159. VITAMIN B₁ IN VERTEBRATE MUSCLE

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(Received 19 August 1940)

THE amounts of vitamin B₁ in the muscular tissues of animals of different species are not the same [Elvehjem & Arnold, 1936; Leong, 1939; Schultz *et al.* 1939]. It has also been found that differences may exist between the vitamin B₁ concentrations in different muscles of the same animal. Although the amount of vitamin B₁ present in muscle depends to some extent on the diet of the animal in question, it seems possible that some of these variations may possess a more fundamental significance. In the present work an attempt has been made to survey the distribution of vitamin B₁ in vertebrate muscles with a view to the elucidation of certain of its functions which are at present somewhat obscure.

EXPERIMENTAL

Method. Throughout the investigation the total vitamin B₁ content of the tissues examined has been determined by the modification of the thiochrome method, details of which have already been published [Pyke, 1939].

The distribution of vitamin B₁ in the muscles of different species of vertebrates

The amount of vitamin B₁ in the muscles of the pig is so greatly in excess of that found in other readily available domestic and experimental animals that a survey was made of as many species as possible in the hope that some other animal might be found whose vitamin stores equalled those of the pig, in which case a clue might be furnished to the function of the high muscular vitamin B₁ level. The animals listed in Table 1 were apparently normal specimens and were killed immediately before analysis.

Table 1. *Distribution of vitamin B₁ in vertebrate skeletal muscle; normal animals*

Name	Muscle	Vitamin B ₁ (i.u. per 100 g.)
Pig (<i>Sus domesticus</i>)	Various	170-510
Hedgehog (<i>Erinaceus europaeus</i>)	Various	59-103
Blotched genet (<i>Genetta tigrina</i>)	Leg	95
Goose (<i>Anser domesticus</i>)	Various	13-67
Rabbit (<i>Oryctolagus cuniculus</i>)	Various	9-63
Kid (<i>Capra hircus</i>)	Various	23-63
Fowl (<i>Gallus domesticus</i>)	Various	19-57
Rat (<i>Rattus norvegicus</i>)	Various	2-35
Sheep (<i>Ovis aries</i>)	Leg	34
Ox (<i>Bos taurus</i>)	Gluteus maximus	23
Two-wattled cassowary (<i>Casuarus bicarunculatus</i>)	Leg	25
West African wood owl (<i>Strix woodfordii nuchalis</i>)	—	23
Emu (<i>Dromiceius hollandiae</i>)	Leg	15
Bengal vulture (<i>Pseudogyps bengalensis</i>)	—	11
Horse (<i>Equus caballus</i>)	Various	6
Binturong (<i>Arctitis binturong</i>)	Leg	4
Hare (<i>Lepus europaeus</i>)	Leg	Nil

Table 2 shows the vitamin B₁ contents of specimens taken from animals dead from the diseases listed in column 3. These and many of the samples in Table 1 were made available by the kind co-operation of the Secretary of the Zoological Society, London.

Table 2. *Distribution of vitamin B₁ in vertebrate skeletal muscle; diseased animals*

Name	Muscle	Cause of death	Vitamin B ₁ (i.u. per 100 g.)
Ring-necked parakeet (<i>Psittacula manillensis</i>)	—	Impacted cloaca	86
Pileated guan (<i>Penelope piliata</i>)	Breast	Enteritis	86
Collared peccary (<i>Pecari tajacu</i>)	Various	Pneumonia	38-57
Black-footed penguin (<i>Spheniscus demersus</i>)	Breast	Inanition	42
South American flamingo (<i>Phoenicopterus chilensis</i>)	—	Old age	38
Lion cub (<i>Felis leo</i>)	Leg	Pneumonia	36
Scarlet ibis (<i>Eudocimus rubra</i>)	Breast	Endocarditis	30
Grey squirrel (<i>Sciurus carolinensis</i>)	Leg	Intestinal obstruction	29
White-tailed mongoose (<i>Ichneumia albicauda</i>)	Leg	Endocarditis	19
Black duiker (<i>Cephalophus niger</i>)	Leg	Pneumonia	19
Californian sea-lion (<i>Zalophus californianus</i>)	Breast	Pneumonia	19
Persian gazelle (<i>Gazella subgutturosa</i>)	—	—	17
Crested pelican (<i>Pelicanus crispus</i>)	Breast	Peritonitis	15
Crowned pigeon (<i>Goura cristata</i>)	Breast	Pneumonia	15
Red brocket (<i>Mazarma americana</i>)	Leg	Pneumonia	12
Ocelot (<i>Felis pardalis</i>)	Leg	Old age	11
Garnett's bush baby (<i>Galago garnettii</i>)	Leg	Rupture of the liver	11
King penguin (<i>Aptenodytes patagonica</i>)	Breast	Mycosis	10
Chimpanzee (<i>Pan satyrus</i>)	Abdominal	Dysentery	4-10
Rhesus monkey (<i>Macacus mulatta</i>)	Various	Tuberculosis	4- 8
Pig-tailed monkey (<i>Macacus nemestrina</i>)	Leg	Post-partum haemorrhage	8
Hybrid pheasant (<i>Tragopan blythi</i> × <i>T. satyra</i>)	Breast	Nephritis	6
Spectacled cayman (<i>Caiman crocodilus</i>)	Leg	Tuberculosis	6
Two-spotted palm civet (<i>Nandinia binotata</i>)	Leg	Osteomalacia	4
Nylghau (<i>Boselaphus tragocamelus</i>)	Leg	Tuberculosis	4
Ard vark (<i>Orycteropus afer</i>)	Abdominal	—	4
Silver-grey opossum (<i>Trichosurus vulpecula</i>)	Leg	—	4
Wart hog (<i>Phacochoerus aethiopicus</i>)	Leg	Carcinoma	2
Crested porcupine (<i>Hystrix cristata</i>)	Leg	Gastric ulcer	Nil
Black cayman (<i>Caiman niger</i>)	Breast	Pneumonia	Nil
Australian long-nosed crocodile (<i>Crocodylus johnsonii</i>)	Tail	Pneumonia	Nil
Southern anaconda (<i>Eunectes murinus</i>)	—	Enteritis	Nil

While the random nature of the samples and the pathological condition of the specimens listed in Table 2 make it unjustifiable to draw definite conclusions, it appears that the muscles of the domestic pig are unusually rich in vitamin B₁. The possibility that this superiority of pig muscle is due to the consumption of a diet exceptionally rich in vitamin B₁ must be borne in mind; nevertheless it has been shown by Leong [1939] that no matter how much vitamin B₁ is fed to guinea-pigs, fowls, pigeons or rats, the highest concentration in the muscle is

120 i.u. per 100 g., attained by the pigeon. The maximum figure for the rat reported by Leong was 60 i.u. per 100 g. In the present work a concentration of 35 i.u. per 100 g. only was reached. It seems probable, therefore, that the pig has an innate capacity for retaining vitamin B₁ in its muscles not possessed by other animals.

The variation in vitamin B₁ content of the muscles of random pigs obtained from bacon factories in different parts of the country is shown in Table 3.

Table 3. *Normal variation of vitamin B₁ in pig muscle*

Breed or description	Vitamin B ₁ (i.u. per 100 g.)
Imported frozen from Melbourne	170
Berkshire (black)	170
Large white	260
"	260
"	270
"	290
"	290
"	300
"	310
"	340
"	350
"	390
Imported frozen from Queensland	340
Large white × Saddleback	340
Middle white	400
Essex	460
Pig 113 (from Cheshire)	460
Pig 98 (from Cheshire)	510
Pig 91 (from Cheshire)	510

Variation in vitamin B₁ content of different muscles

Up to the present it has merely been shown that even within the variations due probably to differences in diet the vitamin B₁ content of the muscles of the pig is substantially higher than that found in other animals. Differences of a less striking nature also appear between other species. From Table 4, however, it can be seen that vitamin B₁ is by no means evenly distributed between the muscles of the individual animal.

Table 4. *Vitamin B₁ (i.u. per 100 g.) in different muscle groups*

Muscle group	Horse	Kid	Pig
Psoas	6	4	300
Longissimus dorsi	6	15	210
Abdominal muscle	6	34	220
Pectoral muscle	6	38	260
Tongue	6	58	85
Fore leg, ventral	4	30	150
Fore leg, dorsal	6	46	120
Hind leg, ventral	4	23	180
Hind leg, dorsal	6	63	150

When tissues such as muscle contain less than 10 i.u. of vitamin B₁ per 100 g. considerable skill in titration is necessary to obtain accurate figures. In the case of the horse it can, therefore, only be said that its muscles contain a uniformly low level of vitamin B₁. In other animals, however, it is clear that differences exist in respect of individual muscles and a study was therefore made of the

vitamin B₁ content of the psoas, a muscle primarily concerned with movement, and of the longissimus dorsi, the principal function of which is the maintenance of posture. Pig muscles were used.

Comparison of the amounts of vitamin B₁ in psoas and longissimus dorsi of the pig

A litter of 9 Wessex Saddleback pigs was divided into 2 groups at weaning. Group A was fed on a normal commercial meal containing 190 I.U. vitamin B₁ per 100 g. Group B, which was housed under identical conditions, received the same meal with the addition of enough crystalline vitamin B₁ to raise its concentration to 850 I.U. per 100 g. The pigs were slaughtered at 6½ months of age and a cross-section of the carcass was taken at the level of the last rib through (1) outer back fat, (2) inner back fat, (3) longissimus dorsi, (4) psoas, (5) flare fat. In none of the samples from the fatty layers could more than a trace of vitamin B₁, never exceeding 6 I.U. per 100 g., be detected. The figures for the muscle samples are shown below in Table 5. In this table is included, besides the figures for the 2 groups of littermate animals, a third group of figures obtained from swill-fed pigs of similar age which were provided through the kindness of Prof. John Hammond, of the School of Agriculture, Cambridge. While no analysis of the swill was made-it almost certainly contained less vitamin B₁ than the cereal meal fed to group A.

Table 5. *Vitamin B₁ content of psoas and longissimus dorsi of pigs fed at different vitamin B₁ levels*

	Longissimus dorsi (I.U. per 100 g.)	Psoas (I.U. per 100 g.)
Swill-fed pigs	160 170	230 200
Group A (190 I.U. vitamin B ₁ per 100 g. food)	285 285 285 315 315	450 485 400 430 455
Group B (850 I.U. vitamin B ₁ per 100 g. food)	315 315 340 285	455 430 485 455

This experiment shows that the amount of vitamin B₁ in the psoas of the pig is consistently greater than that present in longissimus dorsi. Comparing groups A and B, one can conclude that a normal pig ration supplies all the vitamin B₁ that the animal is capable of storing. While the swill-fed group is not strictly comparable, since its members belonged to a different breed, it appears that the reduction in muscle vitamin B₁ is due to deficiency in the diet. However, even at this lower nutritional level, the excess of vitamin B₁ in psoas over that in longissimus dorsi is still maintained.

Vitamin B₁ in cardiac muscle

The vitamin B₁ contents of individual specimens of hearts from animals of different species are listed in Table 6. In general it appears that the concentration of vitamin B₁ in cardiac muscle is higher than that found in skeletal muscle. Although variations exist from one species to another, there is some degree of uniformity in the vitamin B₁ levels found.

Table 6. *Vitamin B₁ in heart muscle (I.U. per 100 g.)*

Kid	170
Hen	150
Rabbit	150
Pig	140
Hedgehog	130
Horse	80-130
Sheep	110
Rat	100
Goose	70

When cardiac muscle, however, is examined in greater detail, it is found that the vitamin B₁ is not uniformly distributed throughout the heart. Figures are given in Table 7 from which it appears that in the two species examined the concentration of vitamin B₁ is higher in ventricular muscle than in auricular.

Table 7. *Vitamin B₁ distribution within the heart (I.U. per 100 g.)*

	Pig	Horse
Auricles, right	125	34
left	115	40
Ventricles, right	170	74
left	200	80

Vitamin B₁ in red and white muscle

There is some evidence that red muscle contains more vitamin B₁ than white. For example, the psoas of the pig is a darker red than the longissimus dorsi and contains more vitamin. In the case of the rabbit, it is difficult at all times to measure variation between muscles since in many cases the absolute level of vitamin B₁ may be very low. Results from 3 animals, however, are shown in Table 8.

Table 8. *Vitamin B₁ (I.U. per 100 g.) in the red and white muscles of the rabbit*

	Red muscles	White muscles
Rabbit 1	Soleus (right leg)	Vasta lateralis (right leg)
	Soleus (left leg)	Vasta lateralis (left leg)
	Pectineus (pooled)	Gluteus max. (right)
		Gluteus max. (left)
Rabbit 2	Soleus, crureus (pooled)	Gastrocnemius
	Pectineus	Longissimus dorsi
Rabbit 3	Soleus	Vasta lateralis
	Pectineus	Gracilis

The state of vitamin B₁ in muscle

If muscle is minced, shaken vigorously with water or pepsin-HCl solution and samples are immediately taken for the thiochrome test, figures are obtained which give the amount of free vitamin B₁. The remainder of the sample can then be digested with the pepsin-HCl solution and a second aliquot removed for test. This second figure has been tentatively assumed to be free plus "residual" vitamin B₁, while the final result after an additional digestion with takadiastase has been assumed to comprise free plus "residual" vitamin B₁ plus cocarboxylase. Cocarboxylase may break down after the death of the muscle and although it does not appear to do so in the acid pepsin solution it is possible that the second determination made after 16 hr. digestion in pepsin-HCl includes a proportion of the cocarboxylase originally present. The following experiments must, therefore, be accepted with this possibility of error in mind.

A sample of pig psoas examined within a few minutes of the death of the animal was found to contain a total of 455 I.U. per 100 g. of vitamin B₁. Of this total, 11 I.U., or 2.4 %, were free vitamin B₁. Cocarboxylase, calculated as vitamin B₁, amounted to 170 I.U., or 37 %. Aliquots of the minced muscle were allowed to stand at room temperature, protected from evaporation, and were analysed periodically. The results are shown in Fig. 1, from which it can be seen that cocarboxylase breaks down to free vitamin B₁. Ochoa [1939] has demonstrated that tissues contain an enzyme capable of phosphorylating vitamin B₁. This enzyme may also catalyse the reverse reaction and thus break down cocarboxylase. The fact that a proportion of the cocarboxylase in the present experiment still remained in pig muscle 24 hr. after death is in agreement with the finding of Westenbrink & Dorp [1940] that the enzyme is inhibited by the accumulation of free vitamin B₁.

The breakdown of cocarboxylase in this manner during the autolysis of muscle does not appear to be universal in all muscular tissues. The results of an experiment on horse's heart muscle are shown in Table 9.

Fig. 1. Breakdown of cocarboxylase in minced pig psoas allowed to stand at room temperature.

Table 9. *Partition of vitamin B₁ compounds during autolysis of horse's heart (I.U. per 100 g.)*

Time after death hr.	Free vitamin B ₁	Cocar- boxylase	Residual vitamin B ₁	Total vitamin B ₁
1.5	4	63	13	80
4.5	4	55	21	80
24	8	23	55	86

From a suggestion of Ochoa [Banga *et al.* 1939] it seems probable that "residual vitamin B₁" is in fact vitamin B₁-monophosphate. If this is the case, autolysis of heart muscle of the horse results in the breakdown of cocarboxylase to form the monophosphate, whereas in pig psoas cocarboxylase is completely dephosphorylated to free vitamin B₁. In the pig's muscle, however, the phosphate group is apparently not removed from the "residual" or monophosphate-vitamin B₁.

DISCUSSION

The results which have been presented in this paper raise possibilities of considerable interest. A hypothesis which might be worthy of further investigation, is that vitamin B₁ in muscle is specially concerned with the glycogen cycle. The muscles of the horse, which are consistently low in vitamin B₁, contain high percentages of glycogen even after prolonged exercise [Mathews, 1939], whereas pig muscles, which are exceptionally rich in vitamin B₁, contain small amounts of glycogen which are rapidly depleted by exercise [Bate Smith, 1937]. Again, it has been shown that the psoas muscle of the pig contains more

vitamin B₁ than longissimus dorsi. In agreement with this result, Callow [1938] has deduced by his technique of measuring the "ultimate pH" that in practically all of 30 pigs there was less glycogen in the psoas than in longissimus dorsi.

The results of the experiments on cardiac muscles and on red and white rabbit muscles supply additional evidence. It appears that there is more vitamin B₁ in the ventricles than in the auricles of the hearts of pig and horse. If vitamin B₁ is inversely related to the glycogen level one would expect to find less glycogen in the ventricles than in the auricles, and, according to Cruikshank [1936], this is the case. The work on red and white muscles, if it can be confirmed, is also interesting. It appears that the red muscles of rabbits contain more vitamin B₁ than white muscles. Noll & Becker [1936] have shown that red rabbit muscle only contains 65–80 % as much glycogen as white muscle. Red muscle contains more sarcoplasm than white and the granules of glycogen are found in the sarcoplasm and not in the fibrils. It seems possible, therefore, that vitamin B₁ is also present in the sarcoplasm.

Williams & Spies [1938] have reviewed the literature on the present knowledge of the relationship between vitamin B₁ and glycogen. Much of it is inconclusive but certain experiments with pigeons show that deficiency of vitamin B₁ raises the level of glycogen in the liver.

SUMMARY

1. The vitamin B₁ contents of the muscles of a number of animals of different species have been determined. The muscles of the pig appear to be several times richer in vitamin B₁ than those of any other animal.

2. Variations occur in the amount of vitamin B₁ in different muscles in the animal body. In the pig there is more vitamin B₁ in psoas than in longissimus dorsi.

3. Heart muscle contains more vitamin B₁ than is normally found in skeletal muscle. There is a higher concentration of vitamin B₁ in the ventricles than in the auricles.

4. Red muscles of rabbits appear to contain more vitamin B₁ than white muscles.

5. If muscle is allowed to stand after death cocarboxylase breaks down and free vitamin B₁ appears in pig's muscle, while vitamin B₁-monophosphate is probably formed in horse's heart.

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