Compared with the total, the amount of manganese (if any) which is attached to water-soluble phosphatases is negligibly small.

SUMMARY

1. Spectrographic studies and determinations based on periodate oxidations show that the manganese content of ox femur is of the order 0.27–0.51 p.p.m. depending on the type of bone, compact

or cancellous. Irrespective of the type this corresponds to about 1.4 p.p.m. expressed on the ash.

2. Most of the manganese is associated with the inorganic matrix, but a small proportion is bound to the organic matrix.

3. Little manganese, if any, accompanies the water-soluble alkaline phosphatase.

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Manganese in Rabbit Tissues

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Although many papers have appeared on the manganese contents of the tissues of various animals, in no case has a survey been recorded of the concentrations in all the tissues of a single animal.

Such a survey might provide a better clue to function than comparisons of the manganese contents of tissues from different individuals or species.

EXPERIMENTAL

Animals and materials

The rabbit was chosen for this investigation because it is of a convenient size for obtaining reasonable amounts of small bodies such as the pituitary and pineal glands. Four animals were used; samples of most tissues were obtained from an adult female chinchilla and of the other tissues from the remaining three. Specimens of incompletely separated adrenal cortex and medulla, thymus and thyroid were obtained from a resting adult female chinchilla; specimens of mammary gland, thyroid and lung were obtained from a lactating female albino, and samples of pancreas, thyroid, testes and spinal cord from an adult male chinchilla. Liver samples from each animal were used as controls. The pineal and pituitary glands from the last two animals were examined. The separation of the duodenal mucosa and muscular tissue was only approximate. All dissections were made with carefully cleaned stainless-steel instruments.

Using portions of kidney and liver from the first rabbit, homogenates were prepared and dialysed in cellophan bags against changing glass-distilled water for 10 days, toluene being added as preservative. The residue in each bag was divided into two portions and the Mn content of each determined.

Method of analysis

Mn was determined by a modification (Fore & Morton, 1952*a*) of a method described by Kun (1947) based on the catalytic oxidation of diethylaniline by Mn^{++} and periodate to give a coloured product absorbing at 470 m μ . The experimental error is from ± 10 to $\pm 20\%$ depending on the size of the sample.

RESULTS

The findings on rabbit tissues are recorded in Table 1. The manganese contents based on fresh tissue weight are listed in decreasing order in Table 2. For comparison, (a) the values reported in the literature for each rabbit tissue have been averaged, and (b) the values reported for each type of tissue irrespective of species have been averaged. The published figures for the rabbit were obtained from papers by Bertrand & Medigreceanu (1912*a, b*; 1913), Ellis, Smith & Gates (1947), Lorenzen & Smith (1947), Lund, Drinker & Shaw (1921), Ray (1938) and Richards (1930). The figures in the right-hand column of Table 2 are averages obtained by attaching the same weight to all the published figures which can be traced. The number of papers consulted is about thirty-five and a full list of references would take up too much space.

The results of the dialyses are given in Table 3, and if the figures 2.0 and 1.2 p.p.m. are accepted for the manganese contents of the liver and kidney homogenates, respectively, it appears that in the liver 74% of the manganese, 72% of the dry matter

Table 1. *Experimental data on rabbit tissues*

Tissue	Fresh tissue wt. (g.)	Dry matter (%)	Ash as		Manganese content in p.p.m. in		
			% of fresh tissue	% of dry tissue	Fresh tissue	Dried tissue	Ash
Pituitary	0.0078	12	—	—	3.9	34	—
Pituitary	0.0071	14	—	—	1.4	9.7	—
Pituitary	0.0063	24	—	—	1.8	7.6	—
Grey hair	0.713	90	1.5	1.6	0.99	1.1	67
White hair	0.816	87	0.56	0.65	0.98	1.1	170
Hide	1.32	46	0.72	1.6	0.38	0.82	53
Fascia	4.97	23	1.1	4.7	0.024	0.10	2.2
Spleen	1.51	22	1.0	4.5	0.22	0.97	21
Bone marrow	0.640	53	0.99	1.8	0.045	0.087	4.7
Ileum	0.723	18	1.1	6.0	1.7	9.6	160
Kidney (one)	7.11	26	1.3	4.9	1.2	4.7	97
Duodenum	1.41	20	1.2	6.1	1.1	5.6	92
Mucosa	1.03	18	1.2	6.8	1.6	8.6	130
Muscularis	2.22	17	0.91	5.5	0.50	3.1	55
Salivary gland	1.42	22	1.6	7.1	1.4	6.2	88
Stomach	1.75	23	1.1	4.7	1.0	4.4	95
Caecum	1.08	17	0.79	4.7	0.82	4.9	100
Bile	1.61	20	1.7	8.5	0.48	2.4	28
Heart	1.06	22	1.0	4.5	0.28	1.3	28
Liver	3.84	30	1.6	5.3	2.0	6.7	130
Liver	3.03	29	1.5	5.3	2.2	7.6	140
Liver	2.79	24	1.2	4.9	2.0	8.4	170
Liver	3.29	25	1.2	4.9	2.2	8.7	180
Pancreas	0.225	74	1.1	1.5	1.6	2.1	140
Pancreas	0.297	26	1.5	5.8	1.6	6.2	110
Adrenals (two)	0.342	49	1.5	3.1	0.67	1.3	44
Cortex	0.290	42	1.1	2.7	0.56	1.3	50
Medulla	0.027	39	—	—	0.60	1.5	—
Ovaries (two)	0.684	23	1.5	6.3	0.60	2.6	41
Mammary gland	1.88	62	0.31	0.50	0.25	0.41	82
Mammary gland (lactating)	0.738	45	1.2	2.7	2.2	4.8	180
Brain	2.75	22	1.5	6.9	0.36	1.6	24
Muscle	2.07	23	1.2	5.0	0.13	0.56	11
Pineal	0.0061	15	—	—	3.8	26	—
Pineal	0.0018	—	—	—	0.83	3.3	—
Gall bladder	0.431	27	1.7	6.6	0.91	3.4	53
Nerve	0.178	41	1.4	3.5	0.086	0.21	6.1
Thyroid	0.0798	34	1.3	3.7	0.29	0.84	23
Thyroid	0.122	33	0.25	0.75	0.21	0.64	85
Thyroid	0.112	26	1.3	4.7	0.21	0.79	17
Testes (two)	0.0421	32	—	—	0.36	1.2	—
Spinal cord	0.393	31	1.7	5.5	0.39	1.3	23
Lung	0.868	18	0.99	5.4	0.011	0.061	1.1
Thymus	0.018	67	—	—	0.45	0.67	—

and 92 % of the ash was dialysable. In the kidney 41 % of the manganese, 49 % of the dry matter and 88 % of the ash was dialysable.

DISCUSSION

Although simple distribution studies of elements or compounds have in some instances provided valuable clues to specialized function the figures given in Table 2 do not indicate a localized role. The ubiquitous occurrence of manganese in the animal body suggests a role in general cell metabolism as opposed to metabolic processes characteristic of particular tissues. Such a role for manganese would not of course exclude other roles restricted to those tissues with a specially higher manganese content.

Dialysis of kidney and liver homogenates showed that some of the manganese is loosely held and some of it very firmly combined. If it is assumed that in all tissues having somewhat higher manganese contents, a portion of the manganese is dialysable, then the already narrow range for total manganese (Table 2) becomes even narrower for the 'bound' manganese. The small range of manganese concentrations is consistent with a general metabolic role. The fact that little or no dialysable manganese is present in ox retinas which contain only 0.13 p.p.m. of manganese (see Fore & Morton, 1952c) supports the idea that it is only those tissues relatively rich in manganese which contain the element in dialysable form.

Indications have been obtained in other work (Fore & Morton, 1952b) that some of the manganese in bone and bone marrow is attached to the cell membranes. It is therefore interesting to consider the average proportion of manganese to protein in the tissues. If 0.5 p.p.m. (Table 2) is taken as the approximate tissue content of non-dialysable

protein would need to have a molecular weight of the order 10^6 . This figure, though large, is not impossible so since, for example, the muscle protein myosin has a molecular weight of that order. The concept of manganese attached to the cell membrane is not entirely new. Albert, Rubbo, Goldacre & Balfour (1947) suggested that oxine inhibited bacterial

Table 2. *Rabbit tissues arranged in order of decreasing manganese content*

Tissue	Mn content in fresh rabbit tissue (p.p.m.)	Averages of figures recorded in the literature for rabbit	Average of figures recorded in the literature for a wide range of species	Tissue	Mn content in fresh rabbit tissue (p.p.m.)	Averages of figures recorded in the literature for rabbit	Average of figures recorded in the literature for a wide range of species
Bone (long)	—	3.5	3.3	Adrenal medulla	0.60	—	—
Pituitary	2.4	—	2.5	Adrenal cortex	0.56	—	—
Pineal	2.3	—	—	Duodenal muscularis	0.50	—	—
Mammary gland (lactating)	2.2	—	0.26	Bile	0.48	—	0.90
Liver	2.1	1.9	2.5	Thymus	0.45	—	1.6
Pleum	1.7	—	—	Spinal cord	0.39	—	0.60
Duodenal mucosa	1.6	—	—	Hide	0.38	0.20	0.40
Pancreas	1.6	2.3	1.9	Intestine	—	0.38	0.45
Salivary gland (submaxillary)	1.4	—	1.1	Testes	0.36	0.40	0.50
Kidney	1.2	1.1	1.2	Brain	0.36	0.60	0.40
Duodenum	1.1	—	1.9	Uterus	—	0.35	0.33
Stomach	1.0	0.90	0.80	Heart	0.28	0.21	0.34
Colon	—	1.0	0.75	Mammary gland (resting)	0.25	—	0.14
Hair	0.99	1.2	2.4	Thyroid	0.24	—	0.55
Gall bladder	0.91	1.0	1.1	Spleen	0.22	0.55	0.40
Caecum	0.82	0.43	0.43	Muscle	0.13	0.14	0.18
Salivary gland (parotid)	—	—	0.70	Milk	—	—	0.045
Intestine (small)	—	0.70	0.45	Bone marrow	0.045	—	<0.050
Adrenals	0.67	—	0.40	Blood	—	0.03	0.040
Lymph nodes	—	—	0.65	Fascia	0.024	—	—
Ovaries	0.60	—	0.55	Lung	0.011	0.47	0.20
				Urine	—	0.00	0.004
				Parathyroid	—	—	—

Table 3. *Retention of manganese after dialysis of rabbit liver and kidney homogenates*

Wt. of tissue homogenate (g.)	Dialysis residue		Ash on dry matter (%)	Mn from sample (μmg.)	Mn (p.p.m.) on	
	Dry wt. (g.)	Ash wt. (mg.)			Dry wt.	Ash wt.
13.9 g. liver	0.593	8.6	1.5	4060	6.9	470
	0.583	9.6	1.6	3410	5.8	360
Totals	1.177	18.2	—	7470	—	—
Means	—	—	1.6	—	6.4	420
5.67 g. kidney	0.342	4.5	1.3	2270	6.6	510
	0.402	4.8	1.2	1750	4.4	360
Totals	0.744	9.3	—	4020	—	—
Means	—	—	1.3	—	5.5	440

manganese and 10% as an average for protein in tissue, then the proportion of manganese with respect to the protein is 5 p.p.m. There will thus be 55 parts of manganese to 11 million parts of protein and assuming one (or two) molecules of protein per atom of manganese a protein of very high molecular weight is implied. If the manganese is, however, preferentially attached to the cell-wall protein such

growth by combining with essential metals in the bacterial surface, and Gale (1949) has shown that oxine inhibits glutamic acid assimilation in bacteria by combination with a metal in the cell, probably manganese.

The relatively high manganese contents of liver and lactating mammary gland could be accounted for, at least in part, by a high arginase content, and

the somewhat high manganese content in the tissues of the intestinal tract might similarly be attributed to manganese present as part of the peptidase system.

SUMMARY

1. The manganese contents of rabbit tissues have been determined, and the results compared with those obtained by previous workers.

2. The range covered by the results is narrow,

especially as some of the tissues relatively rich in manganese contain much of it in dialysable form.

3. It is suggested that manganese participates in general cell metabolism while attached to the cell-wall protein.

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Manganese in Eye Tissues

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As part of a programme of research on the biochemistry of vision the manganese contents of the eye tissues of the ox have been determined. The only similar study appears to be that of Tauber & Krause (1943), who used an extension of the periodate method (Wiese & Johnson, 1938) on samples obtained by taking tissues from the eyes of a large number of oxen. The method used here is the modified catalytic method (Fore & Morton, 1952a); it is sufficiently sensitive to be applicable to the tissues from single eyes.

EXPERIMENTAL

Left and right eyes from each of two oxen were studied separately. After removing muscle, etc., attached to the sclera, each eye was dissected using carefully cleaned stainless-steel instruments. Separation of the tissues was complete except perhaps for the conjunctiva, and minute fragments of iris tissue were sometimes present in the sample of vitreous humour. The optic nerve was cut out of the sclera, leaving no nerve tissue in the sclera sample, from which choroid pigment was carefully removed. The cornea, lens, sclera, optic nerve and conjunctiva were washed with glass-distilled water and touched on filter paper (Whatman no. 50) before weighing. The retina, choroid and the vitreous and aqueous humours were placed directly into crucibles.

The iris, which adheres to the delicate membrane retaining the vitreous humour, was touched on filter paper to remove traces of vitreous humour.

In a later experiment a retinal homogenate was prepared from about forty ox retinas, toluene added and the brei dialysed against changing glass-distilled water. The Mn contents of samples removed from the cellophan bag at 70 and 180 hr. were determined by the modified catalytic method.

RESULTS

The results of the manganese determinations of the eye tissues are given in Tables 1 and 2. In Tables 3–5 the tissues are arranged in order of decreasing manganese content alongside the values obtained by Tauber & Krause, who did not examine the aqueous humour nor the contents on an ash-weight basis.

The results of the dialysis experiment are given in Table 6. The weight of the homogenate used for dialysis expressed as dry weight (*) was calculated using the percentage dry weight (not given) obtained for two samples of the homogenate. Then, from the manganese content of these samples and the calculated dry weight, the absolute amount of manganese in the homogenate used for the dialysis (†) was obtained.