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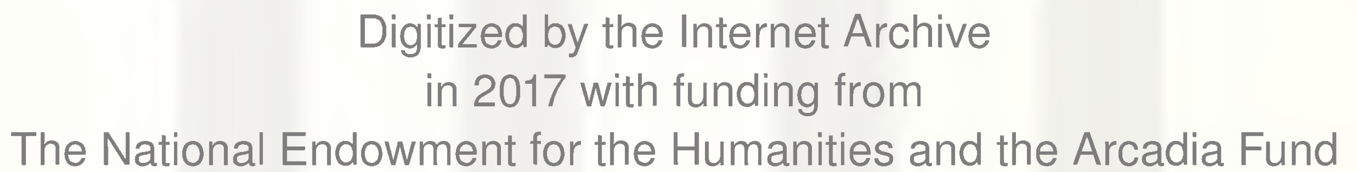
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STUDIES OF THE INHIBITION OF HISTIDINE DECARBOXYLASE *IN VIVO* BY NOREPINEPHRINE AND DOPA

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Histamine is a biologically active amine abundant in many tissues of both animals and man. It has been suggested that histamine plays an essential role in many physiologic processes and responses such as gastric secretion, tissue growth and repair (1, 2) and reaction to tissue damage. Furthermore, histamine has been implicated in several pathological conditions such as peptic ulcer, asthma, allergies, anaphylactic shock, histamine headache, and urticarial reactions (3, 4). Finally, it has been suggested that histamine may function as a neurotransmittor (5, 6); this possibility will be discussed in some detail below.

Despite the many suggested physiologic and pathologic roles of histamine, the factors controlling its synthesis are not completely understood. The mechanism of histamine synthesis by decarboxylation of histidine is well known. Histidine decarboxylase, with pyridoxal phosphate as cofactor, is generally considered responsible for synthesizing the majority of tissue histamine. Most workers agree that there are at least two types of histidine decarboxylases. Evidence for the existence of specific histidine decarboxylase, the prototype of which is prepared from rat fetuses, and non-specific aromatic L - amino acid decarboxylase, the prototype of which may be extracted from guinea pig kidney, has been discussed at length by several authors (7,8,9, 10). There may be, however, more than two enzymes capable of decarboxylating histidine, although all known mammaliam histidine decarboxylases are similar to these two (7). A complete discussion of this aspect of histamine synthesis is not within the scope of this paper. In the rat histamine synthesis is primarily

catalyzed by the specific enzyme (7, 8).

Apparently there are at least three pools of histamine in the body, and there may be several. Examples are the histamine in mast cells which has a half life of approximately 50 days (11), the rapidly turning-over pool of histamine in the gastric and intestinal mucosa with a half life of less than 3 hours (12), and a slowly turning-over tissue pool of histamine not in mast cells (12).

Important as it is, the knowledge of the mechanism of histamine synthesis and physiological pools in which histamine exists does not unveil the factors which are directly responsible for the rate of histamine synthesis and thus the quantity of histamine available at any given time for biological activity. In 1959 Schayer (13) reported that when histamine was released from rats by compound 48/80, there followed shortly thereafter an increased histamine forming capacity (HFC) in the skin. The term HFC was used to imply either the production of more histidine decarboxylase or simply the greater activity of histidine decarboxylase. Since histamine release by 48/80 correlated well with degranulation of mast cells, Schayer postulated that the increased HFC was due either to increased mast cell mitosis (production of more histidine decarboxylase) or release of inhibition of histidine decarboxylase (greater histidine decarboxylase activity). Schayer suggested that this inhibition of histidine decarboxylase, if it occured, might be due to a part of the granule containing the histamine, or something contained in the granule such as serotonin or histamine itself. In 1964 Kahlson reported increased HFC in gastric mucosa following histamine depletion with gastrin pretreatment (14). Since gastric mucosa contains no mast cells, Kahlson went a step further than Schayer and suggested that it

was indeed histamine that inhibited its own synthesis. Since then Kahlson has continued to promote the concept of a negative feedback control over histamine synthesis based primarily on much further evidence that whenever histamine is released from a tissue, HFC increases. He has recently reported this phenomenon to occur during the natural release of histamine in anaphylactic shock (15).

Although the negative feedback theory for the control of histamine synthesis seems attractive as an explanation of the increased HFC in a tissue following histamine release, definite proof for or against the theory was lacking until Levine (16) showed that high concentrations of histamine do not inhibit histidine decarboxylase _in vitro_. This evidence does not completely rule out the possibility that histamine inhibits tissue HFC, but it makes the simple explanation of such an inhibition working directly through the synthesizing enzyme quite unlikely.

Evidence has been reported that naturally-occurring inhibitors of histidine decarboxylase activity may exist in gastric tissue of the rat (14). The precise nature of these inhibitors is not known. When histamine is released from tissue, other amines also may be released (17), and Levine (10) suggested that perhaps another amine was responsible for histidine decarboxylase inhibition. Such a relationship seemed quite reasonable in that many of the physiologic responses to amines such as serotonin, epinephrine, and norepinephrine (NE) are antagonistic to those of histamine (3, 18). Levine studied this possibility _in vitro_ (10) and found that the naturally occuring amines, NE and dihydroxyphenyl ethylamine (dopamine), and the amino acid dihydroxyphenylalanine (dopa) effectively inhibited histidine decarboxylase in concentrations of 5×10^{-3}M. At this concentration serotonin inhibited histidine decar-

boxylase only 19%, while epinephrine, histamine, tyramine, and tyrosine had less than a 5% inhibitory effect. NE, dopamine, and dopa, on the other hand, caused 100% inhibition, which was felt to be reversible with the "characteristics of competition with the cofactor, pyridoxal-phosphate, and also with substrate."

NE is the neurotransmitter for most post-ganglionic synapses of the sympathetic nervous system and thus plays an essential role in many physiologic processes. With the finding that NE inhibits specific histidine decarboxylase _in vitro_, it becomes of great interest whether NE inhibits histamine synthesis _in vivo_ and what effect such an inhibition would have on normal physiology.

Code (16) has recently reviewed the evidence and concluded that histamine is very likely a chemical mediator of gastric secretion during vagal stimulation. Levine (19) has corroborated this by showing that histamine is essential for gastric secretion. It has also been strongly suggested that histamine mediates active reflex vasodilitation (5). These studies allow for speculation on the possibility that histamine may function as a neurotransmitter for portions of the parasympathetic system.

The sympathetic nervous system is generally antagonistic to functions of the parasympathetic system, and it seems not unreasonable that this antagonism might be mediated through inhibition of synthesis of a parasympathetic neurotransmitter by NE, the sympathetic neurotransmitter.

If the assumption is made that histamine functions as a neurotransmitter for portions of the parasympathetic system, the missing link in this sympathetic-parasympathetic antagonism theory becomes the basis

for this paper, *i.e.*, does NE functionally inhibit histamine synthesis *in vivo*.*

An attempt was made to raise and lower tissue levels of NE in heart and stomach by pharmacologic means. If NE does indeed inhibit histamine synthesis *in vivo*, then one would expect to see an inverse relationship between tissue levels of NE and tissue and urine levels of histamine. However, changes in tissue histamine accompanied by opposite changes in urinary histamine would suggest that NE affects release of histamine rather than synthesis of histamine. For example, if increased tissue NE inhibited histidine decarboxylase, both tissue and urinary histamine would decrease. However, if increased tissue NE caused release of histamine, tissue histamine would decrease while urinary histamine would increase. Tissue and urinary histamine did not change as predicted in response to changes in NE. On the basis of *in vitro* studies (10) showing dopa to be an effective inhibitor of histidine decarboxylase, dopa was given to rats in an attempt to inhibit histamine synthesis *in vivo*. Administration of dopa likewise failed to alter urinary histamine.

MATERIALS AND METHODS

Female Sprague-Dawley rats weighing between 135 and 155 grams were used in all experiments. In order to raise NE levels in heart and stomach, single intraperitoneal injections of pargyline hydrochloride

*Admittedly, answering this question would not definitively rule in or rule out the antagonism theory since NE would not only have to physiologically inhibit histamine synthesis *in vivo*, but would have to do so at the nerve endings of tissues exhibiting the antagonism phenomenon and, furthermore, do so in a length of time compatible with observed physiologic changes in these tissues.

(MO - 911) dissolved in sterile isotonic saline in a dose of 50 mg of base compound per kg body weight were given. All rats received 1.5 ml of either pargyline solution or sterile saline (controls). Controls, receiving only sterile saline injections, were included in all experiments. Forty-eight hours prior to treatment the rats were switched from normal diets to a sucrose and saline diet (8% sucrose and 0.2% saline) (20). The sucrose and saline diets were given to allow for empty stomachs and a narrower range of values for both NE and histamine in gastric mucosa. At various time intervals following drug injection, all rats, including controls, were sacrificed by a blow on the head. The glandular portion of the stomach and the heart ventricles were quickly removed, weighed, and frozen. Tissues from treated and control rats were assayed simultaneously for NE.

The same procedure was repeated using alpha-methyl tyrosine (@-MT) to lower tissue NE (27). The only differences in the procedure from that described above for pargyline were that the dose of @-MT was suspended in the 1.5 ml of saline with 0.07 ml of a 20% solution of Tween-80. It was necessary to administer @-MT in this suspension since solution cannot be effected at pH greater than 1.5 or less than 9.0. It was felt that solutions of these pH extremes might alter tissue NE levels and/or histamine levels by stress or direct irritation.

Using the graphs plotting tissue levels of NE against time following drug injection (figs. 1, 2), the injections of pargyline and @-MT were repeated, this time measuring levels of tissue histamine.

Then pargyline and @-MT were again administered and the rats were placed in metabolic cages and fed only water. Three hours after drug injection a 24 hour urine collection was begun. Each urine sample was

collected in a glass beaker with 0,5 ml of 3N HCl added to stabilize the urinary histamine.

Next urinary histamine was measured under conditions just described except that the rats were fed normal diets.

Finally dopa was given intraperitoneally in 3 doses of 120 mg/kg 8 hours apart. A 24 hour urine collection was begun one hour after the first injection, and urinary histamine was measured.

ASSAYS

All assays were of a fluorometric type. An Aminco-Bowman spectrophotofluorometer was employed to measure fluorescence. Tissue NE was assayed by the method of Crout, *et al.* (21) using iodine as the oxidizing agent in the final process for effecting fluorescence. Tissue histamine was measured by the method of Shore, *et al.* (22) with the substitution of H_3PO_4 for HCL to terminate formation of the fluorescing compound (23). Urinary histamine was assayed by the method of Oates, *et al.* (9) with the same modification described for the tissue histamine assay.

RESULTS

Tissue levels of NE were successfully increased and decreased as shown in figs. 1 and 2. The pargyline dose of 50 mg base per kg body weight was chosen on the basis of previous work (24) showing this to be the optimal dose for raising concentrations of tissue NE. This finding was confirmed in preliminary studies. Higher doses of pargyline result in less than peak elevations in NE, due perhaps to a release phenomenon (24). The suspension of α-MT in Tween-80 was apparently well absorbed since tissue levels of NE were significantly decreased and none

of the suspension was seen in the peritoneal cavity 8 hours after injection. Despite the alterations in tissue NE, no changes in tissue histamine were found (Table 1). Also, urinary histamine remained unchanged after administration of @-MT (Table 2). However, after treating with pargyline, urinary histamine content increased (Table 2). Those rats which were fed a normal diet excreted the same amounts of histamine as the rats fed a sucrose and saline diet although the variation among rats was greater. Intraperitoneal dopa had no effect on urinary histamine (Table 3).

DISCUSSION

If these _in vivo_ findings had correlated with Levine's (10) _in vitro_ findings, an inverse relationship should have been obtained between tissue levels of NE and histamine. That is, high concentrations of NE should have inhibited histidine decarboxylase, resulting in a low histamine content and vice versa. In view of the _in vitro_ studies just referred to, I shall assume that under proper conditions NE can inhibit histidine decarboxylase. Thus it would seem that there are two major possible explanations for the results of this study. First, histamine synthesis was indeed affected, but, for various reasons which will be discussed, the effects were not detected by the methods utilized. Second, histamine synthesis was not affected in these experiments.

First Explanation: Histamine synthesis was affected.

Perhaps tissue content of NE was not changed enough. The pool of NE most likely to affect histamine synthesis if a relationship exists between the sympathetic and parasympathetic system is that pool at the adrenergic nerve endings. This pool represents a small fraction of the total NE measured in these experiments. Thus the gross changes in tissue

NE brought about in these studies may not reflect a significant change in the NE at nerve endings. Perhaps during maximal sympathetic system stimulation NE levels at nerve endings transiently rise far outside the usual range.

Tissue histamine concentration also remained unchanged when NE levels were decreased. This would seem to indicate that under relatively normal physiologic conditions NE does not inhibit histidine decarboxylase or histamine levels would have been increased when NE was decreased. From this we may conclude that if NE were the sole inhibiting factor in histamine synthesis, histamine would be manufactured at maximal rates when the usual concentrations of tissue NE prevail. This seems very unlikely in view of Kahlson's work (14, 15) showing that histamine forming capacity goes up markedly following various stimuli. Again, however, we have the problem of whether or not NE levels were decreased sufficiently. I know of no way to resolve this question other than to refer to the NE levels of the untreated animals which were consistently above those of the treated animals.

One might postulate that, as histamine synthesis was blocked, histamine catabolism and release mechanisms adapted to the new situation, causing the net amount of histamine in a tissue to remain constant. However, any change in tissue histamine release or catabolism rates should have been evident in urinary excretion of histamine. In fact, no change in urinary excretion of histamine occurred when tissue NE was decreased, and urinary histamine increased rather than decreased when tissue NE was elevated.

Unfortunately, however, urinary histamine measurements do not entirely reflect the amount of histamine produced by the animal's tissues

since over half of the histamine excreted in rat urine is produced in the gut by bacteria which decarboxylate ingested histidine (25). On the other hand, averages of twenty-four hour urinary histamine measurements of groups of rats remained quite constant from day to day, which tends to make more attractive the idea that urinary histamine should reflect any changes in tissue release of histamine. Female Sprague-Dawley rats were chosen for these experiments because they excrete a significant portion of their histamine unchanged (25). This greatly facilitated measurement since assays of histamine metabolites are quite tedious. If urinary histamine excretion does reflect accurately that histamine released from tissues and thus indirectly that histamine manufactured by the animal, the twenty-four hour urinary measurement would be likely to detect changes in histamine production too small to be detected by measuring histamine in a single tissue. Urinary histamine excretion did not change after treatment with @-MT, and, thus, I must conclude that when NE levels were reduced, histamine synthesis was not affected.

When pargyline was administered, urinary histamine excretion increased. Since pargyline raises tissue NE levels, histamine concentration in tissues and urine were expected to decline rather than increase if NE inhibited histidine decarboxylase *in vivo* as it has been shown to do *in vitro*. Histamine levels in the tissues did not change after pargyline treatment, and, thus, the increase of urinary histamine must be explained by a mechanism other than the effects of NE or pargyline on production or release of histamine from tissues. Pargyline was selected for the experiments over other monoamine oxidase inhibitors because it reportedly does not inhibit diamine oxidase and thus should not interfere with histamine metabolism. If, however, pargyline does

inhibit diamine oxidase as do the various hydrazine monoamine oxidase inhibitors, the formation of imidazole acetic acid and its riboside would have been blocked in these experiments. With this metabolic pathway blocked presumably more histamine would be excreted in the unaltered form rather than as a metabolite. Since only free urinary histamine was measured in this study, blocking diamine oxidase would have resulted in a falsely elevated reflection of total urinary histamine excretion. Further studies should be done to determine if pargyline does indeed inhibit diamine oxidase.

Histamine is metabolized by two major pathways (3). One pathway involves methylation of histamine and then oxidation by monoamine oxidase. The other pathway utilizes diamine oxidase and is not dependent upon monoamine oxidase. Pargyline would be expected to block the pathway dependent upon monoamine oxidase, but presumably the only result would be a shunting of more histamine to the pathway not utilizing monoamine oxidase. It is possible, however, that blocking only one major metabolic pathway could lead to the excretion of more histamine in the free form. This again would lead to misinterpretation of the total excreted histamine as measured in these experiments.

<u>Second Explanation: Histamine synthesis was unaffected in these Experiments.</u>

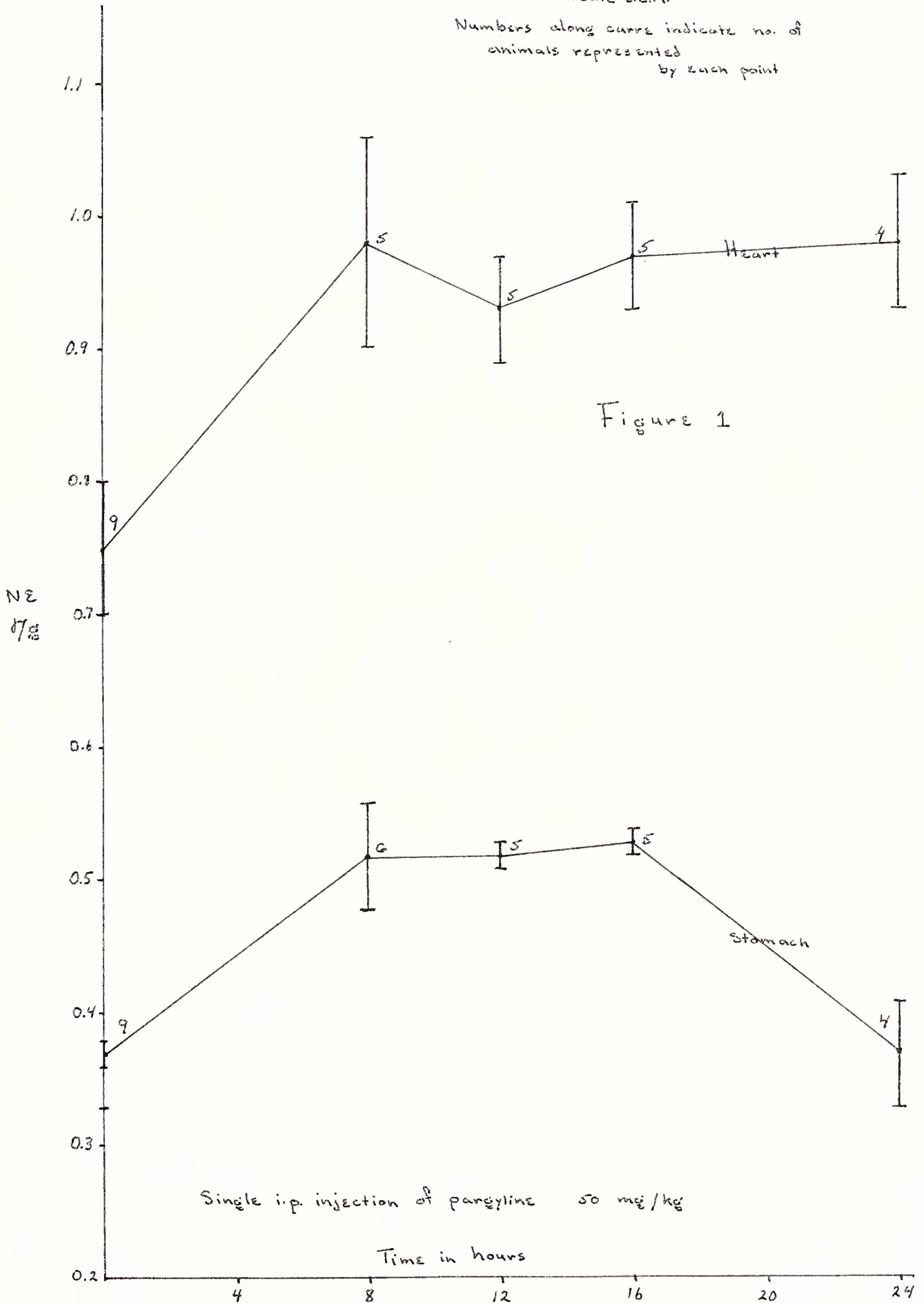
It is rather widely accepted that the NE which serves as neurotransmitter for the sympathetic system is confined to granules near the nerve ending until an impulse reaching the nerve ending releases the NE from the granules (26). What is not known is what portion of the total NE measured in a piece of tissue such as heart or stomach is confined to granules. If in the sympathetically unstimulated tissue the

majority of NE resides in granules, then it seems likely that administering a drug such as pargyline would serve to increase the number of NE containing granules or further saturate existing granules. In either case the increased amounts of NE would be unavailable to any histidine decarboxylase which, as far as is known, exists only in the soluble fraction of the cell. The same, of course, would be true for decreased amounts of NE caused by inhibition of NE synthesis by @-MT. In the usual physiologic situation, however, sympathetic stimulation causes release of NE from the storage granules. It seems reasonable that NE released from the granules would be more likely to come into contact with tissue histidine decarboxylase than NE confined to the granules, no matter what its concentration. If histamine is a neurotransmitter for certain parasympathetic nerves and if NE does inhibit histamine synthesis in vivo, it would seem plausible for such inhibition to occur following sympathetic stimulation and granule release of NE. More plausible in that, as previously mentioned, many effects of sympathetic stimulation are opposite to those of parasympathetic stimulation. For example, sympathetic stimulation causing release of NE in stomach wall would result in decreased histamine production and lowered gastric acid. A change in the total amount of NE in the stomach without release from storage granules would not affect histamine synthesis. Such an hypothesis should certainly be tested experimentally. One possibility would involve isolation of an organ such as a heart or stomach with an intact sympathetic nerve attached. The tissue would be placed in a nutritive physiologic environment to maintain viability. Radioactive histidine placed in this environment would be taken up by the tissue and decarboxylated to form radioactive histamine which could be measured. Stimulation of the attached sympa-

thetic nerve would release NE to inhibit distidine decarboxylase. Controls without stimulation of the nerve would, of course, be included. By using labeled histidine true synthesis could be measured rather than just changes in total histamine content which might be altered by histamine release or block of release. A system such as this would be rather tedious to set up, but should provide more definitive answers.

An easier but far less exact experiment might be performed using an intact animal and causing sympathetic stimulation followed by measurement of tissue and urinary histamine. Disadvantages of such an experiment would include variable sympathetic stimulation from animal to animal and unknown effects of stress on histamine metabolism and release.

Finally, a word must be said about the administration of dopa. Dopa is metabolized *in vivo* to dopamine and norepinephrine (18). *In vitro* dopa and its metabolites dopamine and norepinephrine were all found to be good inhibitors of histidine decarboxylase (10). By giving dopa intraperitoneally, one would have expected to have loaded the rats with potent histidine decarboxylase inhibitors. This method would seem to circumvent many of the possibilities discussed above in explaining why altering tissue levels of NE pharmacologically did not affect histamine synthesis. Dopa and dopamine exist normally in the soluble fraction of the cell (18) and would be available to affect histidine decarboxylase. The fact that the administration of dopa did not result in decreased urinary histamine is evidence that either the methods employed in these experiments were not suitable for detecting any effect on histidine decarboxylase or, for reasons which are not apparent, those compounds found to inhibit histidine decarboxylase *in vitro* do not operate as inhibitors *in vivo*.


Brackets indicate S.E.M.
Numbers along curve indicate no. of animals represented by each point
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
NE γ/g
Heart
Figure 1
Stomach
Single i.p. injection of pargyline 50 mg/kg
Time in hours
4
8
12
16
20
24

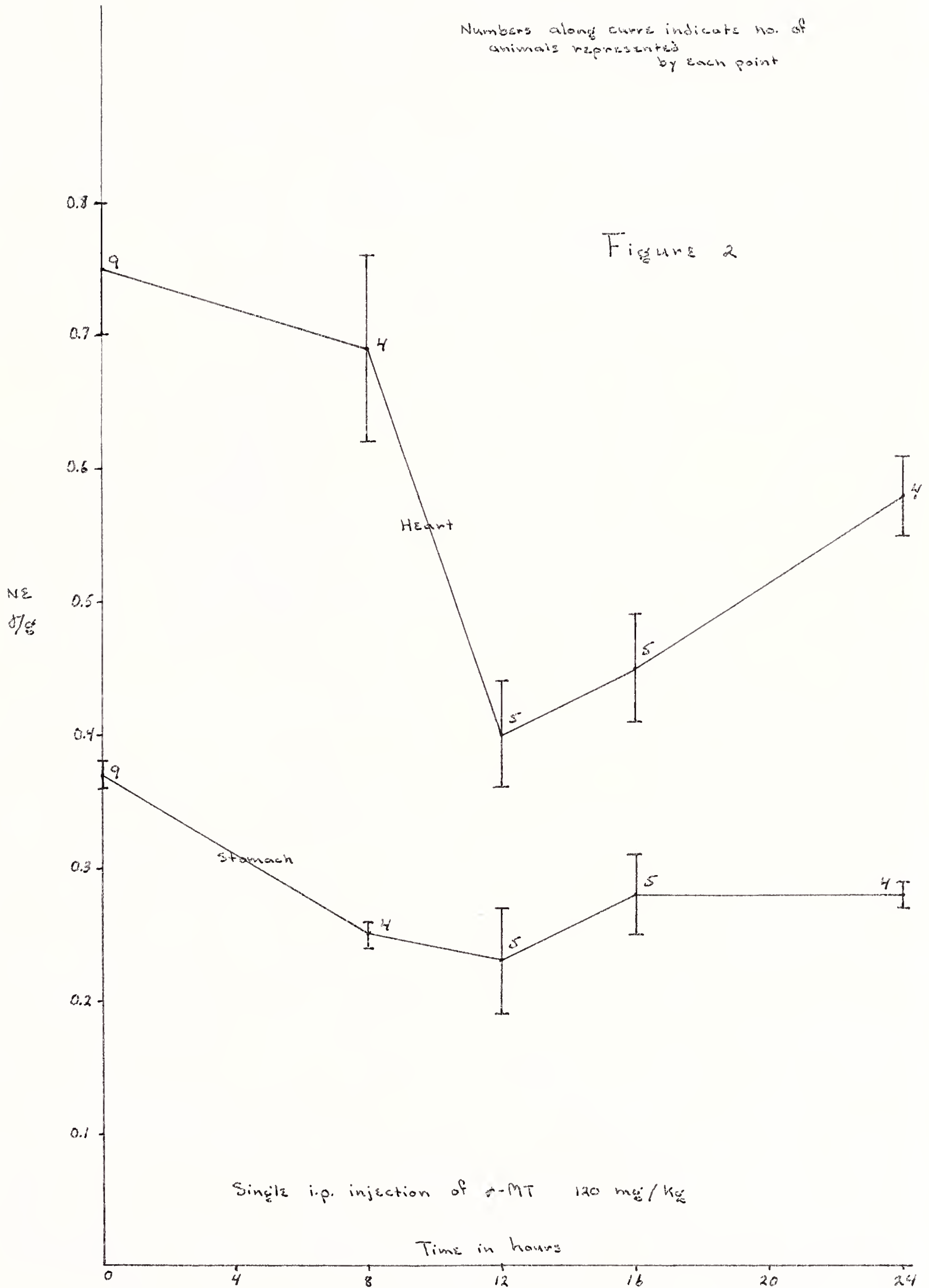

Brackets indicate S.E.M.
Numbers along curve indicate no. of animals represented by each point
Figure 2
NE
γ/g
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Heart
Stomach
9
4
5
5
4
9
4
5
5
4
Single i.p. injection of α-MT 120 mg/kg
Time in hours
0
4
8
12
16
20
24

Table 1

Tissue Histamine in γ/g ± S.E.M.

Drug	Stomach	Heart	Time after Injection	No. of Animals
Pargyline	24.9 ± 1.4	2.2 ± 0.2	12 hours	21
Control	25.0 ± 1.8	2.5 ± 0.3	12 hours	13
α-MT	24.2 ± 2.0	2.0 ± 0.2	15 hours	13
Control	21.7 ± 1.9	2.0 ± 0.2	15 hours	11

Single intraperitoneal injections. Pargyline dose: 50 mg base/kg body wgt.
α-MT dose: 120 mg base/kg body wgt.

Table 2

24 Hour Urinary Histamine in γ/g ± S.E.M.

Drug	Urinary Histamine	No. of Animals
Pargyline	32.4 ± 1.4	18
Control	20.8 ± 0.9	16
α-MT	20.1 ± 0.9	18
Control	18.2 ± 1.0	16

Single intraperitoneal injections. Pargyline dose: 50 mg base/kg body wgt.
α-MT dose: 120 mg base/kg body wgt.

Table 3

24 Hour Urinary Histamine in γ/g ± S.E.M.

Drug	Urinary Histamine	No. of Animals
Dopa	27.3 ± 4.2	6
Control	21.8 ± 4.5	6

3 intraperitoneal injections at hours 0, 8, 16.
Urine collections from hour 1-25.
Dopa dose: 120 mg base/kg body wgt.

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