THE ERYTHROPOIETIC ACTION OF GERMANIUM DIOXIDE.

II. The Source of the Erythrocythemia Produced by Germanium Dioxide in the Albino Rat.

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(Received for publication, November 9, 1921.)

In a previous publication it has been shown that germanium dioxide is non-toxic to the mature female albino rat when injected subcutaneously in amounts up to 180 mg. per kilo of body weight. This is a quantity equivalent to a dose of nearly 11 gm. for a 60 kilo man. The measure of the toxicity was survival after the administration of the compound. In no case did any animal receiving the germanium dioxide succumb or even present obvious symptoms of disturbance.[1]

A systematic study was then made of the effect of the compound on the erythrocyte and white cell content of the normal rat blood.[2] It was found that, regardless of sex, a marked and valid increase in the red cells in the circulation followed the injection of relatively small amounts of germanium dioxide solutions. In view of certain gross findings at autopsy of the rats used in these experiments, and because of the persistence of the effect for many days after the injections, we considered ourselves justified in concluding that germanium dioxide is an erythropoietic agent of remarkable potency.

In this report we propose to present the completed evidence on which that belief is based. The material used was obtained from the rats serving as subjects in the preceding paper.[2]

[1] Hammett, F. S., Müller, J. H., and Nowrey, J. E., Jr., *J. Pharmacol. and Exp. Therap.*, 1922 (in press).

[2] Hammett, F. S., Nowrey, J. E., Jr., and Müller, J. H., *J. Exp. Med.*, 1922, xxxv, 173.

A review up to 1915 of the data and literature concerned with the phenomenon of erythrocythemia has been published by Lamson.[3]

The cause of any given erythrocythemia may be apparent, such as a decrease in the plasma volume, or real, as, for example, a production of new cells, the division of red cells, or a release from deposits. Lamson in studying the erythrocythemia produced by the injection of epinephrine came to the tentative conclusion that the imbibition of fluid by the liver and consequent decrease in plasma volume is a factor of considerable importance.[4] However, since Lamson and Keith[5] have reported that the plasma volume and the erythrocyte content of the blood may vary independently of each other, it would appear that their theory has yet to be proved by further experimentation. Because of the incompleteness of the evidence, and in view of our studies of the bone marrow of rats treated with germanium dioxide, we consider that, for the present at least, the question of the relation of the plasma volume to the germanium erythrocythemia is of relatively minor importance.

Similar considerations apply to the question of the possibility of the observed erythrocythemia arising from either a division of red cells already in the circulation or by release from deposits within the organism.

It may be noted here that the germanium dioxide erythrocythemia differs from that produced by Lamson,[6] with epinephrine in that it is lasting in nature, while that following epinephrine is but transitory. While it is true that we have records of two rats with a persistent red cell increase 5 weeks after a single injection of germanium, and of eleven others showing an erythrocythemia 11 and 14 days after the last injections, yet the objection can be raised, and justly, that this persistence of the effect may well be due to a gradual release of the stimulating compound from a protoplasmically bound condition arising at the time of injection. This would imply a marked stimulating power by relatively minute amounts of germanium. Certain data,

[3] Lamson, P. D., *J. Pharmacol. and Exp. Therap.*, 1915, vii, 169.

[4] Lamson, P. D., *J. Pharmacol. and Exp. Therap.*, 1920–21, xvi, 125.

[5] Lamson, P. D., and Keith, N. M., *J. Pharmacol. and Exp. Therap.*, 1916, viii, 247.

[6] Lamson, P. D., *J. Pharmacol. and Exp. Therap.*, 1916–17, ix, 129.

however, indicate that, in man at least, it is necessary to administer a certain minimum amount before a detectable rise in red cells is obtained. This amount is in excess of what might be considered to be released from the tissues during the persistence of effect observed in our studies, per unit of time. This point, however, can only be decided by investigation.

If the erythrocythemia produced by germanium dioxide is actually the result of an erythropoietic action of the compound, evidence should be found in one or all of the blood-forming organs—spleen, liver, and bone marrow—of a stimulation to new red cell formation. Also young erythrocytes should be found in the circulating blood.

Gross inspection of the tissues at autopsy showed that the livers of the germanium-treated rats were of a reddish purple color, while the livers of the controls were a tobacco-brown. The spleen in the rat is normally a very dark red, and no differences in the appearance of this organ in the two groups could be detected. When the marrow of the long bones was exposed it was seen that in the controls this tissue was light coffee color while that of the test animals was maroon.

Small segments of the liver and the spleen were cut from as near the same locality as possible from these organs in all the rats, tests and controls, and were fixed in formaldehyde. The femurs were removed, cracked open so as to expose the marrow, fixed in formaldehyde, and carried through the dehydrating process to absolute alcohol. The marrow was then removed from as near the middle portion of the shaft of the bone as possible and was transferred to chloroform with the other tissues and embedded in paraffin. The animals of any given series were all autopsied within a few minutes of each other, and the fixation, dehydration, and embedding were carried on simultaneously so that errors due to differences in technique would be eliminated.

The sections of the livers and spleens were stained with hematoxylin and eosin. Methylene blue and eosin, and Goodpasture's polychrome stain, without the preliminary permanganate and oxalic acid treatment, were used for the bone marrow.

The sections of the livers of the germanium-treated rats showed no evidence of the resumption by this organ of its original erythropoietic function. There was present, however, in most cases an apparent dilatation of the hepatic capillaries, in which were many erythrocytes

but no nucleated red cells. This relative engorgement of the liver with red corpuscles which did not occur in the controls—and which explains the gross findings at autopsy—rather effectively speaks against the idea that the erythrocythemia is due to liberation from the liver of red cells that might be considered to be held therein, particularly since in the control livers the absence of erythrocytes in the capillaries is noticeable. In fact the increased number of red cells in the livers of the test rats might give rise to the opinion that the actual number of circulating cells observed was not a complete index of the new red cell formation. However that may be, it is certain that, in so far as these observations go, the liver is not the organ causing the erythrocythemia, although it is in some way as yet unknown affected directly or indirectly by the administration of the germanium dioxide.

Study of the sections of the spleens from the test animals also shows no evidence of the taking on of the original function of red cell formation. Nor are there any indications of an increased red cell destruction by the splenic phagocytes. Whether or not an increased red cell destruction accompanies the germanium erythrocythemia can only be determined by investigation. The impression is given that there is a slightly greater erythrocyte congestion in the sections of this tissue from the germanium-treated rats as compared with the controls, but it is not marked enough to be decisive. There also seems to be a more dense concentration of cells in the Malpighian corpuscles of the spleens of the test rats. However, this too is not sufficiently definite to be decisive. It is thus evident that the spleen is not the source of the increased red cell content of the circulating blood, although possibly the content is affected in some way or other, either directly or indirectly, by the administration of germanium dioxide.

In the sections of bone marrow of the germanium-treated rats there was ample evidence that the compound had produced a stimulation in formation of new red cells in that tissue over and above that present in the marrow of the controls. Not only were there more centers where the nucleated erythrocytes were in evidence, but there were more of these types of cells per unit area. Counts were made of the nucleated red cells in twenty fields, under the oil immersion lens, of longitudinal sections cut through the greatest diameter of the pieces

TABLE I.

Counts of the Nucleated Erythrocytes of the Bone Marrow.

Series No.	Sex.	Controls.	Test rats.			
			No. 1.	No. 2.	No. 3.	Mean.
2	Males.	187	242	232		237
	Females.	231	532	620	524	559
3	Males.	254	476	545	611	544
	Females.	308	477	448	492	472

removed from the bones. The field for counting was defined by an eyepiece micrometer. The results are given in Table I. They demonstrate conclusively that the erythrocythemia produced by the injection of germanium dioxide solution takes its origin from a stimulation of erythrocyte formation in the bone marrow.

With such a marked erythrocyte formation one would expect to find evidence in the circulating blood of young red cells. In fact, the presence of such cells in the blood would be a satisfactory confirmation of the bone marrow findings. In none of the rats used in these experiments, tests or controls, could there be found in the blood any cells that could be identified as nucleated erythrocytes. Nevertheless, in the smears from both the controls and the germanium-treated rats there were many polychromatic staining erythrocytes,

TABLE II.

Relative Number of Polychromatic Staining Erythrocytes in the Smears.

	Preliminary period.		During germanium administration.	
	Controls.	Test rats.	Controls.	Test rats.
Mean	49.0	40.0	59.0*	79.0
Standard deviation	6.4	16.2	12.9	17.6
Probable error of mean	1.6	2.4	2.8	2.1

* This slight increase which is by no means comparable with that obtained with the test animals confirms the slight rise in erythrocytes in these animals reported in the preceding paper.[2]

or young red cells. In order to determine whether or not there were more of these cells in the blood of the test animals as compared with the controls, a systematic count was made of their occurrence in the smears made for the differential leucocyte counts. The results are given in Table II. They represent the means, the standard deviations, and the probable error of the means of all the smears. It should be noted that every time the blood was taken for counting as reported in the preceding paper[2] smears were also taken for this purpose.

This table shows that there is a statistically valid increase in the number of erythrocytes taking the polychromatic stain in the blood of the germanium-treated rats. This confirms the bone marrow findings.

For purposes of completeness differential counts were made on the smears of all the rats. The normal values are to be published elsewhere. A comparison of the distribution of the white cells of the test rats during the preliminary period with that occurring during the germanium administration and with the controls showed that no changes occurred of sufficient magnitude to be significant.

SUMMARY AND CONCLUSIONS.

A histological comparison of the liver, spleen, bone marrow, circulating young erythrocytes, and differential count in mature male and female albino rats receiving germanium dioxide with their litter controls not receiving this compound was made.

It was found that the livers of the test animals in most cases showed a condition of capillary dilatation and that more erythrocytes were in these capillaries than were in those of the controls. There was no evidence of any red cell formation by the liver.

The spleens of the test rats gave the impression of being slightly more congested and of having a slightly more dense concentration of cells in the Malpighian corpuscles than those of the controls. There was no evidence of an increased red cell destruction nor was there any evidence of splenic erythropoiesis.

In the bone marrow of the rats which had received the germanium dioxide injections there was evidence of a marked stimulation in formation of nucleated erythrocytes, in that many more of these cells were found here than in the marrow sections of the controls.

The circulating blood of the test rats contained more young red cells as demonstrated by the increased number of erythrocytes taking the polychromatic stain than did the blood of the controls.

No noteworthy differences in the values for the various types of white cells in the circulation determined by the differential count could be found between the two groups.

Using, then, as an acceptable criterion of erythropoiesis an increase in the number of erythrocytes in the circulation which is accompanied by an increase in the number of young red cells, and an increased number of nucleated erythrocytes in the bone marrow, we consider ourselves justified in concluding that germanium dioxide is a potent erythropoietic agent and the source of the erythrocythemia produced by this compound is the increased production of red cell precursors by the bone marrow stimulated to increased activity by the compound used.