9. *Biochemical Studies on Vitamin B₁₂. VI.* *Relation of Vitamin B₁ and Vitamin B₁₂ to Energy* *Metabolism in Cattle*

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In 1950, R. W. Vilter¹⁾ reported studies on the relations of vitamin B₁₂, folic acid, thymine, uracil and methyl group donors in persons with pernicious anemia and related diseases, and stated that vitamin B₁₂ may be essential to the formation of nucleic acid and nucleoprotein through a biochemical chain reaction in vivo.

Afterwards, in the same year in Japan, further confirmation for these researches was made by Dr. S. Ito²⁾, University of Nagoya and a distinct increase of ATP was observed in the case of bone marrow culture.

In the mean while, Dr. N. Sakurai³⁾ reported several excellent hematological researches of energy metabolism in race-horses, and macrocytic anemia was observed in animals after energy work, while there was an increase of red corpuscles at rest. However, in his publication, no further studies were made from the view-point of vitamin-biochemistry.

In 1939, Lipmann⁴⁾ stated that pyruvate metabolic system in animal tissue may be in the following relation:

Guided by these papers, the present authors have recently attempted a study on relation between energy metabolism and vitamin B₁₂ as follows:

(1) Experiments on Energy Metabolism with Cattle⁵⁾ put on a Tread-mill

In carrying out this experiment, a horizontal tread-mill was first adjusted to 0.75 meters per second in velocity. Two young cattle (♀ 2 years of age, non-identical twins) were used: One cattle (A) was put on the tread-mill, carrying a load (20 kg) and restless walking was continued for 4.5 hours, and the variation of vitamin B₁ content in blood was estimated during this energy work. The

other one (B) was kept in a shed at rest and served as negative control. 28% decrease of vitamin B₁ in blood was observed in animal (A) at the end of experiments and 13% in control (B).

(2) Blood Count in Cattle put on Tread-mill⁵⁾⁶⁾

The above tread-mill used in 1st experiment was again adjusted to 6° in acclivity and animals (A, B) were alternately used for the following experiments. One was placed on the same condition carrying a load (20 Kg) and restless working for 4.5 hours, and the other serving as negative control.

Table I
Variation of red blood cell counts during the experiments

			Before experiment	After experiment
Exp. 1.	Exp. animal	A	$770 \times 10^4/\text{mm}^3$	$660 \times 10^4/\text{mm}^3$
	Control	B	790	780
Exp. 2.	Exp. animal	B	692	530
	Control	A	675	961
Exp. 3.	Exp. animal	A	956	649
	Control	B	811	820
Exp. 4.	Exp. animal	B	635	580
	Control	A	777	830

Fig. 1. Variation of the red blood cell counts and of the diameter of red corpuscles

From the above result, it was found that the animals put on the tread-mill indicated severe symptom of macrocytic anemia.

(3) Vitamin B₁₂ Content in Blood of Cattle
put on the Tread-mill

The variation of vitamin B₁₂ content in blood was estimated.

To 5 cc blood from jugular vein was added 10 mg. Na-citrate and the solution was treated with 1 % solution of papain-diestase (2:1) at 37°C for 44 hours. After removing protein with 10 % metaphosphoric acid, vitamin B₁₂ in blood was determined by Euglena bioassay.

Table II
Variation of vitamin B₁₂ content in energy metabolism

		Before experiment	After experiment	% of variation
Exp. 1.	Exp. animal A	8.8 mr %	8.4 mr%	- 4.6
	Control B	8.0	10.3	+27.7
Exp. 2.	Exp. animal B	8.2	6.7	-18.3
	Control A	7.5	8.0	+ 6.7

Vitamin B₁₂ in blood of animals put on energy work indicated a decrease, but results in animals at rest showed rather an increase.

Summary

- (1) The content of vitamin B₁ decreased in cattle put on tread-mill.
- (2) The red blood cell counts in animals put on energy work indicated a decrease and the diameter of red corpuscles increased.
- (3) Vitamin B₁₂ in blood indicated a decrease in energy metabolism.

From these findings, vitamin B₁₂ seems to be essential to energy metabolism in the following relation:

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(All in Japanese except*)

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