# CLXXIV. MANGANESE IN RELATION TO NUTRITION.

By MARION BROCK RICHARDS.

(*From the Rowett Research Institute, Aberdeen.*)

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It has been recognised for many years that certain inorganic elements, even when present in extremely small amounts, can exercise a marked effect on the growth and development of plants. Since the pioneer work of Pasteur and of Raulin on the mineral nutrition of microbes and of fungi, such as *Aspergillus niger*, much research on similar lines has been carried out, and emphasis has been laid on the importance of the minute chemical constituents in plant physiology and in agriculture generally [*e.g.* Bertrand, 1912].

Vitamin work of recent years has proved the enormous importance in animal physiology of food constituents which are present in the diet only in minute amounts. This raises the question as to whether certain mineral elements, also occurring in traces in food, and known to occur in small amounts in blood and tissue, may not prove to be of significance in the physiology of animals, as they have been shown to be significant in that of plants.

Of these "trace" minerals, manganese has received special attention since Bertrand [1897] formulated his theory that this element is the true active principle of the oxidases. Although this theory was afterwards challenged, it may be regarded as proved that manganese is indispensable for normal plant growth. This indispensability, according to Samuel and Piper [1929], is now recognised to such an extent that manganese is included as a matter of course in a complete nutrient solution for water-culture work, although toxic effects may ensue if the amount present is too large. In contrast with manganese, certain other elements, such as copper and arsenic, are exceedingly poisonous to plants, and do not seem to stimulate growth even when they are applied in the smallest quantities [Brenchley, 1914].

So far little is known with regard to the action of manganese in living creatures, but a few briefly reported experiments [Levine and Sohm, 1924; McHargue, 1926; McCarrison, 1927; Bertrand and Nakamura, 1928], dealing with the effect of additions of this element on the growth of rats or mice, indicate the necessity for fuller knowledge. In the following pages will be found numerous analytical data relating to the manganese content of various materials of plant or animal origin. The analyses have been carried out by a procedure based on the Willard and Greathouse periodate method, of which full details are published elsewhere [Richards, 1930].

# PART I.

## THE MANGANESE CONTENT OF PLANT REPRODUCTIVE ORGANS.

The universal presence of manganese throughout the vegetable kingdom has been frequently demonstrated. It has been shown moreover that it concentrates specially in the parts of the plant that are most active chemically [Jadin and Astruc, 1913; Bishop, 1928]. Although a high percentage of manganese has been reported by various workers in the seeds of plants [Wester, 1921], there are practically no figures available for the manganese content of the reproductive organs, save two examples by Bertrand and Rosenblatt [1922]. In view of the possibility that manganese might be concerned in the processes of reproduction both of plants and of animals, it seemed of interest (*a*) to obtain further figures for plant reproductive organs; (*b*) to find whether any significant difference occurs between male and female organs; and further (*c*) to trace any change that may occur in the manganese content of the seeds during the process of ripening. In Tables I and II will be found the manganese content of the reproductive organs for several species of plants.

Table I. *Reproductive organs of plants.*

(mg. Mn/100 g. dry matter.)

| Plant | Stage of bloom | Female organs | | Male organs | |
|---|---|---|---|---|---|
| Willow (species unknown) I | — | Whole bloom | 35·4 | — | |
| | | Pistils | 22·0 | | |
| | | Bracts | 75·3 | | |
| II | Advanced. Pollen dispersed | — | | Whole bloom | 136·0 |
| | | | | Anthers | 80·0 |
| | | | | Bracts | 159·5 |
| III | — | Pistils | 15·4 | — | |
| Tall sedge (*Carex aquatilis*) | Advanced. Pollen dispersed | Whole bloom | 36·7 | Whole bloom | 28·4 |
| Poppy (large red) | Bud unopened | Pistils | 1·44 | Stamens | 1·73 |
| Laburnum | All stages—from unopened buds to flowers just fading | Pistils | 2·76 | — | |
| White tulip | Advanced. Pollen almost gone | Pistils | 0·95 | Anthers | 3·76 |
| | | | | Filaments | 1·91 |
| Red tulip | I. Flowers just fully out—no free pollen | Pistils | 1·59 | Anthers | 3·70 |
| | | | | Filaments | 1·58 |
| | II. Pollen ripe | Pistils | 1·52 | Anthers | 3·97 |
| | | Ovules | 2·97 | Pollen | 2·91 |
| | | Capsules | 1·25 | Filaments | 1·54 |
| | III. Flowers faded | Pistils | 1·55 | Anthers | 3·58 |
| | | Ovules | 2·87 | Filaments | 1·68 |
| | | Capsules | 1·34 | | |

The data in Tables I and II show that there are wide differences in the manganese content of the reproductive organs of different plants. These differences cannot be accounted for on the score of family, for the lupin and laburnum,

Table II. *Reproductive organs of blue lupin at different stages of development.*

(mg. Mn/100 g. dry matter.)

| Stage of bloom | Female organs: Whole pistil | Seeds | Pods | Male organs | |
|---|---|---|---|---|---|
| I. Tiny buds. No free pollen | 28·7 | — | — | Anthers | 34·7 |
| II. (*a*) Larger buds (*b*) Flowers fully out | (*a*) (*b*) 29·7 | — | — | (*a*) Pollen (*b*) Pollen | 27·4 29·9 |
| III. Flowers not faded, but pollen dispersed | 28·1 | — | — | — | |
| IV. Flowers faded. Pods 0·75–1 cm. long | 29·0 | 30·3 | 28·9 | — | |
| V. Pods 1–1·75 cm. | 37·8 | 69·5 | 34·0 | — | |
| VI. Pods 2–2·5 cm. | 40·3 | 112·5 | 31·6 | — | |
| VII. Pods 2·75–3·5 cm. | 37·1 | 122·8 | 26·8 | — | |
| VIII. Pods 3·5–4 cm. | 34·6 | 119·9 | 25·0 | — | |
| IX. Pods 4·5–5 cm. | 30·4 | 100·7 | 20·9 | — | |
| X. Pods further developed | 21·3 | 48·5 | 14·9 | — | |
| XI. Seeds ripe. Pods bursting open | 22·4 | 20·5 | 23·8 | — | |

both belonging to the Leguminosae, show a striking difference. Nor can they be connected with the size of the plant, since the willow and laburnum, small trees of approximately the same size, show a similar difference. It will be noted that of the few results available, those with very high manganese content, *viz.* lupin, willow, and sedge, are all plants which occur naturally in the neighbourhood of abundant water-supply, and may thus be regarded as water-loving, though not aquatic plants in the true sense. It is therefore of interest to note that a very high percentage of manganese has been reported in many true water-plants, such as *Zostera marina* and *Trapa natans* [Kobert, 1883], and that submerged plants of this nature (*e.g. Elodea*) show in a marked degree adsorption of colloidal manganese compounds, when exposed to light in solutions containing manganese, a phenomenon which has recently been closely investigated by Gicklhorn [1927]. The water-loving character of the above plants may thus be not unconnected with the unusually high manganese content of their reproductive organs.

There is apparently no very wide difference between male and female reproductive organs of the same plant as regards their content of manganese. This is seen in the figures for sedge, poppy, red tulip, and lupin. The figures for male and female willow catkins can scarcely be compared, as they are probably from entirely different species.

There is a rapid increase in the manganese percentage in the seeds of the lupin as the seeds develop, up to a certain point, after which this increase fails to keep pace with the storage of organic matter. For the pods the maximum percentage is reached a stage or two sooner than is the case with the seeds. Table III shows that, although the percentage of manganese in the seeds and pods does not maintain its increase to the end, the total manganese for each pistil, both in the seeds and in the surrounding pods, goes on increasing very markedly up to the final stage of the completely ripe seed.

Table III. *Total Mn content of developing pistil of lupin.*

| Stage | No. of pistils analysed | Total Mn per pistil in mg. Complete pistil | Seeds | Pod | Ratio $\frac{\text{Pod Mn}}{\text{Seed Mn}}$ |
|---|---|---|---|---|---|
| I | 250 | 0·000135 | — | — | — |
| II | 250 | 0·000256 | — | — | — |
| III | 200 | 0·000477 | — | — | — |
| IV | 100 | 0·00280 | 0·00028 | 0·00252 | 9·00 |
| V | 90 | 0·0070 | 0·0014 | 0·0056 | 4·00 |
| VI | 64 | 0·0135 | 0·0041 | 0·0094 | 2·29 |
| VII | 40 | 0·0224 | 0·0079 | 0·0145 | 1·84 |
| VIII | 32 | 0·0318 | 0·0113 | 0·0205 | 1·81 |
| IX | 24 | 0·0452 | 0·0178 | 0·0274 | 1·54 |
| X | 24 | 0·0492 | 0·0214 | 0·0278 | 1·30 |
| XI | 12 | 0·0872 | 0·0341 | 0·0531 | 1·56 |

It will be seen that the manganese content per pistil increased more than 640 times between Stages I and XI, and that while at Stage IV (the point where separation of seeds from pods first became practicable) the total amount of manganese in the pod was 9 times that in the seeds, the relative amount in the seeds increased very rapidly during the next few stages, so that by Stage VII the ratio pod Mn/seed Mn had fallen to 1·84. This rapid increase of manganese in the seeds seems to point definitely to some essential function of manganese in plant development.

*Ash content of ripening seeds.* The large increase in the manganese content of the ripening seed suggests the question whether the ash content behaves similarly. Not many data are available for the variation in the mineral constituents of a plant at different stages of development. From a few examples quoted by Czapek [1925] it would appear, as might be expected, that the percentage content of ash diminishes steadily during seed-ripening, but that the absolute values show an increase. Thus, for the ears of *Avena sativa* at successive stages, the figures given by Arendt [1860] are:

| | | | | |
|---|---|---|---|---|
| Ash % | 3·89 | 3·67 | 2·82 | 2·68 |
| Total ash for ears of 100 oat-plants | 15·66 | 25·70 | 31·86 | 34·29 |

A similar increase was found by Schjerning [1906] for the ash content of ripening barley. In the 3 examples given, the ash content of a given number of barley-grains was roughly doubled between the stage of green ripeness and that of complete ripeness. While these increases in total ash are by no means so great as those found for the manganese content of the lupin seeds, it must not be overlooked that the stages for which figures are given may not be comparable in the different cases.

No instance has been found in which, for seeds, the percentage of one mineral constituent increases during seed-ripening while the percentage of the total ash decreases, but Samuel and Piper [1929] give an example of this nature for the oat plant at different stages of growth. They found a continuous decrease in ash, from 11·59 % to 6·91 % (6·17 % when finally harvested), while the percentage manganese content showed a distinct increase at the age of 12–16 weeks.

This was followed by a steady decline until the plant was ripe, but even then the proportion of manganese was only a little less than at the time of the first sampling. The increase recorded is by no means so striking as in the case of the seeds of the lupin, and it would be interesting to trace the ash and manganese content for the ears of the oats, for comparison with those of the whole plant. The results found for barley differed from those just described for oats in that there was a continuous decrease in the percentage of manganese as the plants approached maturity.

*Analogy between plant and animal kingdoms.* The striking increase in the manganese content of the seeds of the lupin during the process of ripening, suggested that a similar effect might be observed in the animal kingdom. In Table IV will be found the results for the manganese content of 4 series of hen's eggs arranged in order according to the different stages of development from the ovary with the tiniest ova to the completely formed egg (Egg 7). The eggs were taken from the bodies of 4 hens, aged 60–62 weeks, which had all been similarly fed. The table shows the total manganese content in mg. per egg.

Table IV.

| Hen | Ovary | Small ova | Eggs 1 and 2 (per egg) | Egg 3 | Egg 4 | Egg 5 | Egg 6 | Egg 7 |
|---|---|---|---|---|---|---|---|---|
| 1 | 0·0022 | | 0·002 | 0·004 | 0·0055 | 0·0086 | 0·0101 | 0·0098 |
| 2 | — | 0·001 | 0·002 | 0·0043 | 0·0076 | 0·0106 | — | — |
| 3 | — | 0·0005 | 0·0013 | 0·0023 | 0·0065 | 0·0077 | 0·0101 | 0·0126 |
| 4 | 0·0016 | 0·0018 | 0·0013 | Lost | 0·0064 | Lost | 0·0110 | 0·0126 |

It will be seen that the total manganese of the developing egg shows a steady increase similar to that obtained for the ripening lupin seed, though on a smaller scale. With regard to the percentage of manganese, in two cases a maximum was reached about the middle of the series, followed by a decrease. In the third series the highest figure was found in the last two eggs of the series, while in the last case the percentage remained practically constant throughout. The average value for the yolks of all sizes and all series was 0·066 mg. per 100 g. moist substance. It may be noted here that no connection could be traced between the fertility of hen's eggs and their manganese content. Different series of eggs—fertile, infertile, and "dead in the shell"—showed approximately the same range of variation in manganese content, and the same average percentage of manganese.

## PART II.

### The manganese content of foodstuffs.

For our present knowledge of the manganese content of foodstuffs in common use we are indebted mainly to two papers recently published in America by Lindow and Peterson [1927] and by Skinner and Peterson [1928]. In order to get some idea of the variations that may be expected in foodstuffs from different districts and different countries, American figures have been included, when available, for the substances in the present list. The majority of the substances were obtained from the Duthie Experimental Stock Farm Food-store. In other cases the place of origin is indicated.

Table V. *Manganese content of foodstuffs*[1].

| Substance | Mn mg./100 g. dry matter | Figures by Peterson etc. |
|---|---|---|
| *Grains and farm feeds* | | |
| Barley, whole | (1) 1·85 | (1) 1·62 |
| „ „ | (2) 2·17 | (2) 1·90 |
| „ ground | 1·88 | — |
| Blood meal | 0·49 | 0·89 |
| Cotton seed meal | 2·69 | 1·99 |
| „ Bombay | 1·81 | — |
| „ cake, decorticated | 2·42 | — |
| Cotton cake, Egyptian | 2·05 | — |
| Earth-nut cake, undecorticated, ground | 4·7 | — |
| Earth-nut (pea-nut), West Africa | 1·87 | 1·60 |
| Fish-meal | (1) 0·77 | — |
| | (2) 1·08 | — |
| | (3) 1·12 | — |
| Kaffir grain, white, West Africa | 1·57 | 1·59 |
| Linseed, ground | 3·42 | 4·94 |
| „ cake | 4·2 | — |
| Locust beans | 0·78 | — |
| Maize (1) | 1·11 | Yellow corn |
| „ (2) | 0·78 | 0·53 |
| „ (3), West Africa | 0·70 | 0·54 |
| „ (4), Kenya | 0·70 | — |
| „ ground, (5) | 0·93 | — |
| Meat and bone meal | 1·36 | 1·00 |
| Millet, Kenya | (1) 2·49 | Japanese millet |
| | (2) 2·40 | 3·56 |
| Oats, whole | 5·6 | 4·21, 3·18 |
| Oatmeal | (1) 6·9 | — |
| | (2) 6·6 | — |
| Oat straw | 5·2 | 8·71 |
| Pasture | (1) 9·0 | — |
| | (2) 19·0 | — |
| | (3) 14·7 | — |
| Pasture, South England | (1) 10·0 | — |
| | (2) 11·0 | — |
| Rape meal | 10·9 | 5·11 |
| „ cake | 6·9 | — |
| Rice, polished | 1·44 | 1·20 |
| „ husked, unpolished, West Africa | 1·79 | Rice, brewer's 1·68 |
| Soya bean, ground | 5·9 | Soya bean seed 2·95 |
| „ cake | 4·9 | „ „ hay 8·41 |
| Sunflower meal, decorticated | 7·2 | — |
| „ „ semi-decorticated | 4·9 | — |
| „ cake, ground | (1) 5·8 | — |
| | (2) 5·5 | — |
| Tapioca | 0·78 | — |
| Wheat, whole | 3·1 | 3·7, 5·45 |
| „ ground | 4·9 | — |
| „ embryo (hand-picked) | 39·0 | — |
| „ „ (commercial) | 16·8 | — |
| „ bran | 13·8 | 10·10 |
| | | 14·04 |
| | | 14·04 |
| „ middlings or sharps, various | 10·4–16·2 | Wheat middlings |
| | | (flour) 12·96 |
| | | (standard) 15·58 |

[1] I desire to acknowledge my indebtedness to Miss B. W. Simpson and Mr G. W. Leeper for assistance with the analyses.

Table V (*continued*).

| Substance | Mn mg./100 g. dry matter | Figures by Peterson etc. |
|---|---|---|
| *Vegetables etc.* (grown locally, unless otherwise indicated) | | |
| Banana, Kenya ... ... ... ... | 1·88 | 3·33 |
| Beans, Kenya ... ... ... ... ... | 2·36 | 1·22, 1·87, 2·96 (different varieties) |
| Beetroot ... ... ... ... ... | 2·17 | — |
| Cabbage ... ... ... ... ... | 1·50 | 1·06 |
| Carrot ... ... ... ... ... ... | 1·67 | 0·60 |
| Leek ... ... ... ... ... ... | 0·44 | — |
| Lettuce... ... ... ... ... ... | 13·3 | (1) 21·62<br>(2) 10·00 |
| Marrow, Kenya ... ... ... ... | 2·49 | — |
| Onion, various, England ... ... ... | 0·72–1·44<br>av. (6) 0·98 | 0·79<br>— |
| Peas, Kenya ... ... ... ... ... ... | 2·51 | 1·94 |
| ,, ... ... ... ... ... ... | 1·15 | — |
| ,, (pods) ... ... ... ... ... | 2·70 | — |
| Potato, peeled, various, England ... ... | 0·40–0·76<br>av. (15) 0·61 | 0·45<br>— |
| ,, ,, ,, ... ... | (1) 1·18 | — |
| ,, peeled ... ... ... ... | 0·58 | — |
| ,, unpeeled ... ... ... ... | 1·43 | — |
| ,, sweet, Kenya ... ... ... | 2·78 | 0·54, 1·96 |
| Spinach, Kenya ... ... ... ... | 8·9 | 8·65 |
| Turnip ... ... ... ... ... ... | 0·36 | — |
| Yam, Kenya ... ... ... ... ... | 0·20 | — |
| *Miscellaneous* | mg./100 cc. | |
| Milk (cow's) ... ... ... ... ... | (1) 0·004<br>(2) 0·004 | —<br>— |
| ,, South Scotland ... ... ... ... | 0·005 | — |
| ,, Kenya ... ... ... ... ... | 0·004/100 g. | — |
| | mg./100 g. moist wt. | |
| Eggs (hen's) ... ... ... ... ... | 0·020—0·048<br>av. (62) 0·032 | —<br>— |
| | mg./100 g. dry matter | |
| Yeast, dried ... ... ... ... ... | (1) 0·95<br>(2) 0·74 | — |
| Marmite ... ... ... ... ... | 0·08 | — |

## Discussion.

*District Variation.* (*a*) Comparison of the present figures with those of the American workers shows that only in a few cases is there fairly close agreement in the percentages of manganese, *e.g.* barley, earth-nut (pea-nut), Kaffir grain, rice, wheat bran, wheat middlings and spinach. When considerable differences do exist however there is a general tendency for the results to be of the same order of magnitude. This is indicated by the figures for lettuce, ground linseed, wheat and onions.

(*b*) The question of district variation has been studied more closely in connection with the manganese content of potatoes and onions. A number of samples were available, collected from various counties in England, ranging from Cumberland to Cornwall. Analyses of these samples showed that, irrespective of variety, the variation in manganese content is not very great from district to district. Of 16 samples of potatoes analysed, 15 gave figures falling within the range 0·40–0·76 mg., with an average of 0·61 mg./100 g. of manganese. Only one sample differed considerably from the rest, giving a content of 1·18 mg. All these samples were peeled before analysis, as if for human consumption, and it seems possible that in this particular case the potatoes may have been peeled more thinly than usual. Support is lent to this idea by the fact that a sample of local potatoes when analysed unpeeled, after thorough washing, gave a manganese content of 1·43 mg., while the same potatoes, peeled, gave 0·58 mg., a result agreeing closely with the average of 0·61 mg. for the various English potatoes. A similar difference is seen in the figures given by Lindow and Peterson [1927] for scraped and unscraped parsnips, for which the manganese content is 0·20 and 1·39 mg. respectively. The figure for unpeeled potatoes, *viz.* 1·43 mg., may be compared with the average of 1·40 mg. found by Bode and Hembd [1921] for 16 varieties of potatoes (20 samples). In the absence of direct information on the point, it may be assumed that in this case the potatoes were analysed unpeeled. For onions, the percentage variation is approximately the same as for potatoes. The manganese contents of the 6 samples analysed were 0·72, 0·76, 0·83, 1·01, 1·14 and 1·44 mg., giving an average of 0·98 mg./100 g.

Exact agreement in samples from different sources is scarcely to be expected when varying soil conditions are taken into account. There can be little doubt, in view of the results reported in various culture experiments, that the absorption of manganese by a plant may be influenced to a considerable extent by the amount of available manganese in the nutrient solution or soil. Thus Passerini [1906], in pot experiments with lupins, observed that the addition of manganese carbonate to a sand already containing 0·0002 % of manganese increased the manganese content of the dry matter of the plants nearly 7-fold. So also Samuel and Piper [1929] found that the manganese content of healthy oat plants in water-cultures increased from 14·8 to 95·7 parts per million dry matter, as the manganese content of the solution was increased from 1 in 10,000,000 to 1 in 400,000, while the diseased plants from manganese-deficient solutions contained 12 parts per million or less. A similar wide variation for manganese content under different conditions is seen in the figures given by Skinner and Peterson for sugar-beet tops, which when grown in the field contained 71·2 mg./100 g. of manganese, when grown in a greenhouse only 10·3 mg.

On the other hand, Headden [1915] found that the amount of manganese present in different varieties of wheat was very nearly constant, irrespective of the soil. Of 33 samples analysed, consisting of many varieties grown under widely different manurial conditions, two-thirds of the number gave a manganese content of 4–5 mg., the average for all the samples being 5·1 mg./100 g. Again, the results found by Bode and Hembd [1921] for different varieties of potatoes show in general how constancy is maintained in different fields, although in one case a 50 % difference was observed for the same variety in two fields. With certain species the manganese content of the plant appears to be fairly constant, regardless of variations in soil conditions, whereas with others fairly wide variations have been recorded. Our knowledge is at present too scanty to permit of correlation between soil conditions and manganese content of the herbage growing thereon.

*Manganese content of pastures.* Pastures have a high manganese content. For 5 samples given the average is 12·7 mg. per 100 g. dry matter. A number of samples that were available for analysis have given figures much higher than this, but as it is not certain that precautions were taken in collecting the samples to avoid all soil contamination, the results are not recorded.

*Distribution of manganese in the wheat grain.* The figures for the manganese content of the whole wheat grain, and of the various preparations from it, show that the by-products are richer in manganese than the whole seed. The whole grain contains only 3·1 mg., while bran has 13·8 mg. per 100 g., and the various grades of sharps or middlings vary from 10·4 mg. and 10·6 mg. in white and red sharps, to 14·0 mg. and 16·2 mg. in brown sharps and in the coarse middlings respectively. This concentration of manganese in the outer layers of the grain, which is noted also by Skinner and Peterson, was demonstrated by McHargue [1914] for wheat and a considerable number of other seeds. The very high percentage of manganese in *wheat embryo, viz.* 39·0 mg., is worthy of note, in that it offers a further indication of the importance of manganese in the germination of the seed [see Pichard, 1898; McHargue, 1923].

*Manganese content of milk and colostrum.* The manganese content of milk is very low, amounting to only 0·004 mg. per 100 cc. It will be noted that, as with plant-products, the value remains remarkably constant for milks from different neighbourhoods. Samples from Kenya, and from the south of Scotland, gave practically the same figure as the local milk. Colostrum has a higher manganese content than milk, though the differences here recorded are not so great as that in the single example given by McHargue [1924], where the amount of manganese in the colostrum was nearly 7 times as great as the amount in the milk a month later.

Table VI. *Manganese content of colostrum.*

(mg. Mn/100 cc.)

| | Cow I | Cow II | Cow III |
|---|---|---|---|
| 1st milking | 0·007 | 0·005 | 0·009 |
| 2nd ,, | 0·005 | 0·004 | 0·006 |
| 3rd ,, | 0·007 | 0·004 | 0·003 |

## PART III.

### Manganese and the animal organism.

It has been found that the occurrence of manganese is as widespread throughout the animal kingdom as in the plant world. Bertrand and Medigreceanu [1913, 1, 2] have not only shown its presence in the blood and organs of man and the higher animals, but have shown that it occurs also in all the zoological groups. They draw attention however to the excessive poverty of the animal species, as compared with plants, in respect of their manganese content. This is doubtless true if the content of the total organism is considered, since animal muscle is low in manganese whilst the green leaves of plants are particularly high, but if individual animal organs are considered, *e.g.* liver and pancreas, the difference between the plant and animal kingdoms is much less pronounced. In exceptional cases moreover the animal organism as a whole can surpass most of the plants, *e.g.* Bradley [1910] has found that certain molluscs contain as much as 1 % of manganese in the dry tissue, the manganese being present in rather uniform amount, and showing no such large fluctuations as would be expected were the occurrence of the element adventitious.

*Effects of manganese intake on the animal organism.* With regard to the effects of manganese intake, it appears that in general the animal organism shows a great tolerance for manganese compounds. Toxic results following the injection of manganese salts have been reported in the case of such animals as dogs, rats, rabbits, and guinea-pigs, and Findlay [1924] obtained a toxic effect by feeding a large amount of manganese chloride to rats, but the bulk of the evidence seems to show that when ingested *per os*, even in fairly large amounts, manganese compounds have no toxic effect. Thus it has been proved by experiments on dogs and on pigeons [Reiman and Minot, 1920, 2; Oettingen and Sollmann, 1927] that even prolonged feeding of large amounts of manganese ores fails to produce any significant changes in the manganese content of the blood and tissues or to cause any pathological symptoms. With regard to the cases of poisoning that sometimes occur among workmen in manganese factories, these authors suggest either that certain individuals are specially susceptible, or that the ores when inhaled as a dust are much more poisonous than when taken in the food. The tolerance of animals for ingested manganese compounds is borne out by growth experiments with pigs, in which the present author found no toxic symptoms following daily ingestion of 3·5 g. of manganese citrate for nearly 9 months. Information is scanty regarding the effect of additions of manganese to a diet deficient or lacking in this element. The few experiments on rats and mice already referred to are rather inconclusive in their results, owing in most cases either to the small number of animals in the groups, or to the short duration of the experiment. In a later paper, McCarrison [1928] reports a result which may be of considerable significance. He describes a type of goitre which is produced in rats by a deficiency diet, but is not related to iodine deficiency. The condition does not occur if manganese chloride is added to the diet in a suitable proportion. Again however the data are scanty.

Some recent work in America deals with the question of manganese in its relation to anaemia, but the results reported by different authors are contradictory. Titus, Cave and Hughes [1928] found that the addition of manganese and copper to a milk-iron diet produced a quicker response as regards haemoglobin building than did either copper or manganese alone. On the other hand, Waddell, Steenbock and Hart [1929] failed to confirm this finding.

## Present work.

### *Manganese requirement.*

In dealing with the feeding of the "trace" elements (such as manganese) to farm animals, it is difficult to devise a basal ration which will be sufficiently low in the element concerned to make it possible to see any effect from addition of its salts. It is of course impracticable to feed such animals on a synthetic diet, and the amount of manganese present in ordinary food-stuffs, such as might naturally be fed to growing animals, is probably large enough to satisfy the animal's requirement. Thus, in a growth experiment on pigs conducted at this Institute over a period of 10–11 months, no difference as regards growth and general health could be detected between the groups receiving amounts of manganese citrate varying from 0·4 to 3·5 g. per pig daily, although the basal ration of maize, barley, fishmeal, potatoes, and separated milk, contained only about 1/17th of the amount of manganese present in a more normal pig-feeding diet of sharps, oatmeal, fishmeal, and separated milk. It seems therefore that the manganese requirement, if any, of the pig is small, and that it is satisfied by an intake of about 1 part in 180,000, the amount present in the basal ration of the above experiment, although it is possible that continuation of such an experiment for another generation might bring to light differences between the groups with differing manganese intake.

The difficulty of supplying direct experimental evidence as to the efficacy of manganese in promoting the growth and well-being of animals makes it necessary to rely largely on the indirect evidence afforded by the analysis of animal organs under varied conditions. Such evidence is offered in the following pages, in which are summarised the results of analyses of organs from different species of animals, and in some cases at different stages of growth.

### *Manganese content of animal organs.*

In the tables which follow all results are expressed as mg. Mn per 100 g. moist tissue.

Table VII gives the manganese content of various organs of different species, including the chief farm animals. In most cases the range of variation is given as well as the average.

In Table VII only average figures have been included for the liver, pancreas, and reproductive organs, as these will be discussed more fully later. Omitting these organs, it will be noted that figures for manganese content are high for the duodenum, and fairly high for the kidney and submaxillary salivary glands, while for the remaining organs, *e.g.* spleen, heart, brain, lung, thyroid, and muscle, they are on a distinctly lower level.

Although the number of analyses done for each animal is not large, the table indicates that for these organs of lower manganese content there is a fairly close agreement among the different species, except that the organs of the fowl give somewhat higher values—a point noted also by Bertrand and Medigreceanu [1913, 1]. Thus the figures (expressing Mn in mg. per 100 g. moist tissue) for

Table VII.

(mg. Mn/100 g.)

| Organ | Pig | Ox | Sheep | Fowl | Man |
|---|---|---|---|---|---|
| Liver | Av. 0·393 | 0·250 | 0·277 | 0·303 | 0·175 |
| Pancreas | Av. 0·207 | 0·171 | 0·205 | 0·222 | 0·115 |
| Kidney | 0·075–0·157 | 0·115–0·151 | 0·071, 0·175 | 0·204–0·227 | 0·068–0·083 |
| | Av. (7) 0·109 | Av. (3) 0·136 | Av. (2) 0·123 | Av. (3) 0·216 | Av. (5) 0·077 |
| Duodenum | 0·084–0·272 | — | 0·288, 0·333 | — | 0·092 |
| | | | Av. (2) 0·311 | | |
| Salivary glands | | | | | |
| Submaxillary | 0·105 | 0·110 | 0·114 | — | 0·091 |
| Parotid | 0·086 | 0·050 | — | — | — |
| Testis | Av. 0·053 | 0·028 | 0·080 | 0·053 | — |
| Ovary | Av. 0·054 | — | 0·056 | 0·046 | — |
| Gall-bladder | 0·033–0·103 | 0·023–0·088 | 0·216–0·270 | — | — |
| | Av. (4) 0·071 | Av. (4) 0·052 | Av. (4) 0·234 | — | — |
| Bile | 0·031–0·097 | 0·011–0·058 | 0·123–0·191 | — | — |
| | Av. (4) 0·052 | Av. (4) 0·031 | Av. (4) 0·164 | — | — |
| Spleen | 0·027–0·046 | 0·016 | 0·036 | 0·062 | 0·008–0·025 |
| | Av. (4) 0·036 | — | — | — | Av. (4) 0·018 |
| Heart | 0·018, 0·021 | 0·028 | 0·029 | 0·067, 0·071 | 0·021 |
| | Av. (2) 0·020 | | | Av. (2) 0·069 | |
| Lung | 0·028 | 0·016 | — | — | 0·038, 0·041 |
| | | | | | Av. (2) 0·040 |
| Thyroid | 0·031, 0·076 | 0·037 | 0·036 | — | 0·019–0·077 |
| | Av. (2) 0·054 | | | | Av. (3) 0·049 |
| Muscle (thigh) | 0·015 | 0·015 | — | — | 0·017 |
| Blood | — | — | 0·005 | 0·004 | — |

the kidney in the pig, ox, sheep, and man, are 0·109, 0·136, 0·123, and 0·077 respectively, while for the fowl the value is 0·216, which agrees with Bertrand's figure of 0·217. Even closer agreement is found for the submaxillary salivary gland, muscle and heart.

*Manganese content of liver and pancreas.* Table VII shows that for all the species considered the organs highest in manganese are the liver and pancreas. The results for these are therefore given in greater detail in Table VIII.

Table VIII.

(mg. Mn/100 g.)

| | Pig | Ox | Sheep | Fowl | Rabbit | Man | | Fish |
|---|---|---|---|---|---|---|---|---|
| Liver | 0·408 | 0·234 | 0·249 | 0·328 | 0·259 | 0·189 | *Monk-fish | 0·076 |
| | 0·377 | 0·289 | 0·238 | 0·275 | 0·234 | 0·213 | Coal-fish | 0·079 |
| | 0·448 | 0·247 | 0·263 | 0·328 | 0·245 | 0·130 | Cod | 0·086 |
| | 0·381 | 0·223 | 0·336 | 0·327 | 0·292 | 0·195 | Haddock | 0·140 |
| | 0·370 | 0·256 | 0·300 | 0·255 | 0·247 | 0·147 | Hake | 0·196 |
| | 0·355 | — | — | — | 0·274 | — | | — |
| | 0·412 | — | — | — | — | — | | — |
| Mean | 0·393 | 0·250 | 0·277 | 0·303 | 0·259 | 0·175 | | 0·115 |
| Pancreas | 0·190 | 0·147 | 0·234 | 0·239 | 0·233 | 0·136 | | — |
| | 0·197 | 0·167 | 0·153 | 0·205 | — | 0·102 | | — |
| | 0·200 | 0·178 | 0·212 | — | — | 0·106 | | — |
| | 0·186 | 0·192 | 0·220 | — | — | — | | — |
| | 0·214 | — | 0·208 | — | — | — | | — |
| | 0·252 | — | — | — | — | — | | — |
| Mean | 0·207 | 0·171 | 0·205 | 0·222 | 0·233 | 0·115 | | — |

* Monk-fish—*Lophius piscatorius*; coal-fish—*Gadus virens*; cod—*Gadus callarias*; haddock—*Gadus aeglefinus*; hake—*Merlucius vulgaris*.

The most striking point is the remarkable constancy found in the manganese content of the same organ in the different individuals of one species. Some indication of this was seen in a few results of Bertrand and Medigreceanu, [1913, 1], and the observation is amply confirmed by the numerous instances above recorded. While the table thus brings out the fact of the constancy within the species, it shows that for the organs of higher manganese content, variations among the different species are more marked than was the case for the organs already considered.

Thus the liver of the pig, (with approximately 0·4 mg. per 100 g. moist tissue), is distinctly richer in manganese than that of the fowl, sheep, rabbit, and ox, and the figures for these species are again considerably higher than those for man and for various kinds of fish. The low figure for human liver agrees with that of Reiman and Minot [1920, 1], who found 0·170 as the mean of 13 results, and for fish-liver two values given by Bertrand and Medigreceanu [1913, 1] are also low—0·040 for monk-fish, and 0·089 for dog-fish. In view of the possible connection between manganese and anaemia, the low manganese content of the liver of fish, as compared with that of land animals, seems worthy of mention, since it has been found in anaemia experiments [Robscheit-Robbins and Whipple, 1926] that fish-liver, in contrast with the liver of ox, pig, or fowl, is almost inert as a dietary factor in haemoglobin production.

For the pancreas the differences among animals of different species are less marked, the pig, sheep, fowl, and rabbit giving approximately equal values. Ox-pancreas however gives a somewhat lower figure, and human pancreas, like human liver, contains little more than half the percentage of manganese found in the corresponding organs of the other species considered. This low value for human pancreas is supported by the results of Reiman and Minot [1920, 1], who give an even lower figure, namely 0·076 as a mean of six results. As far as the author is aware, no attention has yet been drawn to the relatively high manganese content of the pancreas in most species, and in fact very few analyses of this organ seem to have been carried out. Bertrand and Medigreceanu [1913, 1] give figures only for the dog, the seal, and a species of dog-fish, and though the latter two are high—0·166 and 0·271 respectively—he makes no mention of the pancreas among the organs of principal functional importance highest in manganese.

*Effect of manganese feeding on the liver and pancreas.* Growth experiments on pigs, in which successive groups received increasing amounts of manganese, provided material for determining whether these additions to the diet caused any noticeable difference in the manganese content of the organs (Table IX). Two or three animals were slaughtered from each group, after a period of 3 months on their respective diets. In Group E, the animals had received a small amount of added manganese for two months (0·0135 g. Mn per pig daily), but the dose was increased to 0·47 g. a month before slaughter.

It will be seen that, associated with the manganese feeding, there is a greater irregularity in the figures than was observed in the case of the normal

Table IX. *Effect of manganese intake on liver and pancreas.*

| Group | Ration | Mn in liver mg. per 100 g. | Mn in pancreas mg. per 100 g. |
|---|---|---|---|
| A | Basal ... ... ... ... | 0·456 | 0·174 |
| | | 0·395 | 0·265 |
| B | Basal + 0·054 g. Mn per pig daily | 0·494 | 0·198 |
| | | 0·418 | 0·186 |
| C | Basal + 0·135 g. Mn ... ... | 0·551 | 0·272 |
| | | 0·299 | 0·221 |
| D | Basal + 0·27 g. Mn ... ... | 0·547 | 0·249 |
| | | 0·493 | 0·153 |
| | | 0·435 | 0·176 |
| E | Basal + 0·47 g. Mn ... ... | 0·348 | 0·302 |
| | | 0·524 | 0·172 |
| | Mean | 0·451 | 0·215 |

animals. On account of this irregularity and the small number of analyses available no group averages are given. It seems that the manganese feeding has had no very apparent effect on the manganese content of the pancreas. The figures for the different animals show only slight deviations from the normal range, *viz.* 0·186–0·252, and the average for all the animals, 0·215, is very close to the normal average of 0·207. Nor has there been any very decided effect on the liver. While a few of the individual figures are higher than normal, especially in the two highest manganese groups, there is no evidence of any very marked accumulation of manganese in this organ.

*Excretion of manganese in the bile.* Little information is available regarding the extent to which manganese is absorbed from the alimentary canal, or regarding its path of excretion from the body, although Wichert [1860] showed that numerous heavy metals, including manganese, passed into the bile. Reiman and Minot [1920, 2] have recently furnished reliable information on the absorption and excretion of manganese when administered *per os*. They have shown that manganese ores are soluble in gastric juice and that manganese is absorbed into the blood stream. Further, they have demonstrated that the manganese content of bile is markedly increased after ingestion of manganese (in the form of franklinite), and they conclude that "the bile is at least one important path of elimination of ingested and absorbed manganese". As evidence in confirmation of the biliary excretion of manganese, it may be stated that excessively high figures (3·52 and 0·785 mg./100 g.) for manganese have been obtained in the bile of two animals which died of intestinal obstruction.

*Deviations from the normal in manganese of liver and pancreas.* (*a*) *Anaemic pigs.* Abnormally low figures for manganese of liver and pancreas were obtained in the case of two pigs which died from anaemia. The low value for the liver may be accounted for by the change in the hepatic tissue which has been shown to take place as a sequel to iron deficiency, the centre of the lobules being destroyed and replaced by blood [McGowan, 1926]. It was thought that possibly the deviation from the normal in the manganese content of these organs might be due to the age of the animals, the normal figures having been obtained from animals about 10 months old, while the pigs in question were only 5–6 months. But this is evidently not the case, as is seen by comparison with the figures obtained for animals of similar age in the control group of the manganese feeding experiment.

Table X.

(mg. Mn/100 g.)

| Animal | Age (months) | Liver Mn | Pancreas Mn |
|---|---|---|---|
| Anaemic pigs | (1) 6 | 0·103 | 0·130 |
| | (2) 5 | 0·092 | 0·113 |
| Pigs from control group of Mn experiment | (1) 6½ | 0·456 | 0·174 |
| | (2) 6½ | 0·395 | 0·166 |
| Normal pig (mean) | 9–10 | 0·393 | 0·207 |

(*b*) *Young pigs.* The low figures obtained for liver and pancreas in some piglets of the manganese experiment, which died at birth or within a few days, seem to require further investigation.

Table XI. *Liver and pancreas of piglets from Mn experiment.*

| | Liver | | Pancreas (omitting pigs <2 days old) | |
|---|---|---|---|---|
| Group | Range | Average | Range | Average |
| A | 0·100–0·192 (7) | 0·144 | 0·078–0·130 (4) | 0·097 |
| B | 0·139–0·174 (4)<br>0·316 (1) | 0·161 (4)<br>0·192 (5) | 0·122 (1) | 0·122 |
| C | 0·130–0·138 (3) | 0·134 | 0·116–0·143 (2) | 0·130 |
| D | 0·101–0·168 (5)<br>0·268 (1) | 0·143 (5)<br>0·164 (6) | 0·117–0·167 (3) | 0·140 |
| E | 0·124–0·174 (2) | 0·149 | | |

(The figures in brackets indicate the number of animals for which the range or average is given.) It may be a question of age, but the higher results obtained in two cases, *viz.* 0·316 (Group B) and 0·268 (Group D), seem to point against this. On the other hand, the low value may be connected with the fact that practically all the surviving piglets in this experiment suffered from iron deficiency. Of course if there is any connection between the anaemic condition and the low manganese content of the organs, the experiment proves that addition of manganese to the diet of the sow is not sufficient in itself to prevent anaemia in the young pigs, since all the groups, even those with the highest manganese, show similar figures. But it seems possible that the deficiency of the ration may in some way have interfered with the assimilation of manganese.

(*c*) *Foetal pancreas.* It will be noted that in the preceding table no pancreas figures have been included for piglets less than 2 days old. It is a curious fact that abnormally high percentages of manganese are found in the pancreas of the pig foetus, and of young pigs which have died at birth, or within a day or two after birth (0·6–0·8 mg./100 g. occurs frequently, and occasionally the figure is even higher). The total amount of manganese involved is small however and it is difficult to judge whether the point is of any significance.

*Manganese content of reproductive organs.* Next to the liver and pancreas, chief attention has been given to the reproductive organs, in view of the possible connection between manganese and reproduction suggested by the steady increase of manganese in the ripening seeds of plants and in the developing yolk of the hen's egg.

Table XII. *Mn content of reproductive organs.*

| | Pig | Ox | Sheep | Fowl |
|---|---|---|---|---|
| Testis | 0·039–0·063 | (1) 0·022 (7 weeks) | 0·035–0·086 | 0·053 (3) |
| | Av. (20) 0·053 | (2) 0·029 (5 months) | Av. (16) 0·059 | — |
| | — | (3) 0·029 (5 months) | 0·123–0·285 (4) | — |
| | — | (4) 0·029 (15 months) | Av. (20) 0·080 | — |
| | — | (5) 0·031 (3½ years) | | — |
| | | Av. 0·028 | | |
| Piglets of Mn experiment | | | | |
| | Group A 0·046 | — | — | — |
| | „ B 0·048 | — | — | — |
| | „ C 0·041 | — | — | — |
| | „ D 0·045 | — | — | — |
| | „ E 0·041 | — | — | — |
| Epididymis etc. | 0·020–0·046 | (1) 0·041 | 0·020–0·076 | |
| (residue of | — | (2) 0·022 | — | — |
| testicle) | — | (3) 0·045 | — | — |
| | — | (4) 0·053 | — | — |
| | — | (5) 0·060 | — | — |
| Ovary | 0·040–0·067 | — | 0·041–0·070 | Ovary 0·046 |
| | Av. (3) 0·054 | — | Av. (2) 0·056 | *Oviduct 0·060–0·078 |
| | | | | Av. (4) 0·070 |
| Uterus | 0·031–0·041 | — | — | — |
| | Av. (3) 0·037 | — | — | — |
| Placenta | 0·056 | (Human) 0·0061–0·0098—Av. (5) 0·0073 | | |

* The high figure found by Bertrand and Medigreceanu [1913, 1] for the oviduct of the hen, *viz.* 0·786, has not been confirmed by the author.

In the case of the pig, sheep, and fowl, several pairs of testes were combined for each analysis, so that each figure is itself an average.

While the amount of manganese in these organs is on a distinctly lower level than that in the liver and pancreas, the constancy with which it occurs seems to be significant. Again it is seen that not only is the figure approximately constant for one species, but the differences between the figures for different species are not great. Such differences as do exist are much smaller than those found in the plant kingdom; *e.g.* compare the difference between the reproductive organs of the ox and the sheep with that between the organs of the laburnum and the lupin (Tables I and II). Plants and animals agree more closely in that male and female reproductive organs contain almost the same percentage of manganese. Just as manganese feeding had little or no effect on the liver and pancreas, so also differences in the amount of manganese in the diet of sows have had no influence on the manganese content of the reproductive organs of their male offspring.

*Effect of age on the manganese content of reproductive organs.* In all cases except the ox, the testes were obtained from animals only a few weeks old. In the case of the ox, material was obtained from animals of different ages. It will be seen that the percentage content of manganese remains practically constant as the animal matures, but this of course implies a large increase in the total amount of manganese present in the organ with increasing age.

*Manganese content of the stomach.* Numerous analyses have been carried out on the stomachs of various animals, but the results have not been included in the general table. While in many cases high figures have been obtained, the variations found are much wider than is the case for most of the other organs analysed. Table XIII gives a summary of the results found for several species.

Table XIII. *Manganese content of stomach of various animals.*

| | Pig<br>Stomach-lining | Rabbit<br>Stomach | Fowl<br>Crop | Man<br>Stomach |
|---|---|---|---|---|
| Cardiac | 0·066–0·408<br>Av. (6) 0·224 | 0·099–0·181<br>Av. (3) 0·135 | 0·299–0·419<br>Av. (4) 0·354 | 0·029 |
| Pyloric | 0·084–0·218<br>Av. (6) 0·133 | —<br>— | Proventriculus<br>0·100–0·209<br>Av. (5) 0·140 | *0·013–0·040<br>Av. (8) 0·026 |
| | | | Gizzard-lining<br>0·149–0·389<br>Av. (8) 0·261 | — |

* Reiman and Minot [1920, 1].

The results for the stomach of man are low, as was the case also with the liver and pancreas, while the fowl gives figures above the average.

*Ruminants.* The analyses for the different parts of the stomach of ruminants are not included in the present paper, as the data obtained cannot be conveniently summarised, and no satisfactory explanation can as yet be offered for the very wide variations observed in the manganese content.

## SUMMARY.

The manganese content of a large number of substances of vegetable or animal origin has been determined.

With regard to the reproductive organs of plants, wide differences are found in different plants, but there is little difference as regards manganese content between the male and female organs of the same plant. Analysis of lupin seeds at various stages of development shows that there is a marked increase in total manganese as the seed grows to maturity. This increase seems to bring further evidence that manganese may be regarded as an essential element for the development of the plant.

The data for the manganese content of foodstuffs indicate that while soil-conditions may have some influence in determining manganese absorption by the plant, samples of a foodstuff from different sources show in general approximately the same content of manganese.

From a consideration of the data for the manganese content of animal organs, it appears that while we may be still in ignorance of the actual function of manganese in nutrition, there is a considerable amount of evidence in favour of regarding this element as one of the essential constituents of the animal organism. Direct proof of the indispensability of manganese for normal growth and development has not yet been furnished, and in considering the possibility of its importance, it has to be remembered that manganese may behave in the animal body like silver, mercury, lead, and other heavy metals, and that its presence in organs and tissues may have no real physiological significance. On the other hand, the steady increase of manganese in the developing egg, the invariable presence of the element in such tissues as the reproductive organs, and the constancy of the amount not only in these organs but also in those most closely connected with the processes of assimilation (a constancy which is fairly well maintained when large amounts of manganese salts are added to the diet) are facts which suggest that manganese is no accidental constituent of the organism, but may have some intimate relationship to the vital processes.

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