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The fundamental erythrocytopoietic stimulus.

By H. E. JORDAN and C. C. SPEIDEL.

[*From the Laboratory of Histology and Embryology, University of Virginia, Charlottesville, Va.*]

The problem of determining the factors involved in the stimulation and regulation of erythrocyte production has been attacked both from the morphological and physiological point of view. Following detailed studies on both normal and experimental animals some hematologists, *e. g.*, Maximow[1] and Danchakoff[2], have concluded that the differential factor responsible for the development of an erythrocyte from a lymphoid hemoblast is intravascular location. However, the specific feature of intravascular location that stimulates erythrocytopoiesis, whether the mechanical condition of envelopment by endothelial wall, or certain chemical substances in the blood, remains unidentified.

In regard to the alleged instances of extravascular erythrocytopoiesis among birds and mammals, Danchakoff is inclined to believe that many, if not all, of these should be interpreted as cases in which the primary stimulus is nevertheless applied intravascularly, the hemoblasts thus receiving an initial and irreversible bias in the direction of an erythrocytogenic differentiation which is later completed extravascularly. In this connection it may be noted that Doan,[3, 4] and Cunningham and Doan[5] have recently pointed out that the capillary network in the bone marrow of birds and mammals is much more extensive than is commonly believed, and suggest that much of the supposed extravascular erythrocyte formation may in reality be intravascular. Latta[6], however, claims to find true primary extravascular

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1 Maximow, A., *Arch. mikr. Anat.*, 1909, lxiii, 444.

2 Danchakoff, V., *Am. J. Anat.*, 1918, xxiv, 1.

3 Doan, C. A., *Johns Hopk. Hosp. Bull.*, 1922, xxxiii, 222.

4 Doan, C. A., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 260.

5 Cunningham, R. S., and Doan, C. A., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 262.

6 Latta, J. S., *Am. J. Anat.*, 1921, xxix, 159.

erythrocytopoiesis in the intestinal wall of the rabbit. Accordingly, he substitutes for "intravascular conditions", the condition of "favorable relation to transudations from the blood plasma", and he believes that there is something in the balance of chemical substances in the blood plasma which determines erythrocytopoiesis. The evidence from histological studies indicates that close relation to the blood stream, and very slow blood flow, are chief conditions necessary for red-cell differentiation.

Among the results obtained by various investigators studying the problem experimentally from the functional viewpoint may be mentioned the following: Carnot and Deflandre[7] have suggested the presence of a "hemopoietin" and a "hemolysin" in normal blood in a certain balance. They believe that an increase in erythrocyte formation is brought about by any stimulus that will cause the hemopoietin to predominate, and conversely that any stimulus causing the hemolysin to predominate will result in decrease in erythrocyte number. There has, however, been no confirmation of their theory concerning the presence of a specific hemopoietin. Mansfield[8] working with thyroidectomized animals, some of which were experimentally rendered anemic, and some transferred to high altitudes, came to the conclusion that the thyroid gland was necessary for the proper functioning of the erythrocytopoietic tissues. Dallwig, Kolls and Loevenhart[9] demonstrated that oxygen lack, or fall in oxygen tension, is an effective stimulus to the red bone-marrow, resulting in increased erythrocyte production. Drinker[10] in a recent review of the subject states that at present the idea, unsatisfactory as it is, of oxygen want as the stimulus for the production of red blood-corpuscles must be accepted.

In any experimental analysis of the factors determining erythrocytopoiesis, a clear distinction must be made between (1) the fundamental stimulus (or stimuli) which, by disturbing the balance of normal metabolism, causes increase or decrease in erythrocytopoietic activity; (2) the basic morphological and physico chemical conditions which are necessary for the success-

[7] Carnot, P., and Deflandre, C., *Compt. rend. de la Soc. biol.*, 1906, cxliii, 384.

[8] Mansfield, G., *Pflüger's Arch.*, 1913, clii, 23.

[9] Dallwig, H. C., Kolls, A. C., and Loevenhart, A. S., *Am. J. Physiol.*, 1915, xxxix, 77.

[10] Drinker, C. K., *Oxford Med.*, 1920, ii, 509.

ful operation of the fundamental stimulating agent. To the second category belong the spatial conditions of "intravascular location" with slow blood flow, and "favorable relation to transudations from the blood plasma."

From several lines of study, data have been obtained which suggest the nature of the fundamental stimulating agent, given favorable spatial conditions, which operates to effect erythrocytopoiesis. Our data are derived, (1) from a study of the erythrocytopoietic activity accompanying thyroid-accelerated metamorphosis in the larval frog; (2) from a study of the normal seasonal erythrocytopoietic variation (response to temperature changes) in the adult frog; and (3) from a study of the erythrocytopoietic effect following the creation of an erythrocyte deficiency in the frog by means of cardiac aspiration or saponin treatment.

After thyroid treatment of frog larvæ the erythrocytopoietic activity of the spleen is markedly increased.[11] The mechanism by which this result is brought about appears to be as follows: (a) The thyroid extract causes increase in general metabolic rate. (b) This leads to increased formation of acid metabolites including especially carbon dioxide (and other waste materials of cell activity), with resulting increased hydrogen ion concentration. Helff[12] has shown that the carbon dioxide—oxygen exchange increases steadily during the first week or ten days after thyroid treatment, and our histological studies have shown that there is a marked increase in erythrocytopoietic activity during this time. (c) The increased concentration of carbon dioxide acts as a hormone to stimulate more rapid respiratory rate and more rapid erythrocyte differentiation. Both of these rate changes represent merely two aspects of the general physiological adaptation of the animal to the new high metabolic level.

From the standpoint of "usefulness" it is clear that an increased metabolic rate with resulting increased respiratory and erythrocytopoietic rate means: (a) More rapid internal respiration; (b) more efficient transport of oxygen and carbon dioxide between respiratory organ and body tissues; (c) more efficient external respiration. The importance of the erythrocyte, in each of these processes, as furnishing the chief material basis, is ob-

[11] Jordan, H. E., and Speidel, C. C., *J. Exp. Med.*, 1923, xx, 529.

[12] Helff, O. M., PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 34.

vious. Erythrocytopoiesis is accomplished in those localities which are most favorable for the process, *i. e.*, endothelium-lined spaces where the blood current is slow, as in the sinuses of the spleen, kidney and liver. This differentiation of lymphoid hemoblasts which are in the blood vessels, into erythrocytes is followed by the migration into the blood vessels of other lymphoid hemoblasts, which up to this time have been located extravascularly.

Instead of thyroid extract, the extrinsic factor initiating an increase in erythrocytopoiesis may be temperature rise. In the adult frog erythrocytopoietic changes accompany seasonal temperature changes. In winter, during hibernation, the spleen is relatively quiescent, little erythrocytopoiesis taking place. The carbon dioxide output must be coincidentally quite low. With the transition to spring the average level of body temperature again rises, bringing about an increase in metabolic rate. This again initiates the chain of reactions described above; increased carbon dioxide concentration, increased respiratory rate, and increased erythrocytopoiesis. Conversely, in the transition period from late summer to autumn the temperature falls, bringing about a decrease in metabolic rate. This means a decrease in carbon dioxide formation and in rate of respiration, with consequent lessening of the stimulus for erythrocyte differentiation. As a result, many lymphoid hemoblasts and their lymphocyte precursors fail to differentiate into erythrocytes, thus adjusting the rate of erythrocytopoiesis to fall conditions. In many autumn frogs (28 out of 75) the spleen shows a variable number of prominent white nodules. Upon microscopic examination these are found to be areas of degenerating lymphocytes. In others the adjustment may be made without this wholesale disintegration of lymphocytes. Such nodules have not been seen in our spring specimens.

Experimental hemorrhage brought about by aspiration of blood directly from the heart of the frog causes an erythrocyte (and plasma) deficiency which results in a stimulation of the rate of erythrocytopoiesis.[13] Both erythrocytes and blood plasma function in the transportation of carbon dioxide. A deficiency of these leads to greater concentration of carbon dioxide, with resulting stimulation of the erythrocytopoietic tissues, until a

[13] Jordan, H. E., and Speidel, C. C., *Am. J. Anat.*, 1923, xxxii, 155.

more normal carbon dioxide balance is regained. A similar result follows administration of the hemolytic toxin, saponin.

An experiment by Danchakoff[14] bears on our problem. She finds that an intense stimulation of erythrocytopoiesis occurs in the spleen of the chick embryo if a spleen graft is made on the allantois at the ninth day of incubation, a time when the vascular development of the spleen primordium renders it suitable for erythrocytopoietic activity. On the other hand, if the graft is made at an earlier date, before the spleen primordium has become vascularized, there results a defective system of splenic vessels and sinusoids, and a differentiation of the splenic cells into granublasts instead of erythoblasts. In the first case we interpret the erythrocytopoietic stimulation as a result of the increased carbon dioxide concentration in the body fluids of the embryo, following the interference with the respiratory and circulatory functions of the allantois by the graft. In the second case, although the allantoic functions have been interfered with as before, the vascular development of the spleen has not proceeded far enough for this region to offer a favorable locus for erythrocytopoiesis. The conditions are, however, favorable for granublastic differentiation, *i. e.,* the morphological conditions of extravascular location and relatively meagre blood supply, together with the fundamental stimulus of tissue lysis (proteolysis). This matter is discussed in another paper.[15]

In each of the cases cited, there is an extrinsic or relatively remote factor (the active principle of the thyroid extract, change in temperature, depletion of blood, and injury to the allantois) which has caused a variation in the concentration of carbon dioxide in the blood. It is this change in carbon dioxide concentration which is believed to be the more immediate fundamental stimulating cause of change in erythrocytopoietic rate.

In the experiments of Dallwig, Kolls and Loevenhart[9] the decrease in oxygen tension of the respired air is followed by increased carbon dioxide accumulation. The oxygen lack in the respired air is the more remote factor, the carbon dioxide in the body fluids is the more immediate factor, in bringing about the increased erythrocytopoietic activity in the bone marrow. As to just what the mechanism is by which carbon dioxide acts to

[14] Danchakoff, V., *Am. J. Anat.*, 1916, xx, 255.

[15] Jordan, H. E., and Speidel, C. C., *Am. J. Anat.*, 1924, xxxiii (in press).

stimulate the erythrocytopoietic tissues nothing is known. Dallwig, Kolls and Loevenhart, finding also that carbon dioxide concentrations of from 0.5 per cent to 1.0 per cent cause some stimulation of the bone marrow, suggest that the mechanism of this stimulation is to be found in the acid properties of carbon dioxide and its consequent power to decrease oxygen fixation.

That certain of the nitrogenous waste substances formed in metabolic activity, *e. g.*, ammonia, may be concerned in the stimulation of erythrocytopoiesis must be admitted as possible. However, certain other observations have led the writers[15] to the provisional conclusion that the products of protein metabolism constitute the fundamental stimulus that induces a lymphoid hemoblast to differentiate into a granular leucocyte. Thus, the fundamental stimulus for erythrocytopoiesis is regarded as coming from carbohydrate and fat metabolism, the stimulus for granulocytopoiesis as coming from protein metabolism.

Given lymphoid hemoblasts in suitable locations differentiating at a certain rate into erythrocytes, the data from the rather extreme conditions studied suggest that an increased concentration of some product of cellular metabolism, probably carbon dioxide, accelerates the rate of erythrocytopoiesis, decreased concentration retards it. The inference follows that in normal metabolism the more immediate fundamental stimulus for erythrocytopoiesis is the presence of a certain concentration of this metabolite.

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What determines the color of the light of luminous animals?

By E. NEWTON HARVEY.

[*From the Physiological Laboratory, Princeton University, Princeton, N. J.*]

A luminous ostracod (species unknown) occurs in the sea about Jamaica, B. W. I., whose light is more yellow than the Japanese ostracod, *Cypridina hilgendorfii.* Like the Japanese form, the Jamaican form exhibits the luciferin-luciferase reac-