Intracellular Thioctic Acid and Coenzyme A following Vanadium Treatment

It was suggested in a previous communication[1] that the fall in coenzyme A liver concentrations in animals treated with vanadium might be due, at least partially, to a reduction in the number of —SH groups available. This hypothesis was based on the following facts: (1) The toxic effects of vanadium may, to a great extent, be attributed to modifications in reactions connected with sulphur compounds, especially those of the thiolic type[2]. (2) The lack of —SH groups, and particularly of sulphurated amino-acids, lowers the coenzyme A content in the liver[3]; in this case, a fall in thioctic acid, the connexion of which with coenzyme A is well-known, also occurs in the tissues[4].

These investigations were undertaken on the basis of the above considerations to assess the effects of vanadium treatment on the liver content and intracellular distribution of thioctic acid and coenzyme A.

The experiments were performed on rats bred by us, of average weight 120–150 gm., fed on a diet of known composition[1] starting 7 days after the beginning of the experiment. Vanadium was administered mixed with their diet, at the level of 0·05 per cent, in the form of sodium metavanadate ($NaVO_3 \cdot 4H_2O$) for 14 days. At the end of this period, the animals were killed by bleeding, and their livers removed for analysis.

Some analyses were carried out on the whole liver homogenate, and some on fractions obtained by centrifugation at 500 r.p.m. (nuclei), 3,000 r.p.m. (mitochondria) and 20,000 r.p.m. (microsome particles), in accordance with the standard procedure.

The following determinations were then made: coenzyme A[5]; thioctic acid[6]; and proteins[7]. The results are given in Table 1.

In the whole homogenate more thioctic acid disappeared than coenzyme A; the sharpest reduction occurred in the mitochondrial fraction, where thioctic acid and coenzyme A were respectively 86 and 52·5 per cent below normal; in the remaining homogenate fractions, variations in both thioctic acid and coenzyme A, were far less marked than those observed in mitochondria.

These results would suggest a slow-down in the biosynthesis of coenzyme A, caused, at least in part, by the non-decarboxylation of pantothenylcysteine to pantotheine, which process is known to require pyridoxal phosphate as the prosthetic group: indeed, vanadium would seem to affect this reaction[8].

The fall of thioctic acid concentrations in the tissues might be regarded partly as a clue to a more widespread alteration in the metabolism of sulphurated substances, and partly as the result of the lack of coenzyme A, which is essential for restoring the dithiolic form of the lipothiamide.

In other words, the fall in coenzyme A concentrations in the livers of animals treated with vanadium might be the result of two different actions exerted (1) on pyridoxal-phosphate and, (2) on the metabolism of sulphurated substances. This would appear to be confirmed by the preliminary results of investigations now being performed in this Laboratory; treatment with pantothenic acid and/or cysteine causes only a slight increase of coenzyme A concentrations in the liver of rats kept on a diet supplemented with vanadium, so a substantial degree of protection appears to be provided by pantotheine.

Table 1. Thioctic acid and coenzyme A in liver homogenate fractions from animals treated with vanadium (average values ±S.E.)

Homogenate Fraction	Thioctic Acid: control (8)*	μgm./gm. Protein: treated (10)	Coenzyme A: control (8)	U/100 mgm. Protein: treated (10)
Whole	8·33±0·39	2·37±0·18	91·6± 4·7	50·8±3·8
Mitochondria	29·37±1·64	4·16±0·25	260·6±11·5	124·1±8·6
Nuclei	4·98±0·18	3·58±0·20	67·1± 3·1	59·8±5·0
Microsome	—	—	23·3± 1·8	11·0±0·9
Supernatant	4·57±0·21	2·44±0·13	51·8± 2·4	35·4±2·7

* Number of animals given in brackets.

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Aug. 10.

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Deaminases of Adenylic Acid and Adenosine in Rat Kidney

Hitherto it has been generally accepted that only muscle, blood and nervous tissue contain a specific adenylic deaminase[1,2]. Recently, however, we have succeeded in separating adenylic deaminase from adenosine deaminase by using rat kidneys.

Rat kidney homogenate prepared in 0·1 *M* potassium chloride containing 0·039 *M* borate buffer *p*H 7, produced both ammonia and inorganic phosphate when incubated with muscle adenylic acid. When the homogenate was fractionated by differential centrifugation, the phosphatase activity was localized mainly in the mitochondrial fraction (sedimenting between 3,000 and 16,000 *g*). This fraction, however, was practically devoid of deaminase activity (Table 1). After the centrifugation of the mitochondrial fraction there remained a clear reddish liquid (cytoplasm). When dialysed against distilled water at 0°C. for 24 hr., the cytoplasm separated into a water-insoluble fraction (cytoplasmic globulins) and a water-soluble

Table 1. Deamination and Dephosphorylation of Adenylic Acid by Rat Kidney Fractions

Fraction present in the incubation mixture		μmole P/mgm. protein-N	μmole NH_3/mgm. protein-N
Whole homogenate	*a*	4·42	3·42
	b	3·23	1·89
	c	3·98	2·58
Mitochondrial fraction	*a*	14·70	1·71
	b	7·23	0·0
	c	8·85	0·80
Cytoplasmic albumins	*a*	1·40	2·68
	b	1·33	0·56
	c	1·05	1·11
Cytoplasmic globulins	*a*	1·21	5·65
	b	1·43	5·45
	c	1·82	7·87

Rat kidneys were homogenized in 7·5 vol. of 0·1 *M* potassium chloride containing 39 m*M* borate buffer *p*H 7, the homogenate centrifuged at 3000 *g* for 10 min., and the sediment disregarded. The supernatant was centrifuged at 16000 *g* for 15 min. The sediment thus obtained is called the 'mitochondrial fraction'. The clear supernatant solution was further separated in two fractions by dialysis against distilled water for 24 hr.: the water-soluble 'cytoplasmic albumins', and the water-insoluble 'cytoplasmic globulins'. The letters *a*, *b*, *c*, indicate different animals.

The incubation mixtures contained 0·025 *M* succinate buffer *p*H 6, 0·005 *M* adenylic acid, 0·1 *M* potassium chloride and the appropriate kidney cell fraction. Incubations were terminated after 30 min. at 25°C. by the addition of trichloroacetic acid to the final concentration of 7·5 per cent. In the filtrates inorganic phosphate was estimated by the method of Gomori (ref. 3) and ammonia according to Russel (ref. 4) by Conway's microdiffusion method. The results, each representing an average from two samples obtained from one animal, were expressed in terms of μmoles/mgm. protein-nitrogen.