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Studies on esinophils in bone-marrow tissue culture of the human sternum Part 2. Study on the causative factor of eosinophilia in hookworm disease by means of bone-marrow tissue culture with a special reference to the relationship with allergic reaction

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Studies on esinophils in bone-marrow tissue culture of the human sternum Part 2. Study on the causative factor of eosinophilia in hookworm disease by means of bone-marrow tissue culture with a special reference to the relationship with allergic reaction*

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Abstract

From these results it is but natural to assume that the antigen-antibody reaction is involved in the phenomenon, eosinophilia. The antigen in this instance is the filtrate of hookworm emulsion, and the serum of hookworm disease as well as the bone marrow can be thought to contain the antibody. In any case, so long as the medium contains the serum or bone marrow or both of them obtained from the patient of hookworm disease, eosinophilia and the acceleration in the motility of eosinophils are brought about in the growth zone by addition of the filtrate of hookworm emulsion. Therefore, as for the mechanism inducing hookworm eosinophilia, it may be interpreted that the patient of hookworm disease is repeatedly sensitized by the antigen arising all probability from the metabolic products of hookworms or from the dead bodies of the worms; and producing the antibody in tissues and blood, thus the antigen-antibody reaction is elicited in vivo as long as hookworms live in the human body so that the increase in the mitosis and the acceleration in the motility of eosinophils in the bone marrow are brought about with the resultant continuous discharge of a large quantity of eosinophils from the bone marrow parenchyma into the sinusoids, thereby inducing eosinophilia in the peripheral blood.

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**STUDIES ON EOSINOPHILS IN BONE-MARROW TISSUE
CULTURE OF THE HUMAN STERNUM**

**PART 2. STUDY ON THE CAUSATIVE FACTOR OF
EOSINOPHILIA IN HOOKWORM DISEASE BY MEANS
OF BONE-MARROW TISSUE CULTURE WITH A
SPECIAL REFERENCE TO THE RELATIONSHIP
WITH ALLERGIC REACTION**

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In hookworm disease marked eosinophilia can be recognized in peripheral blood, bone marrow or in other tissues, but as for the mechanism inducing eosinophilia opinions are varied and no fixed theory is available. For example, there are such theories as; (1) proteins of hookworms or their metabolic products possess a certain stimulants; (2) in addition to the direct stimulation on eosinophils by the toxin contained in the hookworm, eosinophilia is caused by substances produced in the serum of patient, which accelerate the increase in number of eosinophils; and (3) allergic theory.

We have previously made a report on the relationship with eosinophilia and antigen-antibody reaction in our bone-marrow tissue culture. Namely, in the bone-marrow tissue culture of guinea pigs, when the antigen-antibody reaction (using bovine serum) is induced in the medium, a marked eosinophilia and an acceleration in the wandering velocity of eosinophils can be recognized in the growth zone, reaching the maximum 9 to 12 hours after the start of culture. It is obvious that there is an intimate relationship between eosinophilia and the antigen-antibody reaction. An increase in the mitosis of immature eosinophils have been observed in the stained specimens of explant. From the foregoing facts we have assumed that when the antigen-antibody reaction takes place in the body, eosinophils in the bone marrow parenchyma increase in number by mitosis on one hand and the wandering velocity of these eosinophils is accelerated on the other, and consequently a great quantity of eosinophils is continuously discharged from the bone-marrow parenchyma into the sinusoids of bone marrow, thus bringing about eosinophilia in the peripheral blood.

Therefore, from a similar viewpoint we have attempted to find out a clue to the causative factor of hookworm eosinophilia by determining the differences in the reaction-behaviors of eosinophils between bone-marrow tissue culture of normal person and that of patient with hookworm disease, with addition of the emulsion filtrate of fresh hookworms, and by observing the cell density and motility of those eosinophils wandering out into the growth zone.

MATERIALS AND METHODS

The filtrate of hookworm emulsion is an aseptic liquid prepared from the emulsion of fresh hookworms collected after deworming and after filtering by Seitz' apparatus. The sera for the culture are the serum of normal person and the serum of the patient with eosinophilia in the peripheral blood. The method of culture is the same as in Part 1, a simplified method of bone-marrow tissue culture.

RESULTS

The bone-marrow tissue culture of normal person to which the filtrate of hookworm emulsion is added (Figs. 1, 2). When the serum of

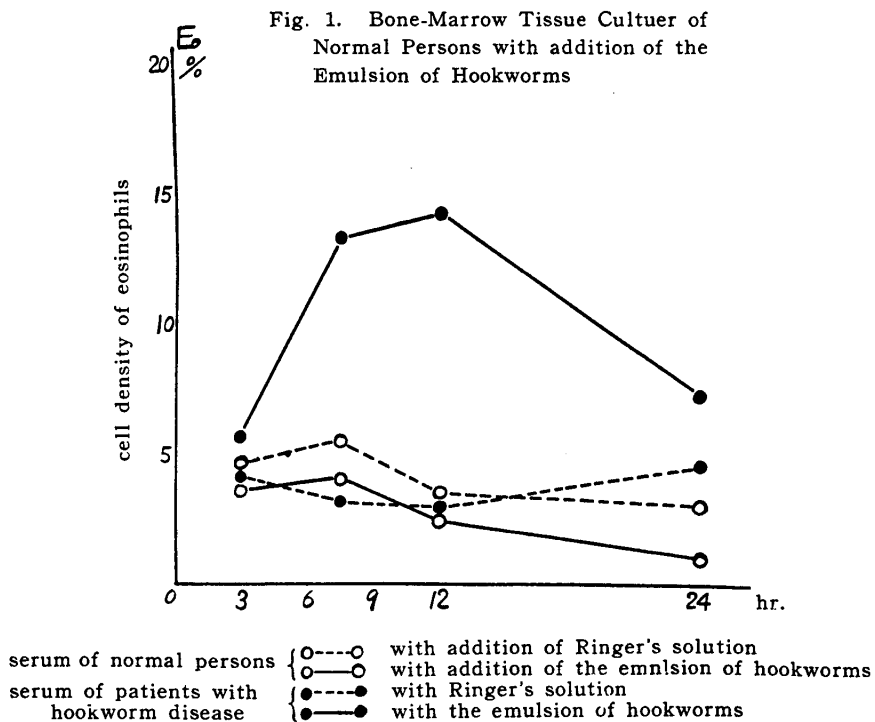
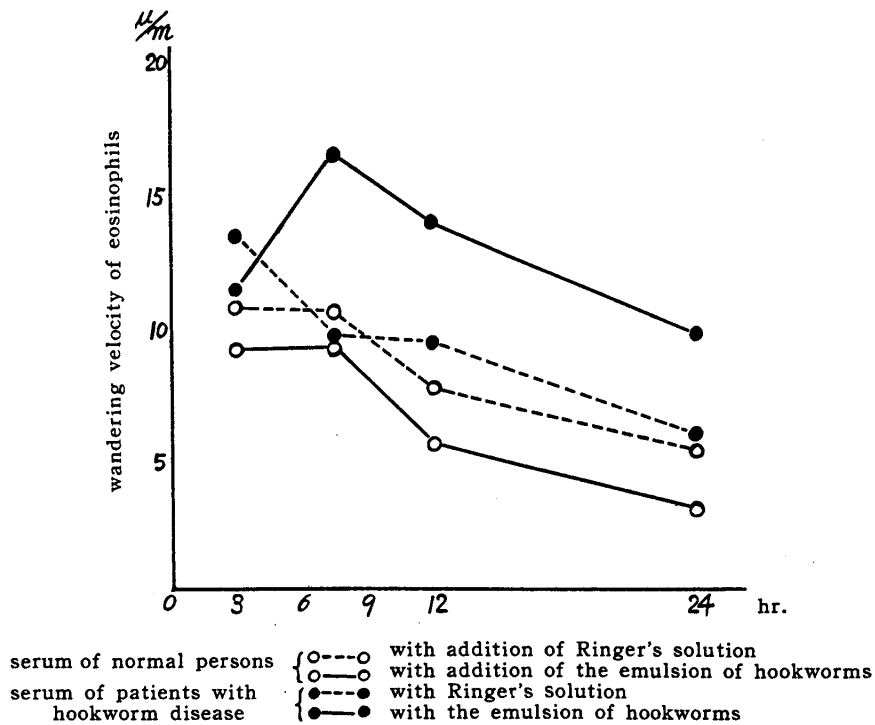


Fig. 2. Bone-Marrow Tissue Culture of Normal Persons with Addition of the Emulsion of Hookworms

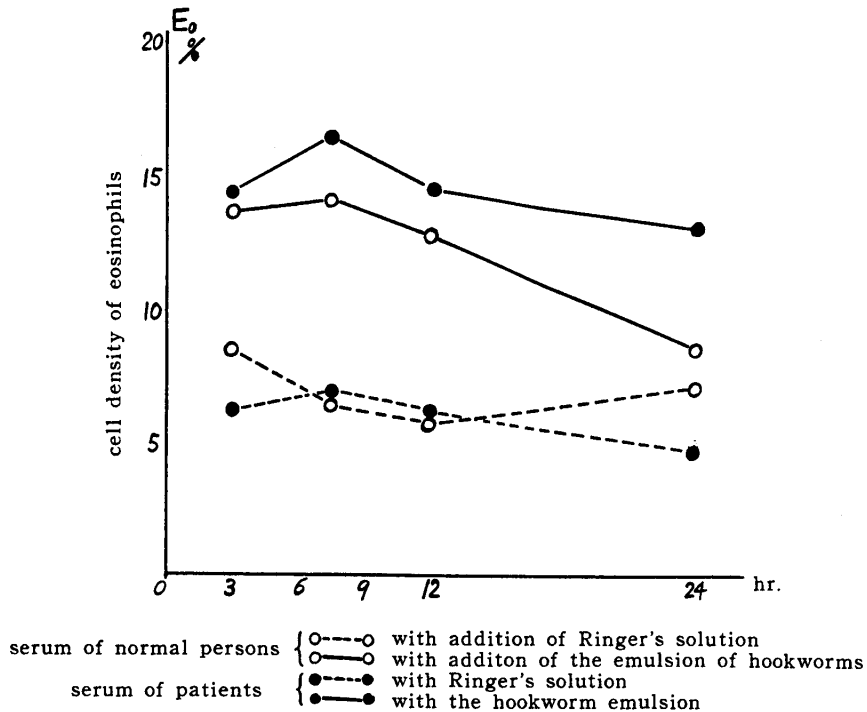


normal person is used in the culture, the cell density of eosinophils in the growth zone is rather lower than that of the control (with addition of Ringer's solution to the medium), and the wandering velocity is also somewhat diminished. In contrast to this, when the serum of the patient with hookworm disease is used, a marked eosinophilia and an acceleration in the wandering velocity of eosinophils can be recognized, reaching their maximum 9 to 12 hours after the start of culture.

In the present experiment when the serum of the patient with hookworm disease is used, irrespective of addition of hookworm emulsion filtrate, eosinophils of Type I-B can be observed in about 20 per cent of wandering eosinophils.

The bone-marrow tissue culture of hookworm disease to which the filtrate of fresh hookworm emulsion is added (Figs. 3, 4). Whatever the serum may be used, the number of eosinophils are markedly increased by the addition of the filtrate. Moreover, as for the change in the

Fig. 3. Bone-Marrow Tissue Culture of Patients with Hookworm Disease, with Addition of Hookworm Emulsion

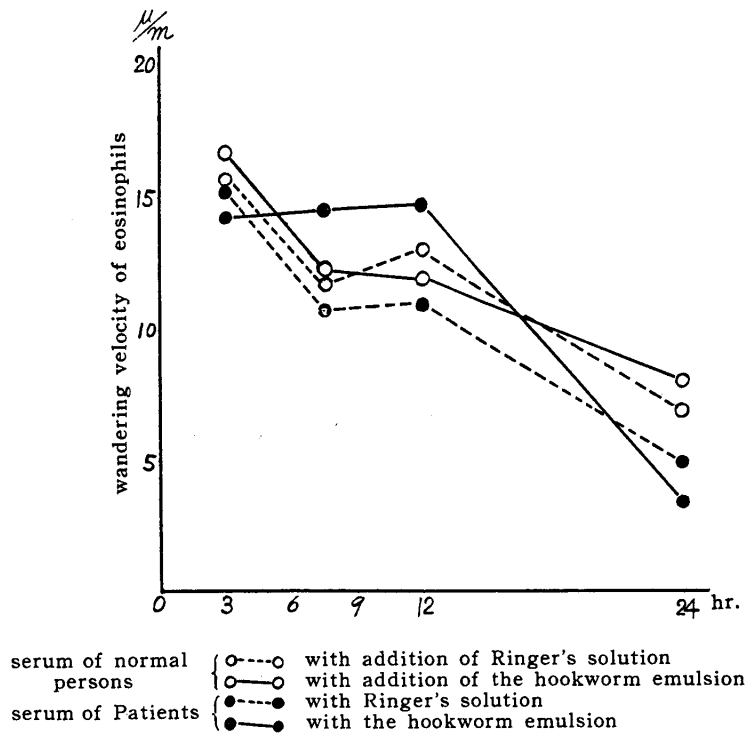


wandering velocity of eosinophils, the wandering velocity of eosinophils in hookworm disease being accelerated to start with as compared with that in the case of normal person, it is difficult to find any significant difference from that of the control with addition of Ringer's solution, but a still further increase in the wandering velocity can be observed when the serum of hookworm disease is used.

SUMMARY

From these results it is but natural to assume that the antigen-antibody reaction is involved in the phenomenon, eosinophilia. The antigen in this instance is the filtrate of hookworm emulsion, and the serum of hookworm disease as well as the bone marrow can be thought to contain the antibody. In any case, so long as the medium contains the serum or bone marrow or both of them obtained from the patient of hookworm disease, eosinophilia and the acceleration in the motility of eosinophils are brought about in the growth zone by addition of the filtrate of hookworm

Fig. 4. Bone-Marrow Tissue Culture of Patients with Hookworm Disease, with Addition of Hookworm Emulsion



emulsion. Therefore, as for the mechanism inducing hookworm eosinophilia, it may be interpreted that the patient of hookworm disease is repeatedly sensitized by the antigen arising all probability from the metabolic products of hookworms or from the dead bodies of the worms; and producing the antibody in tissues and blood, thus the antigen-antibody reaction is elicited *in vivo* as long as hookworms live in the human body so that the increase in the mitosis and the acceleration in the motility of eosinophils in the bone marrow are brought about with the resultant continuous discharge of a large quantity of eosinophils from the bone marrow parenchyma into the sinusoids, thereby inducing eosinophilia in the peripheral blood.

CONCLUSIONS

With the addition of the filtrate of hookworm emulsion to the bone-marrow tissue culture of normal person and the patient with hookworm

disease the authors obtained the following results: Namely, for the causative factor of eosinophilia in hookworm disease the allergic reaction to the protein or the metabolic product of the hookworms seems to play an important role, and as the result of the antigen-antibody reaction the motility of the bone-marrow eosinophils is markedly accelerated, and eosinophilia in the growth zone of the bone-marrow tissue culture results.

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