On the Manganese Content of Transplanted Tumours.

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(Communicated by Sir J. R. Bradford, K.C.M.G., Sec. R.S. Received November 21, 1912,—Read January 23, 1913.)

(From the Laboratory of the Imperial Cancer Research Fund.)

The occurrence of manganese in plants is well known. Its quantitative distribution and biological significance have been carefully studied from many points of view. A few examples of the more recent, especially experimental work, illustrating some of the biological properties of this metal, may be mentioned. Bertrand* showed that there exists a close relationship between the activity of vegetable oxydases and the amount of manganese present. In a series of very exact experiments with *Aspergillus niger*, the same author demonstrated that the presence of manganese is necessary to the formation of conidia of this mould,† and also that the rapidity of its growth may be largely influenced by the quantity of manganese added to the culture medium.‡

The study of manganese in animals is far less advanced than in plants. Since the food-stuffs contain manganese, it is obvious that this element is continuously introduced into the animal body. The detection of manganese in animal tissues has been the subject of repeated investigation during the last 70 years. The conclusions, however, which the earlier authors have drawn are very contradictory, undoubtedly attributable mainly to the insufficiency and the defects of the methods and the technic used for the detection and estimation of this element.§

Recently Bertrand and Medigreceanu applied Bertrand's colorimetric method for estimating the manganese in organic substances‖ to an extensive analytical study of this metal in normal animals. By means of this method manganese can be estimated even when present in very small quantities, 2/1000 mgrm., and with an error not exceeding 10 per cent.

Manganese was thus found to be a normal constituent of the organism throughout the animal kingdom.¶ The invertebrates usually show relatively

* G. Bertrand, 'Comptes Rendus,' 1897, vol. 124, p. 1032.

† 'Bull. Soc. Chim. France,' 1912, Ser. 4, vol. 11-12, p. 494.

‡ *Ibid.*, p. 400.

§ See Bertrand and Medigreceanu's article, 'Bull. Soc. Chim. France,' 1912, Ser. 4, vol. 11–12, p. 656.

‖ 'Bull. Soc. Chim. France,' 1911, Ser. 4, vol. 9, p. 361.

¶ See Bertrand and Medigreceanu, 'Comptes Rendus,' 1912, vol. 154, pp. 941, 1450 ; 1912, vol. 155, p. 82.

much larger quantities of manganese than the vertebrates, and of the vertebrates the mammals contain the smallest amounts—a few hundredths of a milligramme per 100 grm. of the total weight of the organism—while the birds, reptiles, batrachians, and fishes show 5–10 times as much.

The quantitative distribution in the different organs, tissues, and animal products, especially of the higher classes that have been studied, is very interesting. The blood, for example, contrary to the claims of most previous investigators, has been found to contain much smaller amounts of manganese than sometimes admitted, usually only a few hundredths of a milligramme per litre. · The hæmoglobin of horse blood contains no manganese. Of the organs and tissues of principal functional importance higher manganese values have been met with in the liver (0·265–0·416 mgrm. per 100 grm.) and in the kidneys (0·063–0·238). Lower values are found in the muscular tissue ($<$ 0·005–0·018), the nervous tissue ($<$ 0·005–0·036), and the lungs (0·006–0·023). The organs of the birds are generally richer in manganese than those of mammals, and the highest value obtained has been for the oviduct of birds (0·786–2·201).

It may be mentioned that the grey matter of the ox brain is much richer (0·022) in manganese than the white ($<$ 0·005), and also that, in general, the heart and muscles of the tongue contain larger amounts of this metal than the trunk muscles and the muscles of the extremities.

Among the organs or tissues of minor functional importance, the hair, plumage, and nails contain relatively large amounts of manganese (0·111–3·214).

The milk is very poor in manganese, although apparently richer than the blood. In the egg-white (fowl and duck) they were unable to detect this metal, even when analysing 100 grm. of the fresh substance. The yolk seems to contain all the manganese present in the egg.

Considering the ubiquity of manganese throughout the animal kingdom, and its remarkable distribution in the various tissues, these authors emphasise the importance that it probably has as a catalytic agent of living matter. Again, the wide differences shown to obtain between the amount of manganese found in plants and in animals is an observation which may have considerable importance. The quantities of manganese present in the various organisms and tissues may very well be taken into consideration in studying the problems of the origin of species, as well as those of biochemical adaptation to the medium, in interpreting the influence of vegetarian and flesh diets, and finally in drawing deductions as to the nature of the physiological soil.

In connection with Bertrand and Medigreceanu's work on manganese, it

seemed of interest to study its occurrence and quantitative distribution in tumours. The transplantable tumours (mouse, rat, dog) were chosen for the purpose. These kinds of tumours are at present the best known as regards their biological and morphological properties, and the most suitable for an exact and rapid orientation.

The tumour strains analysed belong to both the principal morphological groups—carcinoma and sarcoma. Each strain shows different morphological and biological properties.*

Technic.

As already mentioned in the introductory part, the estimation of manganese was made by Bertrand's colorimetric method. It consists essentially in converting the manganese present in the sulphate ash of the organic substance into permanganic acid, in oxidising the ash dissolved in concentrated nitric acid with potassium persulphate in the presence of silver nitrate, and in comparing the intensity of its rose-pink to violet colour with the colour of standard solutions of the same acid prepared in a similar way. The details of the method were followed exactly as given by Bertrand and Medigreceanu.†

Of the tumour tissue to be analysed quantities not exceeding 100 grm. were first dried at 100° C. and then incinerated at the lowest possible temperature, using sulphuric acid for the destruction of the final traces of carbon. The sulphate ash was then dissolved in concentrated hydrochloric acid, again treated with a little sulphuric acid, and finally heated until the appearance of white fumes of sulphuric acid denoted the absence of hydrochloric acid. The residue was then dissolved in 10 c.c. of 25-per-cent. nitric acid, and if necessary the undissolved part of the ash allowed to precipitate. A few drops of 10-per-cent. silver nitrate was then added to the clear solution, the tube warmed, and its contents oxidised with a few decigrams of potassium persulphate.

The greatest care was always taken to avoid introducing impurities containing manganese into the samples for analysis, and pure reagents were used throughout.

It may also be mentioned that the small quantities of blood contained in the tumours do not influence the analytical results, for it was found that 25 grm. of mouse blood treated in the same way did not show any visible trace of manganese. This fact fully agrees with the previous findings of Bertrand and Medigreceanu, who observed only traces of manganese in larger

* Full details on the tumours analysed may be found in the 'Fourth Scientific Report on the Investigations of the Imperial Cancer Research Fund,' London, 1911, Taylor and Francis.

† 'Bull. Soc. Chim. France,' 1912, Ser. 4, vol. 11–12, p. 656.

Mouse Tumours.

Tumour strain.	Age of tumours, in days.	Weight of the fresh material examined, in grammes.	Milligrammes of manganese.		Remarks.
			Found in the sample.	Per 100 grm. of the fresh substance.	
Adeno-carcinomata and Carcinomata.					
63 (1) 65 D	28	60	0·006	0·01	39 mice. Average weight: mice 17 grm., tumours 1·6 grm. Tumours generally cystic.
(2) 65 D, 66 D, 73 A	18–50	21	0·002	0·009	10 mice. Average weight: mice 15·4 grm., tumours 2·1 grm.
91 (1) 30 B, 43 A	28–82	28	0·003	0·01	10 mice. Average weight: mice 18 grm, tumours 2·8 grm. Necrotic and cystic.
(2) 44 A	19	47	0·004	0·008	23 mice. Average weight: mice 16 grm., tumours 2 grm.
199 42 A, 43 A, 44 A	29–69	22	0·003	0·013	11 mice. Average weight: mice 15·8 grm., tumours 2 grm.
37 54 H, 50 M, 61 F, 65 D, 69 D	48–130	46	0·006	0·012	13 mice. Average weight: mice 17 grm., tumours 3·5 grm.
100 50 E, 51 E, 51 F	44–68	20	<0·002	<0·01	13 mice. Average weight: mice 16 grm, tumours 1·5 grm. Large cysts, containing opalescent liquid.
286 32 B, 33 A, 34 A	22–69	14	<0·002	<0·014	12 mice. Average weight: mice 17 grm., tumours 1·1 grm.
Sarcomata.					
100 75 B	13	66	0·003	0·004	24 mice. Average weight: mice 15 grm., tumours 2·7 grm.
92 53 A, 54 A, 58 A, 60 A	23–180	26	<0·002	<0·007	13 mice. Average weight: mice 22 grm., tumours 2 grm.

Rat Tumours.

Tumour strain.	Age of tumours, in days.	Weight of the fresh material examined, in grammes.	Milligrammes of manganese.		Remarks.
			Found in the sample.	Per 100 grm. of the fresh substance.	
Carcinoma.					
F.R.C. 75 A, 78 A	42–105	18	<0·002	<0·01	5 rats. Average weight: rats 64 grm., tumours 3·6 grm. Very necrotic.
Sarcoma.					
J.R.S. (1) 77 A	73	50	0·003	0·006	1 rat. Weight: rat 107 grm., tumour 52 grm.
(2) 76 A, 78 A	52–90	80	0·005	0·006	2 rats. Average weight: rats 83 grm., tumours 41 grm.
(3) 80 A	19	55	0·005	0·009	16 rats. Average weight: rats 74 grm., tumours 3·4 grm.
(4) 75 A, 77 A, 79 A	39–123	(*a*) 50 (*b*) 86	0·005 0·007	0·01 0·008	5 rats. Average weight: rats 98 grm., tumours 30 grm.
Dog Tumour.					
Lympho-Sarcoma ?.					
Generation 40 A	27	22	<0·002	<0·003	3 dogs. Average weight: tumours 7 grm.

quantities of the blood of several mammals and birds. It serves also as a control to the purity of the reagents used. The analytical results in the several tumour strains examined are shown in the adjoining table.

Summary and Conclusions.

As a general conclusion it may be stated that the quantities of manganese found in transplanted mouse and rat tumours, whether carcinomata or sarcomata, and also in the so-called lymphosarcoma of the dog, are very small —they vary between 0·004 and 0·012 mgrm. per 100 grm. of the fresh material.

In order to obtain an idea of the comparative amounts of manganese contained in the normal mammary gland and the epithelial tumours derived from it, two manganese estimations were made of normal mouse mamma. In the first, 15 grm. of lactating mamma were analysed. Only 0·004 mgrm. was found, *i.e.* 0·026 mgrm. manganese per 100 grm. For the second determination, 14 grm. of resting mamma were incinerated. The amount of manganese present was 0·002, *i.e.* 0·014 mgrm. per 100 grm. Though the comparison be not strictly exact, nevertheless the figures obtained allow this general conclusion to be drawn, that the epithelial transplantable mouse tumours developing in the mammary gland do not contain a larger amount of manganese than their normal mother tissue.

Furthermore, there are not very marked differences in the percentage distribution of manganese between carcinomata and sarcomata. In connection with this observation it may be also mentioned that the carcinoma and sarcoma strains of the mouse tumour "100" do not exhibit appreciable differences in their manganese content.