Manganese in the nutrition and metabolism of the pullet

2.* The manganese contents of the tissues of pullets given diets of high or low manganese content

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1. Groups of pullets were given a diet of high (106–107 μg/g) or low (6–7 μg/g) manganese content and killed either before sexual maturity, at the point-of-lay or after a 6- to 7-month laying period. The birds were dissected into six tissue fractions: skeleton, liver, kidney, ovary and oviduct, skin and feathers, and muscle with remaining tissue. Total Mn and concentration of Mn as μg/g dry fat-free tissue were determined for each fraction.

2. There were no differences in live weight attributable to level of dietary Mn, and no differences in egg production.

3. Mean total body Mn varied among groups over a fairly narrow range (528–738 μg), with the exception of birds given the high-Mn diet throughout the experiment, in which the mean was 2319 μg. This represented an increase in Mn content during egg laying of 244 %. There was no significant difference in the Mn content of birds given the low-Mn diet whether they were killed at the point-of-lay or after the laying period.

4. The effects of treatment on the weight of Mn in each of the tissue fractions are described. The very large increase in total Mn that occurred during egg production in birds given the high-Mn diet was accounted for largely by the increase in skin and feathers (1072 μg Mn).

5. In general terms, the Mn content of liver, kidney and ovary and oviduct together constituted only just over 10 % of total body Mn, the remainder being distributed about equally among skeleton, skin and feathers, and muscle with remaining tissue.

6. There was a close parallel between the concentration of Mn of a tissue and the total weight of Mn it contained except in certain instances when stage of maturity or egg production influenced weight of the tissue.

7. The effects of treatments on the Mn contents of these birds are discussed in relation to the retention of dietary Mn, and the withdrawal from and accumulation of Mn in individual tissues and the whole body.

It has been suggested that a high proportion of total body manganese is present in the skeleton and that this constitutes a substantial store of the element (Underwood, 1962), but no information is available from which the proportion in the skeleton could be calculated, nor to show that skeletal Mn can be withdrawn at times of shortage. That the liver may be an important storage site for Mn was indicated by the report of Bolton (1955), who found that the Mn content of the livers of laying birds was greater than that of non-laying birds and that a concentration similar to that in laying birds occurred in immature pullets after the administration of sex hormones.

The object of the experiment reported here was to obtain information on the accumulation in and withdrawal of Mn from certain tissues and whole body of pullets by analysing the tissues of birds killed at different stages of egg production and given a diet of high or low Mn content.

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EXPERIMENTAL

Forty-six light hybrid pullets were reared on a commercial growers diet, and then divided randomly into six groups at 18 weeks of age. Seven birds were killed at 18 weeks of age (group I), seventeen were given the high-Mn diet, ten being killed at the point-of-lay (group H) and seven after 6–7 months of egg production (group HH). Fifteen birds were given the low-Mn diet, eight being killed at the point-of-lay (group L) and seven after 6–7 months of egg production (group LL). A further seven birds were given the high-Mn diet to point-of-lay followed by the low-Mn diet during the same period of egg production (group HL). Groups HH, LL and HL were the birds described in Expt 3 of Hill & Mathers (1968).

The basal low-Mn diet described earlier (Hill, 1965*b*) contained 6–7 μg Mn/g, and the high-Mn diet was prepared by adding manganese carbonate to provide a further 100 μg Mn/g.

The tissues of the birds were separated for analysis into six fractions: skeleton, liver, kidney, ovary and oviduct, skin and feathers, and muscle with remaining tissue. The tissue, or a known proportion of it, was dried and extracted with light petroleum (b.p. 40–60°). The dry fat-free tissues were ground and ashed, or a sample was taken for ashing. The desirability of washing the skin and feathers to remove possible surface contamination was considered but rejected; the matter is discussed later. Mn was determined in the ash by the standard permanganate method (Association of Official Agricultural Chemists, 1945). The total Mn content of each tissue, of the whole bird, and the concentration of Mn in the dry fat-free tissues were calculated.

RESULTS

Live weights and egg production

These values are given in Table 1. The birds increased in live weight during the 7 weeks before egg laying began and during the subsequent 7 months, but there were no consistent effects of level of dietary Mn on live weight.

All the groups of birds that laid for 6–7 months (HH, LL and HL) produced at a high level, and dietary treatment had no significant effect on egg production.

Total Mn content of tissues

Mean values for individual tissues, as well as for the whole body, are given in Table 2.

Total body Mn was less than 1 mg in all groups except HH. The sexually immature pullets (group I) contained 738 μg Mn: they were killed about 7 weeks before egg laying began in the remainder of the birds. Birds given the high-Mn diet to the point-of-lay (group H) contained a slightly, but not significantly, smaller weight of Mn (677 μg), than those of group I ($P > 0.05$). Birds given the low-Mn diet to the point-of-lay (group L) contained significantly less Mn (527 μg) than those of group I ($P < 0.01$). Values for groups H and L represent decreases in Mn content during sexual maturation of 8% and 29% respectively.

Birds that continued on the high-Mn diet during 6–7 months of egg laying (group HH) contained a very much larger amount of Mn (2319 μg) than corresponding birds killed at the point-of-lay (group H, 677 μg): the difference was highly significant statistically and represented an increase in Mn content during egg laying of 244%.

Birds that continued on the low-Mn diet for the egg-laying period (group LL) contained 552 μg Mn, slightly more than those on the same diet killed at the point-of-lay (group L, 527 μg), but the difference was not significant. The difference corresponds to an increase in Mn content of 5%.

Table 1. *Mean values for live weight and percentage egg production of the pullets*

Treatment group	Treatment	No. of birds	Live weight when killed (g)	No. of days in lay	Egg production (%)
I	Sexually immature birds killed at 18 weeks of age	7	1439	—	—
H	Birds killed at point-of-lay, after receiving the high-Mn diet from 18 weeks of age	10	1647	—	—
L	Birds killed at point-of-lay, after receiving the low-Mn diet from 18 weeks of age	8	1656	—	—
HH	Birds killed after 7 months of egg production having received the high-Mn diet from 18 weeks of age	7	1869	200	86
LL	Birds killed after 7 months of egg production having received the low-Mn diet from 18 weeks of age	7	1854	207	83
HL	Birds killed after 7 months of egg production having received the high-Mn diet from 18 weeks of age to point-of-lay and the low-Mn diet during 7 months of egg production	7	1943	202	83
	SE of difference between means		—	±4·61	±3·89

Changing the level of dietary Mn from high to low at the point-of-lay (group HL) gave a total body Mn value of 577 μg, not significantly lower than for corresponding birds killed at the point-of-lay (group H, 677 μg), but a difference that represents a decrease during egg laying of 15%.

The weight of Mn in the skeleton (Table 2) of birds given the high-Mn diet during egg laying (group HH) was very large (564 μg) and significantly greater ($P < 0{\cdot}001$) than weights of the remaining five groups (126–180 μg), among which there were no significant differences. There was a fairly close relationship between the Mn content of the skeleton and of the whole body, skeletal Mn constituting about 25% of the total for all treatments.

Total liver Mn contents in groups given the low-Mn diet (L, LL and HL) were similar (30·6, 40·5 and 35·6 μg respectively) and significantly lower than in the remaining three groups (I 81·5, H 70·1 and HH 70·7 μg). This distribution of values with treatment contrasts markedly with that for the skeleton; it is illustrated by the wider range of values for liver Mn expressed as a percentage of the total (3·1 for group HH to 11·1 for group I). The exceptionally low value for group HH (3·1%) was caused by the liver content remaining fairly constant while total body Mn increased greatly.

Table 2. *Mean weights (μg) of manganese in individual tissues of the pullets and the proportions they represent of the whole*

	Skeleton		Liver		Kidney		Ovary and oviduct		Skin and feathers		Muscles and remaining tissue		
Treatment*	Wt	% of total	Wt	% of total	Wt	% of total	Wt	% of total	Wt	% of total	Wt	% of total	Total wt in whole body
I	153	20·7	81·5	11·1	11·2	1·5	0·82	0·1	219	29·7	272	36·9	738
H	180	26·6	70·1	10·3	15·8	2·4	26·7	4·0	193	28·5	191	28·2	677
L	126	23·9	30·6	5·7	13·7	2·7	19·2	3·6	138	26·1	200	38·0	528
HH	564	24·3	70·7	3·1	26·0	1·1	62·5	2·7	1265	54·5	330	14·2	2319
LL	179	32·4	40·4	7·4	14·4	2·5	27·6	5·1	168	30·4	122	22·1	552
HL	172	29·8	35·6	6·2	15·1	2·6	26·3	4·5	159	27·6	169	29·3	577
SE of difference between means	±30·7	—	±7·66	—	±1·46	—	±5·70	—	±53·4	—	±31·9	—	±77·3

* See Table 1. † These are approximate values based on a mean of 7·7 birds/treatment.

Table 3. *Mean concentration (μg/g) of manganese in individual dry fat-free tissues of the pullets and the dry fat-free weights (g) of these tissues*

	Whole body		Skeleton		Skeletal ash		Liver		Kidney		Ovary and oviduct		Skin and feathers		Muscle and remaining tissue	
Treatment*	Concn	Wt	Concn	Wt	Concn	Wt	Concn	Wt	Concn	Wt	Concn	Wt	Concn	Wt	Concn	Wt
I	1·91	386·0	2·16	71·3	4·58	33·5	11·88	6·93	5·98	1·88	3·11	0·26	1·83	120·4	1·46	185·2
H	1·55	439·9	2·13	84·6	4·15	43·5	10·30	6·95	8·10	1·95	2·66	11·05	1·61	123·5	0·91	211·8
L	1·14	454·8	1·47	87·9	3·12	42·0	4·50	7·02	7·70	1·67	1·22	14·71	1·08	126·4	0·89	217·1
HH	5·45	425·8	7·83	73·5	12·72	45·4	9·28	7·90	10·56	2·49	3·18	19·80	11·43	111·1	1·48	211·1
LL	1·25	442·1	2·54	70·8	4·24	42·1	4·88	7·93	6·02	2·41	1·14	23·80	1·24	128·2	0·59	208·9
HL	1·24	467·4	2·47	65·8	4·24	40·8	4·74	7·44	6·21	2·44	1·20	22·40	1·21	128·2	0·70	241·2
SE of difference between means†	±0·125	±23·9	±0·458	±5·27	±0·743	±3·13	±0·890	±0·824	±0·424	±0·218	±0·315	±2·77	±0·356	±8·46	±0·118	±14·4

* See Table 1. † These are approximate values based on a mean of 7·7 birds/treatment.

The Mn content of kidney and ovary and oviduct varied with treatment somewhat similarly to that of the skeleton; values for group HH were considerably greater than for the other groups. The Mn content of skin and feathers also gave similar treatment differences to the skeleton but the difference between group HH and the remaining groups was much larger than for other tissues. The Mn content of the skin and feathers of group HH was six to nine times greater than that of other treatment groups, while for the skeleton, kidney, and ovary and oviduct the corresponding differences were only two- to three-fold. The very large skin and feather Mn content of group HH (1265 μg) represents an extremely high proportion (54·5%) of the total body Mn. The possibility that surface contamination contributed to this value is discussed later.

The Mn content of muscle and remaining tissues did not follow closely the pattern of treatment effects described for other tissues. Both groups killed at the point-of-lay (H and L) contained significantly less Mn than the initial group (I), and the two low-Mn groups killed at the end of the egg-laying period (LL and HL) contained slightly less than those killed at point-of-lay (H and L); also unlike other tissues except the liver, the value for muscle and remaining tissue of group HH (330 μg) was only moderately greater than those of other groups and not significantly different from that of group I (272 μg).

From values for individual tissues calculated as percentages of total body Mn, it is evident that fairly wide variations occurred between treatments in the proportion of the total contributed by a particular tissue, but in general it will be noted that Mn in liver, kidney, and ovary and oviduct constituted only just over 10% of the total and the remainder was found in approximately equal proportions in the skeleton, the skin and feathers, and in the muscle with remaining tissue.

Mn concentration

Mean concentrations as μg/g dry fat-free tissue are given in Table 3, together with weights of tissue and the standard errors of differences between two means. In general, the effects of treatment on the weights of individual tissues were small, and were related to stage of maturity or egg laying, not to level of dietary Mn. In consequence of the generally small effects on weight of tissue, differences in concentration of Mn between the treatment groups reflected fairly closely those described above for weight of Mn.

The mean concentration of Mn in dry fat-free tissue of the whole body varied between 1·14 and 1·91 μg/g for all groups except HH, which had a much higher value (5·45 μg/g). Among groups in the lower range of values, group I (1·91 μg/g) had a significantly greater concentration than the remainder.

The concentration in dry fat-free bone was greater than in the whole body, the difference varying from twice as great for groups LL and HL to only slightly greater for group I. The effects of treatment on skeletal Mn were in some instances greater in terms of concentration than in terms of weight, there being differences between treatments in weight of dry fat-free bone and in percentage of ash. A marked example of this effect is observed when values for group H are compared with those for HL: total Mn contents were similar, 180 and 172 μg respectively, but concentrations in dry fat-free bone differed significantly (2·13 and 2·47 μg/g respectively), while in

consequence of a substantial difference in percentage of ash (H 52% and HL 62%) concentrations in ash were similar (4·15 and 4·24 μg/g respectively).

In the liver, concentration of Mn was generally high (4·5–11·9 μg/g) in comparison with that of the whole body, and kidney Mn concentration was also high (6·0–10·6 μg/g). There were differences in the weight of dry fat-free kidneys, birds killed after 6–7 months of egg production (groups HH, LL and HL) having significantly heavier kidneys than those killed earlier (groups I, H and L), giving significantly different concentrations of Mn in some instances where none existed for weight. A striking example of this effect occurred between groups L and LL: weights of Mn were similar (13·7 and 14·4 μg), but weights of tissue, 1·67 and 2·41 g, and concentrations, 7·70 and 6·02 μg/g respectively, differed significantly.

The ovary and oviduct were not rich in Mn, the concentration being 1·1–3·2 μg/g. There was a tendency for the weight of dry fat-free tissue to be greater for low-Mn groups than for the corresponding high-Mn groups, giving a greater difference between H and L groups for concentration than for weight of Mn.

The concentration of Mn in skin and feathers was low, 1·1–1·8 μg/g for all groups except HH, for which the value was remarkably high, 11·4 μg/g. In muscle and remaining tissue Mn concentration was low for all groups, 0·6–1·5 μg/g. In this tissue, as with liver, and ovary and oviduct, but unlike bone, kidney, and skin and feathers, the concentration of Mn for birds given the high-Mn diet throughout the experiment (group HH, 1·48 μg/g) was no greater than for birds killed at the start of the experiment (group I, 1·46 μg/g).

DISCUSSION

At the outset it is necessary to appreciate the problem alluded to earlier, namely the possibility that the values found for the Mn content of skin and feathers may be in error owing to surface contamination, by food in particular. The value most suspect is for group HH, 11·43 μg/g dry fat-free tissue, that was very high by comparison with values of 1·08–1·83 μg/g for the remaining five groups. The HH birds had received the high-Mn diet longer (about 250 days) than any other birds given the same diet (about 50 days) and therefore the opportunity for contamination was greatest. However if this were an explanation of the high concentration for these birds some tendency for contamination with the high-Mn diet might have been expected in the skin and feathers of group H birds given this diet for about 50 days before being killed at point-of-lay, but their mean value (1·61 μg/g) represented a decrease in concentration, the initial group (I) having 1·83 μg/g.

From two birds, not part of this experiment, that had been laying for about 6 months on a diet similar to the high-Mn diet used here (100 μg added Mn/g) the skin and feathers of each bird were halved along the mid-line and one half washed five times with water and the other half left unwashed. The washed samples contained 2·72 and 3·27 μg/g dry fat-free tissue, considerably less than the unwashed samples 10·57 and 6·92 μg/g, and suggested that the high values were given largely by surface contamination, but it is known that Mn does not form stable bonds with organic compounds (Chemical Society, 1957; Hill, 1965*a*) and it is possible that some at least of the Mn

removed by washing could be properly regarded as part of the tissue. It was decided to analyse unwashed samples in the present study, since there was no way of determining whether washing gave a more or a less accurate assessment of Mn content. It may be noted that when the balance method is used to determine retention any contamination of skin and feathers that occurs from the feed is recorded as part of the retained fraction. Two further points may be mentioned that support the validity of the high values obtained for group HH. First, for these to arise from feed contamination would require just over 10 g of the feed to be adhering to the skin and feathers, whereas no gross contamination of this order was observed, and secondly, individual values for that group were fairly uniform (10·08–13·30 μg/g) whereas a larger range might be expected if marked contamination had occurred.

The results obtained with this comparative slaughter method can be discussed in terms of changes in composition, or of gains or losses of Mn, on the assumption that birds killed at one stage were representative of the birds on the same dietary treatment killed later.

An important premise underlying this experiment was that, when egg laying begins in birds given a high-Mn diet, the tissues contain a large potential reserve of Mn for use during egg production when the diet has a low Mn content. It is difficult to know what quantity of Mn would constitute a 'large reserve', but at the point-of-lay in birds given the high-Mn diet the total body Mn content was surprisingly small—677 μg. If the Mn content of an egg is taken as 10 μg (Hill & Mathers, 1968), this amount represents about seventy eggs, but when similar birds were changed to a low-Mn diet at the point-of-lay they contained 577 μg after the production of 170 eggs, a loss of only 100 μg Mn (the Mn content of about ten eggs). This suggests either that the low-Mn diet was able to supply almost sufficient Mn for egg production (1700 μg: 1600 μg from the diet and 100 μg from the tissues), or that only 100 μg could be withdrawn without seriously disturbing metabolism and that a smaller amount than 10 μg reached each egg. This second possibility is supported by results for the other two low-Mn groups: they both contained similar amounts of Mn (528 and 552 μg) to that given above, though one was for birds given the low-Mn diet for only 50 days before the point-of-lay, and the other for birds given the low-Mn diet for the whole experiment (50 days before laying and a 200-day laying period). These considerations suggest that at the point-of-lay the potential reserve of Mn in the bird is fairly small, probably the total Mn content of about ten eggs. In this experiment a low-Mn diet was imposed only at the point-of-lay or a few weeks before. Had a change from high- to low-Mn diet been made after a period of 6–7 months egg production, the picture might have been quite different, since the birds accumulated a very large amount of Mn during such a period of egg production (1642 μg).

The decrease in Mn content (100 μg) that occurred during egg laying in birds given the high-Mn diet to the point-of-lay and the low-Mn diet during egg laying was largely from the liver (one-third) and skin and feathers (one-third), and most of the rest from the muscle and remaining tissue. Only 8 μg of the 100 μg was contributed by the skeleton, an unexpectedly small proportion in view of the deduction made by Underwood (1962) that skeletal Mn represents a substantial store of the element. For

all tissues other than liver, there was an accumulation of Mn during egg laying in birds given the high-Mn diet. In some tissues this increase was large (skin and feathers (1072 μg) and skeleton (384 μg)), and had these birds been subsequently given the low-Mn diet for a period of egg production some of this Mn may have become available. The liver was exceptional in that no accumulation occurred during egg laying on the high-Mn diet, and the maximum amount available from this organ was about 35 μg.

The total content of Mn in the liver was of the same order as that found by Bolton (1955), but this author found a lower value for non-laying than for laying and oestrogen-treated immature birds, whereas the livers of the immature birds in the present experiment contained more Mn than those of any of the sexually mature groups.

A consideration of changes in total body Mn provides evidence of greater retention of dietary Mn during egg laying than before the point-of-lay. In birds given the high-Mn diet an increase of 1642 μg Mn occurred during the 200-day laying period, and 170 eggs were laid representing a similar weight of Mn (170×10 μg), giving a total retention of about 3200 μg. At this rate, 800 μg would be retained in 50 days, but birds given the same diet for 50 days before egg laying gained no Mn: indeed a small but non-significant loss occurred (61 μg). Values for birds given the low-Mn diet provide similar evidence for a greater retention during egg laying than before sexual maturity, and the increase in Mn content of eggs observed in a previous experiment during a 2-month laying period indicated that retention increased as the laying period advanced (Hill & Mathers, 1968).

As estimated above, birds in this experiment given the high-Mn diet retained about 16 μg Mn/day during egg production. If a higher value is taken for the Mn content of an egg (Underwood, 1962) this may be increased to 25 μg, and if the daily feed intake be taken as 100 g (unpublished findings) 25 μg represents about 0·25% of intake. In an earlier short-term experiment in which radioactive Mn was administered to birds that had been given a high-Mn diet, 0·35% of a dose was found in the liver (Hill 1965): this represents about 1% in the whole bird (Mathers & Hill, 1967). These estimates made by different methods do not tally exactly, but they are fairly close by comparison with the 32% (1600 μg/day) retention obtained from a balance experiment by Brown & McCracken (1965). No explanation of this apparent discrepancy can be given, though the difficulties involved in the balance method for mineral studies discussed by Duncan (1967) may be relevant.

From observations described earlier on the shells of eggs produced by birds in the present experiment (Hill & Mathers, 1968), and the results for Mn given here, it appears that, although at the start of egg laying shell thickness was related to tissue Mn content, this was not so after 6 months of egg laying. The first shells produced by high-Mn and low-Mn groups had thicknesses of 77·6 and 70·0 mg/cm^2 respectively, and corresponding concentrations of Mn in ovary and oviduct were 2·66 and 1·22 μg/g, while shells produced after 6 months had thicknesses of 73·3 in group HH, 68·0 in group LL and 74·8 in group HL, and corresponding Mn concentrations were 3·18, 1·14 and 1·20 μg/g respectively. Further, on considering changes in shell thickness and tissue Mn content for birds given the high-Mn diet throughout the experiment, shell thickness tended to decrease with time and tissue Mn to increase.

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