Infuences of adrenalin, cortisone and ACTH on eosinophils in bone marrow tissue culture (With some suggestions for examining the anterior pituitary function)

Sinro Yamamoto*
Infeluenes of adrenalin, cortisone and ACTH on eosinophils in bone marrow tissue culture (With some sug-gestions for examining the anterior pituitary function)*

Sinro Yamamoto

Abstract

1. Adrenalin, when acting directly on eosinophils, brings about a diminution in the wandering velocity of eosinophils but it has no influence on the number of the cells. Judging from the movement patterns of eosinophils this drug acts as to impede the motive function. 2. Acting directly on eosinophils, cortisone markedly decreases the wandering velocity of these cells and also brings about the diminution in the number of the cells. Likewise from the movement patterns of eosinophils, this drug markedly impedes the motive function of the cells. 3. ACTH (adrenocorticotropic hormone), when acting directly on eosinophils, enhances the wandering velocity of eosinophils but it in no way affects the number of eosinophils. Judging from the eosinophil movement patterns, this drug markedly promotes the motive function. 4. Although adrenalin brings about a decrease in the number of peripheral eosinophils in hypophysectomized dogs, the rate of the decrease is less than that observable in the case of normal dogs. 5. Cortisone brings about no significant change in the number of peripheral eosinophils in hypophysectomized dogs, but is induces a decrease in peripheral eosinophils of normal dogs. 6. ACTH acts as to decrease the number of peripheral eosinophils to an equal degree in both hypophysectomized and normal dogs. 7. When cortisone is administered simultaneously with Adrex, a marked decrease in peripheral eosinophils is brought about in hypophysectomized dogs. 8. By means of the bone-marrow tissue culture of hypophysectomized dogs it has been confirmed that the blood plasma of hypophysectomized dogs lacks an essential factor for cortisone to induce eosinopenia in peripheral blood. 9. The decrease in eosinophils of peripheral blood induced by cortisone has been proved to be dependent upon the presence or absence of the pituitary body. 10. Taking the decrease in peripheral eosinophils by cortisone administration as the criterion, the author has carried out clinical observations with this method and obtained anticipated results.

*Copyright ©OKAYAMA UNIVERSITY MEDICAL SCHOOL
In 1939 Dalton recognized a decrease in the number of eosinophils under a stress, and since then it has been clarified by Vogt\(^{29}\), Long\(^{13}\), etc., that a decrease in the number of peripheral eosinophils is brought about by epinephrine. Concerning this phenomenon Long presented a hypothesis of the stimulation of anterior pituitary lobe. In 1948 Thorn\(^{26}\) indicated that the decrease in eosinophils is induced by adrenocortical hormone excreted when adrenocorticotropic hormone (ACTH) is administered. Later Recant\(^{19}\) et al. advocated the test with adrenalin for determining the function of adrenal cortex. Now, it is a well-known fact that cortisone has an action as to induce eosinopenia\(^{4,18,28}\) and also that cortisone is excreted by the action of ACTH. There are numerous literatures with respect to the mechanisms of ACTH, cortisone, and adrenalin, inducing eosinopenia of peripheral blood, but there still remain many unsolved problems on the nature of the actual mechanisms, especially so with respect to their relation to the pituitary body.

With the purpose to clarify the mechanisms of adrenalin, cortisone, and ACTH involved in eosinopenia and especially their relation to the pituitary body, the author performed a series of bone marrow tissue cultures of rabbits and hypophysectomized dogs. Then observations were carried out to elucidate the influences of these drugs on the movement of eosinophils wandering out of the explant, and at the same time numerical changes in eosinophils were followed.

**MATERIALS AND METHODS**

For the experimental animals, 55 male adult normal rabbits weighing about 1.5 kg each and 72 male adult normal dogs weighing about 10 kg each were used. Of them those showing the peripheral leucocytes...
Influences of Adrenalin, Cortisone, and ACTH on Eosinophils

within the normal range and those proven to be negative to parasitic tests (Anchylostoma duodenal and filaria) were selected. The blood plasma mixed with heparin is taken always on the day of the experiment; namely, about 15 ml blood is drawn from the heart of the rabbit in the early morning following a day’s fasting into a syringe containing heparin, and this mixture of blood and heparin is centrifuged at 3,000 rpm for 15 minutes and the supernatant is transferred to an asceptic test tube. As for chick embryo extract, embryonated egg 9-day old is made into brei by Fisher’s homogenizer and the homogenate so obtained is centrifuged at 3,000 rpm for 15 minutes and the supernatant is extracted.

Drugs used are adrenalin of Sankyo Seiyaku, cortisone of Merck, U. S. A., Armour’s ACTH (adrenocorticotropic hormone), and Adrex, pig adreno-cortical preparation of Sankyo Seiyaku.

The bone marrow tissue culture is used mainly for the present experiment (8) and all tools and containers needed are sterilized for 6 hours at 100°C in a dry-heat sterilizer. It is needless to say that all procedures are carried out as asceptically as possible.

Bone marrow is aspirated from the left femur of the animals killed instantaneously in the early morning of the experiment day but fasted one day previously and the bone marrow is taken into a syringe filled with Ringer’s solution. The mixture of heparin and blood plasma is used as the medium, and to this medium 0.01 ml of chick embryo extract or 0.01 ml spleen extract of the hypophysectomized dog is added as the growth promoting substance, and the bone marrow is cultured under a coverglass (Carrel and Fisher). Whereas drugs, ACTH, cortisone and adrenalin, are adjusted with respective solvent in quantities shown in Figures 1—8 so as to contain 0.01 ml each and are then added to the medium; for the cortisone addition, 0.01 ml of the solvent used with cortisone, stirred and mixed by supersonic waves; and for the ACTH addition 0.01 ml physiological saline solution.

As for the steps of observations these are observed under a light microscope kept in a warm box at 37°C at the intervals of 3, 6, and 12 hours after the start of the tissue culture. Abbe’s illuminator is used to observe the growth area, and the area is measured by planimeter from time to time. As for the estimation of the Wandering velocity of cells morphological pictures of individual cells are taken every 30 seconds and the distance covered by the cell center is recorded for 3 minutes and the average distance covered by an individual cell per minute is calculated from the computations of the curvemeter. The measurements of the cell density are taken in three zones of the central, intermediate, and pe-
ripheral zones, and the ratios of the number of eosinophils wandering out to 50 pseudoeosinophils (or neutrophils) are taken as the cell density.

The patterns of eosinophil movement are observed under a light microscope along with those of the respective control, and also micro-cinematographs of them are taken, and by projecting these pictures on the screen their types are studied again.

For the hypophysectomy the method employed at Okinaka Internal Medicine, Tokyo University\(^1\) and that of ISHIKAWA\(^2\) have been referred to and the monolateral operation of the temple has been performed. Our method differs somewhat in that the skin is opened medially and the superior attachment of the temporal muscle is removed as much as possible and is cut away leaving temporal arteries and veins intact. The endocranium is cut crosswise and by lifting the base of brain with a special spatula designed by the author, the pituitary body is taken out.

As for the counting the number of eosinophils, peripheral eosinophils in Hinklemann's solution are actually counted with Fuchs-Rosenthal computer, and smears of peripheral blood are studied as well. The percentage of decrease in the number of eosinophils is calculated from the ratios between the number of eosinophils before the administration of drug to that after the administration. In the experiments with dogs adrenalin is injected into the skin, and cortisone, ACTH, and adrex are injected into the gluteal muscle as shown in Figures.

RESULTS

Case A: Observations on the influences of adrenalin, cortisone, and ACTH on the leucocyte series with a special reference to eosinophils, in the bone marrow tissue culture of normal rabbits:

In these experiments studies were carried out on the actions of adrenalin, cortisone and ACTH on eosinophils themselves and attempts were also made to elucidate the mechanism of eosinopenia in bone marrow. The results to follow are the average values of 5 cases each.

The case treated with adrenalin (Fig. 1).

Adrenalin was added in the amounts from 0.01 mg to 0.005 mg. However, the wandering velocity of eosinophils shown in Fig. 1 is all those treated with 0.002 mg, and in this instance the wandering velocity is lower than that of the control from the onset of the culture. At 6 hour it is \(6.9 \mu/m\) as against \(15.4 \mu/m\) of the control and at 24 hours it is only \(1/2\) to \(1/3\) that of the control. The wandering velocity of pseudoeosinophils, on the other hand, hardly shows any significant difference between
Influences of Adrenalin, Cortisone, and ACTH on Eosinophils

Fig. 1. Case Loaded with Adrenalin

Wandering Velocity of Eosinophils

\[
\begin{align*}
\text{Control} & \quad \text{Loaded with 0.002 mg Adrenalin} \\
\text{Adrenalin (0.01 mg ~ 0.0005 mg) was used}
\end{align*}
\]

Wandering Velocity of Pseudoeosinophils

the control and the case treated with adrenalin. With respect to the values of the eosinophil count and of the growth area, though the adrenalin treated cases show slightly lower values, here again no significant difference can be recognized between adrenalin treated ones and the control.

The cortisone treated cases (Fig. 2).

This group was treated with cortisone in the amount ranging from 0.25 mg to 0.002 mg, but the values presented in Fig. 2 are those treated with 0.05 mg cortisone. The wandering velocity of eosinophils is markedly lower from the onset of the cortisone treatment when compared with that of the control. The value at 6-hour culture is 2.7 μ/m as against 10.2 μ/m
of the control, and at 24 hours the difference between the two becomes still more marked. However, no marked difference can be recognized between the wandering velocity of pseudoeosinophils of the experimental group and that of the control. That is to say, the wandering velocity of pseudoeosinophils (treated with cortisone) is as low as under 1/3 that of the control and also the growth area in the case treated with cortisone is somewhat smaller than that of the control.

In the case treated with ACTH (Fig. 3) ACTH was used in the amounts ranging from 0.1 mg to 0.017 mg but the values shown in Fig. 3 are all based on the cases treated with 0.05 mg.
Influences of Adrenalin, Cortisone, and ACTH on Eosinophils

Fig. 3. Case Loaded with ACTH

Wandering Velocity of Eosinophils

Wandering Velocity of Pseudoeosinophils

--- Control

--- Loaded with 0.05 mg ACTH

ACTH (0.1 mg ~ 0.017 mg) was used

As can be seen from the figure, the wandering velocity of eosinophils treated with ACTH is markedly accelerated as 10.2μ/m against 4.2μ/m of the control at 3 hour culture, and even at 24-hour incubation this tendency is still maintained. On the other hand, the wandering velocity of pseudoeosinophils in the case treated with ACTH, is accelerated in the early stages as compared with that of the control, but later the velocity becomes less than that of the control, hardly revealing any significant difference between the two. With regards the eosinophil count and the growth area, there can be recognized no significant difference between those treated with ACTH and those of the control.
Case B: Observations of the effects of adrenalin, cortisone, ACTH on the movement patterns of individual eosinophils:

The effects of adrenalin, cortisone, and ACTH on the movement patterns of eosinophils have been observed under the light microscope kept in a warm box along with those of the control, and obtained the following results.

As there is no significant difference in the movement patterns of eosinophils both in the case treated with these drugs and those untreated ones, the movement patterns are discussed summarily. By dividing the movement stages into the initial stage, the most active stage, (after 3—6 hours of incubation), and the slowing-down stage of the movement some differences in the eosinophil movement patterns between the treated and the untreated ones may be pointed out in the following manner.

With respect to the control shown in Photo. 4, each cell has its motile stage and the resting stage, and when the movement is accelerated, the length of the motile stage is prolonged, thus shortening the resting stage and increasing its speed of progression. Matured eosinophils like other matured leucocytes, move about by their pseudopodia, and the migration of a cell is completed when granules and the nucleus are transferred into the pseudopodium. The shape of the cells is mostly irregular during the motile stage, but it is spherical during the resting stage. Naturally in the most active phase, the resting stage where cells maintain a spherical shape is quite brief, and in this stage the pseudopodium is extended and projected in the direction of the cell movement and granules move very actively, gathering in the forward direction followed by the nucleus. In this instance most cells as a whole present a semi-circular shape. As the cell movement becomes gradually less active, the pseudopodial movement coincidentally is slowed down, making the pseudopodium pointed and granules movement also less and less active. Occasionally the cells of the control present a semi-circular shape, but the majority are near semi-circular or irregular in shape, showing an active pseudopodial movement. There are detailed reports on the movement patterns of pseudoeosinophils by HIRAKI-WATARI of our department, but these patterns are essentially the same as those of eosinophils.

In the case treated with adrenalin (Photo. 1) the constriction of the cell body of eosinophils is more marked than that of the control, and the pseudopodial movement is slower and the majority of the cells show pseudopodium with a narrow round tip. Also most of the cells possess fibrous processes at the tip of pseudopodium and cilia at the tail end but the cell granules are less active. Speaking of the movement stage of an
Influences of Adrenalin, Cortisone, and ACTH on Eosinophils

Photo. 1
Eosinophils loaded with Adrenalin
Greater magnification of lower photos

Photo. 2
Eosinophils loaded with Cortisone
Greater magnification
Photo. 3
Eosinophils treated with ACTH

Greater magnification

Photo. 4
Eosinophils Control

Greater Magnification
individual cell, the resting stage is rather prolonged. This is clearly a sign of a fall in the motive function. However, there can be recognized no difference in the movement pattern of the pseudoeosinophils treated with adrenalin and that of the control.

Next, coming to the case treated with cortisone (Photo. 2), the majority of eosinophils maintain spherical shape and are spiraling around even in the most active stage after the culture, and sometimes they project a sharp pseudopodium. And no active pseudopodial movement as observable in the control can at all be seen and the movement of the cell granules are also quite slow. The wandering velocity has, as already mentioned, declined and it is such as can be observed in the cell at the slowing-down stage of movement. However, the movement pattern of pseudoeosinophils differs not much from that of the control, their pseudopodial movement being kept up actively.

In the case treated with ACTH (Photo. 3), at a glance eosinophils present the feature entirely different from the control. Namely, the motile stage of individual eosinophils has been lengthened and these cells are very active in their movement. The pseudopodium is wider at the forward end with granules arranged uniformly and the nucleus following these granules, and the cell body presents an appearance as if it has become a
pseudopodium itself and the majority of these eosinophils are of a radial form. Individual granules within the cell body advance forward in the manner as if to outstrip one another, and the forward speed of the cell is considerable, indicating an acceleration in the cell function. However, there can be recognized no difference between the movement pattern of the pseudoeosinophils treated with ACTH and that of the control.

**Case C:** *Influences of adrenalin, cortisone and ACTH on the hypophysectomized dogs* (Figs. 4, 5, 6).

This experiment has been conducted with the purpose to study the relationship between eosinopenia and the anterior pituitary lobe when treated with adrenalin, cortisone, and ACTH. By administering these drugs in the manner already stated, changes in the number of peripheral eosinophils have been followed and the results are as described below.

When 0.1 ml/kg adrenalin (1/1000) is administered under the skin, the decrease in peripheral eosinophils of hypophysectomized dogs 2 hours after the administration is -23 per cent as shown in Fig. 4 but it becomes -8.4 per cent 4 hours later, whereas in the control (normal dogs) the peripheral eosinophils decrease along with the lapse of time, reaching as low as 45 per cent less 4 hours after the administration. Namely, the decrease in the number of peripheral eosinophils in hypophysectomized dogs is less than that in the control.

When 1.25 mg/kg cortisone is administered intramuscularly to both hypophysectomized dogs and to the control, eosinopenia can clearly be
observed in the control (the value 4 hours after the administration is 46.6 per cent lower), while no significant decrease can be observed in hypophysectomized dogs, the maximum decrease being —13 per cent two hours after the administration and the value 4 hours later is plus 1 per cent as shown in Fig. 5. When two-fold or one half the amount of cortisone, namely, 2.5 mg/kg to hypophysectomized dogs and 0.625 mg/kg is administered to the control, no significant eosinopenia can be recognized in either case as shown in Fig. appendix (1).

When 0.5 mg/kg ACTH is administered intramuscularly to both hypophysectomized dogs and the control marked eosinopenia can be observed in both test animals and the control group as shown in Fig. 6. In this instance the rate of eosinophil decrease in the control is —42.9 per cent while —51.2 per cent in hypophysectomized dogs. When, however, one half the amount, namely, 0.25 mg/kg ACTH is administered to the two groups, both take about the same course as shown in Fig. appendix (2), revealing a slight decrease in the number of eosinophils.

When 1.25 mg/kg cortisone and 20 IU Adrex are administered intramuscularly at the same time to hypophysectomized dogs, marked eosinopenia (a decrease of 55.5 % in the peripheral eosinophils) can be observed as shown in Fig. 7. However, when 20 IU Adrex alone is administered to hypophysectomized dogs, no significant decrease in eosinophils can be recognized (the rate of peripheral eosinophil decrease being 14.5 %).

Case D: Influences of Cortisone and Adrex on the Bone Marrow Tissue Culture of Hypophysectomized Dogs.

As for the material the bone marrow of hypophysectomized dogs was used with two different culture media. By the method mentioned in the preceding section heparin-containing blood plasma of hypophysectomized dogs and the same of normal dogs were prepared, and to these media the same quantity (0.01 mg) of cortisone was added. As the growth promoting substance physiological saline extract of the spleen of hypophysectomized dogs was used, and the culture was carried out under a coverglass and likewise observations were conducted in the same manner as in Case A.

In the preliminary experiment conducted at first without adding cortisone both in the heparin containing plasma of hypophysectomized dogs and in the similar medium of normal dogs about one half to one third the cells wandering out of the growth area are eosinophils and those are moving actively as can be seen in Photo. 5. In the following are described the average values of 4 cases each.

In the case added with 0.01 mg cortisone (Fig. 8) the wandering velocity of eosinophils in the blood plasma of hypophysectomized dogs is
Fig. 6. ACTH Administration (Per Kg 0.5 mg)

Fig. 7. Eosinophilic Reduction Ratio
Adrex, Adrex + Cortisone administered to the hypophysectomized dog

Fig. Appendix

(1) Cortisone administration
(2) ACTH administration

21.5 μ/m at three hours' culture, whereas it is 3.4 μ/m in the case of the medium consisting of blood plasma of normal dogs, showing marked difference between the two. In the case using blood plasma of normal dogs the majority of eosinophils show a spherical shape, clearly indicating a fall in the cell function. At 12 hours' culture number of eosinophils (10.5) in the blood plasma of normal dogs is about 20—30 per cent less than those
Fig. 8. Tissue Culture of a Hypophysectomized Dog's Bone Marrow Loaded with Cortisone

Wandering Velocity of Eosinophils  

Wandering Velocity of Neutrophils

Case of normal dog blood plasma  

Case of hypophysectomized dog blood plasma  

(same dose of cortisone was added)

Number of Eosinophils

Growth area

(16.0) in the blood plasma of hypophysectomized dogs. The wandering velocity of neutrophils in both media reaches respective maximum at 3 hours' incubation, namely, 6.0 \mu/m and 6.3 \mu/m, but in the subsequent observations carried out from time to time there can be recognized hardly any difference in between the two. As for the growth area at 12 hours' culture it is 2.2 in the blood plasma of normal dogs while it is 4.3 in that of hypophysectomized dogs.
Case E: Clinical observations of the cortisone tests on the anterior pituitary lobe function.

On the basis of the results obtained in Cases A to D, namely, from the fact that the eosinopenia inducing action of cortisone in hypophysectomized dog in vivo differs from that in normal in the bone marrow tissue culture of hypophysectomized dogs and that the action of cortisone on eosinophils cultured in the blood plasma of hypophysectomized dogs also differs from that on eosinophils in the blood plasma of normal dogs, it has been assumed that the decrease in the number of eosinophils caused by cortisone may prove to be the criterion for the actual functional state of the anterior pituitary lobe. Therefore, the following clinical observations have been conducted on this assumption. The method employed here is essentially the same as in the case with dogs, i.e. 1.25 mg/kg cortisone was injected into the gluteal muscle of the subjects. For the duration of 4 hours after the injection the actual number of peripheral eosinophils was counted from time to time in the same manner as described previously (Case C), and on the other hand the percentage of eosinophils as against the eosinophil count before the treatment was computed. For the other series of observations 0.5 mg/kg ACTH was injected into gluteal muscle and the decrease in the number of peripheral eosinophils was calculated the same as in the case of cortisone administration. In the following are presented the percentage of decrease in the number of peripheral eosinophils 4 hours after the administration of cortisone and ACTH.

Case No. 1. In the case with pituitary tumor (clinical diagnosis, diabetes insipidus) the decrease in eosinophils due to cortisone amounted to 1.5 per cent and the same treated with ACTH to 52.9 per cent.

Case No. 2. In the case of pituitary tumor (postoperative diagnosis) the decrease in eosinophil count treated with cortisone was 18 per cent while the same treated with ACTH was 36 per cent.

Case No. 3. In the case of adiposogenital dystrophy (clinical diagnosis) the decrease in eosinophil count due to cortisone was 3 per cent while the same due to ACTH was 50 per cent.

Case No. 4. In the case of diffuse symmetrical scleroderma (clinical diagnosis) the decrease in the eosinophil count due to cortisone was 34 per cent while that due to ACTH was 43.3 per cent.

Case No. 5. In the case of Bechet's disease (clinical diagnosis) the decrease in the eosinophil count due to cortisone was 40 per cent and the same due to ACTH was 32 per cent.

From these results it has been clarified that there are some differences between the percentage of decrease in the eosinophil count due to cortisone
and that due to ACTH. Cases 1 to 3 have been suspected of the lowered function in the pituitary body while Cases 4 and 5 have been judged to have the diminished functions in both the pituitary body and the adrenal cortex.

DISCUSSION

Judging from the observations of the influences of adrenalin, cortisone, and ACTH on the leucocyte series, especially on eosinophils, in bone marrow tissue cultures, it has been recognized that adrenalin acts as to inhibit the wandering capacity of eosinophils, and also from the changes in their movement pattern as already described this drug inhibits the movement of eosinophils. From the changes in the wandering capacity and the movement pattern of eosinophils cortisone has been confirmed to act still more inhibitorily on eosinophils than adrenalin. When ACTH is made to act directly on eosinophils, however, it accelerates the wandering capacity of them and judging from their movement pattern it brings about an increase in the motive function. Moreover, adrenalin and ACTH do not bring about any significant change in the number of eosinophils, while cortisone alone has been found to possess an inhibitory action in this respect.

In the bone marrow perfusion tests with adrenalin SUGA of the Hiraki Department of Internal Medicine, Okayama Univ. Med. School, has clarified that adrenalin, acting directly on bone marrow, elicits the detention of eosinophils in the bone marrow and he has further confirmed it in the histological picture of the bone marrow. RUPPEL claims that after the subcutaneous administration of adrenalin the human bone marrow picture revealed the acceleration of the bone marrow function and an increase in the eosinophil production, and he believes eosinopenia caused by adrenalin is due to the impairment of the eosinophil production.

From the author’s own findings it seems that as the results of the direct action of adrenalin on eosinophils the wandering capacity is diminished and that even from the movement pattern there can be seen some sign of the decline in the wandering capacity so that it becomes difficult for eosinophils to migrate into the venous lumen of bone marrow, and thus the detention of eosinophils in bone marrow ensues.

As regards the effect of cortisone GORDON observed a marked decrease in the number of eosinophils in the bone marrow of the albino rats administered with a large dose of cortisone and he attributes this phenomenon to an impairment of the eosinophil production due to cortisone, and the same view is supported by several other workers. From the author’s
experimental results since cortisone, acting directly on eosinophils, impedes their wandering capacity and since it shows an inhibitory action from their movement pattern and exhibits an inhibitory action on the number of eosinophils, it seems that when cortisone is administered in vivo, the migration of eosinophils into the venous lumen of bone marrow is inhibited and at the same time the bone marrow parenchyma is disturbed.

With respect to the effect of ACTH, it is well known that ACTH usually acts in vivo through the mediation of adrenal cortex as attested by Thorn's tests, etc. Concerning the direct effect of ACTH SUGA of our department (Hiraki Internal Medicine, Okayama Univ. Med. School) recognized in his in vitro observation with ACTH added to fibrin-free blood (dog and human) that ACTH has no effect at all on eosinophils and other white corpuscles numerically and qualitatively. Likewise in the series of in vitro experiments using peripheral blood mixed with ACTH CAPE reports that he saw no changes in the morphology of eosinophils. From the author's own results it has been ascertained that ACTH, when acting directly on eosinophils, has no influence on the number of eosinophils but it enhances and strengthens their wandering capacity.

With respect to the effects of three drugs, adrenalin, cortisone, and ACTH on the number and wandering capacity of pseudoeosinophils, none of these drugs brings about any appreciable change. Concerning the actions of adrenalin, cortisone, and ACTH studied with hypophysectomized and normal dogs, in the case with adrenalin the decrease in peripheral eosinophils is less than in the control; in the case of cortisone the control show a significant decrease in eosinophils while no significant decrease in the experimental group; and in the case of ACTH treatment a significant decrease in eosinophils can be recognized equally in both test animals and the control. From these results it is obvious that the actions of adrenalin, cortisone, and ACTH in vivo are dependent on the state of the pituitary body.

With ACTH treatment THORM observed its eosinopenic action, and by adrenalin (epinephrine)-tests on the albino rats previously hypophysectomized but autotransplanted with the same pituitary gland to one of the anterior chambers LONG recognized a decrease in peripheral eosinophils after subcutaneous injection of adrenalin yet no eosinopenia ensued after removing the autotransplanted eye, and also in his adrenalin injection into conjuctiva of the autotransplanted eye he observed a decrease in peripheral eosinophils while no eosinopenia after the similar injection to the eye not transplanted with pituitary gland, proving that adrenalin acts through the mediation of pituitary body. On the other hand, SPEIRS re-
Influences of Adrenalin, Cortisone, and ACTH on Eosinophils

Cognized eosinopenia in 50 per cent of the animals hypophysectomized, and RUPPER\textsuperscript{20} likewise obtained similar results. AKASU\textsuperscript{1} recognized that while ACTH and adrenalin bring about eosinopenia in a similar degree, the adrenalin test on hypophysectomized albino rats yields variable results and ACTH-test gives a fixed decrease in eosinophils, indicating some differences in the actions of the two drugs. Also cortisone is widely known to possess the action to decrease eosinophils\textsuperscript{4,18,28}. In his administrations of various drugs such as ACTH, cortisone, adrenalin, Doca, Doca plus vitamin C, to one and the same patient TAKEDA\textsuperscript{27} recognized the potency of these drugs to be in the descending order of ACTH, adrenalin, Doca plus vitamin C, cortisone and Doca. From these it can be seen that there are some differences in the acting mechanisms of the three drugs, ACTH, cortisone, and adrenalin with respect to their eosinopenic effects in peripheral blood.

Judging from the author's own results the fact that the presence or absence of the pituitary body affects the decrease in the number of peripheral eosinophils seems to suggest that eosinopenia due to adrenalin is in some way associated with the pituitary body, and in this respect eosinopenia caused by ACTH appears to be different from the former. Next, in the elucidation of the question why eosinopenia did not occur in the case of hypophysectomized dogs when treated with cortisone, the results shown by Case D were obtained, i.e. when cortisone acts directly on eosinophils, in the case with normal dogs a marked inhibition of the wandering capacity of eosinophils identical with Case A can be observed, while on the contrary in the case with hypophysectomized dogs no such inhibition at all be recognized. From these results a factor required by cortisone in the mechanism inducing eosinopenia seems to be lacking in the absence of the pituitary function.

Now, when Adrex, a synthetic adrenocorticotropic hormone is injected intramuscularly to hypophysectomized dogs along with cortisone, a decrease in peripheral eosinophils can be observed in an identically same degree as in normal dogs (the control). However, when an equal amount of cortisone and Adrex is administered each independently, no significant decrease in the eosinophils can be recognized. Furthermore, when both Adrex and cortisone are added to the blood plasma of hypophysectomized dogs in the bone-marrow tissue culture of hypophysectomized dogs, the inhibitory action on the eosinophil wandering capacity can be observed in the same degree as in the case of adding cortisone to the medium of normal dog plasma. On the other hand, when either Adrex or cortisone alone is added to such a culture medium, no inhibitory action on the wandering
capacity of eosinophils can be recognized.

From these results it seems that the decrease in peripheral eosinophils in vivo does not occur solely by the action of cortisone but it is brought about by the co-operative action of cortisone and adrenocorticotropic hormone. It follows then that after the hypophysectomy the amounts of adrenocorticotropic hormones in vivo would decrease markedly and consequently the decrease in peripheral eosinophils would not occur simply by the administration of cortisone alone. With the intention to apply this mechanism clinically the author used cortisone for examining the functions of the pituitary body and obtained results anticipated. However, this point requires further clarification by future studies, and concerning the decrease in peripheral eosinophils at the time of cortisone-tests, there arises a problem of the reactivity of the adrenal cortex function.

SUMMARY

1. Adrenalin, when acting directly on eosinophils, brings about a diminution in the wandering velocity of eosinophils but it has no influence on the number of the cells. Judging from the movement patterns of eosinophils this drug acts as to impede the motive function.

2. Acting directly on eosinophils, cortisone markedly decreases the wandering velocity of these cells and also brings about the diminution in the number of the cells. Likewise from the movement patterns of eosinophils, this drug markedly impedes the motive function of the cells.

3. ACTH (adrenocorticotropic hormone), when acting directly on eosinophils, enhances the wandering velocity of eosinophils but it in no way affects the number of eosinophils. Judging from the eosinophil movement patterns, this drug markedly promotes the motive function.

4. Although adrenalin brings about a decrease in the number of peripheral eosinophils in hypophysectomized dogs, the rate of the decrease is less than that observable in the case of normal dogs.

5. Cortisone brings about no significant change in the number of peripheral eosinophils in hypophysectomized dogs, but is induces a decrease in peripheral eosinophils of normal dogs.

6. ACTH acts as to decrease the number of peripheral eosinophils to an equal degree in both hypophysectomized and normal dogs.

7. When cortisone is administered simultaneously with Adrex, a marked decrease in peripheral eosinophils is brought about in hypophysectomized dogs.

8. By means of the bone-marrow tissue culture of hypophysectomized
Influences of Adrenalin, Cortisone, and ACTH on Eosinophils
dogs it has been confirmed that the blood plasma of hypophysectomized
dogs lacks an essential factor for cortisone to induce eosinopenia in per-
ipheral blood.

9. The decrease in eosinophils of peripheral blood induced by cortisone
has been proved to be dependent upon the presence or absence of the pitui-
tary body.

10. Taking the decrease in peripheral eosinophils by cortisone admini-
stration as the criterion, the author has carried out clinical observations
with this method and obtained anticipated results.

REFERENCE
2. CANNON, W. B. and RAPPORT, D.: Studies on the conditions of activity in endocrine
gland, Amer. J. Physiol., 58, 338, 1921
66, 441, 1952
4. ESSELLER, A.F., REANNERT, R.L. and MORAND, L.: Mechanism of glucocorticoid
eosinopenia, Blood, 6, 531, 1954
5. GORDON, A.S.: Relation of adrenal to blood formation in rat, Endocrinol., 49, 497,
1951
     ditto : Tokyo Ijishinshi, 70, 257, 1953
8. HIRAKI, K, OFUJI, T. and WATARI, Z. : Observation of various living blood cells
     by tissue culture of the bone marrow, Acta Medicinae Okayama, 10, 110, 1956
9. HOUSSAY, B.A. Amer. J. Physiol., 76, 551, 1926
10. KARK, R.N. and MUEHRKE, R.C. : Does adrenalin eosinopenia reflect pituitary-
     adenal function in man?, Lancet, 1, 1189, 1952
12. LOHMAYER, G., and HUSSIMAN, H.: Der Klinische wert der Nebennierenrindentest-
    ing mit ACTH und Adrenalin, Klin. Wschr., 31, 1, 1953
13. LONG, C.N.H. and FRY, E.G. : Effects of epinephrine on adrenal cholesterol and
15. NELSON, DH., REICH, L.T. and SAMUELS, L.T.: Isolation of steroid hormone
     from adrenal-vein blood of dogs, Science, 111, 578, 1950
18. QUITTNER, H.: Effect of massive dose cortisone on peripheral blood and bone
     marrow, Blood, 6, 513, 1951
     effect of epinephrine on the pituitary-adrenocortical system, J. of Endocrinol.,
     20, 187, 1959
20. RUPPEL, W.: Kritisches zum Eosinophilien-Test, Schweiz. Med. Wschr., 81, 926,
     1951