THE ERYTHROPOIETIC ACTION OF GERMANIUM DIOXIDE.

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Not only have the substances which by their commonness, such as the salts of sodium, potassium, and calcium, or by their striking and unexpected action on living tissues, such as radium and its derivatives, been intensively studied, but also many observations have been reported of the therapeutic trials and physiological action of the rarer elements. Cerium,[1] cesium,[2] erbium,[3] indium,[4] lanthanum,[5] neodmiumy,[5] palladium,[6] praseodymium,[1] rhodium,[7] rubidium,[8] ruthenium,[9] samarium,[10] selenium,[11] tellurium,[12] thallium,[13] thorium,[14] titanium,[15] vanadium,[16] yttrium,[17] and zirconium[18] have all received some attention from the physiological point of view.

[1] Frouin, A., *Compt. rend. Acad.*, 1920, clxx, 1471.
[2] Kaiser, L., *Arch. nêer. physiol.*, 1918–19, iii, 587.
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[4] Gerber, G., *Compt. rend. Soc. biol.*, 1910, lxix, pt. 2, 104.
[5] Rénon, L., *Bull. et mêm. Soc. mêd. hôp. Paris*, 1920–21, xliv, 602.
[6] Ascoli, M., and Izar, G., *Biochem. Z.*, 1907, vi, 192.
[7] Rémond, A., *Schweiz. Apoth. Z.*, 1919, lvii, 242.
[8] Hanke, M. T., and Koessler, K. K., *J. Biol. Chem.*, 1920, xliii, 567.
[9] Kalle and Co., German Patent No. 289,620, July 31, 1913.
[10] Esnault and Bron, *Bull. et mêm. Soc. mêd. hôp. Paris*, 1920–21, xliv, 606.
[11] Duhamel, deB.-G., *Compt. rend. Soc. biol.*, 1919, lxxxii, 724.
[12] Shie, M. D., and Deeds, F. E., *Pub. Health Rep., U. S. P. H.*, 1920, xxxv, 939.
[13] Pöhlmann, A., *Arch. Dermatol. u. Syph.*, 1912–13, cxiv, 633.
[14] Gudzent, F., and Herschfinkel, *Strahlentherap.*, 1916, vii, 519.
[15] Hanzlik, P. J., and Tarr, J., *J. Pharmacol. and Exp. Therap.*, 1920, xiv, 221.
[16] von Oefele, F., *New York Med. J.*, 1913, xcvii, 78.
[17] Mines, G. R., *J. Physiol.*, 1910, xl, 327.
[18] Hébert, A., *Bull. Soc. chim.*, 1906, xxxv, series 3, 1299.

Germanium is absent from this list. We have been unable to find in the literature any references to therapeutic or physiological studies of this element or its compounds.

EXPERIMENTAL.

The germanium dioxide used in these experiments was supplied by Müller.[19]

It was found that the mature albino rat can withstand the subcutaneous injection of 180 mg. of a 0.4 per cent solution of germanium dioxide per kilo of body weight without fatal result or the appearance of any symptoms whatsoever of a toxic effect. The detailed report of these experiments is now in press.[20] That study was planned as a preliminary to the determination of the effect of germanium on the erythropoietic system because of the interest that has been attached to arsenic in this connection and because germanium occupies a place in the periodic system next to arsenic and in many of its reactions resembles this latter element.

For this study there were used two litters of mature albino rats. In one litter, 150 days old, there were three males and four females. In the other, which was about 175 days old, there were four males and four females. The females had been segregated before puberty and had never been pregnant. All the rats had from birth been on the same varied diet which was continued throughout the investigation. We thus had for the purposes of our experiment two groups of animals of two lots each in which constitutional variability was reduced to a minimum. The relatively low variability within the litter has been shown by Jackson[21] and King.[22] Each lot of rats was kept in a separate cage.

Two preliminary determinations, 7 days apart, were made of the erythrocyte and leucocyte counts of each rat of each lot in order to establish the normal values. One rat of each lot was reserved as control. Weekly counts of the cellular blood components were made

[19] Müller, J. H., *J. Am. Chem. Soc.*, 1921, xliii, 1085.

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

[21] Jackson, C. M., *Am. J. Anat.*, 1913–14, xv, 1.

[22] King, H. D., *Anat. Rec.*, 1915, ix, 751.

of all the rats—controls and tests. The blood on which the counts were made was obtained by cutting off a bit of the tail for each determination. The blood was allowed to flow freely and the first 1 or 2 drops were discarded. This was done under light ether anesthesia as were the injections of the germanium dioxide solution.

The test rats of the first group, both males and females, were injected with 1.2, 1.5, 1.8, and 2.1 mg. of germanium dioxide per kilo of body weight in sterile 0.4 per cent solution on the 8th, 11th, 15th, and 19th days of observation respectively. The solution was made at first slightly alkaline in order to facilitate the dissolving of the oxide. It was then brought back to neutrality by dilute hydrochloric acid. The rats of the second group were injected with ten times the amount given to the first group, on the 8th, 11th, and 15th days of observation. No injections were made on the 19th day because an increased coagulability of the blood had appeared which made difficult the taking of a proper sample. A total of 6.6 mg. per kilo of body weight was injected into the rats of the first group and of 45 mg. into the test rats of the second group.

Two more counts were made of the corpuscles, one on the 22nd and one on the 28th day of the experiment, the last count being made 11 days after the last injection in the first group and after an interval of 14 days in the second group. At this time the experiment was discontinued because the results were adequate, as shown by Tables I to V. The animals were anesthetized with ether and killed by crushing the spinal cord in the cervical region. All values represent the average of two counts made in two counting chambers. In Table V will be found the statistical data concerned with this study. The accepted criterion for observed differences to be statistically valid is that the probable error of the means must be contained at least twice and should be contained three times, for incontestable figures, in the difference between the means. The differences in the erythrocytes between the fore and after periods of the test rats and the differences between the control rats and the test rats are seen to be statistically valid.

The observations conclusively demonstrate that germanium dioxide causes a very marked increase in the number of red cells per cubic millimeter of blood. An increase in the erthyrocyte count of from 1

TABLE I.

The Influence of Small Doses of Germanium Dioxide on the Erythrocyte and Leucocyte Content of the Blood of the Male Albino Rat.

Day.	Control rat.		Test rats.				GeO_2 injected per kilo of body weight.
	R. B. C.	W. B. C.	No. 1.		No. 2.		
			R. B. C.	W. B. C.	R. B. C.	W. B. C.	
							mg.
1	7.45	7.2	8.17	9.6	7.61	8.3	
7	7.76	6.6	8.39	7.7	7.96	9.3	1.2*
10	—	—	—	—	—	—	1.5
14	7.57	6.5	9.26	8.6	8.42	7.9	1.8
18	—	—	—	—	—	—	2.1
21	7.78	7.1	9.60	7.8	10.37	8.6	
28	7.68	7.8	9.21	9.5	10.34	9.3	

* The germanium dioxide solution was injected after the taking of the blood sample for the second count.

In Tables I to IV all values for the red cells are in terms of millions, all values for the white cells in terms of thousands.

TABLE II.

The Influence of Small Doses of Germanium Dioxide on the Erythrocyte and Leucocyte Content of the Blood of the Female Albino Rat.

Day.	Control rat.		Test rats.						GeO_2 injected per kilo of body weight.
	R. B. C.	W. B. C.	No. 1.		No. 2.		No. 3.		
			R. B. C.	W. B. C.	R. B. C.	W. B. C.	R. B. C.	W. B. C.	
									mg.
1	7.73	7.3	7.81	7.3	7.65	8.2	7.67	6.7	
7	7.98	7.7	7.79	7.2	7.86	8.4	7.74	7.4	1.2*
10	—	—	—	—	—	—	—	—	1.5
14	7.90	—	9.38	6.6	9.25	8.6	9.10	8.3	1.8
18	—	—	—	—	—	—	—	—	2.1
21	Killed by acci-		9.68	8.7	10.09	11.3	9.60	7.5	
28	dent.		9.34	7.5	9.58	10.3	8.92	8.6	

* The germanium dioxide solution was injected after the taking of the blood sample for the second count.

TABLE III.

The Influence of Large Doses of Germanium Dioxide on the Erythrocyte and Leucocyte Content of the Blood of the Male Albino Rat.

Day.	Control rat.		Test rats.						GeO_2 injected per kilo of body weight.
	R. B. C.	W. B. C.	No. 1.		No. 2.		No. 3.		
			R. B. C.	W. B. C.	R. B. C.	W. B. C.	R. B. C.	W. B. C.	
									mg.
1	8.64	9.6	9.31	10.2	8.76	10.1	9.38	10.2	
7	8.61	10.4	9.30	12.2	8.70	10.1	9.38	10.1	12.0*
10	—	—	—	—	—	—	—	—	15.0
14	8.72	10.6	11.04	16.8	10.20	12.5	9.82	11.0	18.0
18	—	—	—	—	—	—	—	—	
21	8.95	9.2	10.96	12.2	10.16	8.6	10.79	9.1	
28	8.92	10.4	10.47	10.2	10.40	9.4	11.58	9.5	

* The germanium dioxide solution was injected after the taking of the blood sample for the second count.

TABLE IV.

The Influence of Large Doses of Germanium Dioxide on the Erythrocyte and Leucocyte Content of the Blood of the Female Albino Rat.

Day.	Control rat.		Test rats.						GeO_2 injected per kilo of body weight.
	R. B. C.	W. B. C.	No. 1.		No. 2.		No. 3.		
			R. B. C.	W. B. C.	R. B. C.	W. B. C.	R. B. C.	W. B. C.	
									mg.
1	7.70	13.9	7.38	7.1	9.52	7.0	8.69	8.7	
7	7.81	17.0	7.64	11.8	9.35	10.6	8.60	8.4	12.0*
10	—	—	—	—	—	—	—	—	15.0
14	7.97	20.0	9.20	9.4	10.54	9.1	9.36	9.6	18.0
18	—	—	—	—	—	—	—	—	
21	8.20	18.9	9.40	12.8	11.20	12.4	10.57	9.5	
28	8.01	13.1	12.31	10.3	10.66	10.9	11.56	8.9	

* The germanium dioxide solution was injected after the taking of the blood sample for the second count.

to nearly 5 million cells occurred in every one of the eleven rats which had had injections of this compound. No such response was exhibited by the control rats. Moreover, germanium dioxide does not produce a leucemia as an accompaniment of the erythrocytosis.

The combination of the fact that germanium dioxide is non-toxic and non-corrosive, with the fact that it produces such a marked increase in the number of the erythrocytes in the circulation of the healthy rat gives us the hope that this compound has a specific stimulating effect upon the erythropoietic tissue and will be found of clinical value.

An inspection of the tables will show that there is a tendency for the red count to increase most in those animals in which the original

TABLE V.

Statistical Data Concerned with the Study of the Influence of Germanium Dioxide on the Erythrocyte and Leucocyte Content of the Blood of the Albino Rat.

	Control rats.				Test rats.			
	Initial counts.		Subsequent counts.		Initial counts.		Counts after GeO_2 injections.	
	R. B. C.	W. B. C.	R. B. C.	W. B. C.	R. B. C.	W. B. C.	R. B. C.	W. B. C.
Mean.	7,960,000	9,959	8,170,000	11,533	8,395,000	8,931	10,074,000	9,734
Standard deviation.	406,000	3,452	485,000	4,702	713,000	1,547	2,743,000	1,907
Probable error of mean.	97,000	823	104,000	1,057	103,000	222	322,000	223

cell count is lowest. It is also evident that the small doses cause just as marked and significant a rise as do the larger doses.

Attention should be called to the fact that there occurred a slight but statistically valid increase in the erythrocytes of the controls in each series. This increase is attributed to the possibility of food contamination through excretion of germanium, and is supported by the fact that the effect was not observed in rats used as controls in a similar series of studies with arsenic.

At autopsy marked differences were found in the color of the liver and the bone marrow of the germanium-treated rats as compared

with the controls. The liver of the control was a reddish tobacco-brown color, that of the test animals was a reddish purple. The bone marrow of the control albinos was the color of coffee with cream with here and there a slight tinge of red. That of the germanium-injected rats was maroon. No differences in the color of the spleens were detectable. A paper dealing with the histological findings will be published later.[23]

SUMMARY AND CONCLUSIONS.

Injections were made of a sterile 0.4 per cent solution of germanium dioxide into four lots of mature male and female albino rats. Exact conditions of control were maintained. To two lots there was administered in four doses at intervals of 4 days a total of 6.6 mg. of the oxide per kilo of body weight. To two lots there was given in three doses at like intervals a total of 45 mg. of the compound per kilo of body weight. In each lot there was one rat which served as a control and which did not receive any germanium. A preliminary period of observation was maintained as a further control during which two determinations of the erythrocyte and leucocyte counts of the blood were made 7 days apart on all the rats. Weekly counts were made on all the rats during the progress of the investigation which lasted for 4 weeks, during 3 of which the treated animals were under the influence of germanium. In the first group 11 days elapsed between the last injection and the last count. In the second group the interval was 14 days.

It was found that without exception all of the test rats responded to the germanium dioxide by a marked and sustained rise in the number of erythrocytes in the blood which ranged from 1 to nearly 5 millions.

These results are statistically valid.

There was an apparent tendency for the degree of effect to be related to the initial erythrocyte number, in that with a lower initial count there seemed to take place a greater rise, and *vice versa*.

There was no indication that the larger doses of germanium dioxide exerted a greater stimulating effect on the production of the resultant erythrocythemia than the smaller doses.

[23] Hammett, F. S., and Nowrey, J. E., Jr., *J. Exp. Med.*, 1922, xxxv (in press).

There is evidence that the effect is quick in making its appearance. The rise in the red cell count was found to occur within a week, and after but two injections of the oxide.

Indications were obtained that germanium dioxide tends to increase the coagulability of the blood.

Autopsy findings showed color changes in the liver and bone marrow.

We believe that germanium dioxide is an erythrocytogenic agent and we hope that fruitful results will come from its therapeutic application.