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MONOAMINE OXIDASE ACTIVITY IN PERIPHERAL ORGANS AND ADRENERGIC TISSUES OF THE RAT*

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Abstract—Monoamine oxidase activity *in vitro* was studied in the heart, kidneys, submaxillary glands, and superior cervical ganglia of control rats by the method of Lovenberg and co-workers (*J. Pharmacol.* 1962). Comparison of monoamine oxidase activity in the absence and presence of exogenous aldehyde dehydrogenase revealed nearly saturating amounts of aldehyde dehydrogenase in the liver, submaxillary glands, lungs, spleen, and stomach (fundus, body, pyloric antrum).

In the absence of exogenous aldehyde dehydrogenase, the uterus, ventricles, right atrium, left atrium, and proximal and distal small intestine showed activities of 24, 46, 58, 49, 65, and 68 per cent activity respectively. The activity in the superior cervical ganglia, stellate ganglia, thoracic chains, and retinas was 8·5, 11·0, 27, and 38 per cent. In the presence of exogenous aldehyde dehydrogenase, the greatest monoamine oxidase activity, based on the protein content, was noted in the superior cervical ganglia. On the wet weight basis, ganglionic monoamine oxidase activity was greater than that of all other tissues investigated except the liver.

In the absence of exogenous aldehyde dehydrogenase, the reaction velocity of monoamine oxidase in ventricles, atria and pooled adrenergic tissue was linear for 30 min at the optimum substrate concentration. In the presence of exogenous aldehyde dehydrogenase, the monoamine oxidase reaction rates of the ventricles, atria, and adrenergic tissue were linear for up to 40 min at optimum substrate concentrations. Substrate inhibition became apparent at the highest substrate concentration used (11·67 μmole/ml), and unsaturation of the enzyme was noted at the lowest substrate concentration (0·233 μmole/ml).

In homogenates of the ventricles, atria, and pooled adrenergic tissue, monoamine oxidase activity was inhibited by relatively small increases in enzyme concentration (tissue homogenate). This inhibition was not overcome by a tenfold increase in substrate concentration nor by the doubling of NAD and exogenous aldehyde dehydrogenase. Centrifugation at 50,000 *g* for 25 min localized the inhibitory component(s) in the pellet. The supernatant was devoid of monoamine oxidase activity.

NUMEROUS assays have been devised to study monoamine oxidase activity *in vitro*.[1–6] To us, the method of Lovenberg *et al.*[5] seemed suitable and sensitive enough to measure the activity of monoamine oxidase in rat tissues (including adrenergic ganglia). It had been used in our laboratory to study the activity of this enzyme in immunosympathectomized rats[7] *in vitro*; however, it was found advisable to investigate

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some aspects of the assay in greater detail and to explore the monoamine oxidase activity of peripheral organs and adrenergic tissues of control rats. These investigations are reported below.

EXPERIMENTAL

Preparation of tissues. The processing of tissues and the preparation of crude guinea pig kidney aldehyde dehydrogenase were done according to Lovenberg *et al.*[5] Fresh and frozen tissue homogenates were used as indicated.

Assay procedure

The quantities of tissue used and the composition of the incubation mixture are listed in Table 1. After a preincubation period of 5 min, the substrate, tryptamine, was

TABLE 1. COMPOSITION OF INCUBATION MIXTURE (1·5 ml) FOR ROUTINE ASSAY

0·3 ml homogenate in 0·25 M sucrose (30 mg tissue; for liver 10 mg)*
0·5 ml phosphate buffer, pH 7·2 (125 μmoles)
0·1 ml nicotinamide (30 μmoles)
0·1 ml NAD (3·5 μmoles)*
0·1 ml aldehyde dehydrogenase preparation (or water)*
0·3 ml water*
preincubation period, 5 min
0·1 ml tryptamine (3·5 μmoles)*

* These concentrations were altered as indicated in figures and text.

added and mixed. Aliquots (0·5 ml) of the incubation mixture were immediately withdrawn, and empty, preincubated tubes were filled and incubated for the appropriate time period. The 0·5-ml aliquot which remained in the original tube was used as the zero-minute sample (or tissue blank). The reaction was terminated by the addition of 0·4 ml of 2 N HCl, and the tube was kept in crushed ice or frozen, dependent upon the time elapsing before extraction. In experiments which required more than three aliquots of incubation mixture, all the components were increased in proportion to the desired volume. To correct for traces of monoamine oxidase activity present in the aldehyde dehydrogenase preparation, control assays were performed by substituting 0·25 M sucrose for the tissue homogenate, incubating, and carrying the aliquots of this incubation mixture through the extraction procedure.

Extraction of indoleacetic acid

After terminating the reaction by adding 0·4 ml of 2 N HCl, 7 ml of washed* toluene (Merck, reagent grade) was added, and the tube was agitated by means of a Vortex mixer or in a mechanical shaker and centrifuged. The organic layer (5 ml for routine work, 6 ml for some experiments) was transferred to a second tube containing 1·5 ml phosphate buffer, pH 7·2, 0·25 M. After agitation (mechanical shaker) and centrifugation, the organic layer was aspirated, and an appropriate aliquot of the aqueous layer (phosphate buffer) was measured in an Aminco-Bowman spectrophotofluorometer at 290/370 mμ (uncorrected wavelengths). For some tissues, two toluene extractions were made, and the indoleacetic acid from the pooled toluene fractions was partitioned into the phosphate buffer.

* 1 N NaOH, 1 N HCl, followed by several washes with distilled water.

Experimental design

To ascertain the reliability of the assay, the following investigations were made: (1) Extraction and measurement of added, incubated indoleacetic acid in the absence and presence of various concentrations of tryptamine. (2) Extraction and measurement of indoleacetic acid added after incubation in the absence and presence of various concentrations of tryptamine. (3) Comparison of tissue blanks in the absence and presence of various concentrations of tryptamine. (4) Extraction and measurement of duplicate and replicate aliquots of the incubation mixture.

The following experimental measurements were made: (1) A time study of monoamine oxidase activity in ventricles, atria, and pooled adrenergic tissue without the addition of exogenous aldehyde dehydrogenase. (2) A time study of monoamine oxidase activity in ventricles, atria, and pooled adrenergic tissue in the presence of exogenous aldehyde dehydrogenase. The pooled adrenergic tissue consisted of two superior cervical ganglia, two stellate ganglia, two thoracic adrenergic chains, two splanchnic nerves, and the celiac, cardiac, and superior mesenteric ganglia. These two studies also included changes in monoamine oxidase activity with different substrate and NAD concentrations (0·233, 1·167, 2·33, and 11·67 μmoles tryptamine and NAD per ml incubation mixture). (3) The inhibitory effect on the velocity of the reaction by tissue homogenates (ventricles). (4) The monoamine oxidase activity in peripheral organs and adrenergic tissues in the presence and absence of exogenous aldehyde dehydrogenase. The homogenate of each tissue was incubated in pairs, one containing exogenous aldehyde dehydrogenase, the other an equivalent volume of distilled water. (5) The enzyme activity of four tissues (heart, submaxillary glands, kidneys, and superior cervical ganglia) from six control rats. (6) The protein content of tissue homogenates, measured by the method of Lowry *et al.*[8] Monoamine oxidase activity was reported in terms of the wet weight and the protein content in Tables 2–4, and only in terms of the wet weight in Figures 1–7.

RESULTS

Lovenberg *et al.*[5] reported quantitative (95 to 100 %) recoveries of indoleacetic acid in the presence of tissue if compared with internal aqueous standards but did not report actual recoveries based on the amount of added indoleacetic acid. In the present investigation, recoveries of indoleacetic acid from aliquots of the incubation mixture, added before the toluene extraction, were consistent (47–52%). When two consecutive toluene extractions were performed, 68 to 73% of the added indoleacetic acid was recovered. The use of a mechanical shaker in extractions resulted in somewhat greater recoveries than did the use of the Vortex mixer (standardized procedure), but the use of the shaker sometimes resulted in sample leakage.

Tryptamine did not affect recoveries of indoleacetic acid nor did it appreciably increase the tissue blanks (zero-minute samples). Duplicate aliquots of incubation mixtures, extracted quantitatively with an average variation of $3{\cdot}82 \pm 0{\cdot}55\%$.* Indoleacetic acid added to aliquots of the incubation mixture at 0 min could be recovered quantitatively after 30 min, when compared with recoveries of indoleacetic acid added to aliquots of the incubation mixture prior to the toluene extraction. This verified the stability of the product of the reaction during the incubation period.

Tables 2 and 3 summarize our findings of the monoamine oxidase activity in

* Average of 30 duplicates $\pm$ standard error.

peripheral organs and adrenergic tissues, with and without exogenous aldehyde dehydrogenase. Almost saturating amounts (90%) of endogenous aldehyde dehydrogenase were found in the liver, submaxillary glands, kidneys, lungs, and body of the stomach. In the spleen, fundus, and pyloric antrum, 80 to 90% of the activity was measured in the absence of exogenous aldehyde dehydrogenase. The uterus, ventricles,

TABLE 2. MONOAMINE OXIDASE ACTIVITY OF ADRENERGIC TISSUE AND RETINAS FROM THREE CONTROL RATS (WITH AND WITHOUT EXOGENOUS ALDEHYDE DEHYDROGENASE)*

Tissue	Indoleacetic acid formed per hour per pair of tissue (μmole ± S.E.)		Activity without exogenous AD (%) (range)
	No AD added	AD added	
Superior cervical ganglia	0·006 ± 0·002	0·068 ± 0·010	8·5 (4–12)
Stellate ganglia	0·014 ± 0·003	0·117 ± 0·013	11·6 (9–14)
Thoracic chains	0·044 ± 0·011	0·161 ± 0·019	27·1 (22–35)
Retinas (including ciliary bodies)	0·019 ± 0·005	0·049 ± 0·008	38·3 (23–62)

* Rat body weight: 190 ± 38 *g* (S.E.), ♀; incubation period, 20 min; AD = aldehyde dehydrogenase.

right atrium, left atrium, and proximal and distal small intestine showed an activity of 24, 46, 58, 49, 65, and 68% respectively. In the absence of exogenous aldehyde dehydrogenase, the activity in the superior cervical ganglia and stellate ganglia was 8·5 and 11% and in the thoracic chains and retinas, 27 and 38% respectively. Ventricles and atria did not contain sufficient amounts of endogenous aldehyde dehydrogenase to carry the reaction to completion; but addition of exogenous enzyme nearly doubled the activity in most ventricles and atria. However, it must be emphasized that the enzyme activity in ventricles and right atrium varied to a greater extent than the activity in the other organs. In one of the three experiments, near saturating amounts of aldehyde dehydrogenase were found in the ventricles and the right atrium (82 and 92%), while the activity in the ventricles and both atria of the other two rats was 34 to 60%.

The monoamine oxidase activity *in vitro* in the heart, kidneys, submaxillary glands, and superior cervical ganglia of six control rats (two pairs of littermates and two nonlittermates) shows considerable variation; 2·5-fold variation was found in the activity of submaxillary glands and superior cervical ganglia (Table 4). The greatest monoamine oxidase activity, based on the protein content, was noted in superior cervical ganglia. When based on the wet weight, only the liver had greater activity.

Formation of indoleacetic acid was completely inhibited in all tissue homogenates by the addition of β-phenylisopropylhydrazine (JB-516, Catron)† at a 10^{-3} M concentration.

In the absence of exogenous aldehyde dehydrogenase (Fig. 1), the rate-substrate concentration study shows that for ventricles, atria, and adrenergic tissue the rate is not linear beyond 30 min (40 min for adrenergic tissue). Optimum substrate concentration for atria (16 mg/ml) and for ventricles (20 mg/ml) is 11·67 μmoles/ml, and

† The author wishes to thank Dr. Murray Finkelstein of Lakeside Laboratories for the generous supply of Catron.

TABLE 3. MONOAMINE OXIDASE ACTIVITY IN PERIPHERAL ORGANS OF CONTROL RATS WITH AND WITHOUT EXOGENOUS ALDEHYDE DEHYDROGENASE*

Tissue	Indoleacetic acid formed per hour				Activity without exogenous AD	
	per g wet weight		per g protein			
	No AD	AD	No AD	AD	(%)	(range)
	(μmoles ± S.E.)		(μmoles ± S.E.)			
Liver (3)†	24·30 ± 3·57	27·00 ± 3·78	129·0 ± 18·81	143·0 ± 20·10	90	(89–92)
Ventricles (3)	6·08 ± 0·09	13·30 ± 3·07	34·0 ± 6·38	67·7 ± 5·76	46	(34–82)
Right atrium (3)	5·23 ± 0·63	8·90 ± 1·14	36·9 ± 7·20	61·0 ± 5·77	58	(38–92)
Left atrium (3)	3·62 ± 0·30	7·37 ± 0·60	25·9 ± 4·88	50·5 ± 4·49	49	(37–63)
Duodenum, jejunum (4)	4·97 ± 0·37	7·65 ± 0·88	27·9 ± 1·96	42·4 ± 1·47	65	(57–80)
Ileum (3)	7·40 ± 1·30	10·82 ± 1·70	36·9 ± 4·10	54·0 ± 2·77	68	(63–75)
Fundus (4)	2·44 ± 0·17	2·99 ± 0·29	12·3 ± 0·99	14·7 ± 1·15	82	(70–95)
Body, pyloric antrum (4)	4·64 ± 0·29	5·01 ± 0·35	22·8 ± 1·17	24·7 ± 1·46	93	(91–95)
Pyloric antrum (2)	4·80 ± 0·14	5·56 ± 0·40	24·3 ± 0·96	28·1 ± 0·07	83; 90	
Submaxillary glands (3)	4·46 ± 0·04	4·96 ± 0·16	26·9 ± 4·23	29·3 ± 3·50	90	(86–97)
Kidneys (3)	4·08 ± 0·34	4·40 ± 0·43	25·6 ± 2·60	27·9 ± 3·35	93	(90–95)
Lungs (3)	4·30 ± 0·38	4·74 ± 0·54	25·2 ± 2·43	28·0 ± 3·76	91	(84–95)
Spleen (3)	1·87 ± 0·30	2·28 ± 0·26	10·8 ± 2·14	13·0 ± 1·56	82	(69–94)
Uterus (2)	0·98 ± 0·01	4·03 ± 0·85	7·6 ± 1·46	31·6 ± 4·54	19·3; 31·3	

* Body weights: (4) 240 ± 57g (S.E.), ♀ ♂; (3) 190 ± 38g (S.E.), ♀; (2) 163 ± 47g (S.E.), ♀.
† Animal numbers in parentheses.

TABLE 4. MONOAMINE OXIDASE ACTIVITY OF PERIPHERAL TISSUES FROM SIX CONTROL RATS* (WITH EXOGENOUS ALDEHYDE DEHYDROGENASE)

Tissue	Indoleacetic acid formed per hour		
	per g wet weight (μmoles ± S.E.)	per g protein (μmoles ± S.E.)	per tissue (μmoles ± S.E.)
Kidneys	3·72 ± 0·23	21·7 ± 1·8	
Submaxillary glands	6·50 ± 0·85	38·6 ± 2·9	
Heart	8·98 ± 0·61	52·4 ± 3·7	
Superior cervical ganglia	19·70 ± 2·88	362·0 ± 44·9	0·060 ± 0·009

* Body weight: 139 ± 6 g (S.E.), ♂; incubation period 30 min.

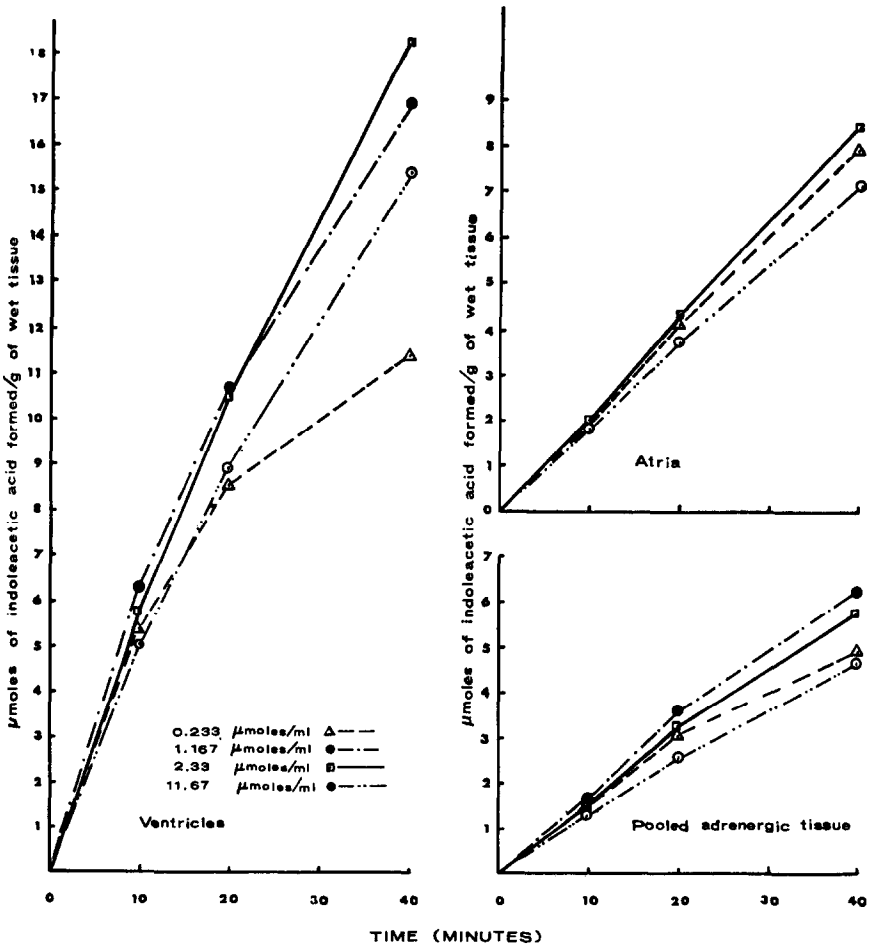

FIG. 1. Effects of substrate concentrations on monoamine oxidase activity (defined as μmoles indoleacetic acid formed per g wet tissue in Figs. 1–5) in rat adrenergic tissue, atria, and ventricles, in the absence of exogenous aldehyde dehydrogenase. Substrate and NAD concentrations: 11·67, 2·33, 1·167, and 0·233 μmoles/ml incubation mixture. Body weight of rat: 250 g, ♀.

for adrenergic tissue (9·4 mg/ml) 2·33 μmoles/ml. At 30 min (40 min for adrenergic tissue) the differences in rates for three substrate concentrations (1·167, 2·33, and 11·67 μmoles/ml) are small for these three tissues. At the lowest substrate concentration (0·233 μmole/ml), the rate becomes very slow after 30 min (40 min for adrenergic tissue), indicating that the enzyme is not saturated.

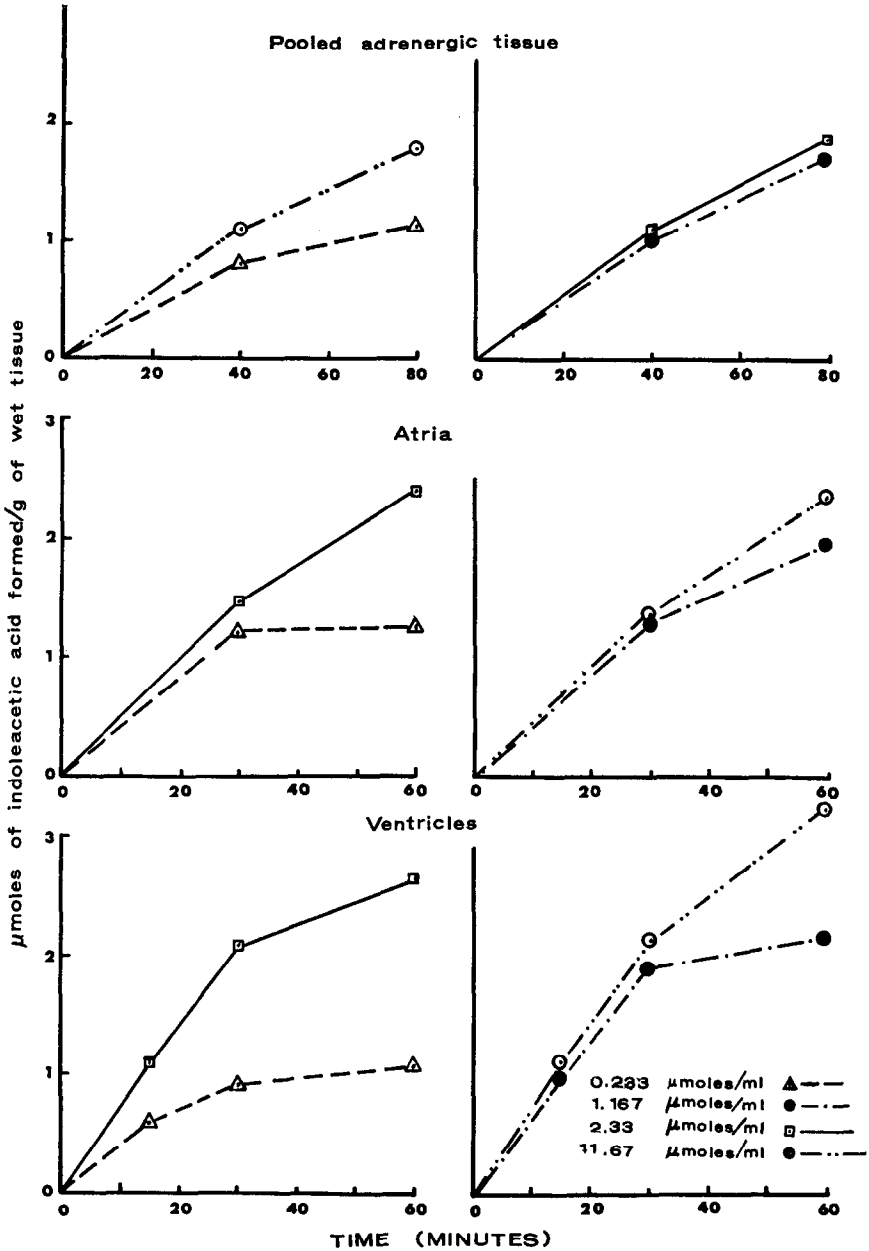

FIG. 2. Effects of substrate concentrations on monoamine oxidase activity in the presence of exogenous aldehyde dehydrogenase. Conditions as in Fig. 1. Identical rates were obtained with atrial homogenates, with substrate and NAD concentrations of 2·33 and 1·167 μmoles/ml. Body weights: 309 and 329 g, ♂,

In the presence of exogenous aldehyde dehydrogenase (Fig. 2), the rate is nearly linear for 40 min at all substrate concentrations, except for the ventricles, at a substrate concentration of 0·233 μmole/ml. There is evidence of substrate inhibition with the highest substrate concentration of 11·67 μmoles/ml. Each point of Fig. 2 represents the mean of two experiments. These two experiments were so similar as to permit averaging the results without including ranges or standard deviations. The tissue concentrations for ventricles, atria, and pooled adrenergic tissue were 20 mg, 6·3 mg, and 5 mg per ml of incubation mixture.

In order to explore the effects of increased enzyme concentration, the lowest substrate concentration (0·233 μmole/ml) was incubated at two tissue concentrations: 20 and 40 mg/ml for ventricles and atria and 12·5 and 20 mg/ml for pooled adrenergic tissue (Fig. 3). An increase in enzyme concentration (tissue homogenate) resulted in inhibition of monoamine oxidase activity in the ventricles, atria, and pooled adrenergic tissue. This inhibitory effect of tissue homogenate was studied in greater

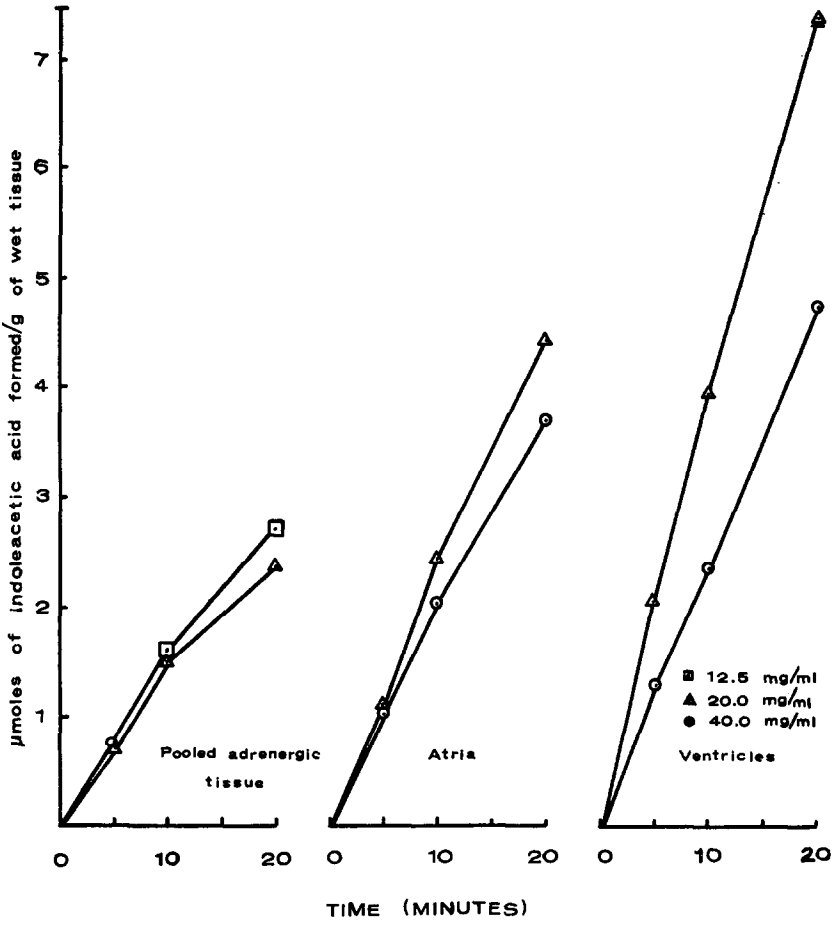

FIG. 3. Effects of tissue concentrations on monoamine oxidase activity in adrenergic tissue, atria and ventricles of the rat. Substrate concentration 0·233 and NAD concentration 2·33 μmoles/ml incubation mixture. Body weights: 360 and 400 g, ♂.

detail in ventricles (Figs. 4–7). Increasing the substrate concentration to 2·33 μmoles/ml of incubation mixture and doubling the NAD and/or the exogenous aldehyde dehydrogenase concentrations did not overcome the inhibitory effect of fresh (Fig. 4) and frozen (Fig. 5) tissue homogenate. Frozen tissue homogenates sometimes showed a faster rate (e.g. Figs. 4 and 5), but this increase in activity could not always be produced by freezing.

The homogenate (200 mg/ml) was centrifuged at 50,000 *g* for 25 min. The supernatant was removed and the pellet dissolved in 0·25 M sucrose to twice the original concentration. Addition of the supernatant (0·1 ml, corresponding to 20 mg of original tissue homogenate) to the incubation mixture did not produce inhibition (Fig. 6, A and B), but addition of the dissolved pellet (0·05 ml, corresponding to approximately 20 mg of tissue) did result in inhibition (Fig. 6, A and C). In this experiment, the inhibitory effect was larger than that produced by 40 mg of ventricle/ml (Fig. 6, C and D). When the dissolved pellet was added to the incubation mixture after 5-min incubation, an increase in rate was noted for the first 5-min period, followed by a marked decrease (Fig. 7, C and D). The total amount of indoleacetic acid formed per 20 min was similar to the amount formed when 40 mg of tissue was incubated. No difference was seen in the rate of the reaction between the preincubated dissolved pellet (at 37° for 10 min) and the dissolved pellet kept at 0° before addition to the incubation mixture.

DISCUSSION

This study shows that the method of Lovenberg *et al.*[5] is a reliable and reproducible assay for monoamine oxidase activity in rat organs *in vitro*. Values for tissue blanks and zero-minute incubation mixtures are low. The substrate, tryptamine, does not interfere with the extraction procedure and the measurement of the indoleacetic acid. The product of the reaction is not destroyed during incubation periods and can be extracted quantitatively. Appropriate blanks correct for the traces of monoamine oxidase activity present in the aldehyde dehydrogenase preparations from guinea pig kidney.

The monoamine oxidase activities in submaxillary glands and superior cervical ganglia of control rats (Table 4) indicate considerable variation (about 2·5-fold). Variability, to a similar degree, has been previously reported for a few rat tissues and tissues from other species.[5]

By using the complete system (with exogenous aldehyde dehydrogenase), the monoamine oxidase activity in one pair of superior cervical ganglia of the rat (2 to 4 mg wet weight) can be measured with ease. The method is sensitive enough to measure the monoamine oxidase activity in a single ganglion, if the volume of the incubation mixture is decreased and/or the extraction procedure is made more efficient. Comparison of the monoamine oxidase activity in the major peripheral organs, in the absence or presence of exogenous aldehyde dehydrogenase, revealed that endogenous aldehyde dehydrogenase is present in sufficient amounts in liver, submaxillary glands, spleen, lungs, kidneys, and stomach to carry the reaction to or nearly to completion. Lovenberg and co-workers reported that rat hearts contain saturating amounts of endogenous aldehyde dehydrogenase. As mentioned in Results, the greatest variability of monoamine oxidase and aldehyde dehydrogenase activities was found in cardiac tissue. The ventricles and right atrium of one rat contained

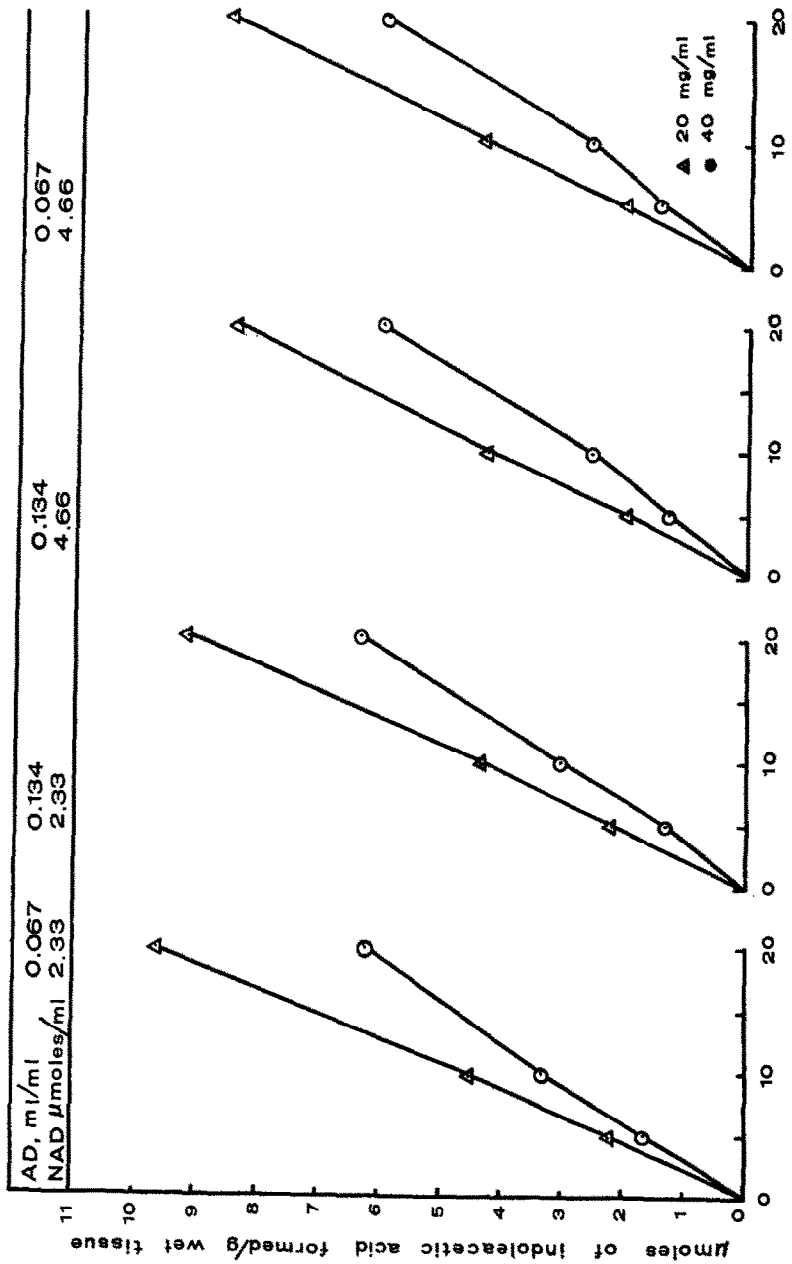

FIG. 4. Effects of changes in tissue concentration, NAD, and exogenous aldehyde dehydrogenase, on monoamine oxidase activity in ventricles of the rat. Substrate concentration 2·33 μmoles/ml incubation mixture. Fresh homogenate was used. Body weight: 350 g, ♂.

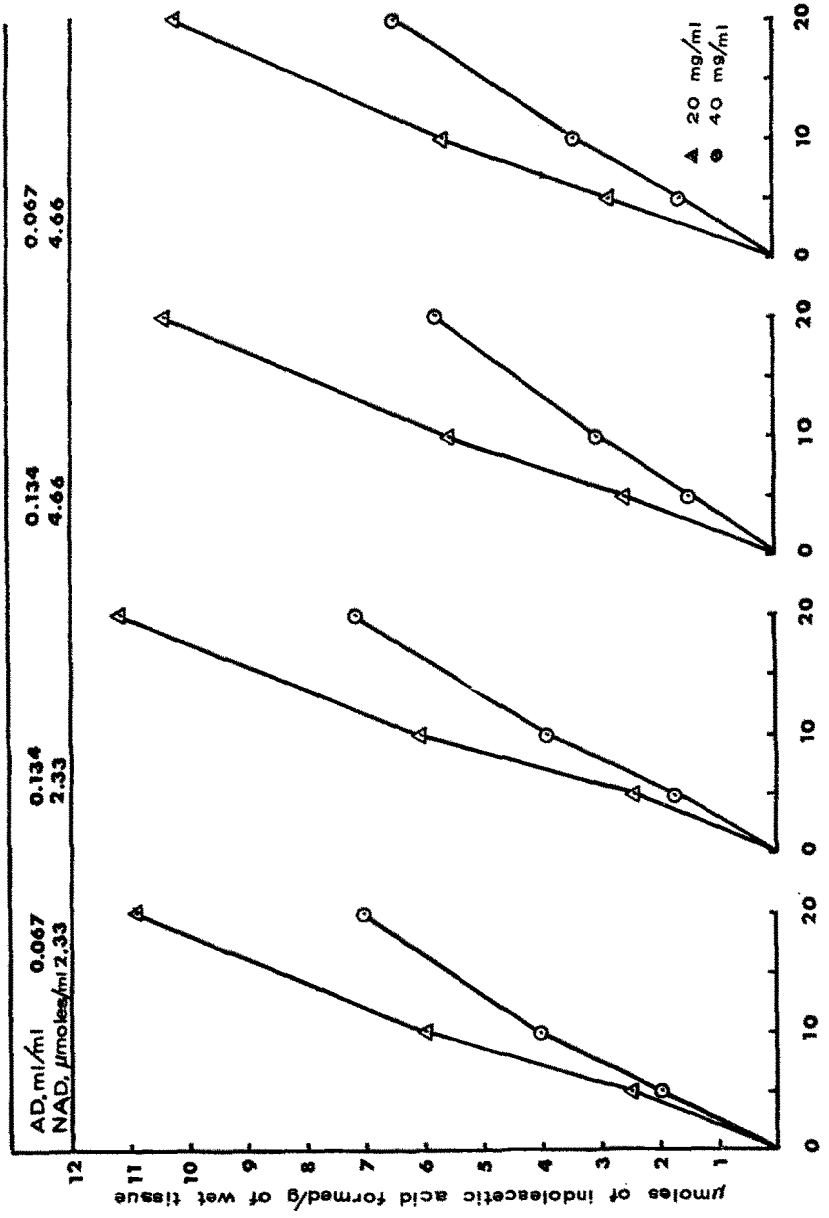

FIG. 5. Effects of changes in tissue concentration, NAD, and exogenous aldehyde dehydrogenase., on monoamine oxidase activity in ventricles of the rat. Substrate concentration 2·33 μmoles/ml incubation mixture. Frozen homogenate was used. Body weight: 350 g, ♂.

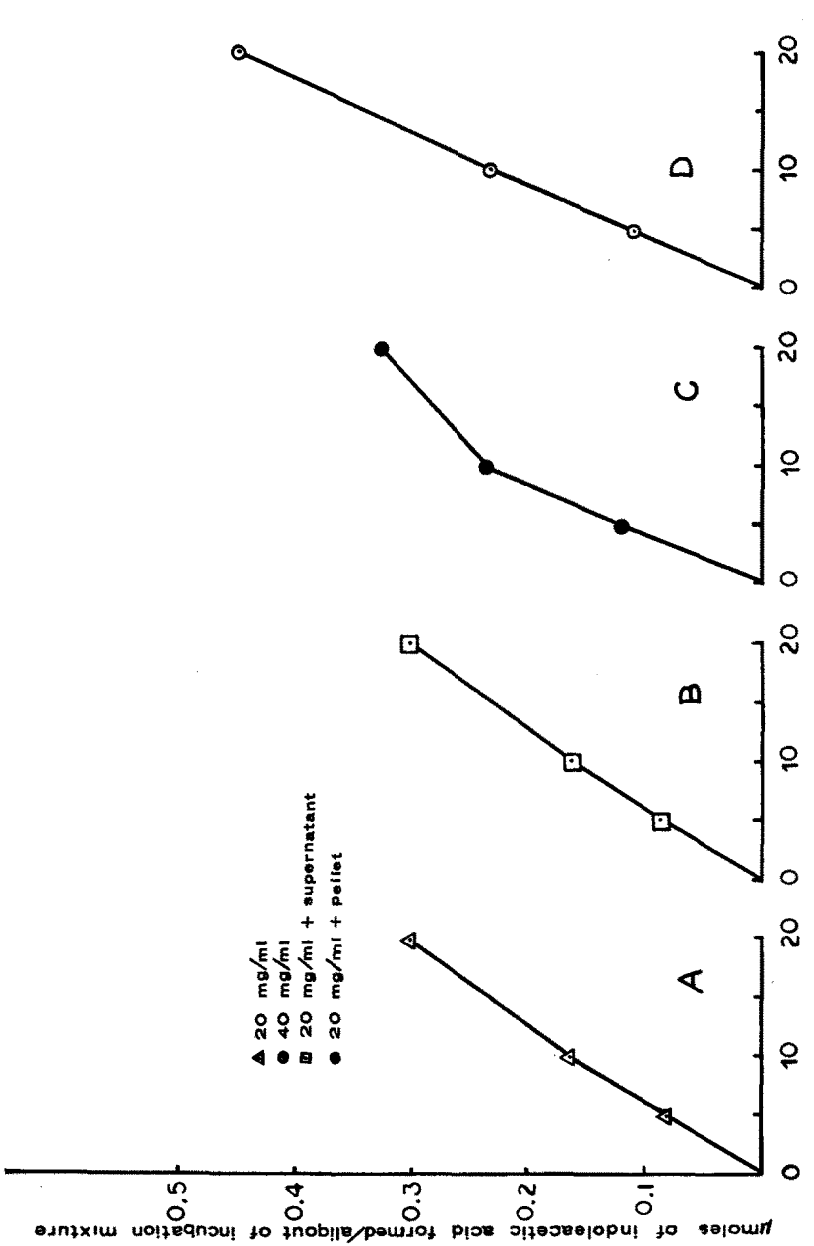

FIG. 6. Effect of increased enzyme concentration by addition of homogenate, supernatant, or pellet (50,000 *g*, 25 min), on monoamine oxidase activity (μmoles indoleacetic acid formed per aliquot of incubation mixture) in ventricles of the rat. Substrate concentration 2·33 μmoles/ml incubation mixture. Body weight: 411 g, ♂.

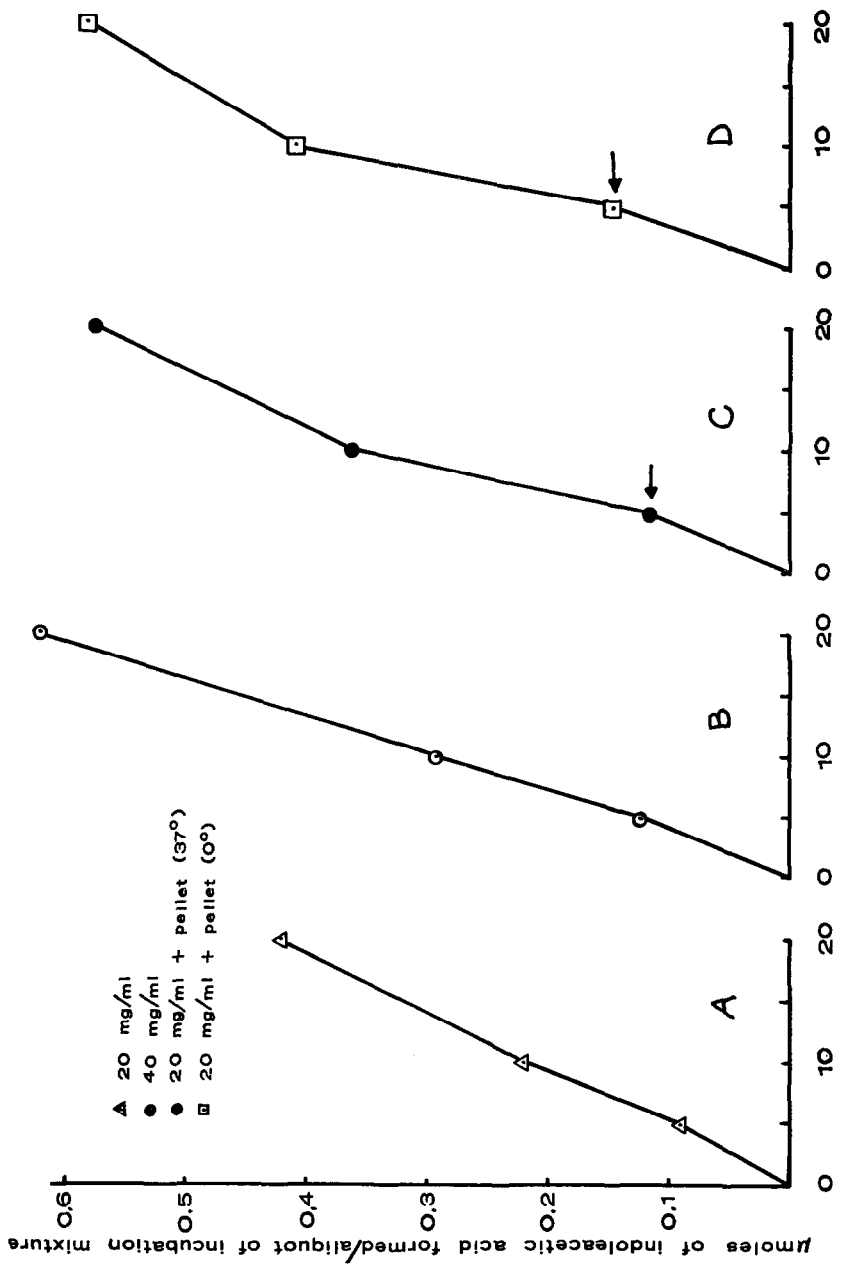

FIG. 7. Effect of increased enzyme concentration by addition of homogenate or pellet (50,000 *g*, 25 min) on monoamine oxidase activity (μmoles indoleacetic acid formed per aliquot of incubation mixture) in ventricles of the rat. Substrate concentration 2·33 μmoles/ml incubation mixture. Arrows indicate time of pellet addition. Body weight: 414 g, ♂.

nearly saturating amounts (82 and 92% respectively) of endogenous aldehyde dehydrogenase, whereas the activity in the ventricles and both atria of the other two rats amounted to only 34 to 60%.

The monoamine oxidase activity in superior cervical ganglia of the rat is considerably higher than the activity previously reported[5] for dog and cat ganglia. The per cent activity in the absence of exogenous aldehyde dehydrogenase compares well with the data reported by these authors (11%). From the publication by the Lovenberg group it cannot be surmised how much tissue was used for the incubations or the length of the incubation period. From Fig. 3 it can be seen that the tissue concentration is critical; beyond a certain point, an increase in tissue homogenate results in smaller amounts of indoleacetic acid formed per gram. A similar relation also holds true for the duration of the incubation period. Comparison of the same homogenates, incubated for 30 and 60 min, showed that in 6 out of 6 submaxillary glands, significantly decreased amounts of indoleacetic acid were calculated after 1-hr incubation.[9] The same observation was made with superior cervical ganglia (5 out of 6) and hearts (4 out of 6), but was not noted in kidneys. These two phenomena may also account for the differences in heart monoamine oxidase activity of the present study and the study by Lovenberg *et al.* The activity in liver previously reported, and that reported by the present study, compare favorably.[5] The possibility, at least as far as ganglionic monoamine oxidase activity is concerned, that species variation (dog and cat versus rat) is responsible for the differences in activity cannot be excluded.[5]

Thoracic adrenergic chains consistently showed possession of more endogenous aldehyde dehydrogenase activity than the superior cervical and the stellate ganglia. It is not known whether this constitutes a true difference or whether it is an artifact, introduced by dissecting techniques. Superior cervical and stellate ganglia are devoid of nearly all nonganglionic tissue (except the sheath) whereas dissected adrenergic thoracic chains contain a considerable amount of extraneous (non-neural) bits of tissue. Furthermore, thoracic chains contain more nerve fibers than the superior cervical and stellate ganglia, and monoamine oxidase and aldehyde dehydrogenase activities have not yet been studied in pre- and postganglionic fibers.

The time study without exogenous aldehyde dehydrogenase (Fig. 1) shows that the substrate concentration is critical. For atria (16 mg/ml incubation mixture) and adrenergic tissue (9·4 mg/ml incubation mixture), the lowest substrate concentration (0·233 μmole/ml) did not saturate the enzyme, whereas the highest substrate concentration (11·67 μmoles/ml) appeared to produce some inhibition. This became apparent at 30-min as well as at 60-min incubation (40 and 80 min for adrenergic tissue). For ventricles (20 mg/ml incubation mixture), the optimum substrate concentration was 11·67 μmoles/ml.

When the effect of substrate concentrations on the velocity of the reaction in the presence of exogenous aldehyde dehydrogenase was investigated, rates were linear, or nearly so, for 40 min, except for monoamine oxidase activity in ventricles at the lowest substrate concentration (0·233 μmole/ml). Substrate inhibition was noted with the highest concentration (11·67 μmoles/ml) for all three tissues.

It is of interest to note that doubling the tissue concentration from 20 to 40 mg/ml resulted in a decrease in reaction velocity. This is quite marked in ventricles and atria, and may be suspected in adrenergic tissues as well (Fig. 3). According to Lovenberg and co-workers, this phenomenon did not occur with homogenates from dog hearts.

Increasing the substrate concentration 10-fold (from 0·233 to 2·33 μmoles/ml) and doubling the NAD and/or aldehyde dehydrogenase concentrations did not overcome the inhibitory effect of tissue homogenates (Figs. 4 and 5). This indicates that the rate-limiting phenomenon is not due to limiting concentrations of components of the incubation mixture but rather to some component(s) present in the homogenate. The inhibitory component of the crude system had been localized in the pellet after centrifugation at 50,000 *g* for 25 min (Fig. 6).

This study provides evidence that the method of Lovenberg *et al.*[5] is well suited to the study of monoamine oxidase activity of rat tissues *in vitro*, particularly in tissues which lack sufficient amounts of endogenous aldehyde dehydrogenase—e.g. adrenergic ganglia. However, it must be emphasized that various aspects of this assay can lead to erroneous conclusions. Substrate and enzyme (tissue homogenate) concentrations are critical, and even moderate changes in either of these two parameters may lead to inhibitory effects. Incubation periods should be kept as short as possible to obtain optimum rates; incubation periods of 5 to 20 min, dependent upon the tissue, are sufficient. The possibility of large variations of enzyme activity in tissues from control rats must be taken into consideration when comparing the activity in control tissues with that in experimental animals.

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