The retention of an oral dose of radioactive manganese in the pullet and the effects on retention of the intake of inactive manganese

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In the growing chick a large proportion, over 95%, of an oral dose of radioactive manganese was found in droppings collected during 72 h after dosing (Mohamed & Greenberg, 1943), indicating a very low retention of this element.

The object of the study now presented was to make similar determinations of retention in the laying pullet and to observe the effects on retention of (1) giving a low-Mn diet for 10 weeks or more and (2) varying the quantity of inactive Mn with the radioactive dose. It soon became evident that retention was very small in all treatments and in consequence, even if differences among treatments occurred, they were unlikely to be detected by the 'difference method'. Therefore treatment effects were sought in the quantities of radioactive Mn present in the liver and blood. A brief report of part of the work has been published (Hill, 1964).

EXPERIMENTAL

Expt 1. Six Rhode Island Red (RIR) × Light Sussex (LS) 6-month-old pullets that had been fed on a high-Mn diet were offered 40 g of the diet mixed with 5 ml of a solution containing 2μc ^{54}Mn as the chloride. When this had been consumed, in about 3 h, the diet without radioactive Mn was offered. Droppings were collected, dried and milled for the 1st, 2nd, 3rd, 5th and 7th 24 h periods after dosing. Samples of 1 g were taken for counting in a large well-type sodium iodide scintillation counter: the volume of standard solution counted was similar to that occupied in the counting tube by 1 g of the dried droppings.

The basal diet used in this and subsequent experiments contained (%): maize meal 66, barley meal 10, white fish meal 6, dried separated milk 6, dried brewer's yeast 6, calcium carbonate 3, tricalcium phosphate 2·5 and salt plus vitamins 0·5. The Mn content was 6μg/g (low-Mn diet), and a supplement to provide a further 50μg/g (high-Mn diet) was added as required.

Expt 2. Five RIR × LS laying pullets aged about 8 months were fed during the following 2½ months on the low-Mn diet, and five similar birds were fed on the high-Mn diet. Then all birds were dosed with ^{54}Mn as the chloride in solution mixed with the food; the diet of low Mn content and without the calcium carbonate and tricalcium phosphate supplements was used for both groups. Schaible, Bandemer & Davidson (1938) showed that bone meal and possibly limestone reduce the availability of Mn. The dose for each bird, 7·7μc in 5 ml solution, was distributed among

ten gelatin capsules that between them contained 10 g of the food. Before dosing, food had been withheld overnight and was offered again 2 h after dosing. Droppings were collected for two 24 h periods after dosing, and at 48 h the birds were killed. The gut contents were washed out and retained along with the two collections of droppings for determination of radioactivity, made as in Expt 1.

Expt 3. Sixteen RIR × LS $4\frac{1}{2}$-month-old pullets that were due to lay in about 4 weeks were divided into two groups, one (treatments 1 and 2) being fed on the low-Mn diet, and the other (treatments 3 and 4) on the high-Mn diet. When the birds had been in lay for about 2 months they were each dosed with 100μc ^{52}Mn as the chloride in 5 ml solution. Half the birds on each feeding treatment were given the solution without added inactive Mn (total 24μg—treatments 1 and 3), and half the solution with 1000μg added inactive Mn (total 1024μg—treatments 2 and 4). There were therefore four treatment groups, each of four birds:

(1)	Previously given	low-Mn diet	dose with	low content of Mn
(2)		low-Mn diet		high content of Mn
(3)		high-Mn diet		low content of Mn
(4)		high-Mn diet		high content of Mn

The 5 ml dose solution was administered to each bird in ten gelatin capsules containing the diet of low Mn content without the supplements of calcium carbonate and tricalcium phosphate, as in Expt 2. Food was withheld for 3 h before and 3 h after dosing.

Blood samples were taken at intervals between dosing and killing, 2 ml being used for the determination of radioactivity. Between four and six samples were taken from each bird; the time varied among birds to cover the whole period from 0 to 48 h, though most were taken during the first 24 h, when the peak of activity occurred. To facilitate sampling, birds were dosed at different times of the day but always in fours, one bird from each treatment.

Droppings were collected for 24 h periods and, after the birds had been killed, gut contents and livers were retained. After drying, and fat extraction of livers, the samples were ground and counted as in previous experiments. Half the birds on each treatment were killed at 24 h after dosing and the remainder at 48 h.

A further sixteen pullets at about the same stage of maturity, but of the reverse cross LS × RIR, were treated in exactly the same manner as that described above. No significant differences were found between results obtained from the two parts of the experiment; thus the results are presented together as those of a single experiment with a total of thirty-two birds, eight on each treatment.

The isotope ^{52}Mn was used in Expt 3 because it was evident from earlier observations that, if reliable counts of blood were to be obtained, the dose of radioactive Mn had to be increased to about 100μc ($\simeq 20 \times 10^6$ counts/min) and it was less expensive and less hazardous to give this dose of ^{52}Mn than of ^{54}Mn.

RESULTS

Droppings and gut contents

Proportions (mean values with their standard errors) of the dose of radioactive Mn found in 24 h collections of droppings up to 7 days after dosing (Expt 1) were:

Day 1	Day 2	Day 3	Day 5	Day 7
91·2 ± 2·1	2·1 ± 0·2	0·68 ± 0·14	0·18 ± 0·11	0·05 ± 0·03

Just over 90 % of the dose appeared in droppings voided during the first 24 h, and by the 7th day very little radioactivity was being excreted, 0·05 % of the dose being voided in the 7th 24 h period.

The recovery of radioactive Mn in the droppings and gut contents of the birds of Expts 2 and 3 is given, as percentages of the dose, in Table 1. In all birds, a very large proportion of the dose was found in the droppings and gut contents, the mean value for droppings of the first 24 h period being 87 % and the total for droppings and gut contents to 48 h, 92 %. Thus retention of radioactive Mn given as the chloride in solution mixed with the food was low, 8 % in the 48 h period of this experiment. There were no significant effects of treatment on retention: thus, if a shortage of dietary Mn for about $2\frac{1}{2}$ months or increasing the Mn content of the dose solution from 24 to 1024 μg affected retention, the effects were small and were not detected by this 'difference method'.

Table 1. *Expts 2 and 3. Radioactive manganese, as a percentage of the dose, in droppings and gut contents of pullets given different Mn intakes before or with an oral dose of the isotope*

(Mean values with their standard errors)

Expt no.	Treatment	No. of birds	Droppings collected during 0–24 h	Droppings collected during 24–48 h and gut contents	Total
2	Previous diet of low Mn content	5	89·8 ± 2·2	4·6 ± 0·6	94·4 ± 1·8
	Previous diet of high Mn content	5	88·1 ± 3·3	7·2 ± 2·5	95·3 ± 1·5
3	Previous diet of low Mn content, dose of low Mn content	8	83·6 ± 4·7	8·4 ± 3·1*	92·0 ± 5·8
	Previous diet of low Mn content, dose of high Mn content	8	83·8 ± 5·6	4·3 ± 1·4*	88·2 ± 5·8
	Previous diet of high Mn content, dose of low Mn content	8	92·7 ± 2·8	2·4 ± 0·2*	95·1 ± 3·2
	Previous diet of high Mn content, dose of high Mn content	8	84·3 ± 3·0	2·4 ± 0·3*	86·7 ± 3·3

* Half the birds of each group were killed at 24 h; for them the value included in the mean is for gut contents only.

Liver

There was no significant difference between liver values for birds killed 24 h after dosing and those for birds killed at 48 h (Expt 3); therefore the results were analysed as a single set.

Treatment means for the ^{52}Mn content of liver are given in Table 2, together with

Table 2. *Expt* 3. *Effect of intake of inactive manganese by pullets on the mean concentration of* ^{52}Mn *in the liver after an oral dose of the isotope, calculated to a dose of* 10×10^6 *counts/min*

	Ll	Lh	Hl	Hh	SE of a treatment mean	Main effect of diet: Change	Main effect of diet: Significance *P*	Main effect of dose: Change	Main effect of dose: Significance *P*
Counts/min per g dry, fat-free tissue	9887	5884	3556	2365	—	—	—	—	—
Log_e counts/min per g dry, fat-free tissue	8·96	8·54	8·05	7·66	±0·213	−0·90±0·213	< 0·001	−0·40±0·213	NS
Mean total counts as:									
% of dose	0·965	0·743	0·438	0·255	—	—	—	—	—
Log_e % of dose	0·26	−0·47	−1·03	−1·44	±0·216	−0·87±0·216	< 0·001	−0·31±0·216	NS

L, previous diet of low Mn content; l, dose of low Mn content; H, previous diet of high Mn content; h, dose of high Mn content; NS, not significant.

Table 3. *Expt* 3. *Effect of intake of inactive manganese by pullets on the mean concentration of* ^{52}Mn *in the blood after an oral dose of the isotope, calculated to a dose of* 10×10^6 *counts/min*

	Ll	Lh	Hl	Hh	SE of a treatment mean	Main effect of diet: Change	Main effect of diet: Significance *P*	Main effect of dose: Change	Main effect of dose: Significance *P*
Mean maximum values:									
Counts/min per 2 ml blood	240	172	152	84	±52·2	—	—	—	—
Log_e counts/min per 2 ml blood	5·10	5·03	4·72	4·11	±0·291	−0·65±0·291	< 0·05	−0·34±0·291	NS
Mean terminal values:									
Counts/min per 2 ml blood	102	89	71	31	±18·6	—	—	—	—
Log_e counts/min per 2 ml blood	4·38	4·40	4·01	3·29	±0·226	−0·74±0·226	< 0·01	−0·35±0·226	NS

L, previous diet of low Mn content; l, dose of low Mn content; H, previous diet of high Mn content; h, dose of high Mn content; NS, not significant.

the results of statistical analysis of the natural logarithms of these. The distribution of values determined the use of logarithms rather than the original values. The proportion of the dose retained in the liver was very low, the means for all treatments being less than 1 %. The percentage in livers of birds fed, before dosing, on the low-Mn diet, 0·85, was significantly greater than the corresponding value for birds fed on the high-Mn diet, 0·35 ($P < 0{\cdot}001$), but the difference in liver retention caused by varying the Mn content of the dose did not reach significance. Radioactivity calculated as a concentration in the tissue gave the same treatment effects, and there was no interaction between treatments.

Blood

Preliminary observations on birds given oral doses of ^{54}Mn showed that a large dose, greater than 2×10^6 counts/min, had to be administered to obtain measurable counts in samples of blood that were sufficiently small to be taken fairly often. In Expt 3 the dose was increased tenfold over the previous maximum used, to about 20×10^6 counts/min, and even this dose gave maximum counts for a few birds of only about 100 per min per 2 ml.

A large range of values was found among birds on a given treatment (cf. SE of treatment means in Table 3), but for individual birds values fell on a fairly smooth curve with maximums mostly at 6–8 h after dosing. From 24 h after dosing to 48 h there was only a small decrease in concentration.

Maximum and terminal concentrations were analysed for treatment effects and the results are given in Table 3. As with the liver, the distribution of values determined the use of logarithms rather than the original values. Values for all birds were analysed together, there being no significant difference between the results for birds killed 24 h and for those killed 48 h after dosing.

The mean maximum concentration was significantly greater ($P < 0{\cdot}05$) in birds fed on the low-Mn diet before dosing than in those fed on the high-Mn diet, and a similar difference, but with $P < 0{\cdot}01$, was found between corresponding mean terminal concentrations. Differences caused by varying the Mn content of the dose did not reach significance and there were no interactions between treatments.

DISCUSSION

The retention of an oral dose of radioactive Mn in the laying pullet was very low, and of the same order as that found in the chick by Mohamed & Greenberg (1943). The mean value for the pullet of 8 % retained in a 48 h period is likely to be in excess of the real value owing to the difficulties of complete collection of the excreta. As noted earlier, the difference method lacks sensitivity when the overall value is low, and the absence of significant treatment effects was not surprising. Although retention was affected by the treatment as judged by the quantities of ^{52}Mn in the liver and blood, the outstanding feature was the low retention of the element, no matter how it was measured, in laying birds that had been fed on a low-Mn diet for about $2\frac{1}{2}$ months. From observations (unpublished) on the egg-shells of these birds it was clear that, in some at least, there was a shortage of Mn. It appears therefore that, even when the

tissues are in need of Mn and the element is given in circumstances that can be expected to favour absorption, only a small proportion, less than 10% of the intake, is retained.

The absence of a difference of retention by birds given a fixed amount of ^{52}Mn but different amounts of inactive Mn is also noteworthy for it suggests that, although the percentage retention of a given intake is very low, quite large amounts can be retained if the intake is high. It is difficult to envisage the physiological processes underlying these results, and further observations are being made.

Though no direct measurements of absorption were made in the experiments described here, the low activity appearing in blood taken shortly after the isotope was given suggests that the absorption of Mn in the pullet was much lower than that of Ca Fe or Co in the chick (Migicovsky & Nielson, 1951; Migicovsky & Jamieson, 1955: Masuhara & Migicovsky, 1963). On the same basis it seems that absorption was modified by the level of dietary Mn given for $2\frac{1}{2}$ months before dosing but not by the Mn content of the dose solution.

SUMMARY

1. Droppings collected from pullets fed on a high-manganese diet contained 91·2% of an oral dose of ^{54}Mn in the first 24 h and very small amounts in subsequent 24 h collections.

2. The mean percentage recovery of radioactive Mn in the droppings and gut contents of laying pullets that had been fed on low- or high-Mn diets, were given a dose of radioactive manganese in solution with small or large amounts of inactive Mn and were killed 24 or 48 h after dosing was 92%. There were no differences among treatments.

3. The mean ^{52}Mn content of the liver was 0·60% of the dose. There was significantly more in the livers of birds fed on the low-Mn diet (0·85%) than in those of birds fed on the high-Mn diet (0·35%), but the difference caused by varying the concentration of Mn in the dose solution was not significant.

4. The concentration of radioactive Mn in the blood, though always low relative to the dose, reached a maximum in most birds 6–8 h after the dose. Treatment effects were similar to those for the liver.

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