time interval between them being registered by an ordinate recorder. The blood was readministered by intravenous drop infusion. Arterial blood pressure, rectal temperature and ear skin temperature (as an index of skin circulation) were recorded.

A typical result is demonstrated in the Figure *C*, where hypothalamic heating gave decreased flow through the muscle and increased skin temperature (secondarily to vasodilatation) with a latency of about 2 min. Blood pressure was slightly lowered. At the end of the heating, the muscle blood-flow returned to the earlier level although the blood pressure was about 20 mm Hg lower. The muscle tension record (not reproduced in the Figure) was constantly at 10 g throughout the period of observation shown in the Figure.

The effects of hypothalamic heating on muscle circulation were as a rule small as compared to those seen in the skin. The effects were probably not due to baroceptive influences since the changes in skin and muscle blood-flow were not always related in magnitude. Vasomotor effects in the muscle could only be demonstrated when it was under some stretch. This may explain why FOLKOW *et al.*[6] did not find any changes in muscle flow during hypothalamic heating, although electrical stimulation reveals the presence of nervous structures capable of mediating such effects[7]. Small additional doses of "Nembutal" which gave a rapid vasodilatation in the skin generally induced a slight increase in blood-flow also in the muscle (*A*), whereas small doses of chlorpromazine sometimes elicited vasoconstriction (*D*). As a rule, however, and regularly in large doses, chlorpromazine also induced vasodilatation.

U. SÖDERBERG

Nobel Institute for Neurophysiology, Karolinska Institutet, Stockholm 60, Sweden, April 25, 1956.

Zusammenfassung

Es wurde gefunden, dass lokale Erwärmung des vorderen Teils des Hypothalamus an mit „Nembutal" narkotisierten Katzen die Durchblutung des Musculus gastrocnemius verminderte, ohne dass messbare Änderungen des Muskeltonus auftraten. Der Effekt erfolgte nur, wenn der Muskel unter einer gewissen Spannung war. Kleine Dosen von Chlorpromazin hatten manchmal ähnliche Wirkung.

[6] B. FOLKOW, G. STRÖM, and B. UVNÄS, Acta physiol. scand. *17*, 317 (1949).

[7] P. LINDGREN, Acta physiol. scand. *35*, Suppl. 121 (1955).

Effects of Pyrithiamine and Oxythiamine on the Thiamine Content of Tissues and Blood Pyruvate in Mice

WOOLLEY and MERRIFIELD, in two consecutive papers[1], postulate a new metabolic function of thiamine not mediated through the cocarboxylase. The experimental evidence which supports this statement is represented by the different *in vivo* actions of two antivitamins, pyrithiamine (PY) and oxythiamine (OT). While both compounds produce a thiamine deficiency in animals, only the PY calls forth the typical neurological disturbances of the athiaminosis in mice, without however modifying the cocarboxylase content of the liver[2] or the blood pyruvate[3]. On the contrary, according to FROHMAN and DAY[4], OT increases both the urinary excretion of thiamine and the blood pyruvate in the rat, but fails to produce the neuromuscular syndrome.

These results led WOOLLEY and MERRIFIELD[5] to the conclusion that PY "in calling forth characteristic manifestations of thiamine deficiency interferes primarily with a function of thiamine responsible for the typical neurological signs and that this function is distant from the one involving cocarboxylase and pyruvate".

In other words, because the PY, administered to mice, does not modify the liver thiamine content and the blood pyruvate, which is notoriously connected with the cocarboxylase activity and produces the typical disturbances of the thiamine deficiency, the authors conclude that the PY interferes with a function of the thiamine indispensable to the nervous tissues and not involving the carboxylase. Instead, the OT would be mainly the antagonist of cocarboxylase.

In order to verify this interesting hypothesis, we repeated and extended WOOLLEY's[2] experiment, determining the thiamine contents not only of the liver but also of the muscle and particularly of the brain, since the new function of the thiamine concerns the nervous tissue.

Thus male mice of 11–14 g body weight were housed in single small cages and reared on thiamine deficient diet of the following composition: Washed, fat-free casein 18%; wheat starch 65%; olive oil 10%; cod liver oil 2%; Osborne and Mendel salt mixture 5%, supplemented with suitable amounts of all the B vitamins and α-tocopherol. In experiment No. 1, 0·5 mg of PY[6], dissolved in 0·02 ml of H_2O, was administered *per os* on the first day, followed by a daily dose of 2 μg of thiamine *per os*: the controls received only 2 μg of vitamin *pro die*.

In experiments No. 2, 3 and 4, the administration of thiamine was discontinued and the animals fed the thiamine deficient diet were treated at the beginning of the experiment with a single dose of PY or OT[7] *per os*, dissolved in 0·02 ml of H_2O, as indicated in the Table. The controls were placed on the same diet. The mice were killed by decapitation, together with their controls, when the neuromuscular symptomatology appeared. In the experiments in which the disturbances did not appear the mice were killed on the 10th day of the experiment.

The blood pyruvate was determined by the micromethod of RINDI and FERRARI[8]: the thiamine (total) contents of the tissues (liver, muscle and brain) were determined by a modification of the classical thiochrome method[9], where the isobutanol extraction was performed with 5 ml of solvent. The method used has been checked on pure thiamine solutions and always gave good results, closely comparable to those obtained with the usual method.

[1] D. W. WOOLLEY and R. B. MERRIFIELD, Fed. Proc. *11*, 458 (1952); Bull. Soc. Chim. biol. *36*, 1201 (1954).

[2] D. W. WOOLLEY, J. biol. Chem. *191*, 43 (1951).

[3] D. W. WOOLLEY and R. B. MERRIFIELD, Bull. Soc. Chim. biol. *36*, 1201 (1954).

[4] C. E. FROHMAN and H. G. DAY, J. biol. Chem. *180*, 93 (1949).

[5] D. W. WOOLLEY and R. B. MERRIFIELD, Fed. Proc. *11*, 458 (1952).

[6] Neopyrithiamine, made by the California Foundation for Biochemical Research, Los Angeles, Cal.

[7] Kindly supplied by Roche Products Ltd., Welwyn Garden City, England.

[8] G. RINDI and G. FERRARI, Exper. (in press).

[9] Association of Vitamin Chemists, Inc., *Methods of Vitamin Assay* (2nd Ed., Interscience Publ., New York 1951), p. 111.

Body weight, blood pyruvate and total thiamine content of the tissues of PY or OT treated mice. Mean values ± Standard error

Experiment No.	Treatment	No. of mice	Body weight, g		Blood pyruvate mg/100 ml	Thiamine (total) content, μg/g			% of mice showing neurological symptoms
			initial	final		liver	muscle	brain	
1	0·5 mg PY + 2 μg B_1	9	12·0 ± 0·60	15·8 ± 0·72	2·10 ± 0·15	3·39 ± 0·27	0·96 ± 0·04	1·58 ± 0·07	0
			0·7 > p > 0·6		0·4 > p > 0·3	0·9 > p > 0·8	0·02 > p > 0·01	0·001 > p	
	Controls (2 μg B_1)	9	11·7 ± 0·32	15·8 ± 0·49	1·92 ± 0·08	3·30 ± 0·25	1·15 ± 0·06	2·46 ± 0·11	0
2	0·5 mg PY	9	13·9 ± 0·22	12·2 ± 0·24	3·08 ± 0·50	2·11 ± 0·13	0·78 ± 0·03	0·74 ± 0·05	100
			0·9 > p > 0·8	0·001 > p	0·1 > p > 0·05	0·1 > p > 0·05	0·9 > p > 0·8	0·001 > p	
	Controls	9	14·1 ± 0·44	14·2 ± 0·40	2·02 ± 0·19	1·69 ± 0·17	0·79 ± 0·06	2·16 ± 0·08	0
3	0·5 mg OT	9	13·0 ± 0·34	14·0 ± 0·45	2·19 ± 0·37	1·34 ± 0·05	0·94 ± 0·06	2·34 ± 0·12	0
			0·6 > p > 0·5	0·5 > p > 0·4	0·8 > p > 0·7	0·01 > p > 0·001	0·5 > p > 0·4	0·9 > p > 0·8	
	Controls	9	12·7 ± 0·36	13·6 ± 0·34	2·07 ± 0·14	1·73 ± 0·09	1·00 ± 0·04	2·35 ± 0·15	0
4	2 mg OT	9	12·1 ± 0·23	12·5 ± 0·19	2·26 ± 0·10	1·23 ± 0·06	0·85 ± 0·02	2·13 ± 0·07	0
			0·4 > p > 0·3	0·6 > p > 0·5	0·5 > p > 0·4	0·001 > p	0·5 > p > 0·4	0·6 > p > 0·5	
	Controls	9	12·5 ± 0·34	12·8 ± 0·47	2·14 ± 0·10	1·67 ± 0·11	0·89 ± 0·05	2·20 ± 0·08	0

The significance of the difference between the means has been determined according to the t of "Student"

As can be seen from the Table, in experiment No. 1, which is the same as that performed by WOOLLEY[2], the mice did not show, during the 10 days of observation, any neuromuscular disturbance; the blood pyruvate and the thiamine content of the liver were not different from the controls. These results closely agree with WOOLLEY's findings[2]. However, in muscle and brain the thiamine content is sharply lowered: the decrease is statistically significant.

In experiment No. 2, where we did not administer the thiamine and used the same dose of PY as in experiment No. 1, all the treated mice showed the neuromuscular syndrome after 6 to 10 days of the antivitamin treatment. Their body weight was lowered, the blood pyruvate increased although not significantly, in comparison with the controls which did not show any neurological disturbance. It is well known, in fact, that in the alimentary athiaminosis of mice the motorial syndrome does never appear.

The thiamine content was unchanged in the liver and in the muscle, whereas it was greatly decreased in the brain. This means that the PY, in our experimental conditions, did not induce a further decrease of the thiamine contents of liver and muscle, already lowered by the deficient diet, but it was very efficacious in reducing to a further extent the brain thiamine level. Actually, the diminution in experiment No. 2 is much greater than in experiment No. 1 where it is likely that the daily administration of thiamine tends to abolish the effects caused on the brain vitamin content by the single dose of PY.

In experiments No. 3 and 4, the mice, treated with two different doses of OT, never showed any neuromuscular syndrome and behaved like the controls. Only in the liver we found a slight but statistically significant decrease of the thiamine content, bearing out in the mice what we had already observed in the rat[10].

The results obtained under our experimental conditions show that PY lowers the thiamine content particularly in the nervous tissue (brain) with a mechanism which is still unknown. The neuromuscular disturbances and the body weight decrease were found only in those animals in which the decrease of the brain thiamine content was conspicuous.

The OT, administered under the same experimental conditions, fails to produce, even in doses 4 times greater than PY, the neuromuscular syndrome or any modifications of the blood pyruvate, of the body weight or of the thiamine contents of both brain and muscle, although it slightly lowers the liver content. Therefore our findings do not support the hypothesis of a new function of thiamine in the nervous tissue. Actually the PY, in the dose used, fails to lower the thiamine level of the liver, according to WOOLLEY's results, but it greatly lowers the brain content, producing the neurological disturbances typical of thiamine deficiency.

On the other hand, the OT shows an action only on the liver, where it slightly lowers the thiamine content without affecting the blood pyruvate.

L. DE CARO, G. RINDI, V. PERRI, and G. FERRARI

Institute of Human Physiology, University of Pavia (Italy), April 27, 1956.

[10] L. DE CARO, G. RINDI, V. PERRI, and G. FERRARI, Int. Z. Vitaminforsch. (in press).

Riassunto

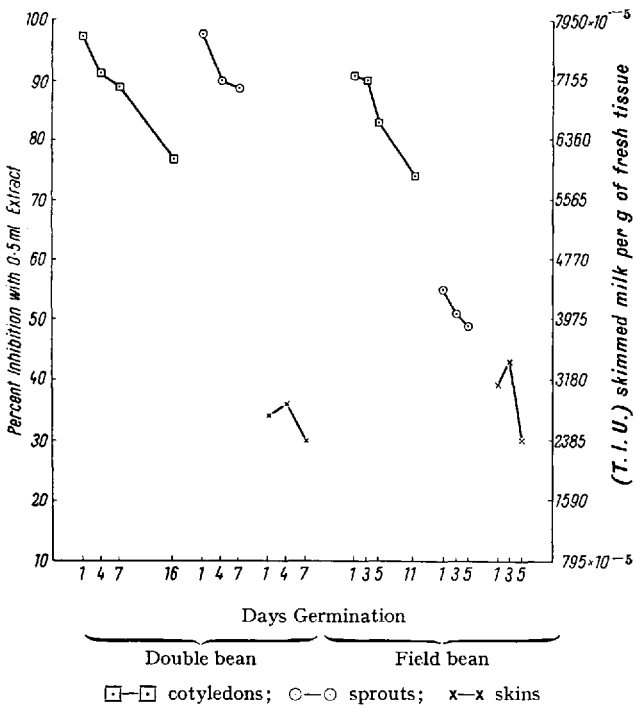
La Piritiamina, somministrata per bocca in una singola dose di 0,5 mg a topolini di 12–14 g che ricevono 2 μg di tiamina *pro die*, non produce la sindrome neuromuscolare caratteristica dell'avitaminosi B_1 e non modifica nè la piruvicemia nè il livello di vitamina nel fegato, mentre lo fa abbassare nel muscolo e nel cervello. Se i topolini non ricevono tiamina, la stessa dose di Piritiamina dà perdita di peso, sindrome neuromuscolare in tutti gli animali trattati e cospicuo abbassamento del livello di vitamina B_1 nel cervello. Nelle stesse condizioni sperimentali, l'Ossitiamina, somministrata *per os* una sola volta in dosi di 0,5 e 2 mg, non dà alcuna sintomatologia neuromuscolare e non modifica nè il peso corporeo, nè il piruvato ematico, nè il contenuto in vitamina B_1 del muscolo e del cervello, solo abbassa il livello vitaminico del fegato.

Questi risultati non sono in favore dell'ipotesi di una funzione della tiamina distinta da quella della cocarbossilasi.

Trypsin Inhibitor in Plant Metabolism

SOHONIE and AMBE[1] have recently reported the crystallization of trypsin inhibitors from the Indian double bean (*Faba vulgaris*) and field bean (*Dolichos lablab*). The same pulses have been further investigated for the inhibitor activity throughout their plant life-cycle. For comparison, the inhibitor activity residing in equal amounts of cotyledons, sprouts and skins of the germinating grains and also of roots, stems, leaves, legumes, etc., of the growing plants has been determined at three stages of development.

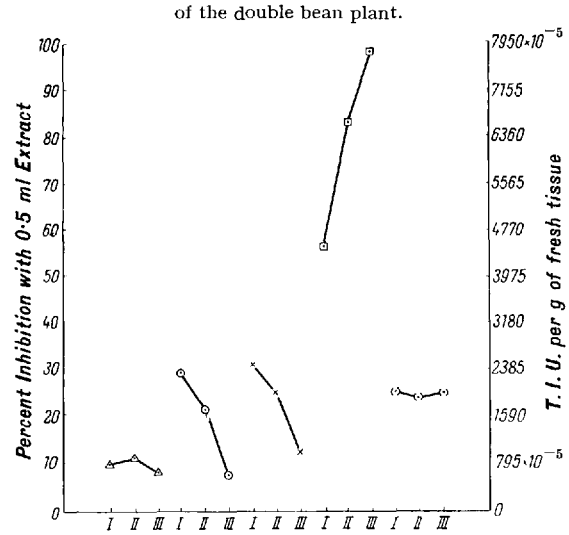
Fig. 1.–Trypsin inhibitor activity of various parts of the germinating double bean and field bean.



[1] K. SOHONIE and K. S. AMBE, Nature *175*, 508 (1955).

The various plant organs were extracted under identical conditions with dilute hydrochloric acid and the inhibition caused due to equal portions of extracts were evaluated. A method similar to that described by ANSON[2] was employed using digestion mixtures of skimmed milk and commercial trypsin (Merck's) at pH 7·6, at 37°C. The inhibitor activities of the various parts of the germinating pulses and their plants have been presented graphically (Fig. 1, 2. 3).

Fig. 2.–Trypsin inhibitor activity of various organs of the double bean plant.



Plant Organs at Three Stages of Development
(I = tender; II = semimature; III = mature)

It is interesting to note that the trypsin inhibitor is present in all the parts of the germinating pulses and their plants at all the stages of growth. A study of the curves depicted here reveals that the inhibitor activity in the different parts of either plant is found to be maximum in the seeds and least in the roots; the leaves, stems, etc., fall in between. The cotyledons and the sprouts show decrease in the inhibitor content with germination, while the skins do not exhibit any significant variations. On the plants, in the same organ, however, with the exception of the legume seeds, the power of inhibition diminishes with maturation. The seeds, on the other hand, show an increase in the inhibiting capacity with development. A parallel investigation on the plants of potato (*Solanum tuberosum*), sweet potato (*Ipomea batatas*), green grams (*Phaseolus aureus*) and Asiatic Yam (*Dioscorea alata*) at different stages of development has also confirmed the same trend of results. The activity of the extracts of leaf-stem portions is found to decrease with maturation whereas the tubers and the seeds exhibit a reverse phenomenon (unpublished).

These results suggest a parallelism between the changes occurring in the inhibitor activity and the protein synthesis in the plant tissues. BURSTRÖM[3] has shown that the latter declines with age; and the same seems to be true, with the exception of the seeds, in the case of the trypsin inhibitor concentrations in different parts of the plants analyzed. The seeds form the storage

[2] M. L. ANSON, J. gen. Physiol. *22*, 79 (1938).
[3] H. BURSTRÖM, Bot. Arch. [B] *30*, 1 (1943).