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Kiyoshi Hiraki*

Hiroshi Sunami†

Hideo Nishishita‡

*Okayama University,

†Okayama University,

‡Okayama University,

Influences of various hormones on the megakaryocyte in bone-marrow tissue culture*

Kiyoshi Hiraki, Hiroshi Sunami, and Hideo Nishishita

Abstract

In our study on the influences of various hormones and various endocrines on the megakaryocyte function by means of bone-marrow tissue culture, we obtained the following. 1. In the repeated administration of ACTH, cortisone, testosterone, progesterone, or thyroxin to guinea pigs, these hormones accelerated the megakaryocyte function, whereas estradiol on the contrary diminished the function. 2. The removal of such endocrines as the pituitary, adrenal, thyroid or testicles will diminish the megakaryocyte function, while removal of the ovaries accelerates it. 3. For the megakaryocytes in the hypophysectomized rats, ACTH acts most effectively to restore their function, followed by cortisone and pulverized thyroid, while testosterone has hardly any effect on the function. 4. ACTH, cortisone, prednisolone, testosterone, progesterone, and pulverized thyroid act directly on megakaryocytes so as to accelerate their function, while estradiol diminishes the megakaryocyte function. 5. For the megakaryocytes in idiopathic thrombocytopenic purpura ACTH is most effective in restoring the function; for the megakaryocytes in hypoplastic anemia cortisone is most effective; and for the megakaryocytes in Banti's disease prednisolone is most effective in restoring the megakaryocyte function.

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**INFLUENCES OF VARIOUS HORMONES ON THE
MEGAKARYOCYTE IN BONE-MARROW
TISSUE CULTURE**

Kiyoshi HIRAKI, Hiroshi SUNAMI and Hideo NISHISHITA

*Department of Internal Medicine, Okayama University Medical School
Okayama, Japan (Director : Prof. K. Hiraki)*

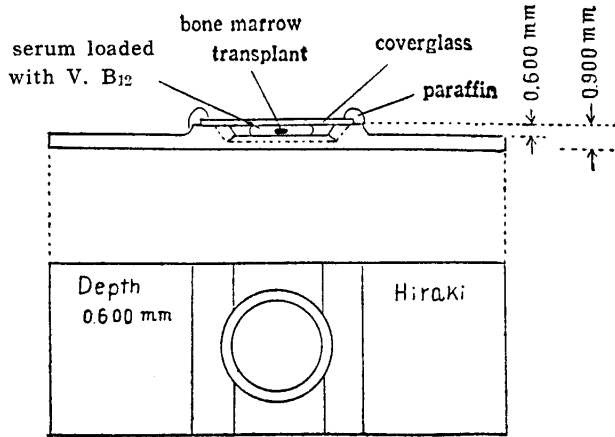
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That the hematopoietic functions of bone marrow are regulated by hormones is clear from the fact that the blood picture changes in patients with disorders of the pituitary, adrenal or thyroid glands and that these disorders can be remedied by hormone treatment or by some appropriate surgical therapy. Moreover, with a marked advance in the field of endocrinology there are numerous new findings on the relationship between the bone-marrow hematopoiesis and endocrines. On the other hand, there are only a few studies of the influences exerted on platelets, the tertiary constituent of blood and on megakaryocytes with smears or slice specimens. However, in 1956 in our bone-marrow tissue culture we^{7,8} confirmed that human megakaryocytes form projections and platelets are split off from the tip of these projections and thereby we realized that this is one of the most satisfactory methods in determining the functions of megakaryocytes. Therefore, with the purpose to elucidate the influences of various hormones and endocrines on the functions of megakaryocytes as well as the effects of ACTH, cortisone, and prednisolone on the megakaryocyte functions in various blood diseases we undertook a series of experiments with this method of bone-marrow tissue culture. In the present report some of new findings are discussed.

EXPERIMENTAL METHODS

The method employed in the present experiment was the simple culture method devised in our laboratory, and observed the functions of megakaryocytes after 18 hours culture (Fig. 1). Materials used were the bone marrow of mature guinea pigs, weighing 300—400g, removed of adrenal, testicles, ovaries, or thyroid gland, another group injected with various hormones, rats weighing 100 to 130g all hypophysectomized three animals each and specimens of the bone marrow from normal persons, patients with Basedow's disease, myxedema, and various blood

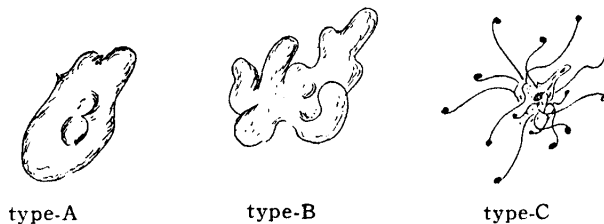
Fig. 1. Simple Cover-Slip Culture Method (devised in our laboratory)



diseases.

In the second series of the experiments the tissue culture was conducted by directly adding various hormones such as ACTH, cortisone, prednisolone, testosterone, progesterone, estradiol, and pulverized thyroid, which were dissolved or suspended in Ringer's solution in various concentrations. In this instance for the control an equal volume of Ringer's solution was used. The observations of the megakaryocytes were carried out in the following way: Megakaryocytes are classified into three types according to their functions as indicated in Fig. 2; namely, type-A those showing a slight deformation of the cell body; type-B those showing pseudopodial movement; and type-C those showing tentacle formation with platelets splitting off. Calculating the percentage of motive megakaryocytes in each of these types against the megakaryocytes appearing in the growth zone, this is taken as the percentage of the motive type megakaryocytes, and the sum of these is taken as the total percentage of motive megakaryocytes (to be abbreviated to TMP hereafter)

Fig. 2. Types of motive megakaryocytes



type-A

type-B

type-C

RESULTS

Pituitary and megakaryocyte functions (Figs. 3, 4):

After injecting daily dosage of 1.25 IU of ACTH per 100 g body weight to guinea pigs for four consecutive days to the total of 20 IU, in their bone-marrow tissue culture of the animals sacrificed immediately afterwards TMP is 1.5 times that of the control, and the percentage of type-C megakaryocytes is 2.5 times that of the control, showing an acceleration of megakaryocyte functions.

In the cells cultured from the bone marrow of the hypophysectomized rats ten days after operation TMP is 3/5 that of the control, and the

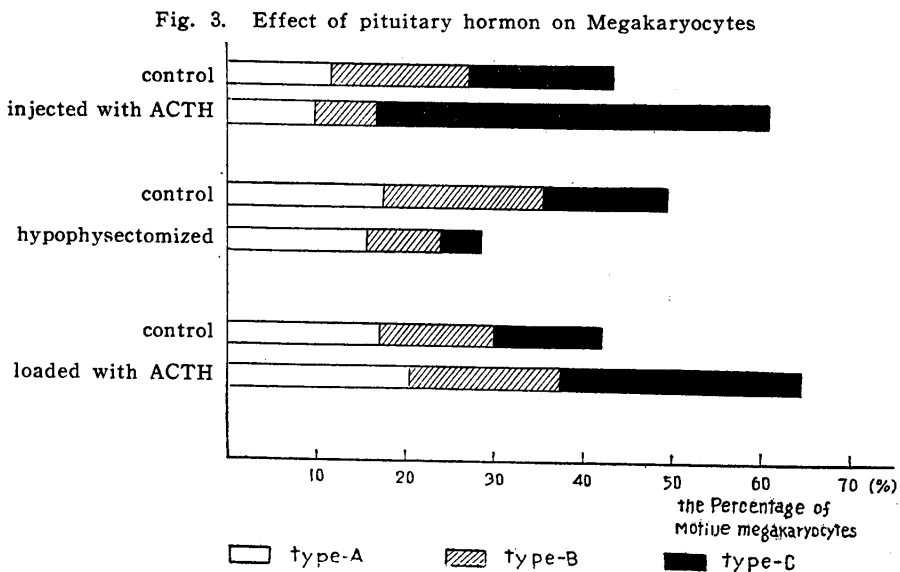
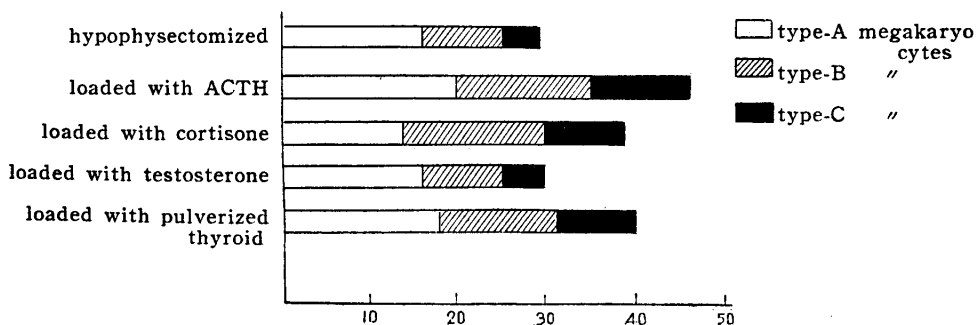


Fig. 4. Effects of ACTH, cortisone, testosterone, and pulverized thyroid on the megakaryocyte function in the hypophysectomized rats



percentage of type-C megakaryocytes is 1/3 that of the control, presenting a fall in the megakaryocyte function.

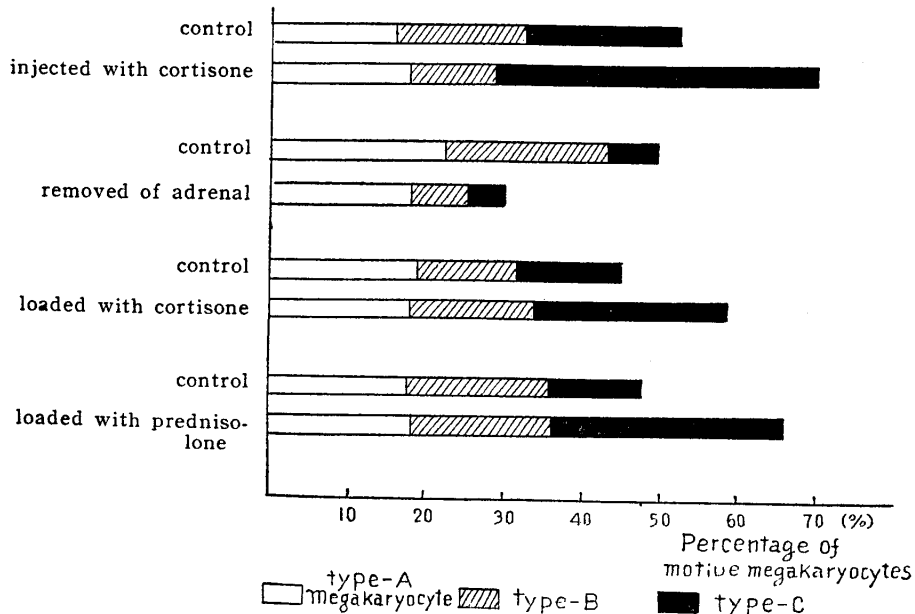
In the culture of the bone-marrow tissue from the hypophysectomized rats in which 0.001 IU/cc ACTH, 1.0 mg/cc cortisone, 0.01 mg/cc testosterone, or 0.02 mg/cc pulverized thyroid was directly added to the culture media, ACTH proved to be most effective in restoring the function of megakaryocytes followed by cortisone and pulverized thyroid. Testosterone has hardly any effect.

In the tissue culture of bone-marrow from normal persons when 0.001 IU/cc ACTH is loaded directly to the medium, TMP is 1.6 times that of the control, and the percentage of type-C megakaryocytes is 2.3 times that of the control, showing an acceleration of the megakaryocyte function.

Adrenal cortex and megakaryocyte function (Fig. 5):

After daily injection of 7 mg cortisone per 100g body weight to guinea pigs consecutively for 7 days to the total of 100mg, and culturing the bone marrow, TMP becomes 1.4 times that of the control, and the percentage of type-C megakaryocytes is 2.1 times that of the control, showing an acceleration of the megakaryocyte function.

Fig. 5. Effect of adrenal cortex hormone on megakaryocyte function



In the tissue culture of bone-marrow from guinea pigs with bilateral resection of adrenals 15 days after operation TMP is found to be lowered

down to $3/5$ that of the control.

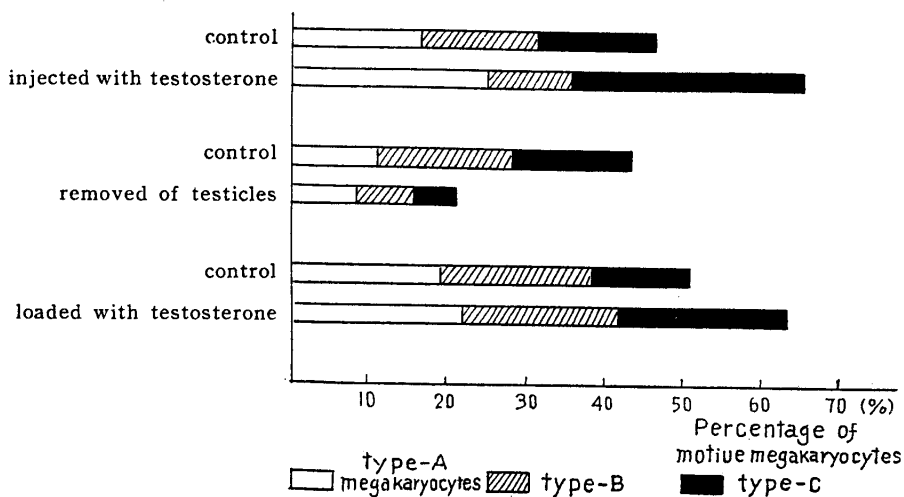
In the tissue culture of bone-marrow from normal persons when 10 mg/cc cortisone is added directly to the medium, TMP is 1.3 times that of the control, and the percentage of type-C megakaryocytes is two times that of the control, revealing an increase in the megakaryocyte function.

In the tissue culture of bone-marrow from normal persons when loaded with 0.1 mg/cc prednisolone, TMP is 1.3 times that of the control, and the percentage of type-C megakaryocytes is 2.2 times that of the control, showing an acceleration of the megakaryocyte function.

Male sexual gland and megakaryocyte function (Fig. 6):

In the tissue culture of bone-marrow from the guinea pigs injected daily with 0.8 mg testosterone per 100 g body weight consecutively for 24 days to the total of 70 mg testosterone, TMP is 1.4 times that of the control, and the percentage of the type-C megakaryocytes two times that of the control, also showing an acceleration of the megakaryocyte function.

Fig. 6. Male sexual gland and megakaryocyte function



In the tissue culture of bone-marrow from guinea pigs removed of testicles 19 days after operation TMP is $1/2$ that of the control and the percentage of the type-C megakaryocytes is $2/5$ that of the control, showing a fall in the megakaryocyte function.

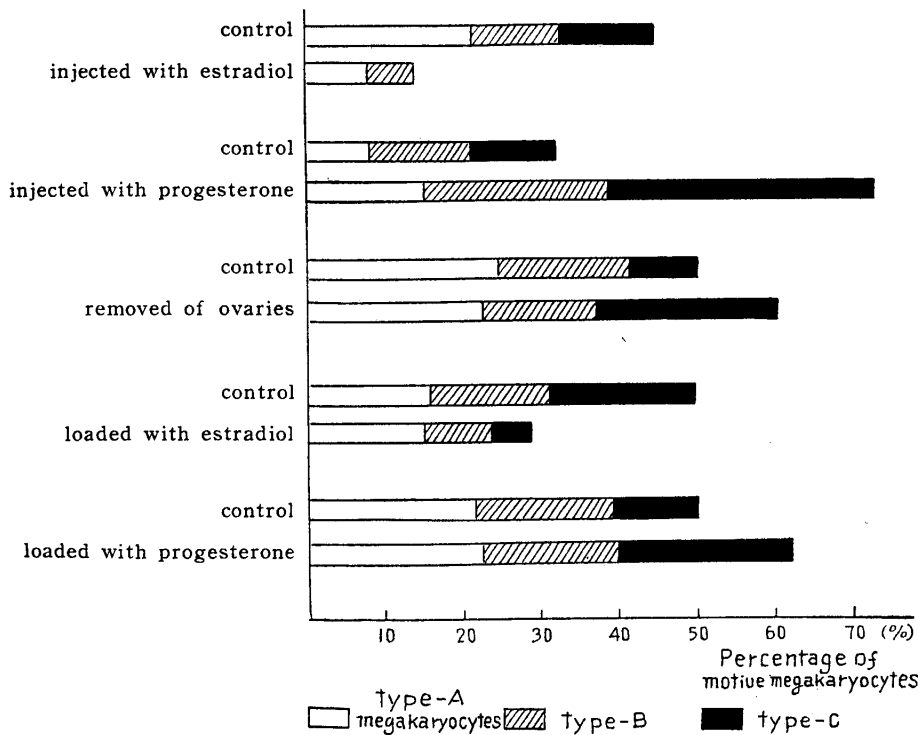
In the tissue culture of bone-marrow from normal persons when directly loaded with 0.0002 mg/cc testosterone, TMP is 1.2 times that of the control, and the percentage of the type-C megakaryocytes is 1.6 times, showing an acceleration of the megakaryocyte function.

Female sexual glands and the megakaryocyte function (Fig. 7):

In the tissue culture of bone-marrow from the guinea pigs injected with daily dosage of 0.12mg estradiol per 100g body weight consecutively for 24 days to the total dosage of 12mg, TMP is lowered to 1/5 of that in the control, and none can be recognized in the type-C megakaryocytes, proving a fall in the megakaryocyte function.

In the bone-marrow tissue culture of the guinea pigs injected with daily dosage of 0.75mg progesterone per 100g body weight consecutively for 8 days to the total of 20mg progesterone, TMP is 2.1 times that of the control, and the percentage of the type-C megakaryocytes is three times the control, showing a marked acceleration in the megakaryocyte function.

Fig. 7. Female sexual gland and megakaryocyte function



In the bone-marrow tissue culture of the guinea pigs 27 days after the removal of ovaries, TMP is 1.2 times that of the control, and the percentage of the type-C megakaryocytes is 2.6 times that of the control, showing an acceleration in the megakaryocyte function.

In the bone-marrow tissue culture of normal persons when directly

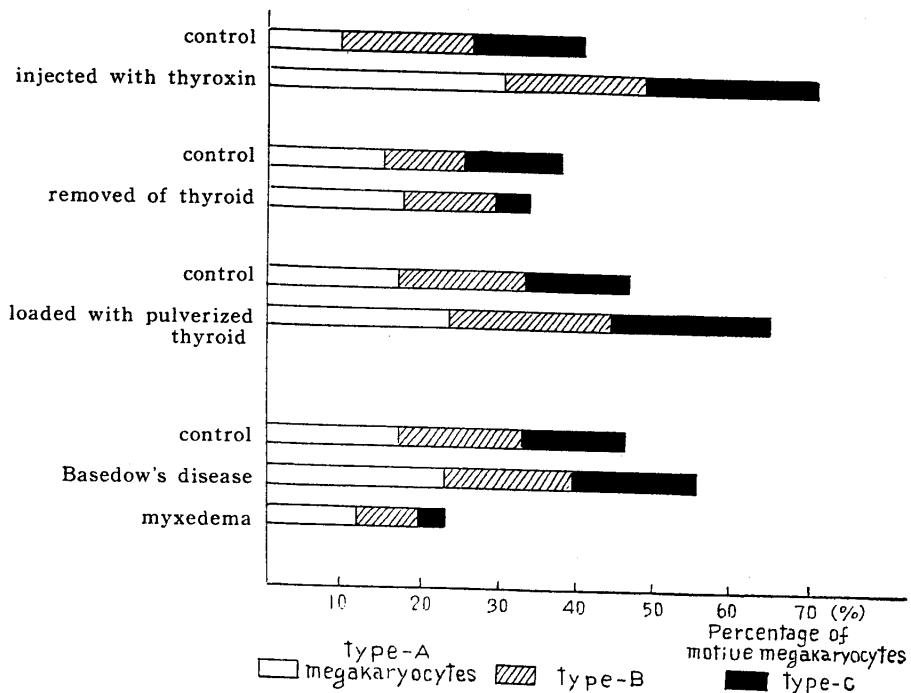
loaded with 0.05mg/cc estradiol, TMP is down to 3/5 that of the control, and the percentage of the type-C megakaryocytes to be down to 1/5 of the control, showing a marked fall in the megakaryocyte function.

In the bone-marrow tissue culture of normal persons when directly loaded with 0.2mg/cc progesterone, TMP is increased to 1.2 times that of the control, and the percentage of the type-C megakaryocytes to be two times that of the control, showing an acceleration of the megakaryocyte function.

Thyroid and megakaryocyte function (Fig. 8):

In the bone-marrow tissue culture of the guinea pigs injected with daily doagse of 0.06 g thyroxin per 100g body weight consecutively for 7 days to the total of 1.6 g, TMP is 1.8 times that of the control, and the percentage of the type-C megakaryocytes is also 1.8 times that of the control, showing an acceleration of the megakaryocyte function.

Fig. 8. Thyroid and megakaryocyte function



In the bone-marrow tissue culture of the guinea pigs 15 days after the removal of thyroid, TMP is down to 4/5 that of the control, and the percentage of the type-C megakaryocytes is 1/3 that of the control, showing a decrease in the megakaryocyte function.

When the bone marrow of normal persons is cultured with direct addition of 0.02 mg/cc pulverized thyroid, TMP is found to be 1.4 times that of the control, and the percentage of the type-C megakaryocytes is 1.6 times that of the control, revealing an acceleration of megakaryocyte function.

In the case of Basedow's disease, TMP is 1.2 times and the percentage of the type-C megakaryocytes 1.3 times that of normal persons, showing an acceleration of the megakaryocyte function.

In the case of the patients with myxedema, TMP is 1/2 and the type-C megakaryocyte percentage to be as low as 1/5 that of normal persons.

Effects of ACTH, cortisone, and prednisolone on the megakaryocyte function in various blood diseases :

When 0.001 IU/cc ACTH, 1.0 mg/cc cortisone, or 0.1mg/cc prednisolone is dissolved in a Ringer's solution and each of these is loaded directly to the medium of the bone-marrow tissue culture of idiopathic thrombocytopenic purpura, hypoplastic anemia and Banti's disease in which the megakaryocyte function is always below the normal level, the following results are obtained.

In the study of 5 patients with idiopathic thrombocytopenic purpura, with the addition of ACTH, TMP is between 1.6 to 3.5 times that of the control, and the type-C megakaryocytes percentage is 1.8 to 9.0 times that of the control, showing the greatest recovery in the megakaryocyte function, followed by the addition of cortisone and prednisolone (Fig. 9).

By the addition of cortisone megakaryocytes recover the function markedly in our study of 8 cases of hypoplastic anemia, which do not usually show the type-C megakaryocytes, but in this instance type-C can be found in as much as five cases of out of 8 (Fig. 10).

In the study of three cases with Banti's disease by addition of prednisolone, TMP is 1.6 to 2.1 times that of the control, while the percentage of the type-C megakaryocytes as high as 6.5 to 7.7 times the control, showing the most remarkable acceleration of the megakaryocyte function, followed by the addition of ACTH and cortisone (Fig. 9).

DISCUSSION

Pituitary and megakaryocytes :

JACOBSON¹⁰ and KOLLER¹¹ agree that ACTH has a regenerating action on platelets, while DAMESHEK⁵ and YOFFEY¹⁶ state that the repeated administration of ACTH will act as to multiply all the bone-marrow cells including megakaryocytes. In our experiment of repeated administra-

Fig. 9. Megakaryocyte function in the bone-marrow tissue culture of idiopathic thrombocytopenic purpura and Banti's disease loaded with ACTH, cortisone, or prednisolone

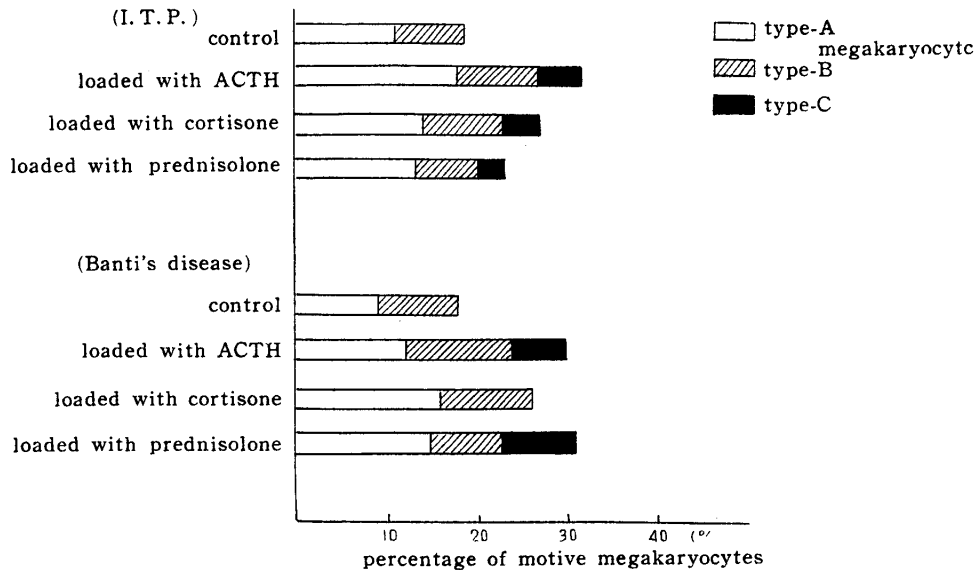
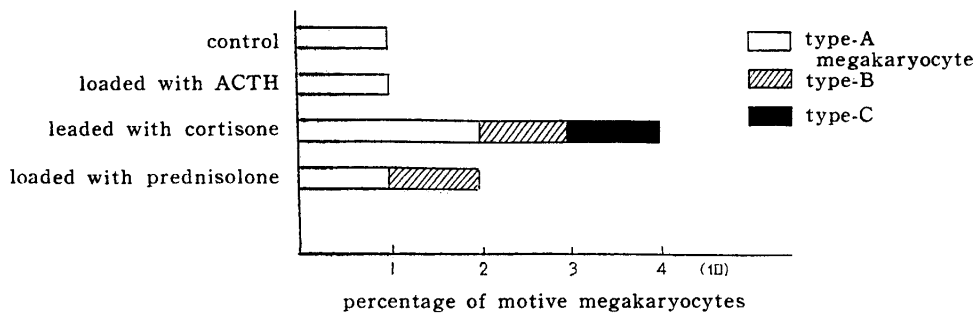


Fig. 10. Megakaryocyte function in the bone-marrow tissue culture of hypoplastic anemia loaded with ACTH, cortisone, or prednisolone



tion of ACTH to guinea pigs, we recognized a marked acceleration in the megakaryocyte function, namely, not only the proliferation of megakaryocytes but also an acceleration in the multiplication of platelets.

Next, as to the behaviors of megakaryocytes after hypophysectomy ADAMS¹ reports a decrease in the number of megakaryocytes after the removal of pituitary body. In our observations on the megakaryocyte function in the rats after hypophysectomy a marked decrease was seen in the function of platelet separation. Furthermore, in the investigations

on the effects of ACTH, cortisone, testosterone and pulverized thyroid made to act directly on the megakaryocytes of the hypophysectomized rats, the megakaryocyte function was restored by the addition of ACTH, cortisone, and pulverized thyroid in that order of the effectiveness, but testosterone had hardly any effect on the megakaryocyte function. This seems to be due to the diminution in the functions of various endocrines after hypophysectomy as well as to differences in the influences of these endocrines on the megakaryocyte function. In other words, the fact that ACTH is most efficacious in the restoration of the megakaryocyte function seems to suggest that the lack in ACTH secretion due to the hypophysectomy plays an important role in the fall of the megakaryocyte function. Moreover, the fact that actions of cortisone and pulverized thyroid are inferior to that of ACTH is because the adrenal and thyroid still maintain their autonomy to a certain degree even after hypophysectomy. That testosterone has hardly any effect on the megakaryocyte function is due to a considerably long period of time required before the diminution of the megakaryocyte function is brought about by disorders in the sexual glands.

Now, as for the direct action of ACTH on megakaryocytes, from the marked acceleration of the megakaryocyte function as observed in our bone-marrow tissue culture of normal persons directly loaded with ACTH, we verified that ACTH possesses a directly stimulating action on megakaryocytes without mediation of the adrenal cortex.

Adrenal cortex and megakaryocytes :

There are many steroid hormones excreted by the adrenal cortex, and among them concerning the influence of cortisone on the bone-marrow picture DAMESHEK and QUITTNER¹⁵ state that cortisone acts as a stimulant to all bone marrow cells including megakaryocytes. Likewise in our repeated injections of cortisone to guinea pigs, we recognized a striking acceleration in the megakaryocyte function, especially a marked acceleration in the platelet separation.

As for the bone-marrow picture after removing the adrenals LEWIS¹² and PILIERO¹⁴ state that it shows hypoplasia, but they do not mention anything about megakaryocytes. In our observations on the megakaryocyte function in the guinea pigs with bilateral resection of adrenals, a marked decrease in the function was recognized. With respect to the direct action of cortisone there is a report by GROSS⁶ about the influences of this hormone on erythroblast multiplication. As regards its influences on the megakaryocyte function by our results, we found cortisone acts markedly as a direct stimulant to megakaryocytes. Similar findings were

obtained with prednisolone. Namely, we confirmed that both cortisone and prednisolone act directly on megakaryocytes as stimulants.

Male sexual glands and megakaryocytes :

DAMESHEK and HOUSSAY⁹ made it clear about the stimulating action of male hormones on bone marrow, but influences of these hormones on megakaryocytes is still obscure. In our repeated administrations of testosterone to guinea pigs we observed an acceleration in the megakaryocyte function. On the other hand, hypoplasia of bone marrow after castration is well recognized by many investigators, but the megakaryocyte function under such conditions has been diminished according to our findings.

Next, in observing the direct action of testosterone on the function of megakaryocytes, testosterone accelerated the megakaryocyte function. Namely, we verified that testosterone acts directly on megakaryocytes as a stimulant.

Female sexual glands and megakaryocytes :

Concerning the influences of ovarian hormones on megakaryocytes CRAFTS⁴ and ARNOLD² recognize that a regressive change is brought about in megakaryocytes by ovarian hormone administration. In our repeated administration of estradiol to guinea pigs we observed a marked fall in the megakaryocyte function. On the contrary, relative to the influences of corpus luteum hormone there are no reports available, but we observed a marked acceleration of the megakaryocyte function in our repeated administration of progesterone to guinea pigs. Namely, we realized that the ovarian hormone and the corpus luteum hormone act antagonistically with one another. From these results, it appears there should be no changes in the megakaryocyte function even after the removal of ovaries since both of these hormones are mainly secreted by the ovary, but actually the function of megakaryocytes has been accelerated after removal of the ovaries. This phenomenon seems to be due to the fact that the ovarian hormone secreted in the ovary is superior to the corpus luteum hormone and moreover, the former constitutes a principal role in the ovarian function.

In observing the direct influences of these hormones on the megakaryocyte function, estradiol diminished whereas the progesterone accelerated the megakaryocyte function. Namely, it has been confirmed that both estradiol and progesterone have a direct action on megakaryocytes.

Thyroid and megakaryocytes :

Concerning the megakaryocytes at the time when the thyroid func-

tion is accelerated, MARKOFF¹³ and AXELROD³ state that numerous megakaryocytes appear in the bone marrow under such a condition. In our repeated administration of thyroxin to guinea pigs the acceleration of the megakaryocyte function has been observed. In the case of Basedow's disease likewise not only an increase of megakaryocytes in number but also an acceleration in the megakaryocyte function have been recognized.

On the contrary, concerning the megakaryocytes at the time when the thyroid function is diminished, AXELROD and BAMATTER report a decrease in the number of megakaryocytes. In our experience with the guinea pigs removed of thyroid we recognized the diminution in the megakaryocyte function, and moreover, we obtained similar result in the case of myxedema.

Next, in the observations of the direct action exerted on the megakaryocyte function by pulverized thyroid, we found an acceleration in the megakaryocyte function. Namely, it has been confirmed that the pulverized thyroid has a direct action on megakaryocytes as a stimulating agent.

Influences of ACTH, cortisone and prednisolone on the megakaryocyte function in various blood diseases :

It has been already reported that the megakaryocyte function is markedly diminished in idiopathic thrombocytopenic purpura but when ACTH, cortisone or prednisolone is directly added to the bone-marrow tissue culture of this disease, ACTH restores the megakaryocyte function most effectively, followed by cortisone and prednisolone, proving that these hormones act on megakaryocytes of this disease as stimulating agents. Therefore, when ACTH was clinically administered to the five patients mentioned above, platelets increased in number in four of these patients. From these results, the increase in the number of platelets observed in the therapeutic application of these hormones is undoubtedly due to the direct action of these hormones on megakaryocytes and to the stimulating action on the platelet separation which has been diminished in diseased conditions.

The megakaryocyte function in hypoplastic anemia is markedly diminished, but when ACTH, cortisone or prednisolone is added directly to the bone-marrow tissue culture of this disease, the megakaryocyte function is restored by cortisone, prednisolone and ACTH in that order of effectiveness. In other words, these hormones have a direct and stimulating action on megakaryocytes in this disease. Moreover, for the restoration of the megakaryocyte function in this disease a considerable quantity of hormone is required and since such a restoration even with

the application of a large quantity of hormone is tedious and insignificant, it is thought that the increase in platelets can not be so marked.

Likewise the megakaryocyte function in Banti's disease is markedly low, and when ACTH, cortisone or prednisolone is added directly to the bone-marrow tissue culture of this disease, the function is restored by prednisolone, ACTH, and cortisone in that order of effectiveness. This fact proves that these hormones has a direct and stimulating action on megakaryocytes in this disease.

CONCLUSIONS

In our study on the influences of various hormones and various endocrines on the megakaryocyte function by means of bone-marrow tissue culture, we obtained the following.

1. In the repeated administration of ACTH, cortisone, testosterone, progesterone, or thyroxin to guinea pigs, these hormones accelerated the megakaryocyte function, whereas estradiol on the contrary diminished the function.

2. The removal of such endocrines as the pituitary, adrenal, thyroid or testicles will diminish the megakaryocyte function, while removal of the ovaries accelerates it.

3. For the megakaryocytes in the hypophysectomized rats, ACTH acts most effectively to restore their function, followed by cortisone and pulverized thyroid, while testosterone has hardly any effect on the function.

4. ACTH, cortisone, prednisolone, testosterone, progesterone, and pulverized thyroid act directly on megakaryocytes so as to accelerate their function, while estradiol diminishes the megakaryocyte function.

5. For the megakaryocytes in idiopathic thrombocytopenic purpura ACTH is most effective in restoring the function; for the megakaryocytes in hypoplastic anemia cortisone is most effective; and for the megakaryocytes in Banti's disease prednisolone is most effective in restoring the megakaryocyte function.

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