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The Copper Content and Cytochrome Oxidase Activity of Tissues from Normal and Swayback Lambs

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Swayback is a non-febrile, ataxic disorder of newborn and young lambs. The pathological changes may include symmetrical cerebral demyelination, degeneration of tracts in the spinal cord and chromatolytic changes in the neurones of the brain-stem nuclei and ventral horn (Innes & Shearer, 1940). Both swayback and the related condition enzootic ataxia, which occurs in Australian lambs, are associated with a deficiency of copper. In Australia this deficiency is due to the low level of copper in the pasture (Bennetts & Beck, 1942). The 'swayback' pastures of this country contain adequate levels of copper (Allcroft, 1952), and it is thought that swayback is caused by a failure in the intermediary metabolism of copper in the ewe, or by the copper in the pasture being in an unavailable form (Hunter, Eden & Green, 1945).

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Recently Gallagher, Judah & Rees (1956*a, b*) have examined the effects of copper deficiency in young rats and hens. It was found that although the copper content of liver, kidney and brain was reduced no demyelination was produced. The two major metabolic disturbances which resulted from this deficiency were loss of cytochrome oxidase activity and the suppression of phospholipid synthesis. This suggested that cytochrome oxidase activity may be reduced in swayback. The present investigation was undertaken to determine the copper content and cytochrome oxidase activity of tissues from normal and swayback animals with particular reference to the brain.

MATERIALS AND METHODS

Tissues were obtained as soon as possible after the animal had been killed and they were sent to us immediately by rail, either in a fresh state or packed in ice. Material was

collected from the abattoir as soon as possible after death. It was then stored at -20° until required. The control lambs had diseases other than swayback and were from areas where the incidence of swayback is very low. These animals were killed and the tissues sent to us immediately. In order to determine whether the tissues had lost cytochrome oxidase activity in the interval between killing and freezing, we dissected two lambs from an outbreak of swayback and the relevant tissues were placed on solid carbon dioxide within 10 min. of death.

The copper content of the tissues was determined by the method of Eden & Green (1940). Cytochrome oxidase activity was measured by the method of Schneider & Potter (1943) with ascorbic acid as substrate. Succinic dehydrogenase activity was determined by the method of Gallagher *et al.* (1956a). Dry weights were determined by drying in an oven at 110° to constant weight. Lipid extraction was carried out on several brain samples by homogenizing in chloroform-methanol (2:1, v/v) and, after filtration, total lipids were determined by weighing each sample to constant weight after evaporation of the solvent. It was found that there was no difference in the total lipid content of samples from normal- and swayback-lamb brains. All the swayback cases were of the delayed type, showing no gross cerebral cavitation.

RESULTS

Determinations of copper content were made on the following tissues: liver, kidney, frontal pole of the cerebrum, whole cerebellum, caudate nucleus and cerebral white matter, which consisted of the corpus callosum and centrum semi-ovale. Cytochrome oxidase activity was determined in the following tissues: liver, kidney, scrapings of frontal cortex, cerebellar peduncle and caudate nucleus.

The mean values for the copper content of tissues

are presented in Table 1 together with values obtained by other workers. Wherever possible two samples were examined from each tissue.

There is a significant difference (P 0.01–0.001) between the values for copper content of grey matter (caudate nucleus) and white matter in the normal lamb.

In order to assess any loss of cytochrome oxidase activity as a result of imperfect storage of some samples, fresh brain and kidney were left for over a day at 25° in a moist atmosphere. There was no

Fig. 1. Cytochrome oxidase in the brain of normal (shaded columns) and swayback lambs. Enzyme activity was determined as described in the text on five normal and five swayback lambs.

Table 1. Copper contents of tissues of normal and swayback lambs

Copper content is expressed as p.p.m. dry wt. ($\pm$ S.E.M. and number of observations). Reference numbers (last column): 1, Present work; 2, Bennetts & Chapman (1937) on enzootic ataxia; 3, Schulz, Van der Merwe, van Rensburg & Swart (1951) on lamkruis, a condition closely resembling swayback; 4, Shearer, Innes & McDougall (1940); 5, Dick (1954).

Tissue	Copper content		<i>P</i>	Reference no.
	Control	Swayback		
Liver	109.08 $\pm$ 12.9 (12)	16.84 $\pm$ 3.77 (9)	<0.0001	1
Liver	360–120	8–3	—	2
Liver	1000–100	24.9–2.2	—	3
Liver	—	14.8 $\pm$ 1.9 (13)	—	4
Whole brain	30.2, 33.6	—	—	5
Whole brain	7.5 $\pm$ 3.0 (30)	8.4 $\pm$ 1.8 (9)	—	4
Kidney	13.45 $\pm$ 1.7 (4)	15.79 $\pm$ 1.5 (10)	0.4–0.3	1
Kidney	11.28	—	—	3
Kidney	14, 15.9	—	—	5
Brain:				
Frontal pole	15.53 $\pm$ 0.71 (12)	5.37 $\pm$ 1.02 (10)	<0.0001	1
Cerebellum	15.75 $\pm$ 1.17 (12)	4.04 $\pm$ 1.5 (11)	<0.0001	1
Caudate nucleus	19.26 $\pm$ 3.9 (6)	1.4, 16	—	1
Cerebral white matter	8.15 $\pm$ 1.81 (4)	1.71 $\pm$ 0.57 (4)	0.01–0.0001	1

loss of cytochrome oxidase or succinic dehydrogenase activity. Furthermore, samples frozen immediately after death in no way differed from the tissues sent to us by rail on ice or in the fresh state. These results substantiate the observations of Greenstein, Werne, Eschenbrenner & Leuthardt (1944), who found that prolonged storage of guinea-pig tissues at -20° or allowing them to stand for 4 hr. at room temperature did not result in loss of cytochrome oxidase activity.

Succinic dehydrogenase activity was determined in samples of brain from a swayback animal and of normal brain. The levels of the enzyme in normal brain did not differ from those in the swayback animals.

Cytochrome oxidase activity in brain samples from swayback and normal lambs is recorded in Fig. 1. These results show a clear difference in the activity of the enzyme in the brains of the two groups. The mean value for cytochrome oxidase activity in the liver of four swayback lambs is $42.1 \pm 12.3 \mu\text{l. of O}_2/\text{hr./mg. dry wt.}$ and for five normal lambs is $63.4 \pm 7.85 \mu\text{l. of O}_2/\text{hr./mg. dry wt.}$ The difference is on the borderline of significance ($P\ 0.2-0.1$). There was no difference in cytochrome oxidase activity of kidney from normal and swayback lambs.

DISCUSSION

Our results show that the copper content of swayback tissues is significantly lower than that of control tissues except for kidney. Our cases of swayback were delayed cases and the animals were fed away from the home farm before we received them. This food presumably had a normal copper content. We think that this may account for the high kidney figures. Shearer, Innes & McDougall (1940) found no loss of copper in the brain of swayback lambs. However, they were working in North Derbyshire and both the swayback and the non-swayback lambs that they examined were copper-deficient with liver-copper contents of 14.8 ± 0.19 and 18.4 ± 0.72 parts/million dry wt. respectively.

Cytochrome oxidase activity has been shown to be reduced in the brains of affected animals (Fig. 1). Since this enzyme was found to be stable at room temperature for at least a day and since the activity of succinic dehydrogenase was normal in the brains of the swayback lambs, it may be concluded that the fall in cytochrome oxidase activity was real and not due to post-mortem autolysis or to necrosis *in vivo*. There was also a small difference ($P\ 0.1-0.05$) between the cytochrome oxidase activity in the livers of normal and swayback lambs. The enzyme activity, however, remained unchanged in the kidney. These changes in cyto-

chrome oxidase activity can be correlated with similar alterations in the copper content of brain and kidney. The liver is the main site of storage of copper and it is not therefore surprising that cytochrome oxidase activities remain high in this tissue even after 85 % copper depletion.

Hurst (1942) produced demyelination in sheep by the administration of potassium cyanide and in monkeys by the administration of potassium cyanide and sodium azide. Both these substances are cytochrome oxidase inhibitors. The absence of neurological lesions in the animals used in experiments of Gallagher *et al.* (1956*a, b*) may be related to the difficulty these workers experienced in reducing brain cytochrome oxidase activity. Hurst (1942) produced lesions only after chronic poisoning. Present evidence would suggest a chronic deficiency of copper and cytochrome oxidase activity in the swayback lambs.

Until recently the role of copper in tissue respiration at the level of cytochrome oxidase has not been clear (Gallagher *et al.* 1956*a*). The original suggestion of Keilin & Hartree (1938) that cytochrome oxidase contains copper has now, however, been confirmed (Wainio, Wende & Shimp, 1958), and Anderson & Tove (1958) have found that copper has a direct function in the synthesis of haem. Our present findings are consistent with this work and also with that of Gallagher *et al.* (1956*a, b*), although the part played by cytochrome oxidase in the demyelinating process in swayback remains to be investigated.

SUMMARY

1. The copper content of brain and liver from swayback lambs was significantly lower than normal.
2. Cytochrome oxidase activity in brain of swayback lambs was also significantly reduced.
3. The relationship between copper contents and cytochrome oxidase activity is discussed.

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