

At any rate it can be stated that the aged phosphorus containing 50% P^{33} gives quite a satisfactory response in comparison with P^{32} alone, and that, for autoradiography—as long as the experiments refer to the usual radiographic procedure in which the specimen is transparent and presents very little self-absorption— P^{33} (or also aged P^{32}) is preferable to pure P^{32} because the “reps” delivered are considerably less than for P^{32} .

This conclusion may be useful in all those types of investigation in which the dosage rate from the same activity of P^{32} would produce radiation damage².

I wish to thank Prof. J. S. MITCHELL for having suggested the consideration of P^{33} , for his valuable advice and for having given me hospitality in the Department of Radiotherapeutics of the University of Cambridge, where this research was carried on.

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Department of Radiotherapeutics, University of Cambridge, March 26, 1956.

Riassunto

Si è sperimentalmente dimostrato che il vecchio radiofosforo contenente il 50% di P^{33} è preferibile al comune radiofosforo P^{32} perchè, a pari attività, presenta effetto fotografico pressochè eguale e dosaggio meno dannoso.

² L. F. LAMERTON and E. HARRIS, J. fotogr. Sci. 2, 135 (1954).

Changes in Muscle Circulation Elicited by Local Heating of Hypothalamus

BARCROFT *et al.*¹ recently demonstrated vaso-motor changes in the muscle of man from changes in environ-

¹ H. BARCROFT, K. D. BOCK, H. HENSEL, and A. H. KITCHIN, Pflüger's Arch. ges. Physiol. 261, 199 (1955).

mental temperature. A decrease in muscle blood-flow of about 55% was obtained by warming the subjects. The results of REIN and SCHNEIDER², formerly criticized by many authors¹, have thus been confirmed. No evidence is, however, given as to whether the effects are elicited from skin receptors, from hypothalamic thermosensitive structures, from baroreceptive influences compensating the vaso-motor reactions in the skin, or from changes in skeletal muscle tone, which is well known to be important for heat production. Recently VON EULER and SÖDERBERG³ found that local heating of hypothalamus caused a marked decrease in gamma motor activity (tested on muscle spindle afferents by the usual method⁴ even without measurable changes in muscle tension. The aim of the present investigation therefore was to see whether hypothalamic heating would influence muscle blood-flow without any changes in muscular tone. The importance of muscle tension was also studied since it is reasonable to assume from DENNY-BROWN's work⁵, that vaso-motor effects may be reflexly elicited by stretch.

In nembutalized cats, local heating of hypothalamus was produced by high frequency alternating current (1 megacycle per s). At the tip of one of the heating electrodes, a thin enamelled advance wire was soldered to form one part of a thermocouple by which the maximal temperature induced by the current could be recorded².

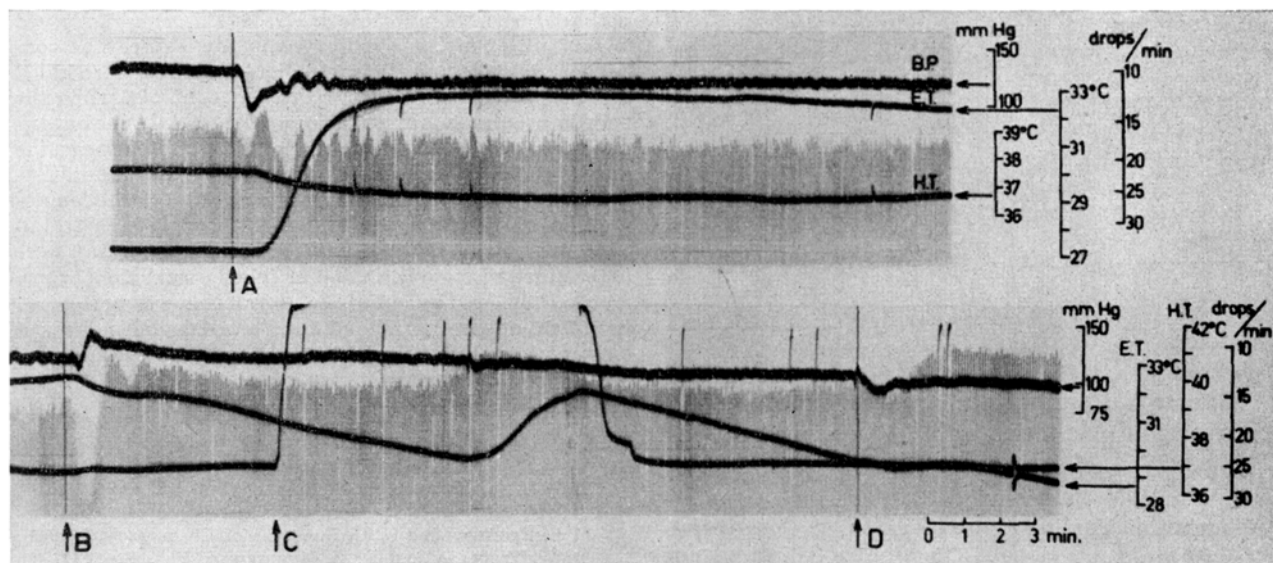
The femur was immobilized, the gastrocnemius muscle dissected free from surrounding tissues and covered with warm paraffin. The Achilles tendon was cut and attached to a sensitive strain gauge. Heparin was given and the popliteal vein cannulated. Ligations were made so that the blood from the canula came from the gastrocnemius muscle only. The blood drops from the free end of the canula were counted electrically, the

² H. REIN and D. SCHNEIDER, Z. Biol. 91, 13 (1930).

³ C. VON EULER and U. SÖDERBERG, Exper. 12, 278 (1956).

⁴ R. GRANIT and B. R. KAADA, Acta physiol. scand. 27, 130 (1952).

⁵ D. DENNY-BROWN, Proc. roy. Soc. [B] 104, 252 (1929).



Cat, 2.5 kg. “Nembutal” (40 mg/kg). BP Blood pressure from femoral artery. ET Ear skin temperature (thermo-couple on right ear). HT Hypothalamic temperature as described in the text. Vertical lines: Blood-flow from the gastrocnemius muscle. Each drop of blood from the muscle vein rapidly carries spot of mirror galvanometer to base of Figure, from which it returns at slower speed so that a steeply rising line is recorded. The length of these lines is directly proportional to time-interval between drops. By joining the upper end of the lines, one obtains inverted tracings of blood-flow, cf. calibration on the right. A 25 mg “Nembutal” intravenously. B 5 µg adrenaline intravenously. C Hypothalamic heating. D 2.5 mg chlorpromazine intravenously. Note slight decrease in muscle flow 2 min after the beginning of hypothalamic heating, coincident with vasodilatation in the skin (increased ear skin temperature).

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 baroreceptive 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 effects⁷. 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.

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

WOOLLEY and MERRIFIELD, in two consecutive papers¹, 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, pyriethamine (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 liver² or the blood pyruvate³. On the contrary, according to FROHMAN and DAY⁴, 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 MERRIFIELD⁵ 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's² 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 PY⁶, dissolved in 0.02 ml of H₂O, was administered *per os* on the first day, followed by a daily dose of 2 μ g of thiamine *per os*: the controls received only 2 μ g 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 OT⁷ *per os*, dissolved in 0.02 ml of H₂O, 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 micro-method of RINDI and FERRARI⁸: the thiamine (total) contents of the tissues (liver, muscle and brain) were determined by a modification of the classical thiochrome method⁹, 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.

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 Neopyriethamine, 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.

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