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.

Mean conversion score ± S. E. Treatment	Hours after drug treatment				
	1	2	3	4	5
CSF + amphetamine 50 μg/animal i.c.	0.8 ± 0.13	1.0 ± 0.15	0.9 ± 0.27	0.8 ± 0.29	0.9 ± 0.31
Methysergide 10 μg/animal i.c. +amphetamine 50 μg/animal i.c.	1.5 ± 0.31*	2.2 ± 0.36**	1.5 ± 0.34	1.0 ± 0.26	1.0 ± 0.21

Each value represents 10 mice and level of significance by Student's t-test is indicated by asterisks; *$p < 0.05$, **$p < 0.01$. Cerebral spinal fluid (CSF) was administered 30 min prior to amphetamine and in equivalent amounts to methysergide.

Amphetamine is known to release dopamine (DA) from dopaminergic neurons[10]; and released DA could enter into serotonergic neurons and release serotonin (5HT)[11], which may serve as the inhibitory modulator, as suggested by its effects as a central depressant. The purpose of this communication is to report our findings with regard to the effects of methysergide, a 5HT receptor blocker, on amphetamine induced convulsions elicited by handling in mice.

Methods. Male Swiss-Webster mice between 19 and 25 g were used as subjects throughout this investigation. They were obtained from Texas Inbred Co. and were stabilized in the animal colony for at least a week before being used. The mice were allowed ad lib access to food and were kept on a 12-h-light-dark-schedule. All testing was done during the light period.

Subjects were intracerebrally injected with amphetamine or artificial cerebral spinal fluid (CSF) according to the procedure of Haley and McCormick[12]. The landmarks used for locating the site of injection were strictly adhered to and we noted the same behavioral effects: quietness for 1 min followed by normal activity after sham injections, injections of physiological saline and artificial CSF. A 27-gauge-needle was used in the injection procedure.

Subjects were assigned randomly 10 to a group and were divided into 4 groups and injected with either CSF alone, CSF plus amphetamine (50 μg/animal i.c.) and methysergide (10 μg/animal i.c.) plus CSF and methysergide (10 μg/animal i.c.) plus amphetamine (50 μg/animal i.c.). In these animals AMP was injected 30 min following the injection of methysergide and convulsions were recorded. The doses of each drug were calculated as the free base rather than as the salt form and at every dose 10 μl was the injected volume. All drugs were dissolved in CSF.

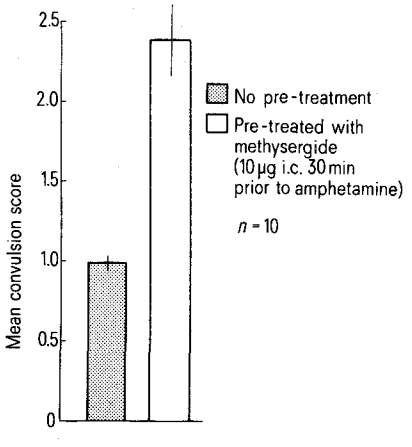
The formula for artificial CSF is: NaCl, 8.98 g/l; KCl, 0.25 g/l; $CaCl_2$, 0.014 g/l; $MgCl_2$, 0.11 g/l; NaH_2PO_4, 0.066 g/l; urea, 0.13 g/l, and glucose, 0.61 g/l. The pH of the solution was adjusted to 7.0 with 0.1 N NaOH and this formulation served as the CSF control.

At intervals of 1 h, for 5 h following these injections, each mouse was tested and scored for 'convulsions on handling' according to the technique described by Goldstein[13]. Briefly, the scoring is as follows:

The observer gently lifts a subject (S) by holding onto the tip of its tail and then looks for a tonic convulsion characterized by the tightening of the facial muscles, flexion of the forelegs, lateral extension of the hindlegs and generalized body tremor. If these signs are observed upon gently lifting the S, then a 'convulsion' behavior has been seen and a score of 3 is assigned to that S. If no 'convulsion' behavior is seen, the S is lifted through a vertical plane of about 3 cm as an additional inducer of the 'convulsion' behavior. If convulsions are observed, a score of 2 is assigned for that S. Failure to observe the behavior after this stage signals the experimenter to spin the S gently through an arc of 180°. If the mouse then 'convulses', a score of 1 is assigned for it. Failure of these handling procedures to produce any 'convulsion' behavior is recorded as zero.

Results. 10 untreated mice had a mean seizure score of 0.26 ± 0.06 and 10 mice receiving artificial CSF alone had a mean score of 0.32 ± 0.07. Mice treated with methysergide at 10 μg/animal i.c. in conjunction with CSF had a mean seizure score of 0.12 ± 0.05 calculated over a 5-h-period. 10 mice treated with methysergide at 10 μg/animal administered intracerebrally 30 min prior to an intracerebral injection of amphetamine at 50 μg/animal had a mean seizure score of 1.44 ± 0.22 whereas animals treated with CSF 30 min prior to a similar injection of amphetamine had a mean seizure score of 0.88 ± 0.37.

The table shows further the effects of methysergide on d-amphetamine-induced convulsions in mice. A significant increase of the convulsive response was obtained at the 1st ($p < 0.05$) and 2nd ($p < 0.01$) h following drug



Effects of methysergide on amphetamine-induced convulsive responce elicited by handling in mice. Methysergide □ injected intraperitoneally at 10 mg/kg 30 min prior to d-amphetamine intracerebrally injected at 50μg/animal ▨. Saline injected intraperitoneally (same volume as methysergide) 30 min prior to d-amphetamine intracerebrally injected at 50μg/animal. At least 10 mice were used in each drug treatment group. Vertical bars indicate standard error of the mean. Convulsive mean score for methysergide plus amphetamine and CSF control plus amphetamine were 2.2 ± 0.36 and 1.0 ± 0.15 respectively.

10 J. Glowinski, in: Effects of Amphetamine on Various Aspects of Catecholamine Metabolism in the Central Nervous System of the Rat. Raven Press, Milan 1970.
11 K. Y. Ng, T. N. Chase, R. W. Colburn and I. J. Kopin, Science *172*, 487 (1971).
12 T. J. Haley and W. G. McCormick, Br. J. Pharmac. *12*, 12–15 (1957).
13 D. B. Goldstein, Psychopharmacologia *32*, 27–32 (1973).

injections. However, although significance was not reached from the 3rd to the 5th h, the convulsive score was higher for methysergide-treated mice compared to control mice receiving CSF prior to an injection of d-amphetamine.

The figure illustrates the peak intensification effect of methysergide on amphetamine-induced convulsions elicited by handling in mice. After 2 h following drug administration, mice pretreated with methysergide (10 µg/animal i.c.) plus amphetamine (50 µg/animal i.c.) had a mean seizure score of 1.0 ± 0.15. These results indicate that methysergide intensified the convulsive response of amphetamine by 63%. These findings provide evidence for an inhibitory role of 5HT in the amphetamine-induced convulsive response.

Discussion. The findings of this study indicate that interruption of serotonergic receptor activity by utilization of methysergide, a serotonergic receptor blocker, results in an enhanced convulsive response induced by d-amphetamine in mice.

It is important to point out that amphetamine has a direct action on serotonergic receptors in a variety of smooth muscles[14,15]. In addition, our findings are in complete agreement with previous experiments reporting enhancement of amphetamine action after interruption of ascending serotonergic pathways[16].

As previously discussed, there has been considerable controversy concerning the mechanisms of amphetamine action on behavior. Along these lines, Havlicek[2] suggested that possibly CA's released by amphetamine may be part of a feedback mechanism that inhibits excessive excitation induced by the direct action of amphetamine. The possibility exists that DA released by amphetamine enters serotonergic neurons[11] and release 5HT which may serve as the inhibitory modulator.

There is considerable evidence that 5HT exerts an inhibitory effect on a variety of behaviors. The depletion of 5HT by lesions or drugs leads to an enhanced wakefulness[17], enhanced lever pressing for intracranial stimulation[18], and enhanced responsiveness to painful stimuli[19]. An alternative explanation is based on the possibility that amphetamine exerts its primary action on the catecholaminergic system. If we assume this to be the primary mechanism of amphetamine-induced excitation, then the released CA's are normally under the inhibitory control of the serotonergic system. Thus, the depletion of 5HT by p-chlorphenylalanine[20], Medial Forebrain Bundle (MFB) lesions or serotonergic receptor blockade (obtained in these studies), reduces the inhibitory influence and allows amphetamine to exert a stronger effect on behavior.

Nevertheless, our experiments further suggests that a full understanding of the behavioral effects of amphetamine must take into account the role of the serotonergic system in the central action of amphetamine.

14 J. R. Vane, in: The Actions of Sympathominetic Amines on Tryptamine Receptors. Little, Brown and Co., Boston 1960.
15 I. R. Innes, Br. J. Pharmac. Chemother. *21*, 427–435 (1963).
16 T. K. Green and J. A. Harvey, J. Pharmac. exp. Ther. *190* (1), 109–117 (1974).
17 M. Jouvet, in: Serotonin and Sleep in the Cat. Academic Press, New York 1973.
18 B. P. H. Poschel and F. W. Ninteman, Physiol. Behav. *7*, 39–46 (1971).
19 J. A. Harvey and L. M. Junger, in: Relationship Between Telencephalic Content of Serotonin and Pain Sensitivity. Academic Press, New York 1973.
20 P. D. Mabry and B. A. Campbell, Brain Res. *49*, 381–391 (1973).

Cyclic nucleotide levels in the perfused rat heart subjected to hypoxia

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Summary. Isolated rat hearts were subjected to hypoxic perfusion on a recirculating Langendorff apparatus. Following a 30-min-period of aerobic stabilization the hearts were perfused for 30 min with media equilibrated with 84% N_2, 12% O_2 and 4% CO_2. At the end of the hypoxic period myocardial concentrations of cyclic AMP and cyclic GMP were determined by radioimmunoassay. Exposure to hypoxia resulted in a significant increase in cyclic AMP ($p < 0.01$) and a decrease in cyclic GMP ($p < 0.05$) as compared to hearts perfused for 60 min with media gassed with 96% O_2, 4% CO_2.

The conversion of a stimulus such as work overload, ischemia or hypoxia to a biochemical signal initiating the increase in RNA and protein synthesis observed in cardiac hypertrophy is not clear. One of the earliest events noted in models of hypertrophy such as pressure overload[3], and cardiomyopathy[4] is an increase in adenylate cyclase activity. The isolated perfused rat heart preparation subjected to hypoxia has been used in this laboratory in series of studies aimed at clarifying the possible sequence of events leading to cardiac hypertrophy. We have demonstrated that exposure of the perfused heart to 30 min of hypoxia results in a 60–100% increase in myocardial RNA synthesis following reoxygenation[5]. The purpose of the present study was to determine whether cyclic nucleotide levels are altered in hearts subjected to 30 min of hypoxia, preceding the increase in RNA synthesis observed in this model.

Materials and methods. Hearts from 230–250 g male Wistar rats (Charles River Laboratories) were perfused on a modified recirculating Langendorff apparatus as

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3 S. S. Schreiber, I. L. Klein, M. Oratz and M. A. Rothschild, J. Mol. Cell. Cardiol. *2*, 55 (1971).
4 K. P. Nair, T. Umali and J. Potts, Am. J. Cardiol. *32*, 423 (1973).
5 L. Gibb, S. P. Bishop, R. Nesher, W. F. Robinson, A. J. Berry and F. A. Kruger, J. Mol. Cell. Cardiol. *8*, 419 (1976).