1964

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Recommended Citation

Waltman, Stephen R., "Catechol-O-methyl transferase and monoamine oxidase levels in rabbit ocular tissues following cervical ganglionectomy" (1964). *Yale Medicine Thesis Digital Library*. 3278.
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CATECHOL-O-METHYL TRANSFERASE AND MONOAMINE OXIDASE LEVELS IN RABBIT OCULAR TISSUES FOLLOWING CERVICAL GANGLIONECTOMY

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CATECHOL-O-METHYL TRANSFERASE AND MONOAMINE OXIDASE LEVELS IN RABBIT OCULAR TISSUES FOLLOWING CERVICAL GANGLIONECTOMY

Stephen Reeves Waltman

Presented to the Faculty of Medicine
in partial fulfillment of the
requirements for the degree
of Doctor of Medicine

Yale University

New Haven, Connecticut

April, 1964

ACKNOWLEDGEMENTS

The author wishes to express his appreciation to Dr. Marvin L. Sears of the Department of Surgery for his continuing interest, inspiration, and guidance.

The author is indebted to Dr. Julius Axelrod of the National Institutes of Health for his suggestions regarding the experimental procedures and for donating some of the compounds used in these experiments.

The technical assistance of Mr. Charles Cintron and the secretarial assistance of Miss Lois Childs is greatly appreciated.

This investigation was conducted under a Fight for Sight Student Fellowship of the National Council to Combat Blindness, Inc., New York, New York.

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INTRODUCTION

The influence of the adrenergic system upon the steady state maintenance of intraocular pressure in normal and diseased eyes has become the subject of recent investigation. In particular, studies of the increase in resistance to outflow of aqueous humor in sympathetically denervated, catecholamine depleted rabbit eyes suggest that under normal circumstances the liberation of catecholamines from structures near the outflow channels exerts a continuous effect upon outflow resistance and intraocular pressure.[23] A puzzling finding, however, is the fact that in normally innervated eyes catecholamines cannot be detected in significant amounts in aqueous humor. It becomes of interest then to study possible modes of inactivation and destruction of the ocular catecholamines since the significant levels present in ocular tissues[24] may exert effects upon intraocular pressure.

Physiological studies by Sears and Barany[23] and Barany[7] have done much to further our knowledge about the role of adrenergic mechanisms in the regulation of intraocular pressure in experimental animals. Using rabbits, these investigators studied intraocular pressure and outflow resistance before and after cervical ganglionectomy and the effects of various adrenergic blocking agents on these parameters. They found an increase in outflow facility 24 hours after ganglionectomy.

Later the resistance became supra normal and still later returned to control levels. The "ganglionectomy effect" was prevented by pretreatment with systemic reserpine or anterior chamber injection of alpha adrenergic blocking agents suggesting that the phenomenon was a manifestation of release of adrenergic material into the aqueous. Using the same preparation, Sears and Sherk[25] found that aqueous outflow resistance was supersensitive to small doses of adrenergic compounds one week after unilateral cervical ganglionectomy. Further studies by these authors indicate that the denervation supersensitivity is much more marked after bilateral ganglionectomy. The mechanism of this supersensitivity has not yet been clarified in these studies.

In the human, Ballintine and Garner[6] and others using topical epinephrine therapy for glaucoma simplex, have indicated that the delayed pressure lowering effect of these compounds is related primarily to an increase in the facility of aqueous outflow. The possibility that adrenergic mechanisms may play a role in the normal regulation of intraocular pressure in man as well as in the rabbit is suggested by these studies.

Metabolic correlates for these physiological observations have not been demonstrated for the rabbit eye.

During the past decades two enzymes have been described which are involved in the metabolic inactivation of circulating

catecholamines. Catechol-O-Methyl Transferase (COMT) is an enzyme that catalyzes the O-methylation of catecholamines using S-adenosyl methionine as a methyl donor.[5] O-methylation has been shown to be the principal pathway of metabolism of administered epinephrine and norepinephrine[1, 22] and studies by Axelrod[2] suggest that COMT may be a major enzyme involved in the inactivation of endogenous catecholamines. Monoamine Oxidase (MAO) is an enzyme that oxidatively deaminates amines. The role of this enzyme in the inactivation of catecholamines is not clear, but Axelrod[3] has suggested that it is mainly concerned with deamination of O-methylated metabolites rather than with catecholamines themselves. Other investigators[16] do not completely accept the subordinate role of MAO and suggest that while O-methylation in the rabbit brain does occur, deamination may be an important pathway for the initial inactivation of norepinephrine. Both enzymes are present in the rabbit brain and it is difficult to decide which plays the more important role in the initial metabolism of catecholamines.

The role of COMT and MAO in inactivation of circulating catecholamines is fairly well accepted, but what part these enzymes play in local inactivation of catecholamines is not clearly delineated. According to Axelrod,[4] the observation that the tissues contain more ^{3}H-metanephrine than ^{3}H-epinephrine within two minutes after intravenous injection of labeled ^{3}H-epinephrine taken together with

the presence of COMT in the organs upon which the amines exert their effects indicates that this enzyme may locally act in the metabolism of these hormones. However, studies by Crout,[13] while confirming the observation that metabolism by MAO and COMT contribute to the removal of circulating hormones in the dog, suggest that physiological inactivation of circulating norepinephrine at receptor sites does not require destruction of the amines by these enzymes. He found that simultaneous inhibition of COMT and MAO produced only a moderate prolongation of the effects of injected norepinephrine, and failed to augment the peak response to this compound. His conclusion that pharmacological inactivation of circulating norepinephrine does not require intact function of MAO and COMT may not, however, apply to norepinephrine released directly into tissues by sympathetic nerve endings. The exact mechanism by which locally released norepinephrine is immediately inactivated is still not completely resolved.

The distribution and relative activity of these enzymes, Monoamine Oxidase and Catechol-O-Methyl Transferase have been studied in many mammalian species.[4] Krishna et al[21] have studied the distribution of MAO in various pooled ocular tissues of the rabbit and cat using a manometric technique. Further studies on this enzyme and on COMT distribution in the eye are lacking at present.

Denervated tissues are known to have an increased sensitivity to the neurohumoral transmitters normally released by their effector nerves. The exact mechanism of supersensitivity of structures deprived of their sympathetic innervation is still not known.[18] Considerable work has been done trying to relate supersensitivity to a decreased ability of the denervated tissue to inactivate the chemical mediators. Studies by Burn and Robinson[9] showed that 9-12 days after removal of the superior cervical ganglion the MAO content of the nictitating membrane of the cat fell to 60% of the control level, but over the next 30 days rose to control levels. Concomitant removal of the stellate ganglion prevented the delayed return to normal levels. These investigators felt that supersensitivity of the cat nictitating membrane to sympathetic amines was well correlated with the decreased MAO content of this tissue. Further studies by Burn et al[8] however, failed to show any change in MAO activity of the cat iris following homolateral superior cervical ganglionectomy, a procedure which renders this tissue supersensitive to norepinephrine. Recently, Crout and Cooper[14] studied myocardial COMT activity after regional neural ablation of the dog heart. Following denervation the catecholamine content of the tissue disappeared and the preparation showed a marked supersensitivity to norepinephrine. The 42% decrease in myocardial COMT suggested that supersensitivity of the denervated dog heart could not be attributed to a marked decrease in COMT activity. This

lack of correlation between enzyme changes and denervation supersensitivity may be due to species variation, but suggests that this theory does not give a satisfactory explanation of all the observed facts.

In view of incomplete knowledge about the enzymatic inactivation of catecholamines in general as well as in the eye and because of the influence of these amines on intraocular pressure this project was undertaken to investigate the distribution of COMT and MAO in the ocular tissues of rabbits to discover possible sites of inactivation of catecholamines. These same enzymes were then studied in rabbits that had undergone bilateral cervical ganglionectomy to decide whether changes in the concentration of these enzymes might play any role in the denervation supersensitivity phenomena demonstrated in this preparation.

METHODS AND MATERIALS

A. Preparation of Tissues

Albino rabbits, mainly males, weighing between 1.6 and 3.2 kg. were used. The animals were killed by the intravenous injection of gallamine triethiodide (Davis and Geck) 5 mg./kg., followed by 5-10 cc. of air. A few animals convulsed, but only briefly. Immediately after death the eyes were enucleated and put into chopped ice. The skull was opened and the optic nerves removed, up to, but not including the chiasm. A specimen of liver was also removed and iced. Eyes were dissected and the various tissues from both eyes of each animal pooled; lenses were removed with their capsules intact. Excess blood and vascular tissues were dissected away from the optic nerves. After blotting to remove excess water the tissues were weighed and then homogenized with five volumes of ice cold 1.15% KCl in an all glass homogenizer and samples were then taken for assay. The average weights of the tissues from each eye were: Iris-ciliary body = 54 mg., Retina - choroid = 72 mg., Optic nerve = 22 mg., Extraocular muscle = 99 mg., and lens = 312 mg.

Tissues used for COMT determinations were centrifuged at 9,000 xg for 12 minutes at a temperature of 0-4° C. Those used for MAO studies were not centrifuged but were kept in ice prior to assay.

Abbreviations

E.O.M. = Extraocular muscle
S.E.M. = Standard error of the mean.

Rabbits were subjected to bilateral cervical ganglionectomy under thiopental sodium (Abbott) anesthesia. Five to seven days later they were sacrificed and the tissues treated in the same manner as the non-operated animals.

B. Catechol-O-Methyl Transferase Assay

Several assay methods were used for the determination of COMT.

The fluorometric assay procedure used is a modification of that described by Axelrod and Tomchick[5]. An aliquot of the soluble supernatant fraction was taken for enzyme assay and the COMT activity determined by measuring the amount of metanephrine formed from epinephrine. The incubation mixture, in a 50 cc. polypropylene centrifuge tube, contained 50 μmoles phosphate buffer pH = 7.8, 10 μmoles $MgCl_2$, 100 mμmoles S-adenosyl methionine, 330 mμmoles of Epinephrine bitartrate and an appropriate amount of enzyme (mouse liver = 20 mg., rabbit liver = 33 mg.) in a final volume of 1.0 ml. Blanks were obtained using boiled tissue extract in place of the enzyme. The mixture was incubated at 37° C for 1 hour and then 0.5 ml. of 0.5M borate buffer, pH=10.0, and 30 ml. of washed Ethylene dichloride was added and the tubes shaken by hand for 5-7 minutes. The aqueous phase was then aspirated and 1.5 cc. of 0.1N HCL was added to the Ethylene dichloride, and the metanephrine back extracted into the aqueous layer. After centrifugation, this layer was aspirated and the amount of metanephrine present was determined using a modification of the Trihydroxyindole method of Haggendal[15] (see details below). The values were corrected for the partition of metanephrine in the above solvent buffer systems (The combined

extraction procedures had a recovery of 40-45%).

The method used for fluorometric determination of metanephrine is as follows:

1) The pH of the acid extract was adjusted to 7.0 (indicator paper) using 1N K_2CO_3. Indicator paper was used as the Brom Thymol Blue originally used was found to interfere with the readings. The volume of the extract was adjusted to 3.2-3.3 ml.

2) Immediately after the pH adjustment, a 1.0 ml. sample was added to 0.5 ml. of buffer (equal parts, 0.6 M citrate and 1 M phosphate, pH=7.2) and an appropriate amount of H_2O.

3) 0.10 ml. of 0.02 M iodine was added and the oxidation allowed to proceed.

4) Three minutes later 0.5 ml. of 5 per cent sodium sulfite containing 2 per cent EDTA was added.

5) 30 seconds later 0.5 ml. of 5 N NaOH was added.

6) After 5 minutes, 0.5 ml. of 10 N acetic acid was added.

7) The final volume was 4.1 ml.

8) Between 2-10 minutes after the acetic acid was added the samples were read in a Turner Fluorometer #110 using an activating wavelength of 436 mμ (Filters #47B and 2A) and a fluorescence wavelength of 525 mμ (Filter #58).

9) A reagent blank containing all reagents was used to zero the instrument.

10) The tissue blank used was the same as the sample except that no iodine was added and therefore no oxidation took place.

11) 0.1 ml. of 0.5 M KCl was added to the metanephrine standard and reagent blank, prior to oxidation, to correct for the added salt in the sample.

12) The internal standard was prepared by adding an appropriate amount of metanephrine to 1.0 ml. of the sample prior to oxidation by the iodine. (See Table 1)

Several important modifications were necessary to adopt the original method of Haggendal[15] to the available equipment and to make it sensitive enough to measure the small yields of metanephrine expected from the ocular tissues. These modifications were as follows:

1) Haggendal used an excitation wavelength of 440 mμ and read fluorescence at 510 mμ or 520 mμ to measure the metanephrine formed. After considerable trial, filters giving wavelengths of 436 mμ and 525 mμ were chosen because they were very close to the original wavelengths and because the results obtained were found to be very reproducible. Distilled water blanks gave a consistently reproducible low value when compared to a black plastic background.

These wavelengths were also very sensitive. Other wavelengths available with the Turner Fluorometer appeared more sensitive, but consistency and specificity were of a lower order of confidence.

2) The final volume of the reaction mixture was 4.1 ml. instead of 3.3 ml. This was necessary to fill the cuvettes to the desired level.

3) Using the present buffer, a pH of 7.2 gave a much more sensitive method then when a pH of 6.5 was used. Sensitivity with the Turner Fluorometer was increased approximately 50 fold using the above filters and a pH 7.2 buffer. Despite the increased sensitivity the reproducibility of the results was excellent.

4) Because we were interested in measuring only metanephrine the fluorescence was measured within 10 minutes after addition of the HAc. The stability of the metanephrine fluorophor was found to be very similar to the values reported by Haggendal and because of this stability and slow linear decay values were not corrected to zero time.

5) As reported by Haggendal citrate-phosphate buffer minimizes the effects of salt on the assay procedure, but to minimize these further an amount of KCl equivalent to the amount formed by neutalization of the sample was added to the blanks.

With these modifications the assay proved to be a reliable one for the measurement of small amounts of metanephrine in solution.

To check accuracy and linearity of the method multiple determinations were done with known amounts of metanephrine ranging from 0.0045μg to 1.125 μg. Approximately 10 readings were made at each point. To obtain reproducible values at low concentrations it was necessary to set the fluorometer at maximum sensitivity. When this was done the higher concentrations used could not be read on the same scale and the sensitivity of the fluorometer had to be decreased. The sensitivity of the instrument can be increased stepwise, each step giving approximately 3 times the sensitivity of the previous one. To facilitate the performance and analysis of these and future experiments three separate ranges of concentrations were run at three different sensitivity settings. Curves were constructed using values:

1. 0.0045, 0.01125, and 0.0225 μgram
2. 0.045, 0.1125, and 0.225 μgram
3. 0.225, 0.5625, and 1.125 μgrams of metanephrine

The average of 10 readings at each of these points was determined and three curves plotted. Straight line curves were obtained in each instance. Using these curves the corresponding amounts of metanephrine were then calculated for each point.

The values are summarized in table 2.

The radioactive tracer assay procedure used for the determination of COMT activity is a modification of that described by Axelrod et al[2]. An aliquot of the soluble supernatant was taken for enzyme assay and the COMT activity was determined by measuring the amount of ^{3}H-metanephrine formed from ^{3}H-epinephrine. The incubation mixture, in a 10 cc. polypropylene centrifuge tube, contained 12.5 μ moles of phosphate buffer pH=7.8, 0.675 μmoles $MgCl_2$, 25 μmoles S-adenosyl methionine, 4.12 mμmoles (250 mμcurie) ^{3}H-epinephrine (Epinephrine - 7 - ^{3}H-hydrochloride from New England Nuclear Corp. was mixed with nonradioactive epinephrine to obtain the desire specific activity) and 50 μliters of enzyme preparation in a final volume of 135 μliters. Blanks were obtained by using boiled liver extract in place of enzyme after experiments showed no differences among boiled liver, lens, or iris. Liver was used because of limited quantities of the other enzyme preparations available. The mixture was incubated at 37° C for 1 hour and then 0.5 ml. of 0.5M borate buffer, pH=10.0, and 6 ml. of a mixture of toluene: isoamyl alcohol (3:2) were added and the tubes shaken by hand for 7-10 minutes. After centrifugation, a 4 ml. aliquot of the organic phase was added to 1 ml. of ethanol and 10 ml. of 0.4% 2,5 Diphenyloxazole and 0.01% 1, 4-bis-2- (5-Phenyloxazolyl-) Benzene in toluene and counted in a liquid scintillation counter.

The values were corrected for the partition of metanephrine in the above solvent buffer system. All assays were done in duplicate. This assay has been shown to be specific for COMT by Axelrod et al.[2]

Another assay procedure, which is perhaps not as specific, was also used. The incubation mixture contained 50 μmoles of phosphate buffer pH=7.8, 1 μmoles $MgCl_2$, 83 mμmoles epinephrine, enzyme preparation, and 3.7 mμmoles of ^{14}C-S-adenosyl methionine, with the ^{14}C label on the methyl group, in a final volume of 210 μliters. After incubation for 60 minutes at 37° C, borate buffer and toluene: isoamyl alcohol were added and the material extracted into the organic solvent was counted in a system identical to the previously described one. The specificity of this assay procedure will be discussed.

Table 1

Fluorometric Assay of Metanephrine

		A	B	C	D	E
		sample	tissue blank without oxidation	metanephrine standard	metanephrine internal standard	reagent blank
Metanephrine 1.1 μg/ml	ml	---	---	0.4	0.2	---
KCL 0.5 M	ml	---	---	0.1	---	0.1
Sample, pH=7.0	ml	1.0	1.0	---	1.0	---
Buffer, pH=7.2	ml	0.5	0.5	0.5	0.5	0.5
H_2O	ml	1.0	1.1	1.5	0.8	1.9
Iodine, 0.02 N	ml	0.1	---	0.1	0.1	0.1
Na_2SO_3 and EDTA	ml	0.5	0.5	0.5	0.5	0.5
NaOH, 5N	ml	0.5	0.5	0.5	0.5	0.5
HAc, 10N	ml	0.5	0.5	0.5	0.5	0.5

The metanephrine in the sample is calculated as follows: the instrument is set to zero with E; $\frac{A-B}{C} \times 0.450 = \mu g$ of metanephrine. Recovery was checked with the internal standard, D.

Table 2

Fluorometric determination of metanephrine

Sensitivity	Metanephrine added (mμgm.)	Reading (average)	Metanephrine found (mμgm.) ± S.D.	Coeff. of variation
30x	4.50	9.7	4.50 ± 0.15	3.3%
	11.25	23.0	11.25 ± 0.88	7.8%
	22.50	45.7	22.50 ± 1.54	6.8%
3x	45.0	11.7	45.0 ± 2.47	5.5%
	112.5	25.2	112.5 ± 5.45	4.8%
	225.0	46.0	225.0 ± 10.95	4.9%
1x	225.0	14.2	225.0 ± 10.5	4.7%
	562.5	30.3	562.5 ± 28.8	5.1%
	1125.0	58.8	1125.0 ± 52.7	4.7%

C. Monoamine Oxidase Assay

The assay procedure used for the determination of MAO activity is essentially that of Wurtman and Axelrod.[27] Duplicate aliquots of uncentrifuged homogenate were used and the MAO activity determined by measuring the amount of ^{14}C-tryptamine converted to ^{14}C-indole acetaldehyde and ^{14}C-indole acetic acid. The incubation mixture in a final volume of 300 μliters contained 100 μmoles of phosphate buffer pH=7.4, enzyme preparation, and 13.77 mμmoles (17.9 mμcuries) of ^{14}C-tryptamine bisuccinate (2.22 mg. of Tryptamine-2- ^{14}C-bisuccinate from the New England Nuclear Corp. was dissolved in 14.5 ml. of ice cold 100% ethanol and 25 μl. of this preparation used per assay.) The solution was stored at -10° C. Approximately 4-8 mg. of tissue were used per assay. This was more tissue than Wurtman and Axelrod[27] used, but because of the lower specific activity of the tissues assayed the assay procedure was shown to be linear over the range of tissue concentrations used. The mixture was incubated in a 10 cc polypropylene centrifuge tube for 20 minutes at 37° C and then 0.2 ml. of 2N HCl was added which lowered the pH to less than 0.5. Six ml. of toluene was then added and the tubes shaken by hand for 5-7 minutes. After centrifugation, 4 ml. of the toluene was added to 10 ml. of phosphor and the samples counted in a liquid scintillation counter.

Blanks were obtained by using boiled liver extract in place of enzyme preparations after experiments failed to show a difference among boiled liver, lens, iris, retina, extraocular muscle and optic nerve. The values obtained were corrected for the distribution of the products in the solvent buffer system used. Wurtman and Axelrod[27] have shown that this assay is specific for MAO since the products formed have been identified as indole acetaldehyde and indole acetic acid and the reaction is inhibited in vivo and in vitro by tranylcypromine, a compound which inhibits monoamine but not diamine oxidase.[26]

The S-adenosyl methionine and the ^{14}C-S-adenosyl methionine used were the generous gift of Dr. Axelrod and had been synthesized by him according to the method of Cantoni.[11] These compounds were stored in and diluted with 0.01M acetate buffer pH=5.5 and were stored at -10° C. Preliminary results with commercially available S-adenosyl methionine iodide were disappointing and inconsistent. The COMT activities of several tissues were determined using S-adenosyl methionine iodide. When these were compared with the activities calculated using Dr. Axelrod's S-adenosyl methionine it was found that the ratios of the activities using these two compounds varied considerably from tissue to tissue. Because of this the S-adenosyl methionine iodide was not used.

RESULTS

A. Fluorometric Assay for Catechol-O-Methyl Transferase

This assay procedure was used in only two tissues, rabbit liver and mouse liver. The results obtained are shown in Table 3.

Table 3

COMT Activity of Liver

Tissue	Metanephrine formed/gram/hour (μmoles)
Rabbit liver	0.10
Mouse liver	1.50

Both results were the average of two assays each done in duplicate. They are in the same range as the values reported by Axelrod and Tomchick:[5] rabbit liver, 0.1 μmole/gm./hr., and mouse liver, 2.3 μmoles/gm./hr. The difference in values for mouse liver may be due in part to a strain difference.

B. Radioactive Tracer Assay for Catechol-O-Methyl Transferase

Tables 4a and 4b and Figure 1 show the COMT values obtained using ^{3}H-epinephrine in the assay procedure.

Using the Student "t" test none of the differences noted in table 4b are significant at a probability level of 0.05.

Without S-adenosyl methionine in the incubation mixture and with liver as the source of enzyme, the yield was 3.5% of the control.

An attempt was made to inhibit the enzyme, in vitro, with pyrogallol (Mallinckrodt Chem. Co.) and the following results were obtained, using liver as a source of enzyme. Epinephrine concentration = 2.60×10^{-5} M.

Pyrogallol concentration	%Yield	%Inhibition
0	100	0
1.5×10^{-6}M	71	29
1.5×10^{-4}M	11	89
1.5×10^{-3}M	0	100

Iridial COMT was also inhibited in vitro by pyrogallol. Over 60% inhibition occurred with pyrogallol at a concentration of 7×10^{-5}M.

Table 4a

COMT Activity of Rabbit Ocular Tissues

(^{3}H-metanephrine formed per gram in one hour (mμmoles)

Normal

Expt.	Retina-Choroid	Iris-Ciliary body	Optic nerve	EOM	Liver
1	10	10	-	-	81
2	18	7	-	4	143
3	12	12	9	5	91
4	24	24	12	7	117
5	9	8	15	3	67
6	11	9	14	3	50
7	9	12	11	3	-
8	12	11	10	3	-
9	11	13	13	5	36
10	14	17	15	3	68
11	13	11	9	2	35
12	14	10	15	4	45

Table 4a (continued)

Ganglionectomized

Expt.	Retina Choroid	Iris-Ciliary body	Optic nerve	EOM	Liver
1	19	16	18	-	114
2	21	10	11	4	84
3	12	18	-	4	52
4	17	12	14	5	88
5	15	13	18	5	50
6	12	14	12	4	37
7	14	10	14	3	54
8	12	11	22	3	83

- indicates that no assay was performed for various technical reasons

Table 4b

COMT Activity of Various Ocular Tissues of the Rabbit

COMT Activity (mμmoles of ^{3}H-metanephrine formed per gram in one hour ± S.E.M.)

Tissue	Control	Ganglionectomized
Retina-Choroid	13.1 ± 1.1 (12)	15.3 ± 1.2 (8)
Iris-ciliary body	12.0 ± 1.3 (12)	13.0 ± 1.0 (8)
Optic nerve	12.3 ± 0.8 (10)	15.6 ± 1.5 (7)
Extraocular muscle	3.8 ± 0.5 (11)	4.0 ± 0.3 (7)
Lens	0	0
Liver	73.0 ± 11 (10)	69.4 ± 9.3 (8)

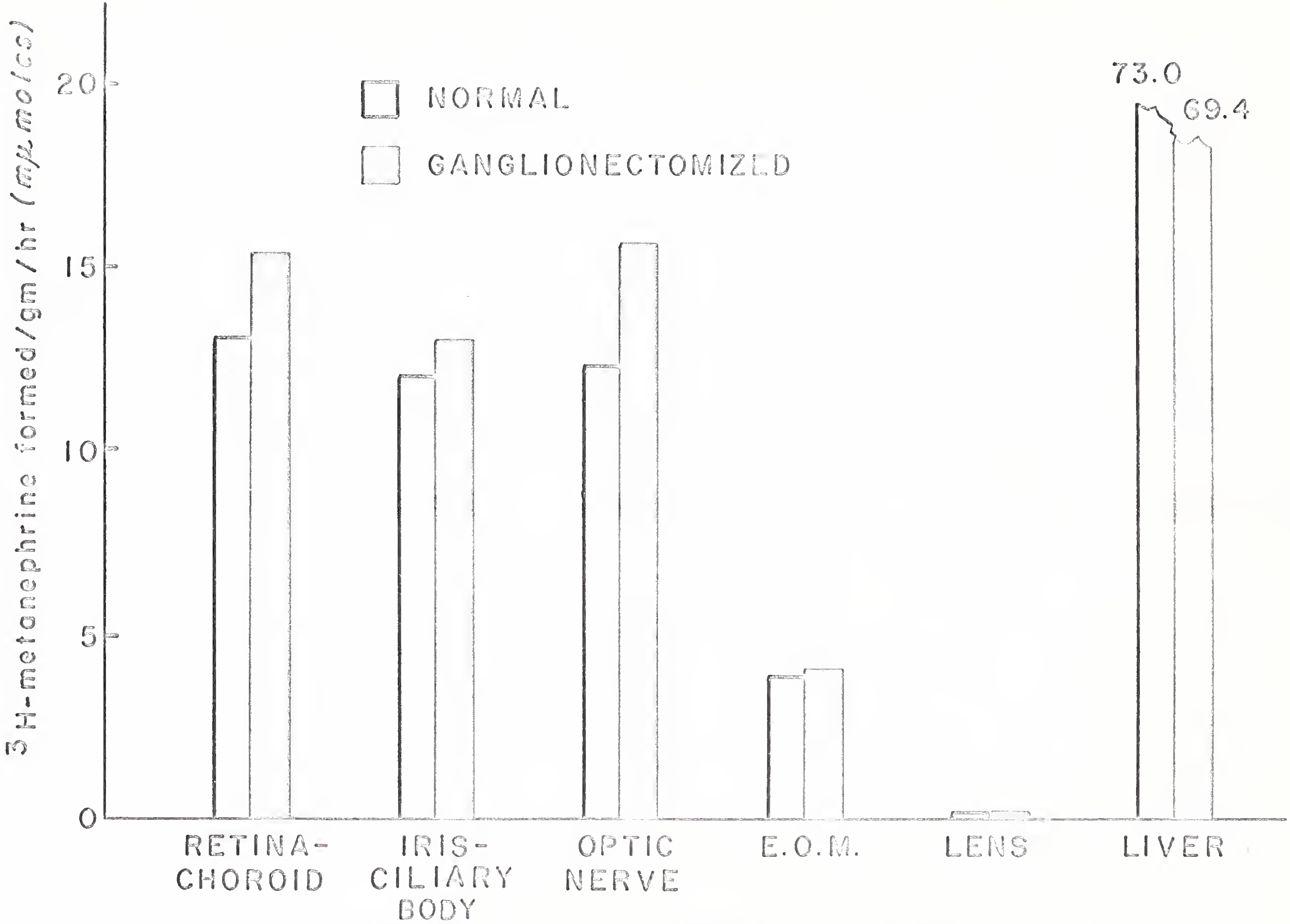

FIG. I COMT ACTIVITY OF RABBIT OCULAR TISSUES

C. Methyl Transferase Activity

Tables 5a and 5b and Figure 2 show the results obtained using ^{14}C-labeled S-adenosyl methionine. Results are reported in mμmoles of CH_3 transferred to substrate and extracted from pH=10.0 buffer/gram of tissue/hour. Using the Student "t" test none of the differences between the control and ganglionectomized animals are significant at a probability level of 0.05.

To determine the specificity of the above method for COMT, epinephrine was omitted from the incubation mixture and the enzymatic activity as a per cent of the control for each tissue was measured. The results are reported in table 6.

Pyrogallol was tried as an in vitro inhibitor of this system. Results with liver were:

Epinephrine concentration = $4\text{x}10^{-4}$M.

Pyrogallol concentration	%Yield	%Inhibition
0	100	0
$1.2\text{x}10^{-6}$M	92	8
$1.2\text{x}10^{-4}$M	31	69
$1.2\text{x}10^{-3}$M	14	86

The enzymatic activity of lens was inhibited 74% using a concentration of pyrogallol of $1.2\text{x}10^{-3}$M.

Table 5a

Methyl Transferase Activity of Rabbit Ocular Tissues

($^{14}C-CH_3$ transferred per gram of tissue in one hours (mμmoles)

Normal

Expt.	Retina-Choroid	Iris-Ciliary body	Optic nerve	EOM	Lens	Liver
1	138	99	71	24	53	496
2	88	104	57	21	46	435
3	86	65	53	14	45	543
4	160	102	78	34	45	600
5	149	70	47	28	-	532
6	161	70	58	29	54	666
7	163	84	77	30	53	554

Ganglionectomized

Expt.	Retina-Choroid	Iris-Ciliary body	Optic nerve	EOM	Lens	Liver
1	44	44	56	-	43	596
2	97	72	15	30	41	527
3	97	97	58	28	56	430
4	114	97	38	23	51	490
5	170	90	84	-	-	-
6	146	104	130	33	46	425
7	153	20	93	38	51	570
8	166	110	54	37	-	490

Table 5b

Methyl Transferase Activity of Rabbit Ocular Tissues

(mμmoles of $^{14}C\text{-}CH_3$ transferred to substrate per gram of tissue in one hour ± S.E.M.)

Tissue	Control	Ganglionectomized
Retina-choroid	135 ± 13.4 (7)	123 ± 15 (8)
Iris-ciliary body	85 ± 7.5 (7)	79 ± 11.3 (8)
Optic nerve	66 ± 4.3 (7)	66 ± 12 (8)
Extraocular muscle	26 ± 2.5 (7)	32 ± 2.3 (6)
Lens	49 ± 1.8 (6)	48 ± 2.3 (6)
Liver	547 ± 28 (7)	518 ± 28 (7)

Table 6

Methyl Transferase Activity Without Added Epinephrine

(as % of values with Epinephrine)

Tissue	%Yield
Retina-choroid	< 5
Iris-ciliary body	< 5
Optic nerve	< 5
Extraocular muscle	< 15
Lens	88
Liver	30

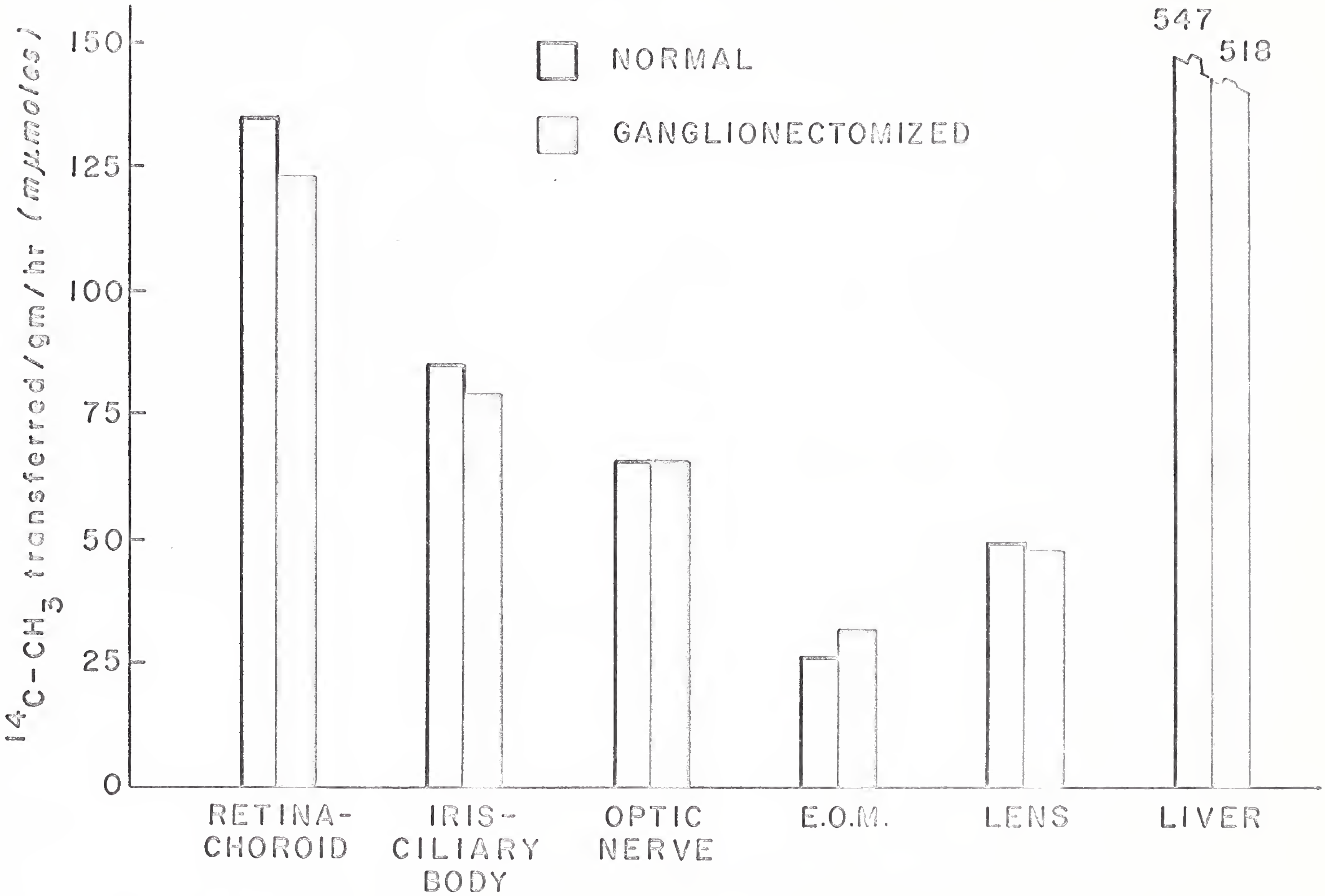

FIG. 2 METHYL TRANSFERASE ACTIVITY OF RABBIT OCULAR TISSUES

Table 7a

MAO Activity of Rabbit Ocular Tissues

(^{14}C-tryptamine oxidized/gram/20 min. (mμmoles)

Normal

Expt.	Retina-Choroid	Iris-Ciliary body	Optic nerve	EOM	Liver
1	196	1230	277	172	1060
2	100	373	190	103	773
3	103	445	190	102	1132
4	212	947	305	110	1187
5	105	340	190	82	600
6	101	304	186	107	1030
7	125	345	204	118	1478
8	130	318	222	89	1015
9	94	271	172	89	1004
10	130	753	278	132	820
11	77	212	114	88	747
12	77	342	156	88	675
13	82	233	148	77	960
14	145	1026	268	129	818

Table 7a (Continued)

Ganglionectomized

Expt.	Retina-Choroid	Iris Ciliary body	Optic nerve	EOM	Liver
1	101	292	175	79	1180
2	88	206	129	79	1087
3	87	264	160	102	803
4	108	280	193	94	987
5	127	342	180	86	837
6	93	258	153	96	985

Table 7b

MAO Activity of Rabbit Ocular Tissues

MAO Activity (mμmoles of ^{14}C-tryptamine oxidized per gram of tissue in 20 minutes ± S. E. M.)

Tissue	Control	Ganglionectomized	
Retina-choroid	120 ± 11 (14)	101 ± 6.2 (6)	N.S.
Iris-ciliary body	510 ± 88 (14)	274 ± 18.2 (6)	$.01 < p < .02$
Optic nerve	207 ± 15 (14)	165 ± 9 (6)	$.02 < p < .05$
Extraocular muscle	106 ± 7.6 (14)	89 ± 3.9 (6)	$.02 < p < .05$
Lens	0	0	
Liver	950 ± 62 (14)	956 ± 55 (6)	N.S.

Table 8

Monoamine Oxidase Inhibition with Tranylcypromine

Tissue	Inhibition (as % of control values)
Retina-choroid	96.5%
Iris-ciliary body	96.1%
Optic nerve	83.5%
Extraocular muscle	96.8%
Liver	93.3%

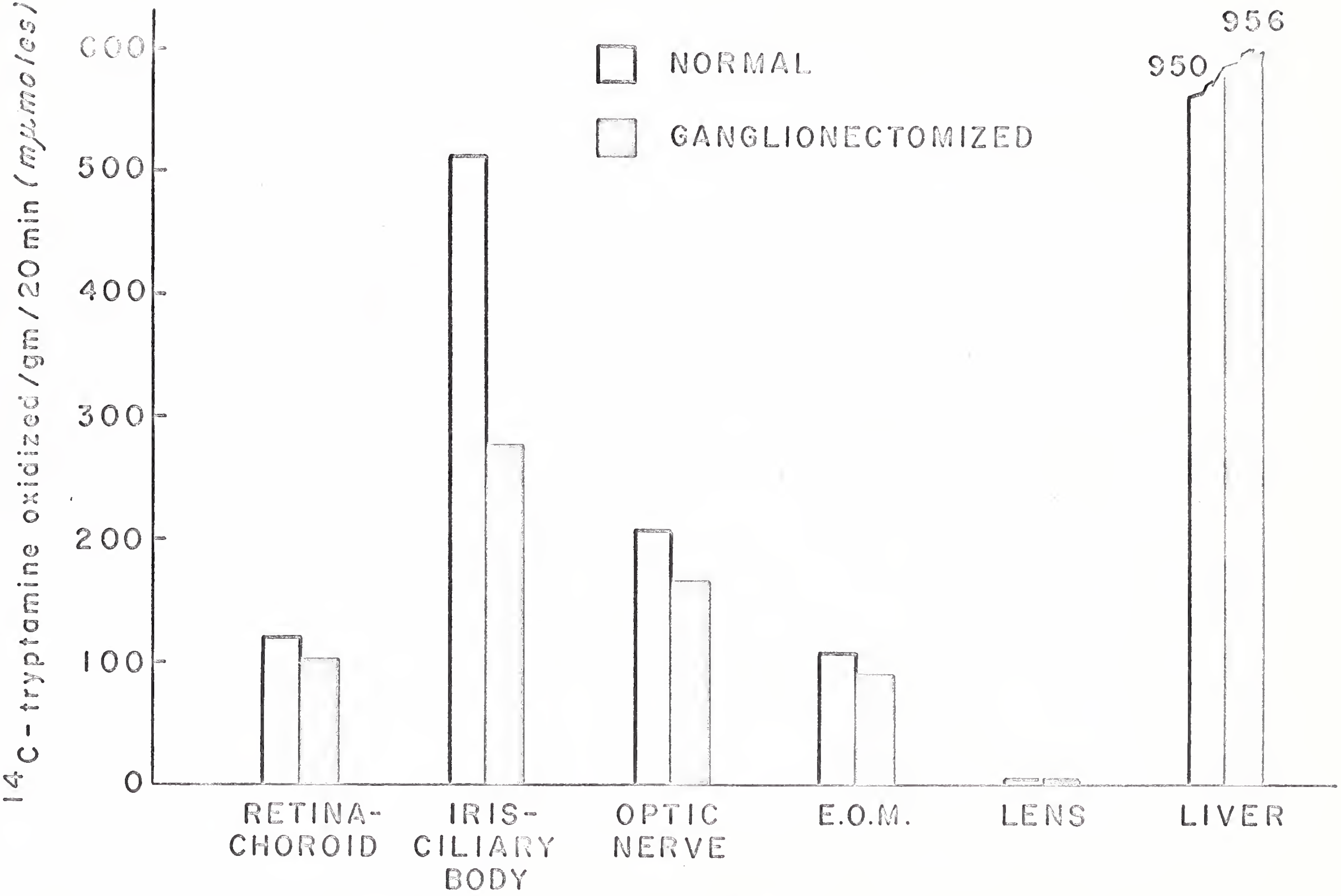

FIG. 3 MAO ACTIVITY OF RABBIT OCULAR TISSUES

DISCUSSION

The results reported show an apparent disparity between the relative COMT and MAO activities of the various ocular tissues. The COMT levels of the different tissues vary, but over a limited range. Iris, retina, and optic nerve all have specific activities within 10% of each other while the lens has no activity and EOM about 30% of the retinal content. The relative MAO activities of the various tissues exhibit greater variation as can be seen in Table 9. These values are in the same range as those of Krishna et al[20] who used pooled tissues from 6-12 rabbit eyes and a manometric technique for measuring MAO.

Table 9

Relative MAO Activity of Rabbit Ocular Tissues*

Tissue	Present Study	Krishna[20]
Retina-Choroid	1.0	1.0
Iris-Ciliary Body	4.25	4.67
Optic Nerve	1.72	1.67
EOM	0.88	0.80
Lens	0	0

*Retina has been arbitrarily assigned a value of 1, and levels for the other tissues derived from this.

To study the possible role of COMT in the immediate inactivation of catecholamines in the eye an attempt was made to inhibit the enzyme using an intravitreous injection of 10^{-3} moles of pyrogallol in a volume of 10 μliters. This was the largest amount of inhibitor that could be placed in the eye in 10 μliters and the initial concentration of pyrogallol in the vitreous was about 1 M. Iris and retina were taken for COMT assay 3 hours and 6 hours after injection, but no inhibition of COMT could be discerned.

Lack of inhibition with pyrogallol could be related to the dilution that the inhibitor undergoes in preparation of the extract, diffusion away from its site of action within three hours, or failure to reach its intimate site of action within the time allowed for the experiment. That the failure to demonstrate in vivo inhibition was not due to an inactive or inappropriate inhibitor was confirmed by in vitro experiments which demonstrated that the pyrogallol used is a potent inhibitor of COMT. The unqiue properties of the ocular system studied make it difficult to apply the results of previous experiments using pyrogallol to this system.

The differences in the levels of COMT and MAO in the various ocular tissues is also noted after denervation. (Tables 4b and 7b). The insignificant effect of bilateral cervical ganglionectomy on COMT activity stands in contrast to the significant 46% decrease in MAO content of iris and 16% decrease in MAO content of optic nerve and EOM. There are several possible explanations for these results.

The enzymes (MAO and COMT) present in the ocular tissues are either associated with the vessels and nerves supplying them or are inherent in the cellular elements of these tissues. The differences in relative activities of the two enzymes suggest that they are either localized in different structures within each tissue or that they are localized in the same structures, in varying amounts, and are differentially affected by denervation. The changes following denervation could be due to a relative decrease in enzyme activity in either of these possible tissue stores. Koelle and Valk[19] have shown that MAO is found in the blood vessels of the nicitating membrane of the rabbit as well as in the ganglion cells of adrenergic fibers supplying this tissue suggesting that this same type of distribution may occur within the ocular tissues. Whether or not MAO is also present in the cellular elements of the ocular tissues is unfortunately not known, but Haggendal and Malmfors[17] have found adrenergic neurons in the inner nuclear layer in the retina of rabbits suggesting that MAO may also be present. No report could be found about the histochemical localization of COMT. Considering all the evidence it is reasonable to suggest that MAO and COMT are localized within the same structures in many tissues and that one or both of these enzymes is also localized alone in other substructures.

It seems logical to conclude from these experiments that while the role of COMT in the immediate inactivation of endogenous catecholamines is still a matter of debate the supersensitivity of the iris and the aqueous outflow facility following cervical sympathectomy is probably not due to a change in the COMT activity of the ocular tissues. The role of MAO is not clear, but in view of the findings of previous investigators that COMT is probably more intimately involved in the immediate inactivation of the amines[4] and that the cat iris, following denervation, is supersensitive to epinephrine without a change in MAO activity[8] it seems reasonable to conclude that the moderate decrease in MAO activity noted after denervation probably has little to do with the observed supersensitivity phenomena. Unfortunately no data on the degree of supersensitivity of the cat iris following denervation is presented in the work of Burn et al.[8] Data about the time course of the supersensitivity and change in MAO content of cat nicitating membrane following denervation is available. At 8-12 days following denervation there was a correlation between these parameters. There was no correlation, however, at 19-33 days. Quantitative histochemical localization of these two enzymes is necessary to ascertain in which elements of these tissues the enzymes are localized and to be certain that intracellular changes in the distribution of these enzymes are not responsible for the observed denervation phenomena.

There appears to be a great deal of species variation in the effect of denervation on the MAO and COMT content of tissues. Crout and Cooper[14] found a 42% decrease in COMT activity of the dog heart following regional neural ablation and Burn and Robinson[9] reported a 40% drop in MAO activity of the cat nicitating membrane after cervical ganglionectomy. In contrast to this Burn et al[8] reported no change in MAO activity of the cat iris following denervation. These reports as well as those of Koelle and Valk[19] and Krishna et al[21] have shown that there is considerable species variation in the relative tissue concentrations of MAO and make it clear that it is impossible to anticipate the effect of denervation on the enzyme content of a given tissue of a given species. The results reported here on the effect of denervation on the enzyme content of the rabbit ocular tissues represent another variation to be considered in future experiments.

The significance of the results obtained using ^{14}C-S-adenosyl methionine in the COMT assay is still unclear. The enzyme measured in iris, retina, optic nerve and EOM would appear to be COMT because depletion of epinephrine from the incubation mixture causes an almost complete reduction in the amount of extractable compound formed. The fact that the system was inhibited, in vitro, by pyrogallol lends further support to the argument. When

epinephrine was excluded from the incubation mixture and liver used as a source of enzyme the activity was 30% of the control. The observation that the liver enzyme was inhibited 85% by 10^{-3} M pyrogallol suggests that the above findings can be explained by variations in the tissue stores of catecholamines. They could also be explained by the presence of an epinephrine activated enzyme that is also inhibited by pyrogallol directly or because the inhibitor interferes with the catalyst role of epinephrine. Treatment with reserpine to deplete the tissue stores of catecholamines would help resolve the question.

The enzyme activity measured in the lens is more complex to analyze. Exclusion of epinephrine from the incubation mixture lowers activity only 5-20% and yet pyrogallol inhibits this enzyme 74% at a concentration of 10^{-3}M. There are many possible explanations for this finding and further studies are needed to determine the compounds formed by the lens with the present incubation system. For now we must conclude that the enzyme measured is a methyl transferase, the specificity of which has yet to be determined.

SUMMARY

1. The levels of COMT in several ocular tissues of the rabbit have been measured.

2. The levels of this enzyme are not affected by bilateral cervical ganglionectomy.

3. The levels of MAO in several ocular tissues of the rabbit have been measured.

4. Bilateral cervical ganglionectomy caused a significant decrease in the MAO content of the optic nerve, extraocular muscle and iris, although the decrease demonstrated for retina was not significant.

5. An incompletely characterized methyltransferase has been measured in the rabbit ocular tissues and its values are unchanged by denervation. There is a significant amount of this enzyme present in the lens.

6. These studies indicate that the enzymes MAO and COMT may be localized in different substructures within the ocular tissues.

7. Denervation supersensitivity phenomena present in the rabbit eye after cervical ganglionectomy are probably unrelated to changes in the tissue concentrations of the enzymes MAO and COMT.

8. A method is described for the measurement of small quantities (4-20 mμgrams) of metanephrine in solution.

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