.

Muehrcke, Lewis and Kark (1952) reported that when 'in vitro' tests were carried out on defbrinated blood a fall in eosinophils occurred when cortisone and compound F were added to the sample. Their results have not been confirmed in a brief study carried out with this object in view.

Two samples of blood were taken from one patient. The first was added to an oxalate anticoagulent mixture, while the other was defibrinated by shaking up with a few small glass beads. The addition of compound F, cortisone free

alcohol, and crystalline cortisone acetate to the samples produced no significant fall in eosinophils after 5 hours incubation. The results are shown in Table V. These show two interesting features; firstly, the defibrinated blood had lost about 50% of its eosinophils compared with the oxalated blood before any hormones were added, and secondly; the greatest drop in eosinophils, about 30%, occurred in the oxalated sample to which cortisone free alcohol was added. This latter was not however considered significant as it was less than 50%.

Hormone used.	Anticoagulant method.	Eosinophil counts. Before Incubation.	After Incubation (5hrs.)
Cortisone Acetate.	Potass.& Amm. oxalate.	1085	924
	Defibrinated.	536	631
Cortisone Free Alcohol.	Potass.& Amm. oxalate.	1085	770
	Defibrinated.	536	618
Compound F.	Potass.& Amm. oxalate.	1085	1005
	Defibrinated.	536	651

Table V. Effect of Hormones on Eosinophils of defibrinated and Oxalated Blood.

4. Conclusions from 'in vitro' studies.

The results obtained confirmed ~~earlier~~ other reports by ~~Forsham~~ Swanson, (1952), ~~Finch et al~~ and Baldridge et al (1951) of similar studies. It is considered that if the eosinophils were destroyed by adrenal hormones, at least one half would have disappeared after 4 hours incubation. Not only did this not occur but there was consistent trend to suggest that a slow but progressive diminution in numbers of eosinophils was

taking place. While admitting that conditions in a test-tube are artificial, the evidence does not suggest that lysis of eosinophils is a satisfactory explanation for their disappearance following suprarenal cortical stimulation. The conflicting evidence on the part anticoagulants and fibrin play, does not clarify the situation.

Studies of "Phase of Reappearance" of Eosinophils.

If all the mature eosinophils circulating in the peripheral blood are destroyed by adrenal steroids, it would be logical to expect the cells reappearing in the blood stream after the adrenocortical stimulation had ceased, to be new and immature ones. On the other hand, if the eosinophils were temporarily removed from the blood stream, and the same cells returned after the increased levels of steroids had fallen, one would expect the returning cells to be older than before. An investigation was carried out to determine whether there was any evidence in support of either of these hypotheses.

During the period following cessation of ACTH administration, 1000 cell differential counts were performed on blood films prepared at frequent intervals, until the total eosinophil count had returned to the level present before the hormone was given. Five patients were studied in this way. To determine the maturity of the eosinophil cells the number of lobes in the nucleus was counted, the cells being recorded as staff, 2-lobed, 3-lobed etc.

The results obtained are shown in Table VI.

Case No.	Time in hours after stopping ACTH	Eosinophil count.	Types of Eosinophil present per 1000 cells.			
			Staff.	2 lobed.	3 lobed.	4 lobed.
1.	0	1069	7	52	28	2
	2	179	2	14	13	1
	4	144	2	12	1	0
	6	332	2	13	4	1
	8	468	1	24	7	1
	13	329	2	15	7	1
	15	505	3	35	10	1
	17	1007	5	42	22	2
2.	0	467	1	75	5	0
	5	0	0	1	0	0
	10	100	0	8	1	0
	14	376	1	24	1	0
	20	485	7	28	4	0
3.	0	53	1	17	5	0
	4	0	0	0	0	0
	7	14	0	1	0	0
	10	40	0	2	2	0
	13	51	0	3	1	1
	30	112	1	13	3	0
4.	0	250	4	33	6	0
	10	37	0	2	0	0
	14	104	1	3	0	0
	18	104	2	11	2	0
	22	183	6	10	5	0
	33	512	6	32	12	2
5.	0		8	29	12	0
	9		0	0	0	0
	21		0	3	2	0
	24	177	0	22	6	0
	27		3	3	3	0
	30	296	1	8	6	0
	36	445	3	12	3	1
	48	559	5	32	9	0

Table VI. Study of Reappearance of Eosinophils after ACTH stopped. At 0 are shown the counts immediately before beginning ACTH.

The first two cases were given an 8-hour intravenous infusion, while the other three cases were given longer courses of intravenous ACTH, infusions running from 12-20 hours in each 24,

for 8 - 10 days. This latter method is more than sufficient to maintain a constant adrenocortical stimulation over the 8 - 10 day period. The results obtained do not show any significant difference in the proportion of staff eosinophils to the more mature cells during the phase of reappearance from that present before the administration of ACTH. In other words, there is no evidence to support an increase in new cells, whether the period of adrenocortical stimulation is 8 hours or 10 days.

In the first two cases the "phase of reappearance" lasted 17 and 20 hours respectively. In the other three cases this period was longer, 30,33 and 48 hours respectively. This can be related to the fairly rapid falling-off of increased adrenocortical secretion after stopping the intravenous flow of corticotrophin. Nelson et al. (1951) found that four hours after a 24-hour intravenous infusion of ACTH was stopped the level of 17-hydroxycorticosteroids in the blood fell from 40 mcgms to 12 mcgms %. Eosinophils were reappearing 6 and 10 hours after the stopping of the ACTH in cases 1 & 2, and 7 and 10 hours after it in cases 3 & 4. This suggests a close correlation between the reduction of adrenal steroid output and the reappearance of the eosinophils.

In cases 3 & 4 the level of eosinophils at the end of the "phase of reappearance" was significantly higher than the levels before ACTH administration. This may be due to a temporary inhibition of adrenocortical secretion. It might also be due to the return to the blood stream of sequestrated eosinophils to whose numbers were added new cells in the bone

marrow until the period of adrenocortical stimulation was ended.

Comment on "Phase of Reappearance" Studies.

These studies do not supply proof of either destruction of eosinophils or sequestration of them from the blood stream. They confirm results reported by Solomon and Humphreys (1951)

In my view when they are considered with the results of the "in vitro" studies they add further evidence against a destruction of the eosinophils. The theory of temporary removal of the cells from the blood stream followed by return after stopping the adrenocortical stimulation, seems more tenable, for two reasons. Firstly in Case No.4, the only case in which a significant change in maturity is seen, there is an increase in older cells after full reappearance of eosinophils has occurred, 14 3 and more lobed cells per thousand compared with 6 per thousand, and secondly, the rapid reappearance of cells of the same maturity as before stimulation.

The first point is not supported by the other two cases in which prolonged adrenocortical stimulation was given. Further comments on this will be made after the account of bone marrow studies.

The rapid reappearance of the eosinophils suggests either a return of cells sequestrated in tissues throughout the body, or the release of relatively large numbers from the bone marrow. In this latter event, evidence of a "build-up" in numbers of eosinophils in the marrow might be expected.

Bone Marrow Studies.

The bone marrow is now recognised as the site of origin of the eosinophils of the blood. This recognition comes only after a considerable difference in opinion among earlier writers, of whom Rud (1947) quotes Max Schultze, Weidenreich and Ehrlich as protagonists of three different points of view. The first regarded eosinophils as derivatives of neutrophils, the second favoured formation of the cells in the tissues, while the third considered the bone marrow as their site of origin. The recognition of eosinophil granules in Promyelocyte and Myelocyte cells, Plates IV and V is strong evidence that blood eosinophils are formed under similar conditions to the neutrophils in the bone marrow.

An investigation was carried out on bone marrow samples, before, during and after a period of adrenocortical stimulation. The technique adopted was to perform sternal punctures and from the marrow samples to prepare films and "squashed granule" smears for detailed study. The films and smears were stained with Wright's Stain. Differential counts were then carried out counting 1000 cells in each case. The same patients were studied as in the investigation of reappearance of eosinophils. In addition the case of Addison's disease was used as a control. Four subjects had an 8 hour intravenous infusion of ACTH, while the two other cases were studied before, during and after a more prolonged period of stimulation of 8 and 10 days respectively.

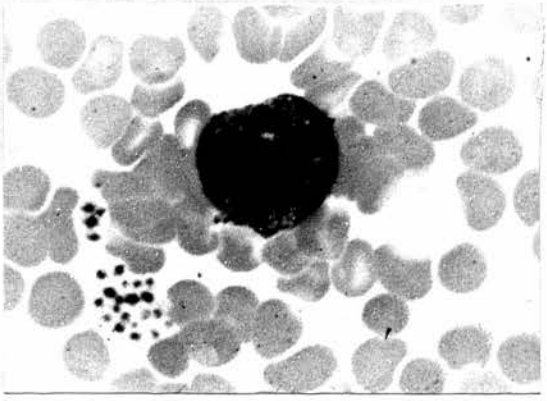


PLATE IV.

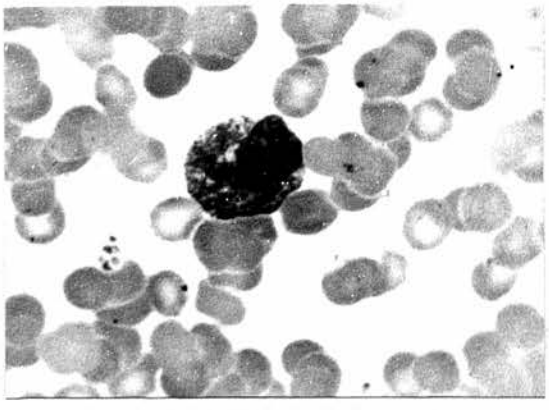


PLATE V.

Study with short period (8 hours) of ACTH administration.

The full differential counts are shown in the Appendix. The figures obtained for all types of eosinophils are shown in Table VII. Case 4 was the case of Addison's Disease, Cases 3 and 4 show very little change after ACTH administration. Case 1 shows a considerable increase in numbers of early eosinophils while Case 2 shows an equally considerable drop in numbers of more mature cells. There is therefore no consistent behaviour discernible on the part of the eosinophils.

Type of Eosinophile Cell.	Case Nos. 1		2		3		4	
	0 hrs.	8 hrs.	0 hrs.	8 hrs.	0 hrs.	8 hrs.	0 hrs.	8 hrs.
Promyelocyte	0	1	0	1	4	0	1	0
Myelocyte.	7	13	10	12	20	6	3	2
Metamyelocytes	4	13	22	10	16	22	2	1
Staff.	5	10	28	34	25	27	5	2
2-Lobed.	13	11	158	80	23	22	25	40
3-Lobed.	0	1	28	19	1	0	3	2
4-Lobed.	0	0	6	1	0	0	0	0

Table VII. Numbers of the various types of Eosinophile Cells in the Marrow before and after 8-hour infusion of ACTH.

Note. The time in hours represents the beginning and end of the 8-hour infusion of ACTH.

Case I was a man who had a persistent leucopenia. The

numbers of polymorphs present before this infusion of ACTH was 31, while after the 8 hour period the figure was 27. When he was given a longer course of ACTH his white count rose to 15,000, 79% polymorphs, from an average count of 2,500, 68% lymphocytes.

Case 2 was a case of Loeffler's Syndrome. His initial eosinophil count was 1,069. This fell to 149 after 8 hours. The marked fall in mature eosinophils in the marrow of almost 50% suggests that the marrow sample contained a proportion of 'peripheral' blood. It is thus difficult to interpret this result.

Cases 3 & 4 had normal blood pictures and marrow films.

Study with prolonged (8-10 days) ACTH administration.

Case I from the above study and a further case (No 5) were investigated. The full marrow differential counts are shown in the Appendix. The details of the numbers of eosinophils per thousand cells are shown in Table VIII.

Type of Eosinophile	Case Nos.			
	1		5	
Cells.	0 hrs.	240 hrs.	0 hrs.	192 hrs.
Promyelocyte.	0	1	3	0
Myelocyte.	6	3	2	2
Metamyelocyte.	6	9	10	2
Staff.	8	8	15	8
2-Lobed.	4	13	20	6
3-Lobed.	1	1	2	0
4-Lobed.	0	0	1	0

Table VIII. Numbers and Types of Eosinophile Cells in the Marrow before and after 8 & 10 days of Adrenocortical Stimulation.

Case 1 here shows very little change. This is striking when one considered that over the 10 day period the polymorphs increased from 3 to 232. Case 5 shows a distinct fall in numbers of eosinophils both immature and mature forms; polymorphs increased from 124 to 313.

The explanation of the different results obtained in these two cases may be due to the chronic leucopenia of Case I. This implies a defect in the marrow. This defect was temporarily overcome by the adrenocortical stimulation. Neutrophils were produced in enormous quantities compared with their pre-ACTH level. Eosinophils remained about their previous level. One might have expected a fairly considerable increase in them, if the adrenocortical stimulation which released the polymorphs had not, at the same time, exerted some damping effect on eosinophil production.

Tentatively, therefore, one might postulate from these two cases of prolonged adrenocortical stimulation, that the hormones produced may cause a decrease in the production of eosinophils.

Marrow 'Squashed Granule' Preparations.

In an attempt to examine pure marrow, granules were crushed and smeared between two slides. The resulting preparations were stained and a variable number of cells were counted. The method of preparation of the smears makes accurate morphological identification very difficult. Eosinophils, however, with their easily recognised granules could be identified. As a check on the method neutrophils

were also counted. Two cases were studied (Nos 2 & 5) and the results obtained are shown in Table IX.

	Case 2. (200 cells counted)		Case 4. (300 cells counted)	
	0 hrs.	8 hrs.	0 hrs.	8 days.
Metamyelocytes.	11	1	48	46
Staffs.	63	72	144	124
Polymorphs.	49	43	82	114
Eosinophil Metamyel.	10	3	3	0
" Staff.	19	25	8	8
" Polys.	48	57	14	8

Table IX. Marrow Squahes before and after ACTH.

There is fairly good correspondence between the numbers of neutrophils before and after ACTH, which suggests that fair samples of cells were taken. It will be noted that, examined in this way, Case 2 shows a slight increase in eosinophils compared with a considerable fall when the films were examined. Case 4 still shows a definite fall but this is less striking. These findings suggest that the examination of films from marrow may give a misleading impression of the numbers and proportions of eosinophils present. The result in Case 2 now indicates that no significant change occurred in the marrow eosinophils.

Marrow Study in Case of marked Eosinophilia.

Recently a case of iododerma with a marked eosinophilia has been reported (Cape 1954). The patient received an almost continuous intravenous infusion of ACTH for 10 days. He had a routine marrow examination 72 hours before the beginning of this therapy, and a second examination 96 hours after it had begun. The results of these are shown in Table X. Unfortunately only 300 cells were counted on the first marrow, and only the eosinophil figures are available. The second specimen was examined by the same methods as those in the cases described above. Full details in Appendix.

Types of Eosinophile Cells.	72 Hours Before ACTH.	96 Hours after ACTH Began.	
Promyelocyte.	6	1	
Myelocyte.	50	14	
Metamyelocyte.	43	23	
Staff.	113	49	
2-Lobed.	(373	57)	
3-Lobed.	(	6)	64
4-Lobed.	(	1)	

Table X. Effect of ACTH on Marrow of Case of Iodoerma and marked Eosinophilia.

During the time between the samplings of the marrow, the eosinophilccount fell from 22, 260 to 0. In spite of

this the marrow still contained 15% of eosinophils of all types, with a proprtionately greater loss of mature cells than immature ones. One must add that in the 72 hours before giving ACTH the eosinophil count fell from 22, 260 to 8,866 and that after the 48 hours of ACTH this had fallen to zero. Fig.11 illustrates that this latter fall was in fact due largely to the ACTH and not merely to the continuation of an existing trend. It will be seen that after the first 10 mg of ACTH had been given there was a gap in time of about 12 hours before the next infusion was administered. During this time the eosinophil level rose from about 1,600 to about 5,400. After resuming ACTH therapy it quickly fell to zero.

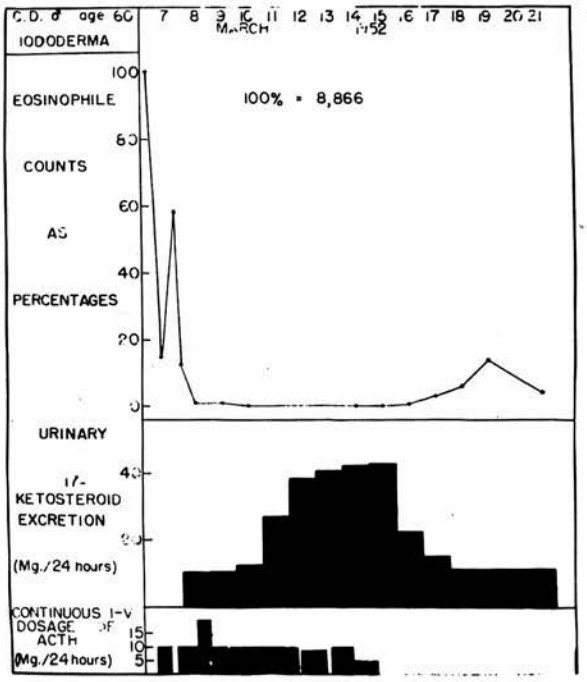


FIG.II.

In this case the eosinophils in the marrow were spared to some extent, the vanishing phenomenon which affected their descendants circulating in the blood stream.

Conclusions from Bone Marrow Studies.

The results obtained indicate :

1. That no significant alteration in numbers of types of eosinophils in the bone marrow can be detected after a short period of adrenocortical stimulation.

2. There may be a significant reduction in numbers of marrow eosinophils after prolonged adrenocortical stimulation. This effect is much less marked than the effect on eosinophils in the peripheral blood. It is suggested that the eosinophils present in the marrow at the beginning of a period of adrenocortical stimulation may be protected from the destructive or sequestrative force of the hormones. At the same time there may be a partial inhibition of the formation of further new eosinophils.

DISCUSSION.

The results obtained in these studies confirm available reports on the subject, with one notable exception (Godlowski 1953), which will be referred to later. The effect of corticotrophin on eosinophils has been known for some time. The minimum effective dose of ACTH required to produce this effect had never been established. This has some importance from a therapeutic standpoint, giving an indication of the dose of ACTH that may be required in a given case. The small amounts of hormone required to produce sufficient adrenocortical stimulation to cause an eosinopenia is of importance in the Thorn Test. In the light of the studies reported above, one can recommend that a satisfactory method of performing this test is by using an intravenous infusion of ACTH at the rate of 1.0 mgm per hour for 8 hours. This requires only 8 mgm of ACTH. It is considered an advantage to perform three eosinophil counts, one at the outset, one at 6 hours, and one at 8 hours after the infusion begins. The response to such a test is a good guide to the state of the adrenal cortex.

In spite of a painstaking series of studies the mechanism by which this eosinopenia occurs is not clear. To review and examine the results obtained, the hypotheses suggested earlier, will now be reconsidered.

1 ? Lysis of eosinophils in the blood stream.

A simple toxic effect of adrenal steroids on eosinophil cells which causes this disintegration is ruled out by the "in vitro" studies. They do not, however, exclude

destruction of the cells by a more complex mechanism "in vitro". In the case of iododerma quoted earlier (Cape 1954), there must have been considerable destruction of eosinophils as the original level was 8,866 compared with a level of about 1000 after the influence of adrenocortical stimulation had ceased. During the 10 days stimulation a large number of eosinophils must have disintegrated.

Whitby & Britton (1947) state that "polymorphs become senile and die in the blood-stream, some may be excreted in the saliva but most are probably destroyed in the reticulo-endothelial system". It is reasonable to suggest that the fate of eosinophils is a similar one. When a prolonged period of adrenocortical stimulation is maintained it is obvious that numbers of eosinophils will come to the end of their natural life during the course of it. If the agent which produced an eosinophilia is simultaneously withdrawn, there will be nothing to produce a recurrence of the eosinophilia, when the adrenocortical stimulation ends. This would account for the results obtained in the iododerma case.

In the group of cases studied during a short period of adrenocortical stimulation, there is absolutely no evidence in favour of a destruction of the eosinophils. They disappear from the bloodstream and reappear again quite rapidly in the same numbers and stages of maturity as before. In the group of cases studied over a longer period, the death of an appreciable number of eosinophils must occur in the normal process of ageing of the cells.

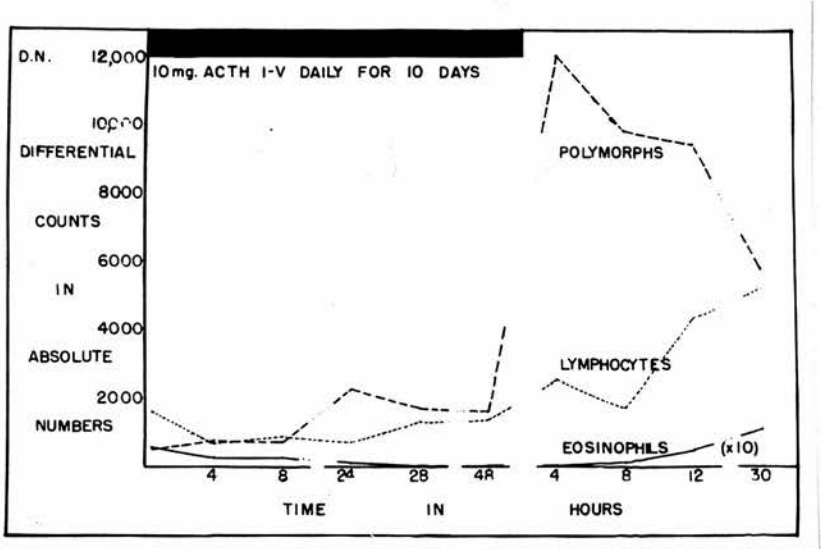
2? Sequestration from the blood stream.

This suggestion holds most appeal. In the studies described there is not a single fact which is against this hypothesis. We know that eosinophils have a predilection for entering the tissues, in such conditions as the Eosinophilia-Pulmonary Syndrome (Apley and Grant 1945). As a site to which such temporary withdrawal may be made the reticulo-endothelial system offers a ready haven. It is interesting to note that, following splenectomy, many people have an eosinophilia for a year or more, the case reported above being an example. Lucia, Leonard and Falconer (1937), however, have shown that a normal ~~eosinopenic~~ leucocyte response to adrenaline occurs in splenectomised animals. Further comment on this subject will be made later.

3? Effect in Bone-Marrow.

The results of the marrow studies indicate that with a short period of adrenocortical stimulation no significant changes can be noted in this tissue. This has been the experience of other authors (Rosenthal et al (1950), Godlowski (1948), Best & Samter (1951)). Finch et al (1951) studied marrow over a 21 day period of treatment with ACTH and found no significant change in eosinophils. The interpretation of the equivocal results found after more prolonged stimulation in the present study is more difficult. To assess it fairly one must consider the behaviour of the white cells as a whole. Fig. 12 illustrates the effect of 10 days continuous adrenocortical stimulation on the white cells of peripheral blood in Case 3, while Table XI gives the counts of the

neutrophil series before and after the 10 day period. Both indicate a considerable increase in polymorph production. Examination of the marrows show that this increase in one member of the granular series is not associated with a similar increase in the eosinophile members of the same series. (vide Table VIII)



The gaps in the lines represent passage of time. The second series of hours signify the time in hours after stopping ACTH. See text.

FIG.12.

Case.	Before ACTH.	After ACTH.
Polymorphs.	302	11,850
Staff.	139	2,100

Table XI. Change in absolute neutrophil cell count per c.mm. before and after 10 days of ACTH in Case 3 (Table VI)

When performing the sternal punctures on the cases

described, it was noted that the marrow was much more vascular after the period of stimulation. Unfortunately total cell counts were not performed on the marrow specimens as the accuracy, and thus the validity, of such counts was thought dountful. There was, however, a definite increase in cellularity after stimulation, noted in the "squashed granule" preparations.

An increase in total cellularity indicates that the alteration in numbers of marrow eosinophils in Case 3 is only a proportional one, but the presence of several "unknowns" makes it difficult to draw any satisfactory conclusions. One can claim that there is no storing up of eosinophils preparatory to a quick release of them into the blood stream at the end of a period of adrenocortical stimulation. In view of the reports of other investigators quoted above this does suggest the probability that bone marrow production of eosinophils continues normally, but that the cells released into the circulating blood are quickly removed.

The work of Godlowski. (1953).

In a book entitled "Enzymatic Concept of Anaphylaxis and Allergy", Godlowski has reported his investigations into the mechanism of hormonally induced eosinopenia. He states quite definitely that adrenal steroids of the C.11-oxygenated group have a "lytic" action on eosinophils of the blood. His views seem based on good grounds and fit into an enzymatic conception of

anaphylaxis and allergy. A discussion of the evidence for and against this conception lies outside the scope of this thesis. His views on the mechanism of eosinopenia following adrenocortical stimulation, however, must be examined in some detail.

He quotes a series of "in vitro" studies (Godlowski 1953A) in which a 50% fall of eosinophils occurred after only 3 hours of incubation. The test was performed using heparinised blood (100 units heparin/ml. blood) and eucortone or cortisone acetate as the hormonal substance. This technique would appear to be almost the same as in our studies. The results obtained were quite different. Muehrcke et al. (1952) carried out similar studies using heparinised blood, and failed to demonstrate any destruction of eosinophils. Godlowski (1953b) explains this by the suggestion that heparin protects the cells, if sufficient is used. The hormones are unable to penetrate to the interior of the cell to produce the proteolytic effect which, Godlowski (1953c) claims, destroy them. This reasoning is not easy to understand, as Muehrcke and his co-workers used <u>less</u> heparin in their studies than Godlowski used in his. From the paper of the former authors one gathers that 40 units of Heparin were used in 3 ml. of blood, while Godlowski used 100 units per ml.

To test his hypothesis, it was decided to repeat a small series of "in vitro" experiments under as similar conditions as possible to those of Godlowski. 10 ml. samples

of blood were taken from five male subjects and each was added to 1000 units of heparin. The resulting samples were each divided into two 5 ml. specimens, one specimen being kept as a control in each case. Various amounts of "supracort" adrenocortical extract or cortisone acetate (alcohol suspension) were added and, after initial eosinophil counts had been performed, all ten specimens were stored at 37°C in a water bath. In this study Randolph's method of staining and counting eosinophils was used, as this was the technique favoured by Godlowski. The results are shown in Table XII.

	Eosinophil counts.	
Hormone used.	Initial.	After 5 hrs Incubation
1. Cortisone acetate 0.2 mgm/cc.	112.	97.
2. Cortisone acetate 0.4 mgm/cc.	422.	389.
3. Cortisone acetate 0.3 mgm/cc.	205.	269.
4. "Supracort" 15 mgm extract/cc.	620.	692.
5. "Supracort" 7.5 mgm extract/cc.	124.	130.

Table XII. The Effect of "Supracort" and Cortisone Acetate on Eosinophils in vitro.

The results are similar to those of all the earlier "in vitro" studies. No fall in eosinophils occurred. In view of this further confirmation of the earlier studies, it is difficult to understand Godlowski's findings. Exact

details of the form in which he used cortisone acetate are not available. The writer has now used the hormone as powdered cortisone free alcohol, crystalline cortisone acetate and the Merck alcohol suspension of cortisone acetate and has consistently obtained the same results. As far as is known, only Godlowski and Muehrcke et al (1952), who used defibrinated blood, have demonstrated a significant drop in eosinophils using a similar technique.

Godlowski (1953d.) also claims that examination of blood films prepared before and after a period of adrenocortical stimulation reveals a marked increase in the numbers of disintegrated cells. His claim is not supported by any detailed comparative counts. These findings are again contrary to those obtained in this study. Over forty 1000 cell differential counts, involving study of about 400 blood films, have been performed on blood collected while the effect of ACTH was present. Comparative counts of disintegrated cells before and after ACTH were not made, as there was never any evidence in the films that a significant increase in numbers of such cells occurred. Padawer and Gordon (1952) describe experiments with rats to which cortisone was given. They obtained a satisfactory fall in numbers of eosinophils in blood, and peritoneal and pleural spaces. They also demonstrated an increase in the numbers of degenerating eosinophils 6 hours after the cortisone administration. The increase from 1.01 - 2.27% of all eosinophils, while significant, is not impressive as an indication that the reduction in numbers of eosinophils was due to destruction.

A further hypothesis of Godlowski's is that not only eosinophils, but all the white blood cells are lysed to a greater, or lesser extent by the adrenal steroids. (Godlowski 1953e). That a lysis of polymorphs should occur seems unlikely in view of the accepted fact that adrenocortical stimulation consistently produces a marked leucocytosis. Godlowski explains this by postulating that the lysed polymorphs release the leucocytosis-promoting factor of Menkin (1939[43]) which, in turn, causes the leucocytosis. Case 3 in the "Reappearance of Eosinophils" study above gives information which does not support this hypothesis. The numbers of neutrophils found in Cases 1 - 4 in "Phase of Reappearance" study (Table VI p28) are shown in Table XIII.

	Absolute polymorph counts/cmm. after ACTH given for			
Case No.	0 hours.	2 hours.	4 - 5 hours.	6 - 7 hours.
1	9,109.	8,814.	7,227.	10,064.
2	2,466		4,340.	
3	302	168	504.	562.
4	7,020	7,098	7,980.	7,326.

Table XIII. Absolute Polymorph counts/cmm. calculated from total white counts and 1000 cell differentials

Case 3 does confirm Godlowski's theory, while case 1 also shows a fall of just over 20% in numbers of polymorphs after 4 hours. The other two cases do not show any drop. Godlowski (1953f) performed perfusion experiments in 4 dogs, two of which were used as controls. Of the two given eucortone in the perfusing blood, one showed a drop in

numbers of polymorphs of about 60% sustained for 4 hours, while the other showed a drop varying between 9 and 11%. In a series of experiments in an isolated section of inferior vena cava, Godlowski (1953g), only one out of the four demonstrated a significant drop in numbers of polymorphs, while in his "in vitro" studies (Godlowski 1953a) only eight out of thirteen results suggested a significant drop in numbers of polymorphs. To sum up these results, one can only say that Godlowski's suggestion is not consistently supported by either the results shown in Table XIII or by his own.~~results~~.

Perhaps the most interesting of Godlowski's observations was on the tissue eosinophilia which he found in the intestinal mucosa. He studied this in the following manner; (Godlowski 1953~~g~~): samples of stomach, duodenum, jejunum, ileum and colon were removed from 17 dogs; 2-3 weeks later, 8 dogs were given an adrenocortical stimulation; 4 hours after this, all the dogs had further intestinal specimens removed; finally, a week later, further intestinal biopsies were performed on 5 of the dogs.

The results show an increase in intestinal eosinophilia between the first two series of biopsies, and a further increase between the second and third series. Godlowski claims that the increases are of the same order in the treated and untreated groups. To assess the significance of this claim, one must consider his experiment in greater detail.

The degree of intestinal eosinophilia was given

as the number of eosinophils found per high power field. To obtain this number, the mean of 90 high power fields was taken, 30 from a section or sections of duodenum, 30 from sections of jejunum and 30 from sections of ileum. There was a considerable variation in the numbers obtained in individual dogs; for example, the average count obtained in the initial biopsies of the eight "treated" dogs was 24, while the same average for the 9 control dogs was 38. There was also a wide variation in the percentage increases between the first two biopsies in different dogs; in the treated group this ranged from + 30% to + 153% with an average of + 75%, in the control group from -13% to + 220% with an average of + 53%. Because of the wide variation from dog to dog the difference in the average increases is not significant. It does, however, suggest that if a larger number of animals had been studied, a significant increase might become obvious.

This suggestion is strengthened if one considers the data in a second way. The average figure obtained at the first biopsy for the numbers of intestinal eosinophils per high power field of the 8 treated dogs was 24. This rose to 42 at the second biopsy, an increase of 75%. The equivalent average figures for the nine control dogs were 38 and 48 an increase of 26%. Examined in this way the difference in the percentage increases gains in significance.

Handling of the gut of experimental animals will produce an intestinal eosinophilia, (Speirs and Heyer.1949). The same procedure will act as a "stress" and cause stimulation of the pituitary-adrenal axis, and consequently an eosinopenia.

It seems possible that the two conditions are connected, and that the second may be the reult of the first. Godlowski (1953a), however, vigorously claims that the two, intestinal eosinophilia and blood eosinopenia, are unrelated, basing his view on the experiment discussed above. The data available is, in fact, insufficient to prove his contention, and even arouses the suspicion that further studies might reveal a significance contrary to his belief.

Roche, Thorn and Hills (1950) have studied the eosinopenia present following operations in man. Their results showed that when a normal recovery took place after the operation the eosinopenia would last for 24-48 hours and return to pre-operative levels would be complete in 3 days. It is a reasonable supposition that the same sequence of events would occur in Godlowski's dogs.

One cam, therefore, suggest that the following may be the sequence of events in Godlowski's experiments.

1. Laparotomy to obtain first set of biopsies with resulting adrenocortical stimulation and blood eosinopenia for 24-48 hours with a return to normal on the third day. During that period of 48 hours eosinophils are being released into the blood stream from the marrow in normal numbers, but owing to a chemotactic effect are immediately drawn into the intestinal mucosa where a condition of tissue eosinophilia quickly builds up.
2. During the 2 to 3 week period before the second stage of this experiment some of the sequestrated eosinophils will die having reached the end of their life cycle, some may be able to return to the blood stream, but most appear to

be held fast where they are. Thus,

3. When the second biopsies are taken all dogs show a degree of intestinal eosinophilia. 4 hours previously eight had received a second adrenocortical stimulation and by the time of taking the biopsies an appreciable number of eosinophils had been removed from the blood. These have been caught in the intestinal mucosa and one would therefore expect a slight increase in levels in the mucosae of the dogs which had the extra adrenocortical stimulation. It is claimed that the results obtained were suggestive of such an increase without having actual statistical significance.

The main reason for this statistical result lies in the wide range of variation in the intestinal eosinophil results from dog to dog. This is attributable to two main causes:

1) The natural individual differences from dog to dog, and
2) The variable responses of individuals to the stress of the first operation, which could not possibly be of precisely the same nature in all the dogs.

One would expect the difference between the increased eosinophilia of the two groups to be slight because the period of eosinopenia following the first operation persisted for 24-48 hours while that due to the adrenocortical stimulation could only have been present from 1-2 hours.

Taking these factors into consideration one can claim that Godlowski's suggestion that this intestinal eosinophilia and blood eosinopenia are unrelated phenomena is by no means confirmed by his experiments. One may even go further and claim that they are suggestive of an association

between the two reactions, although statistically, the suggestion is not significant. More definite information might have been obtained if the period of adrenocortical stimulation before the second biopsy had been prolonged for several days.

Theory of Sequestration of Eosinophils into Intestinal Mucosae.

The results obtained in the various "in vitro" studies described earlier considered with these obtained in the "Phase of Reappearance" and Bone Marrow Studies are all consistent with a sequestration of eosinophils from the blood stream as a result of adrenocortical stimulation. Indeed, from the former one is completely convinced that destruction of eosinophils does not take place. It has always been felt that if one knew the functions of the eosinophils some light would be shed on the whole problem. These functions appear closely associated with "foreign protein" which is perhaps the one type of substance which will consistently produce an eosinophilia. The fact that eosinophils may acquire the antigenic properties of the protein (Godlowski 1948) suggests that in some way the protein is incorporated into the eosinophil cells and in this way it may well interfere with its possible toxic effects.

The commonest mode of entry into the body of protein is through the intestinal tract. It follows that eosinophils will commonly be found in that situation, a conclusion which is confirmed by many observations of Godlowski. He showed (Godlowski, 1953) that by varying the

protein in the diet of animals he could produce variations in the degree of intestinal eosinophilia.

The "Alarm Reaction" is instigated by adrenocortical stimulation. The conception of such a reaction implies a preparation on the body's part to deal with the particular stress which has caused the stimulation. It is consistent with such a hypothesis to suggest that eosinophils congregate in the intestinal mucosa, which may be regarded as their particular battle station. Polymorphs, on the other hand, accumulate in the blood ready to be drawn to any site where they may be called upon to encounter a bacterial or mechanical "stress." Such a distribution of the cells will occur when an artificial stimulation, such as the administration of ACTH, takes place.

The white cells of the blood are generally agreed to arrive at particular sites in response to chemotaxis. By this is meant that some substance at the site develops a strong biochemical attraction for the particular type of cell concerned and draws it to the site. One may pertinently ask what chemotactic influence can exist which will draw eosinophils to the intestine? This is not known. It is, however, accepted that when excessive adrenocortical stimulation occurs, ulcers may develop in the gastro-intestinal tract. It is reasonable, therefore, to suggest that lesser degrees of stimulation will exert some influence on this tract. Part of the result of this could be the release of a substance in the mucosa which exerts a chemotactic influence on eosinophils.

To sum up, one cannot accept as the mechanism of eosinopenia a sacrificial destruction of the cells. If adrenal steroids destroy eosinophils, why does such an action not take place in a test-tube? The conception of eosinophils as useless or even harmful cells has little appeal, and seems contrary to all natural laws. There can, ~~however~~ therefore, be no object in their destruction. The theory of sequestration is consistent with the results of the present studies, and with most of the available reports on the subject. In addition, it offers a reasonable theory of the function of eosinophils following adrenocortical stimulation.

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APPENDIX.

Eosinophil Counts: A modification of the method first used by Dunger (1910). 5 cc. of 2% eosin and 5 cc. of acetone were added to 90 cc. of water to form the diluting fluid. Blood was diluted 1 in 10 in a Hellige white cell pipette. Counts were done within five to fifteen minutes using either a Spencer bright line haemocytometer or a Fuchs-Rosenthal counting chamber. In most cases the latter was used as it allowed a greater depth of fluid to be counted; this meant that the counting of two chambers gave reasonably accurate results, while with the haemocytometer four chambers had to be counted to give comparable accuracy.

Calculation of the quantities of hormones used in the "in vitro" studies.

The amount of ACTH used was calculated as follows: From the observations described in the first section, pp 10-13, it was known that 1 mgm. of ACTH per hour in an intravenous drip was certain to produce a maximum eosinopenic effect in four hours. Thus, without allowing for any loss of ACTH to the extravascular tissues, one can make a crude calculation that 4 mgm. ACTH in 6,000 cc. of blood should be more than enough to produce any effect it might have on the eosinophils. This means 0.003 mgm. in 5 cc. The actual amount of ACTH used was approximately 0.026mgm., or about twenty times the estimated effective amount.

The amounts of cortisone and compound F used were identical. In the first series of incubations these were calculated to give half the equivalent concentration to that produced in the total blood volume by 100 mgm. of the hormones. It was considered, however, that this concentration might be inadequate, asit is possible that stimulation of the adrenal cortex by ACTH may produce a much greater quantity of the hormones. For this reason the second series of incubations were carried out with these hormones, using much larger quantities of them.

The effect of the administration of ACTH on eosinophils.

Details of the data illustrated in Figs. 3 and 4.

Fig. 3. Case E.F. See p. 10.

Infusion number	1	2	3	4	5	6	7
ACTH (mgm/hr)	nil	0.085	0•25	0.5	0.75	1.02	2.23
Eosinophil counts							
Initial	186	189	175	206	254	298	250
After 1 hour							169 (68%)
2 hours	82 (44%)	132 (69%)	90 (51%)	91 (44%)	276 (109%)		229 (92%)
3 hours	101 (54%)		82 (47%)	45 (22%)	96 (38%)	157 (53%)	51 (20%)
4 hours				33 (17%)	108 (42%)	85 (18%)	
5 hours		134 (70%)	74 (42%)	25 (13%)	59 (23%)	50 (17%)	39 (16%)
6 hours	234 (126%)			26 (13%)	59 (23%)		
7 hours		136 (71%)	23 (13%)			25 (8%)	9 (4%)
8 hours	184 (100%)		26 (15%)	17 (8%)	6 (2%)	20 (7%)	

ACTH (Armour) Batch No. J. 26311.

Fig. 4. Case R.G. See p. 11.

Infusion number	1	2	3	4	5	6
ACTH (mgm/hr)	nil	0.05	0.1	0.25	0.25	0.5
Eosinophil counts						
Initial (100%)	301	189	271	256	306	231
After 1 hour		206 (109%)	232 (86%)	160 (63%)	321 (105%)	165 (71%)
2 hours	282 (90%)	206 (109%)	150 (55%)	192 (75%)	267 (89%)	192 (83%)
3 hours	315 (105%)	169 (89%)	202 (75%)	161 (63%)	172 (57%)	112 (48%)
4 hours	259 (86%)	206 (109%)	155 (57%)	119 (46%)	163 (54%)	119 (51%)
5 hours	380 (126%)		67 (25%)	65 (25%)	126 (42%)	68 (30%)
6 hours	164 (54%)	215 (113%)	108 (39%)	39 (15%)	65 (22%)	72 (31%)
7 hours			57 (21%)	33 (13%)		46 (20%)
8 hours	333 (107%)	259 (137%)	88 (32%)	34 (13%)	48 (16%)	43 (19%)

ACTH (Armour) Batch No. K. 30002.

Study of "Phase of Reappearance" of eosinophils.

10 or more blood films were prepared at intervals during and after administration of ACTH. 100 cell differential counts were performed on at least 10 separate films and the 1000 cell counts were calculated from them. A sample is shown below. In the detailed results of the study only the final 1000 cell counts are given.

Sample of determination of 1000cell count.

Polymorph	52	53	48	50	54	53	59	57	50	52	528
Staff	7	2	13	7	10	3	2	8	8	6	66
Lymphocyte	27	31	26	27	25	24	19	20	22	31	252
Monocyte	2	0	2	2	2	6	3	2	11	0	30
Basophil	5	2	3	4	1	5	6	3	3	3	35
Eo. staff	0	3	1	1	0	1	1	0	0	0	7
Eo. 2-lobed	7	7	3	4	2	6	7	6	3	7	52
Eo. 3-lobed	0	2	4	5	5	3	3	3	2	1	28
Eo. 4-lobed	0	0	0	0	1	0	0	0	1	0	2

Case No 1. See Table VI.

Time in hours	0	2	4	6	8	10	12
Eosinophil count	1,069	1,086	676	400	149	179	144
Total W.B.C. count	15,300	13,650	12,250	12,600	13,500	15,350	13,100
Differentials							
Polymorph	528	645	592	639	716	683	679
Staff	66	48	69	60	53	69	44
Lymphocyte	252	162	208	201	185	175	224
Monocyte	30	24	39	26	32	28	28
Basophil	35	18	15	18	10	16	8
Eo. staff	7	3	9	6	0	2	2
Eo. 2-lobed	52	75	45	39	2	14	12
Eo- 3-lobed	28	22	23	12	2	13	1
Eo- 4-lobed	2	3	0	1	0	1	0

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(table continued from previous page)

14	16	21	23	25
332	468	329	505	1007
17,250	14,250	15,350	16,150	15,050
651	658	721	676	618
54	52	45	27	32
202	202	159	194	261
53	48	41	49	29
8	13	9	8	19
2	1	2	3	5
13	24	15	35	42
4	7	7	10	22
1	1	1	1	2

0 hours refers to the start of the ACTH infusion. The infusion continued for eight hours. Eosinophil count refers to chamber counts which were performed at the same time as the differentials from the same samples of blood. The following tables of the other cases are compiled in the same way.

Case No. 2. See Table VI.

Time in hours	0	4	8	13	18	22	28
Eosinophil count	467	109	9	0	100	376	485
Total W.B.C. count	6,850	7,000	7,750	7,350	9,000	8,700	9,600
Differentials							
Polymorph	360	618	742	653	439	452	331
Staff	20	36	18	44	50	36	21
Lymphocyte	430	276	210	229	421	405	544
Monocyte	100	46	28	74	74	74	58
Basophil	9	6	0	0	8	8	7
Eo. staff	1	0	0	0	0	1	7
Eo. 2-lobed	75	16	2	1	8	24	28
Eo. 3-lobed	5	2	0	0	1	1	4
Eo. 4-lobed	0	0	0	0	0	0	0

These two cases received an eight hour infusion of ACTH. The following three were given a more prolonged course, which lasted ten days in Nos. 3 and 5 and eight days in No. 4. The data on Cases 3 and 4 are presented in two parts; in the first section the differentials are shown during the early period of adrenocortical stimulation, while inthe second section are shown the counts taken during the period of reappearance of eosinophils. Only the latter data are available in Case No. 5.

Case No. 3. See Tables XII and VI. Section 1.

Time in hours	0	2	4	6	25	52	96
Eosinophil count	53	26	25	6	0	2	3
Total W.B.C. count	2,320	1,400	1,680	1,520	2,200	3,600	
Differentials							
Polymorph	130	119	300	369	498	397	525
Staff	61	100	186	120	87	67	52
Lymphocyte	683	653	453	475	309	388	304
Monocyte	71	93	44	29	104	147	128
Basophil	32	21	10	6	2	1	1
Eo. staff	1	1	2	0	0	0	0
Eo. 2-lobed	17	12	4	1	0	0	0
Eo. 3-lobed	5	5	1	1	0	0	0
Eo. 4-lobed	0	0	0	0	0	0	0

Section 2. 0 hours represents the time of stopping ACTH.

Time in hours	4	7	10	13	30
Eosinophil count	0	14	40	51	112
Total W.B.C. count	15,000	12,450	15,200	14,500	11,750
Differentials					
Polymorph	787	814	686	645	483
Staff	14	9	18	8	6
Lymphocyte	174	141	262	305	455
Monocyte	25	31	28	35	22
Basophil	0	3	2	1	5
Eo. staff	0	0	0	0	1
Eo. 2-lobed	0	1	2	3	13
Eo. 3-lobed	0	0	2	1	3
Eo. 4-lobed	0	0	0	1	0

Case No. 4. See Tables VI and XII. Section 1.

Time in hours	0	2	5	7
Eosinophil count	250	265	98	42
Total W.B.C. count	10,800	10,350	10,500	9,450
Differentials				
Polymorph	646	680	764	785
Staff	37	34	39	38
Lymphocyte	225	194	132	125
Monocyte	44	50	47	37
Basophil	4	6	4	7
Eo. staff	4	1	1	1
Eo. 2-lobed	33	29	10	4
Eo. 3-lobed	6	5	3	3
Eo. 4-lobed	0	1	0	0

Section 2. 0 hours represents the time of stopping ACTH.

Time in hours after 0.	10	14	18	22	33
Eosinophil count	37	104	104	183	512
Total W.B.C. count	17,000	17,450	16,500	15,700	14,050
Differentials					
Polymorph	589	643	570	581	517
Staff	12	19	17	8	6
Lymphocyte	359	285	342	347	380
Monocyte	29	47	52	55	33
Basophil	0	2	1	0	9
Eo. staff	0	1	2	6	6
Eo. 2-lobed	2	3	11	10	32
Eo. 3-lobed	0	0	2	0	12
Eo. 4-lobed	0	0	0	0	2

Case No. 5. See Table VI.

0 hours represents the time of stopping ACTH.

Time in hours after 0.	9	21	24	27	30	36	48
Eosinophil count			177		296	445	559
Total W.B.C. count	23,050	22,800	17,600	23,700	21,000	23,250	16,400
Differentials							
Polymorph	537	202	185	231	317	298	202
Staff	28	3	5	10	13	19	20
Lymphocyte	356	733	722	694	625	645	691
Monocyte	79	57	60	51	30	19	41
Basophil	0	0	0	0	0	0	0
Eo. staff	0	0	0	2	1	3	5
Eo. 2-lobed	0	3	23	6	8	12	32
Eo. 3-lobed	0	2	5	5	6	3	9
Eo. 4-lobed	0	0	0	0	0	1	0

Bone Marrow Studies. (I)

Patient.	1.		2.		3.		4.	
Time.	0 Hrs.	8 Hrs.	0 Hrs.	8 Hrs.	0 Hrs.	8 Hrs.	0 Hrs.	8Hrs.
Myeloblasts.	6	8	4	8	18	6	2	2
Promyelocytes.	35	34	6	16	14	14	4	8
Myelocytes.	100	97	26	50	53	47	28	25
Metamyelocytes.	113	81	56	64	87	103	81	41
Staff.	183	110	72	166	212	157	197	154
Polymorphs.	31	27	232	295	195	264	226	296
Lymphocytes.	173	153	108	51	137	151	304	326
Monocytes.	17	15	24	2	6	10	22	9
Basophils.	4	1	6	12	3	0	1	4
Prenormoblasts.	13	8	4	3	3	8	3	1
Early Normoblasts.	25	30	10	16	10	7	3	4
Inter.Normoblasts.	98	122	116	44	64	47	23	13
Late Normoblasts.	154	253	66	113	103	102	66	70
Eo.Promyelocytes.	0	1	0	1	4	0	1	0
Eo.Myelocytes.	7	13	10	12	20	6	3	2
Eo.Metamyelocytes.	4	13	22	10	16	22	2	1
Eo.Staff.	5	10	38	34	25	27	5	2
No. 2 L.	13	11	158	80	23	22	25	40
Eo. 3 L.	0	1	28	19	1	0	3	2
Eo. 4 L.	0	0	6	1	0	0	0	0
Plasma.	19	20	8	9	6	6	1	0

1000 Cell Differential Counts on Bone Marrows. 0 and 8 hours refer to the beginning and of ACTH administration.

The above results are those of the study with a short period of adrenocortical stimulation.

Bone marrow studies (II).

Study with prolonged period of adrenocortical stimulation.

Patient No.	1			5	
Time	0 hrs	28hrs	240 hrs	0 hrs	194 hrs
Differentials					
Myeloblasts	34	11	5	10	0
Promyelocytes	25	26	15	19	3
Myelocytes	116	76	75	76	39
Metamyelocytes	77	70	60	122	93
Staff	75	166	178	217	159
Polymorphs	3	42	232	124	313
Lymphocytes	63	102	88	43	140
Monocytes	6	7	6	0	8
Basophils	2	7	0	3	0
Pronormoblasts	9	15	9	5	4
Early normoblasts	38	10	12	17	12
Intermed. "	265	180	78	57	48
Late "	253	246	204	234	174
Eo. promyelocytes	0	0	1	3	0
Eo. myelocytes	6	8	3	2	2
Eo. metamyelocytes	6	6	9	10	2
Eo. staff	8	14	8	15	8
Eo. poly. 2-lobed	4	10	13	20	6
Eo. poly. 3-lobed	1	0	1	2	0
Eo. poly. 4-lobed	0	0	0	1	0
Plasma	0	0	0	20	1

0 hours represents the beginning of ACTH administration, and the time in hours is the number after that time.

Bone marrow studies (III).

Study in case of marked eosinophilia.

Time	72 hrs. before beginning ACTH	96 hrs. after beginning ACTH
Myelocytes		5
Promyelocytes		20
Myelocytes		38
Metamyelocytes		82
Staff		198
Polymorphs		279
Lymphocytes		81
Monocytes		13
Basophils		2
Pronormoblasts		3
Early normoblasts		6
Intermed. "		32
Late "		75
Eo. promyelocytes	6	1
Eo. myelocytes	50	14
Eo. metamyelocytes	43	23
Eo. staff	113	49
Eo. poly. 2-lobed	(	57
Eo. poly. 3-lobed	(-- 373	6
Eo. poly. 4-lobed	(	1
Plasma		11

The results shown in the first column are based on only 300 cell counts and no other data are available at that time. The second column shows results obtained in the same way as in the earlier studies.