is a very complex condition[7,14,25] and casting, which does not ensure a true immobilization[7,26,27].

The observations on fibrillation development in the control muscles are reminiscent of those of Salafsky et al. on the same muscles[5]. Spinal cord section, and in some cases tenotomy or limb immobilization, caused fibrillation in S to develop more slowly and gradually than in the control muscles, thus approaching the pattern observed in the faster AT. According to many authors[17-20,22], prolonged (2–4 weeks) muscular disuse causes the slow muscle to become faster. In a few collateral experiments, we could not appreciate in vitro any change in contractile properties of S after a week of disuse (unpublished observations), but it might be suggested that the 'fast' pattern of fibrillation development represents a precocious, though indirect, evidence of the change in muscle properties. The effects of disuse on the slow components of AT[5,28] could similarly explain the slight change in fibrillation development which was observed also in this muscle.

According to the theory that a neurotrophic factor is cooperating in the control of muscle membrane properties, it would be possible also to speculate that in the disuse pretreated muscle, fibrillation, due to its earlier onset, develops when the peripheral nerve stump is still releasing the trophic substance[29], although at a progressively reduced rate[30], with a resulting slower change in membrane properties. However, this explanation is made doubtful by the fact that tenotomy and casting were practically as effective as cordotomy in accelerating the onset of fibrillation, while they did not consistently affect the subsequent development.

25 A. J. Buller and D. M. Lewis, J. Physiol. *178*, 326 (1965).
26 H. Chor and R. E. Dolkart, Am. J. Physiol. *117*, 626 (1936).
27 D. Y. Solandt, R. C. Partridge and J. Hunter, J. Neurophysiol. *6*, 17 (1942).
28 B. Smith, J. Neurol. Neurosurg. Psychiat. *28*, 99 (1965).
29 J. V. Luco and C. Eyzaguirre, J. Neurophysiol. *18*, 65 (1955).
30 R. Miledi and C. R. Slater, J. Physiol. *207*, 507 (1970).

Thiamine transport by human intestine in vitro[1]

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Summary. Surgical specimens of human gastrointestinal mucosa and muscle were incubated in vitro with thiamine-thiazole-2-^{14}C. Labelled thiamine uptake was uphill in mucosal tissues and downhill in muscle. Small intestinal mucosa accumulated labelled thiamine in a phosphorylated form, while gastric and colonic mucosa, as well as muscular layers of all gastrointestinal segments studied, did not.

Small intestine of several animal species is able to absorb in vivo[2-8] or to transport in vitro[9-15] small amounts of thiamine by an active mechanism[16]. In man, intestinal absorption of thiamine was studied exclusively in vivo[17-20]. The results, recently confirmed by Thomson[21,22] and Levy[23], suggest that, in human intestine, thiamine absorption involves a process saturable by low concentration of vitamin. However, a direct demonstration of an active transport mechanism could be better achieved by using an in vitro technique. This prompted us to choose, in the present research, an in vitro tissue uptake procedure for studying thiamine transport by human gastrointestinal tissue incubated with labelled thiamine. The aim of this investigation was to study: the ability of human intestinal tissue to accumulate labelled thiamine; the contribution to accumulation both of mucosal and muscular layers of gastrointestinal wall; the topographic distribution of the accumulation system along the alimentary canal, particularly the small intestine; the chemical form of thiamine which is accumulated and the functional meaning of accumulation as related to different intestinal segments.

Materials and methods. Specimens of sound gastric and intestinal (duodenum, jejunum, ileum and transverse colon) tissue were obtained from patients at the time of surgical resection for gastric and intestinal ulcer and tumors. Upon removal, the tissues were placed in cold (3–5 °C) Krebs-Henseleit (K-H) bicarbonate buffer, pH 7.4, equilibrated with 5% CO_2 in O_2[24]. Mucosal and muscular layers, thoroughly separated by dissection, were cut into pieces of approximately 200–300 mg and distributed to incubation flasks containing gassed K-H bicarbonate buffer. The time interval from surgical removal of tissues to the start of incubation never exceeded 30 min. Histological tests, performed by light microscope before and after incubation, showed that all tissues were

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2 A. C. Da Silva and A. C. Ivy, Am. J. Physiol. *201*, 185 (1961).
3 A. C. Da Silva, M. G. Vecchia, R. N. Rogano and R. C. De Angelis, 23rd, Int. Cong. Physiol. Sci. Tokyo 1965, p. 220.
4 U. Ventura, E. Giacalone and G. Rindi, Boll. Soc. ital. Biol. sper. *39*, 1443 (1963).
5 B. Gassmann, J. Proll and D. Lexow, Int. Z. Vitaminforsch. *37*, 203 (1967).
6 E. Sandner and B. Gassmann, Nahrung *11*, 583 (1967).
7 G. Sciorelli and U. Ventura, Acta vitamin. enzym. *23*, 231 (1969).
8 D. Polin, E. R. Wynosky and C. C. Porter, Fed. Proc. *21*, 261 (1962).
9 D. Polin, E. R. Wynosky and C. C. Porter, Poult. Sci. *42*, 1057 (1963).
10 K. R. Fox and C. A. M. Hogben, Fed. Proc. *26*, 502 (1967).
11 U. Ventura and G. Rindi, Experientia *21*, 645 (1965).
12 T. Komai, K. Kawai and H. Shindo, J. Nutr. Sci. Vitamin. *20*, 163 (1974).
13 T. Komai and H. Shindo, J. Nutr. Sci. Vitamin. *20*, 179 (1974).
14 A. M. Hoyumpa, H. M. Middleton, F. A. Wilson and S. Schenker, Gastroenterology *68*, 1218 (1975).
15 K. Schaller and H. Höller, Int. J. Vit. Nutr. Res. *44*, 443 (1974).
16 G. Rindi and U. Ventura, Physiol. Rev. *52*, 821 (1972).
17 A. S. Schultz, R. F. Light and C. N. Frey, Proc. Soc. exp. Biol. Med. *38*, 404 (1938).
18 B. Alexander, G. Landwehr and F. Mitchell, J. clin. Invest. *25*, 294 (1946).
19 T. E. Friedemann, T. C. Kmieciak, P. K. Keegan and B. B. Sheft, Gastroenterology *11*, 100 (1948).
20 A. B. Morrison and J. A. Campbell, J. Nutr. *72*, 435 (1960).
21 A. D. Thomson, H. Baker and C. M. Leevy, J. Lab. Clin. Med. *76*, 34 (1970).
22 A. D. Thomson and C. M. Leevy, Clin. Sci. *43*, 153 (1972).
23 G. Levy and R. R. Hewitt, Am. J. Clin. Nutr. *24*, 401 (1971).
24 H. A. Krebs and K. Henseleit, Hoppe-Seylers Z. physiol. Chem. *210*, 33 (1932).

normal, mucosal and muscular layers being perfectly separated and cellular structures intact. Thiamine transport was determined by means of the tissue uptake procedure of Crane and Mandelstam[25]. The tissues were incubated in 20 ml of K-H bicarbonate buffer, pH 7.4 (gassed only at the start of the incubation), for 20 min at 37 °C in a thermostatic shaker. In all tests, the K-H bicarbonate buffer contained 0.2 μM thiamine-thiazole-2-^{14}C (Radiochemical Center, Amersham, England; specific activity 0.1 mCi/1.26 mg). Both in incubation medium and tissues, labelled thiamine was extracted and determined by Rindi and Ventura procedure[26], using for the chromatographic separation of free and phosphorylated thiamine the method of Sharma and Quastel[27]. Radioactivity was measured with a Geiger-Müller low background gas-flow counter (Nuclear Chicago, mod. 512). The standard error of the measurements was less than 2% and efficiency 81%. The counts/min of each sample, corrected for background, were converted into nanomoles (nmoles) of thiamine by dividing by the counts/min of a thiamine-thiazole-2-^{14}C standard. Unlabelled (endogenous) free thiamine of surgical specimens, before incubation, was determined by a modification of the micromethod of Burch et al.[28]. The contents of all the forms of thiamine (labelled as well as unlabelled) were expressed as nmoles per ml of tissue water. In every surgical specimen, water was determined by evaporation to constant weight under vacuum. Labelled total thiamine is the sum of labelled free and phosphorylated thiamine. T/M was calculated as the ratio of labelled total thiamine concentration in tissue and medium at the end of incubation.

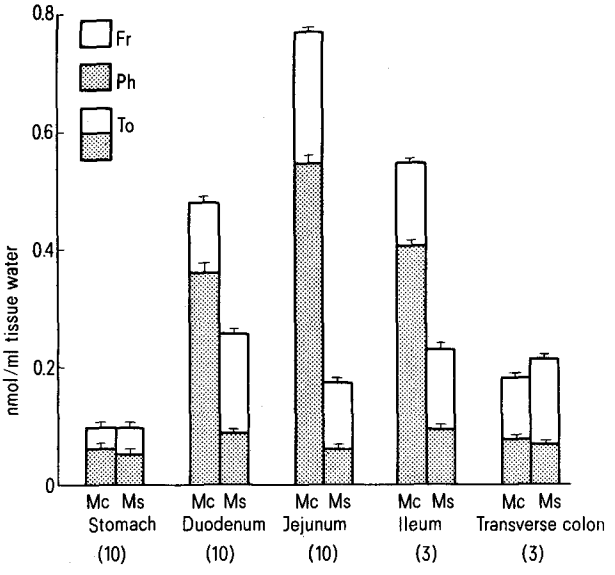

Fig. 1. Mean free (Fr), phosphorylated (Ph) and total (To) labelled thiamine content of human gastrointestinal mucosa (Mc) and muscle (Ms), after 20 min incubation in Krebs-Henseleit bicarbonate buffer, pH 7.4, containing 0.2 μM thiamine-thiazole-2-^{14}C. The vertical lines on top of each bar refer to free and phosphorylated thiamine standard errors. In brackets, numbers of surgical specimens used.

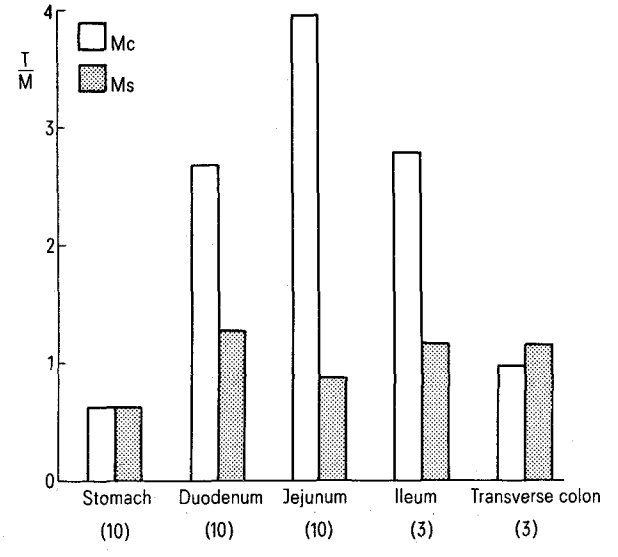

Fig. 2. Mean T/M of human gastrointestinal mucosa (Mc) and muscle (Ms) after 20 min incubation in Krebs-Henseleit bicarbonate buffer, pH 7.4, containing 0.2 μM thiamine-thiazole-2-^{14}C. T/M, nmoles labelled total thiamine of tissue/nmole labelled total thiamine of medium. In brackets, numbers of surgical specimens used.

25 R. K. Crane and P. Mandelstam, Biochim. biophys. Acta, Amst. *45*, 460 (1960).
26 G. Rindi and U. Ventura, Experientia *23*, 175 (1967).
27 S. K. Sharma and J. H. Quastel, Biochem. J. *94*, 790 (1965).
28 H. B. Burch, O. A. Bessey, R. H. Love and O. H. Lowry, J. biol. Chem. *198*, 477 (1952).

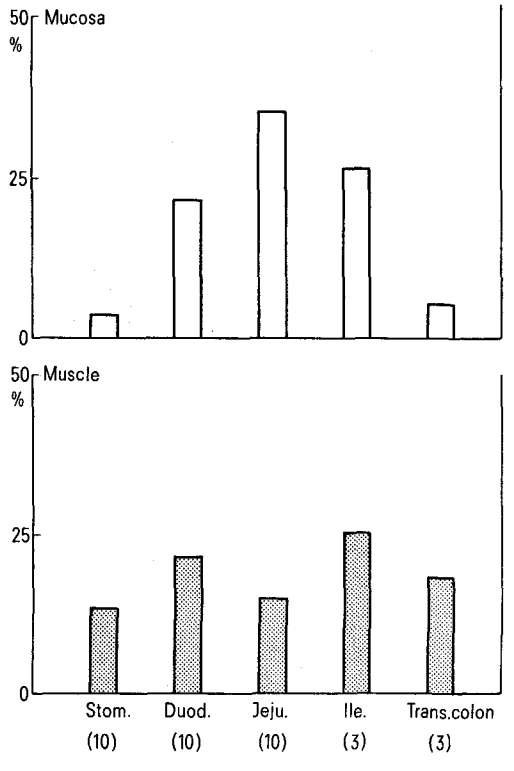

Fig. 3. Labelled phosphorylated thiamine in percentage of all the labelled phosphorylated thiamine found (i.e., sum of contents of single human gastrointestinal segments), after 20 min incubation in Krebs-Henseleit bicarbonate buffer, pH 7.4, containing 0.2 μM thiamine-thiazole-2-^{14}C. Labelled phosphorylated thiamine contents were (nmoles/ml tissue water): 1.43, in mucosa and 0.36, in muscle. In brackets, numbers of surgical specimens used.

Results and discussion. At the start of incubation, unlabelled (endogenous) free thiamine content ranged from 0.40 to 0.21 nmoles/ml tissue water in mucosa and from 0.13 to 0.17 in muscle. Therefore thiamine uptake was uphill in mucosa and downhill in smooth muscle. Only small intestine, particularly jejunum, was able to accumulate labelled thiamine during incubation: neither stomach or transverse colon could do it (figure 1). Intestinal mucosa was specifically involved in the accumulation process, its total thiamine content being constantly higher than that of the respective muscular layer (figure 1). In mucosa thiamine was accumulated in a phosphorylated form (figure 1), its content being always higher than that of free thiamine. Apparently, labelled free thiamine did not accumulate in tissue: indeed, it was almost equally distributed in both mucosal and muscular layers of the tracts studied. In muscle of every tract as well as in mucosa both of stomach and transverse colon T/M values (figure 2) were lower than, or close to, unity, while in small intestinal mucosa they ranged from 2.6 to 3.9. Phosphorylated thiamine, expressed as the percentage of all the phosphorylated thiamine found (i.e. the sum of the contents of the single gastrointestinal segments studied), had in mucosa a regular course with a maximum in jejunum, while in muscle it had an almost identical value from the stomach up to the transverse colon (figure 3).

Present results give direct demonstration of the presence of an active mechanism of thiamine transport in human small intestine, as suggested by in vivo investigations[17–23]. In this respect, human small intestine was similar to that of other animal species which accumulate thiamine in vitro[9–14], mainly in the phosphorylated form. Mucosal and muscular layers had a different power of accumulating and phosphorylating labelled thiamine. Mucosa of small intestinal segments could accumulate against a concentration gradient as well as to phosphorylate thiamine. On the contrary, muscle could phosphorylate thiamine, but not accumulate it: its total labelled thiamine content was constantly lower than, or equal to, that of incubation medium. Further, thiamine mucosal uptake was particularly efficient in jejunum. Therefore, the mechanism of labelled thiamine accumulation and phosphorylation seems to be rather specific both as to tissue type (mucosa) and to intestinal tract (small intestine).

From our results, the conclusion can be reached that the human small intestine is able to transport thiamine, in vitro, by an active mechanism involving its phosphorylation. The mechanism appears to be strictly related to the absorbing structures. The muscular layer of the entire gastrointestinal wall, as well as the mucosal layer of stomach and transverse colon, which are devoided of marked absorbing activity, were unable to accumulate labelled thiamine.

Intensification of amphetamine-induced excitation by methysergide, a serotonergic receptor blocker[1]

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Summary. Methysergide, a serotonergic receptor blocker, was studied to determine its effects against d-amphetamine-induced excitation as measured by convulsions elicited by handling in mice. Significant intensification ($p < 0.01$) of the action of d-amphetamine was observed in mice. These results indicate that reduction in serotonergic activity in the central nervous system enhances excitation induced by d-amphetamine.

Although there are many theories regarding direct or indirect mechanisms of the central excitatory action of amphetamine[2–4], a review of the past literature, tends to favor the indirect theory[5] that amphetamine acts indirectly through the release of norepinephrine (NE). Evidence supporting the indirect NE mediated theory of central amphetamine action was obtained using specific inhibitors of tyrosine hydroxylase, such as α-methyltyrosine (αMT)[6–8]. Findings of this nature showed that the stimulant effect of amphetamine could be blocked even without a marked depletion of catecholamine (CA) stores.

However, Havlicek et al.[2] provided other evidence for the direct stimulant central action for amphetamine. Havlicek and associates proposed that the release of the catecholamines (CA's) after administration of amphetamine and the excitatory effect are independent mechanisms. This suggestion is supported strongly by the findings of their laboratory that, after destruction of CA nerve endings in the brain by 6-hydroxydopamine (6-OHDA), the stimulant effects of amphetamine on locomotor activity are not affected[9]. In addition, a very drastic reduction of functional or storage CA pools in the brain after combined treatment with 6-OHDA and αMT does not inhibit amphetamine-induced excitation.

According to Havlicek et al.[2], the role of CA release induced by amphetamine could be better understood if we consider it as part of a feedback mechanism that inhibits the excessive excitation induced by amphetamine.

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2 V. R. Havlicek, R. Herchl and R. Childiaeva, in: Drug Addiction, vol. 3. Are the Brain Catecholamines Responsible for Amphetamine-Induced Excitation? Ed. J. Singh. Stratton Intercontinental, New York 1974.
3 P. J. G. Mann and J. H. Quastel, Biochem. J. *34*, 414–431 (1960).
4 L. Stein, Fed. Proc. *23*, 836 (1964).
5 H. Weil-Malherbe, Neuropharmacology *9*, 605–606 (1970).
6 A. Weissman and B. K. Koe, Life Sci. *4*, 1037–1048 (1965).
7 A. Weissman, B. K. Koe and S. S. Tenen, J. Pharmac. exp. Ther. *151*, 335–352 (1966).
8 A. Randrup and I. Mankrad, Nature *211*, 540 (1966).
9 V. Havlicek, in: The Mechanisms of Central Amphetamine Action. Winnipeg 1971.