A HISTOCHEMICAL INVESTIGATION INTO THE REGIONAL DISTRIBUTION OF MONOAMINE OXIDASE IN THE BRAIN-STEM OF RABBIT AND CAT

WITH ATLAS OF RELATED CONCENTRATIONS

by

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We accept this thesis as conforming to the required standard

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ABSTRACT

In the last decade, considerable interest has been focused on the role of biogenic amines and their function in the central nervous system. Certain of these, norepinephrine and serotonin, have been suggested as neurotransmitters, and evidence has accumulated that rise in the levels of these amines results in behavioural change. At the same time, it was found that the enzyme, monoamine oxidase (MAO), utilized these compounds as substrates, and that inhibition of MAO resulted in elevated levels of the catecholamines and serotonin.

This knowledge has led to considerable investigation of the areas of brain that might be affected by this inhibition, but beyond the preliminary report of Shimizu et al. (1959) little had been done to determine the histochemical localization of MAO by methods of proven specificity.

A study of the brain-stem has therefore been attempted, to determine the major sites of MAO activity. Rabbit and cat brain-stem have been used, and the histochemical method of Glenner, Burtner and Brown (1959) using nitro-blue tetrazolium. Fresh frozen tissue was cut on the cryostat and sections incubated with this solution, a positive result producing a purple-blue formazan precipitate.

For identification and correlation of brain-stem nuclei, adjacent sections were cut and stained with toluidine blue.

Controls were run in vitro and in vivo with known MAO inhibitors,
as well as by incubation without substrate, application of heat and alteration of pH.

Finally, an atlas has been prepared identifying the sites of MAO activity and suggesting a functional relationship based on these studies. Results indicate that within the brain, the brain-stem itself contains the highest proportion of MAO, which is concentrated within the following regions - choroid plexus, pineal gland, hypothalamus, pituitary, interpeduncular nucleus, habenulo-peduncular tract, dorsal tegmental nucleus, locus coeruleus, area postrema and cranial nerve nuclei, especially the distal portion of the trigeminal nucleus and the dorsal vagal nuclei. The thalamus, inferior colliculi and major fibre tracts were all conspicuously low in MAO. With the exception of the cells of the mesencephalic nucleus of nerve V, activity did not occur within the body of the neuron, but was present in the neuropil of the neocortex and all other positive areas of brain-stem. Certain peculiarities of distribution were noted for the glandular areas of the pineal, pituitary and choroid plexus. In the anterior pituitary and choroid plexus, MAO was found in intracellular granules, but within the pineal and posterior pituitary the activity appeared to lie in a matrix between cells.
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INTRODUCTION

The purpose of this investigation is to explore the specific areas of brain-stem where monoamine oxidase (MAO) is shown to be most active. Only Shimizu et al. (1959) and Smith (unpublished) have attempted any detailed histochemical investigation of cerebral MAO, and since a refinement of Shimizu's technique was available here, it has thus been possible to add to original findings. For this purpose, histochemical methods using nitro-blue tetrazolium have been used, in conjunction with more common histological techniques, and an atlas of the brain-stem has been prepared, supplemented by hand-drawn maps. Controls were run by in vitro and in vivo administration of MAO inhibitors, and an evaluation made of the reaction specificity.

Papez (1929) defines "brain-stem" as that part of the brain which remains after removal of the cerebral hemispheres and cerebellum. In many studies of more recent date, however, the anterior limits are taken no further than the colliculi and may even terminate before them. Thus, in view of the considerable latitude in this respect, it is proposed in this study to describe "brain-stem" as the area bordered by the optic chiasma anteriorly, and by the first cervical nerve posteriorly, excluding the cerebral hemispheres and cerebellum (see plates I, II and III).

The brain-stem has been chosen for examination because of its phylogenetic and functional importance. The primitive brains of vertebrates were little more than brain-stem consisting of cranial reflex mechanisms, which in the mammals became a compact, highly organized structure linking all parts of the old and new brain. In any mixed collection of mammalian
brains, the brain-stem shows remarkable similarity from species to species, so that, bearing in mind the increase in size and complexity of the cortex and related connections in higher mammals, any well-organized brain-stem will exemplify the entire mammalian class. We know at least that this is so anatomically, but we do not yet know whether this is so chemically.

Thus, because the brain-stem is the oldest and most fundamental part of the brain, carrying out reflex arrangements in connection with cranial nerves, regulating conduction systems to the cortex and cerebellum, and engaging in the basic activity of the reticular formation, it is of particular interest to study the chemoarchitecture of this area. Furthermore, it might perhaps be called the "glandular brain" since it is known that in many parts of the brain-stem, hypothalamus, pituitary, pineal and probably some other areas, neurohumoural mechanisms are at work that exert, and are affected by, internal and external environmental influences of all kinds.

Above all, it is here that biogenic amines have been found in the highest concentrations, and upon which monoamine oxidase exerts its effects. It is, therefore, the purpose of this study to explore the brain-stem in order to determine not only the precise regional locations of this enzyme, but also its relative concentrations in the various parts, and to see if some consistent pattern emerges that would indicate any special links between structural units and brain monoamines.
PART I. CHEMISTRY AND PHARMACOLOGY OF MAO

1. History.

The first indication that amines could be deaminated in the body by an enzyme was noted by Hare (1928) who showed that mammalian liver contains a tyramine oxidase. In 1937, Pugh and Quastel observed that melanin-like pigments, firmly bound to tissue, were formed by the oxidation of indolethylamine. They suggested that this reaction might be catalysed by MAO. In the same year, Blaschko et al., Pugh and Quastel, and Kohn, independently concluded that the enzymes oxidizing tyramine, adrenaline and aliphatic amines were identical. Zeller (1951) suggested that this enzyme be called "monoamine oxidase" to differentiate it from diamine oxidase.

In 1953, Blaschko and Hellman confirmed that tryptamine and serotonin (5-HT) were oxidized by MAO, converted to an aldehyde, and finally formed dark brown pigments insoluble in water. They stated that this reaction should also be utilizable as a histochemical technique.

Further work by Blaschko and Philpot (1953), Sjoerdsma et al., (1955), Udenfriend and Titus (1954), Udenfriend et al. (1956), and Weissbach et al. (1957) has shown conclusively that 5-HT is deaminated by tissue MAO to give 5-hydroxyindoleacetaldehyde, which is then converted to 5-hydroxyindoleacetic acid. However, in the presence of an electron acceptor such as tetrazolium salts, the conversion to the acid does not take place, and another pathway is made leading to formation of a formazan pigment precipitate.
2. **Identification and Determination of MAO by Chemical Methods.**

MAO may now be defined (Davison, 1958) as the enzyme which is responsible for the oxidative deamination of monoamines such as the catecholamines, serotonin, tyramine and tryptamine, according to the general equation:

\[ \text{R.CH}_2\text{NH}_2 + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{R.CH}_2\text{NH}_3 + \text{H}_2\text{O}_2 \]

Isolation of MAO has recently become possible, and it is interesting to note that injection of a purified MAO preparation into hypertensive rats results in a prolonged lowering of blood pressure (Davison, 1958). MAO is destroyed by heating for 10 minutes at 50°C., or by a pH below 6 and above 9-10. Its optimum pH appears to be around pH 7.3. It is inhibited by iproniazid (1 x 10^{-4} M) but not by cyanide or isoniazid (1 x 10^{-4}).

Lagnado and Sourkes (1956) postulated an amine dehydrogenase system, requiring a heat-stable co-factor, present in liver and rat brain. Glenner et al. (1959), however, after further investigation using reduction by tetrazolium salts, could find no evidence of such a mechanism. Thus, there are no known co-enzymes or prosthetic groups connected with MAO.

Several methods have been used for determining MAO activity in tissues:

a) **Manometric methods**, using tyramine or tryptamine as substrate, and measuring the oxygen uptake during incubation (Creasey, 1956). The biological activity can best be determined on the washed particulate fraction of homogenates or on dialysed homogenate preparations. This method may then be checked by determining the ammonia or amine liberated during substrate oxidation by the Conway technique.
b) **Measurement of substrate disappearance** (Bogdanski *et al*., 1956, Sjoerdsm et al., 1955). The tissue is homogenized with distilled water, and prior to incubation, serotonin is added with phosphate buffer to pH 7.4. This is followed by spectrophotofluorimetric determinations.

c) Since these procedures are only satisfactory for studying tissues which contain rather large amounts of enzyme, a new method (Lovenberg *et al*., 1962) has been worked out for use in organs with low MAO activity, or very small amounts of tissue such as sympathetic ganglia. This highly sensitive method for measuring MAO *in vitro*, is based on the rate of indoleacetic acid (IAA) formation from tryptamine. The immediate product of the reaction is indoleacetaldehyde, but if excess aldehyde dehydrogenase and DPN are added, indole acetic acid can be obtained from the reaction. It is not often possible to quantify the disappearance of small amounts of tryptamine, but corresponding amounts of IAA can easily be detected since the fluorescent properties of the compound permit assay of microgram quantities (Lovenberg *et al*., 1962).

For all these biochemical assays, similar controls may be used as in histochemical methods, viz., incubation without substrate, application of heat, or incubation with an MAO inhibitor of known potency.

3. **Distribution of MAO**.

The occurrence of MAO appears to be fairly widespread in nature. It is known to occur in plants, although this enzyme may not be identical with vertebrate MAO since it oxidizes not only monoamines but also diamines such as cadaverine and spermine. Plant amine oxidase resembles a diamine oxidase in being inhibited by semicarbazide and cyanide (Kenton and Mann, 1952).
In the animal world, Blaschko and Hope (1957) have shown that MAO is not confined to vertebrates, but has been found in many invertebrates including molluscs.

In invertebrates extensive biochemical studies have been performed showing occurrence of the enzyme in tissues such as liver, kidney, stomach, intestines, adrenals, lung, pancreas, uterus, placenta, spleen and blood vessels, as well as in the central and peripheral nervous system. It is absent in erythrocytes and plasma, and very low levels are found in muscle (Davison, 1957; Langemann, 1944). Nevertheless, although wide variations between species are found, there are consistently high levels in nervous tissue in all species.

In man, recent assays of MAO have been made by Levine and Sjoerdsma (1962) from post-mortem material obtained 8 hours after death, and from jejunal mucosa by capsule biopsy. They found that the MAO of intestinal mucosa greatly exceeded that of any other tissue, but this was in fresh tissue as against post-mortem tissue, which was listed in the following order of activity - liver, kidney, heart, lung, thyroid, adrenal, pancreas, sympathetic ganglion and cerebral cortex. These tests were considered valid in that Davison (1958) considered MAO quite stable even at room temperature for 24 hours. Experiments conducted on brain in this laboratory do not, however, substantiate this finding (see experiment, page 30). It would appear that stability must vary from tissue to tissue, since pineal, pituitary and placenta tested here do not show the rapid deterioration of MAO that is found in brain post-mortem. This may account for the comparatively low levels found in brain by Levine and Sjoerdsma.

Bogdanski et al. (1957) have raised the question of there being
more than one MAO, one of which may be more specific for 5-HT, than, for instance, nor-adrenaline. This would be comparable to the relatively specific and non-specific enzymes involved in acetylcholine metabolism.

The exact intracellular location of MAO has yet to be determined, and histochemical methods are not yet sensitive enough to determine sub-microscopic distribution in all areas. Hawkins (1952) and Cotzias and Dole (1951) have studied the intracellular localization of MAO in rat liver, and consider it to be probably exclusively located in mitochondria. Little systematic work has been reported on other tissues, however, although it is known that MAO is found in the particulate fraction of brain, intestine, lung and kidney. In general, however, it is believed that MAO is found predominantly in mitochondria.

4. MAO in Brain Development.

Shimizu (1959a) and Nachmias (1960) have investigated the activity of MAO in developing rat brain by histochemical and biochemical methods. From their observations it seems that local differences do not become apparent until 3 weeks after birth. At a foetal age of 15 days, almost negative activity was noted, gradually showing conspicuous activity in the locus coeruleus at the 20th foetal day, with generalized activity in other regions. Adult concentrations and localization were present after the age of 3 weeks.

5. Substrate Location, Metabolism and Mechanisms of Action.

Most of the substrates of MAO, the catecholamines, and 5-HT, have been found in granules, many of which, on ultracentrifugation, precipitate below the mitochondrial fraction. Blaschko (1958) felt that these granules might represent a new type of cell organelle, and reports the presence of high ATP wherever catecholamines are high, suggesting
that ATP may be involved in holding adrenaline.

Hagen (1958) reports that these intracellular granules containing adrenaline (A) and noradrenaline (NA) measure about 50 μ in diameter and are smaller than histamine granules. ATP is present in the ratio of 4-3:1. Reserpine causes a depletion; iproniazid appears to protect. Serotonin is also found in granules, as well as in platelets and mast cells of rats and mice. Thus, it is believed that in these sites, the substrates of MAO are "bound" and can only be acted upon by MAO when "free". Precisely what stimulus or mechanism causes the release of these amines is not yet known.

Tables I and II show the mechanism of action of MAO on various substrates. Dixon and Webb (1960) note that monoamines resembling CH$_3$ (CH$_2$)$_n$NH$_2$, which are only slowly attacked by diamine oxidase, are attacked by MAO at a rate which shows an optimum at a chain length which varies with the preparation used. Primary amines are the best substrates; secondary and tertiary amines are slower, while quaternary salts are negative. The action of MAO is prevented by a methyl group on carbon atom 1 of the substrate.

Other enzymes also appear to be active on some catecholamines and other monoamines. Koelle (1958) reports the activity of cytochrome oxidase, dopa decarboxylase (tyrosine oxidase), conjugases, and catechol-0-methyl-transferase, as well as MAO. Although Axelrod (1959) has shown that 0-methyl-transferase may play a significant part in metabolism, it is still held that MAO has a more important biological action.

Barondes (1962) has noted the action of A, NA and 5-HT in stimulating glucose-1-C$^{14}$ oxidation by beef anterior pituitary slices, a property apparently shared by a number of structurally related amines,
TABLE I
Catabolism of Catecholamines Showing Influence of MAO

Tyramine → DOPA → 3,4-Dihydroxyphenylpyruvic acid

Dopamine → 3,4-Dihydroxyphenylacetic acid

N-Methyldopamine → O-Methyldopamine → Homovanillic acid

Noradrenaline → Normetanephrine → 3-Methoxy-4-hydroxyphenylethanol

Adrenaline → Metanephrine → 3-Methoxy-4-hydroxyglycolic aldehyde

Sulfate
TABLE II

Tryptophan Metabolism in Relationship to Serotonin, Melatonin and MAO

\[
\text{Tryptophan} \rightarrow 5\text{-Hydroxytryptophan (5-HTP)} \rightarrow 5\text{-Hydroxytryptamine (5-HT)} \rightarrow \text{MAO (inhibitor)} \rightarrow \text{MAO (inhibitor)}
\]

- 5-Hydroxyindoleacetaldehyde (5-HIAA)
- Formazan ppt.
- 5-Methoxyindoleacetic acid (5-HIAA)
- Conjugates
- N-Acetyl-5-HIAA
- N-Acetyl-5-methoxytryptamine
- Melatonin
- 10-Methoxy harmalaln (MAO inhibitor)
but not by alpha-methylated derivatives. Since alpha-methylated amines are not substrates for MAO, the possibility that these active amines could stimulate glucose oxidation only after reaction with MAO is suggested. Barondes confirmed this by finding that two different MAO inhibitors could block the effect of the active amines. Further, the possibility that the aldehydes generated by MAO might be active was tested, since it is known that the aldehyde metabolites of several of the active amines, and a number of aliphatic aldehydes, share the property of stimulating glucose-1-$^{14}\text{C}$ oxidation, and that the effect of the aldehydes is not blocked by MAO inhibitors. From the fact that such aldehydes can stimulate glucose-1-$^{14}\text{C}$ oxidation, Barondes suggests that the action of MAO may not only be to detoxify and degrade but also to convert neuroamines to an active form.

In view of the consistently high levels of MAO found in the pineal in all species in this study, and the discovery of the hormone "melatonin" in the pineal, it is interesting to note that this substance derives from serotonin. The presence of melatonin has been reported by Lerner et al. (1959) in the pineal gland, and Giarman and Day (1959) have noted the presence of physiologically active amines such as histamine, serotonin and catechol amines, as well as enzymes involved in their formation and metabolism. Axelrod and Weissbach (1961) have observed, in addition, the presence of hydroxyindole-0-methyl-transferase (found only in pineal), imidazole-N-methyl-transferase and catechol-0-methyl-transferase.

Although N-acetyl-serotonin is found to be the best substrate for hydroxyindole-0-methyl-transferase, other hydroxyindoles are methylated but to a much smaller extent (100:12). The observation that
5-hydroxyindoleacetic acid served as substrate for the enzyme is of interest, since Lerner et al. found 5-methoxyindoleacetic acid in bovine pineal glands (Brown, 1959).

Thus, it is becoming apparent that the pineal gland, an organ of which the physiological function has so far been obscure, possesses considerable biochemical activity.

6. Uptake of Substrates in Brain.

Wilson et al. (1962) suggest that a process of active transport may be important in localizing the relatively high concentrations of catecholamines that have been observed (Vogt, 1954) in certain parts of the brain and central nervous system. Although circulating catecholamines are excluded from most of the brain, they do enter the pituitary (Axelrod et al., 1959) as well as certain parts of the hypothalamus in very low concentrations. The pituitary is one of several special regions within the CNS which appear to have no blood-brain barrier (Wislocki and Leduc, 1952; Brodie et al., 1960). It was further shown by Wilson that intravenously administered epinephrine-H₃ was taken up in the pineal body, area postrema and intercolumnar tubercle of the cat, in concentrations considerably in excess of those in parts of the brain lying within the blood-brain barrier. Administration of reserpine to cats greatly reduced the concentration of epinephrine-H₃ appearing in pituitary.

von Euler and Lishajko (1961), studying uptake of catecholamines in adrenergic nerve granules, have also shown that isolated nerve granules can take up the catecholamines, similarly to dopamine uptake in the adrenal medulla (Bertler et al., 1961). The NA is retained in storage granules when the concentration of axoplasm is about 10 μg/ml, then released at lower extra-granular concentration following NA flux through
the axon membrane during nerve stimulation.

These facts are of interest in that MAO concentration has been found, in this study, to be high in the choroid plexus and in those other parts outside the blood-brain barrier where epinephrine-\(H^3\) was taken up, and it could be possible, therefore, that MAO acts in some way to regulate in these regions, the levels of active amines.

7. Inhibition of MAO.

Work on inhibition of MAO was largely pioneered by Zeller (1951, 1952, 1955) and has since become a major method of experimentation. Mann and Quastel, however, had proposed, in 1940, that the stimulant action of amphetamine might be related to the inhibition of MAO. They believed that amine metabolism proceeded via the formation of intermediate aldehydes which were depressants of brain activity. It was thought that inhibition of MAO decreased the formation of such aldehydes and thereby prevented the onset of mental depression. Later workers (Grana, 1959; Schayer, 1953) showed, however, that stimulant doses of amphetamine and related drugs were not sufficient to produce significant MAO inhibition in the intact animal. When Zeller showed the MAO inhibitory properties of the anti-tubercular, anti-depressant drug, iproniazid, the possible relationship between enzyme inhibition and pharmacologic activity was seen. A number of other hydrazine compounds have also been shown to be effective MAO inhibitors (Biel et al., 1959; Chessin et al., 1959; Randall et al., 1959) and have had therapeutic application. Semicarbazide and potassium cyanide, although carbonyl trapping agents, do not inhibit MAO.

It is now generally believed (Horita, 1961; Brodie et al., 1959; Spector et al., 1958) that the inhibition of MAO results in a protection and subsequent accumulation of brain serotonin and catecholamines. Thus,
the primary cause of the anti-depressant response is due to increase in
the levels of these amines. Precisely how the accumulation of amines
produces this effect, is not yet known.

The concentration of stored amines appears to be maintained by
a fine balance between their biosynthesis and detoxication process. The
latter event can occur only after the agents are released from their
"bound" to their "free" forms. The administration of a MAO inhibitor
results in an upset of the balance between synthesis and degradation,
forcing an increase in brain levels of the amine, since synthesis is not
affected.

Among physiological compounds investigated, only weak MAO
inhibitors have been found. The most active is cortisone (Schweppe et al.
1959) and thyroxine, which depressed enzyme activity by only 30-50%.
The degree of inhibition does not influence the amine level, according to
recent work by Gey and Pletcher (1961). Thus, MAO activity does not
influence MAO content of the brain under physiological conditions. The
great excess of MAO suggests a function of rapid inactivation of free
monoamines after release from storage depots.

In regard to MAO inhibition by hydrazines, it has been shown that
these are also effective DAO inhibitors as well (Burkard et al., 1960;
Cohn and Shore, 1960). Most are also potentiators of various pharmaco-
logical agents (Arrigoni-Martelli, 1959; Eltherington and Horita, 1960;
Fouts and Brodie, 1956) and some have sympathomimetic properties, whilst
others are weak adrenergic blocking agents (Griesemer et al., 1955).
Increase of lactic acid has also been noted by Gey and Pletcher (1960).
In addition, many of the anti-MAO hydrazines are effective inhibitors of
5-HTP and dopa decarboxylase (Tabachnick, 1959), thus preventing normal
synthesis of amines after MAO blockade. It has also been found that
depressants such as barbiturates and anti-convulsants, raise the brain
levels of serotonin (Bonnycastle et al., 1957).

New compounds (tranylcypromine, alpha-ethyltryptamine) unrelated
to hydrazines, have, however, been found which produce a long lasting,
probably irreversible blockade of MAO. These, too, have anti-depressant
effects, thus lending support to the hypothesis that MAO inhibition can
be responsible for the mechanism of "psychic-energising". Horita (1961)
warns that in administering any MAO inhibitor, "variation in the route
of administration may give different patterns of MAO inhibition, in
different animals and in different species."

8. Psychiatric Implications.

Many theories have been advanced regarding the psychotropic
effects of over- or under-supply of MAO substrates in Brain, and it has
thus been postulated that absence or dysfunction of the enzyme could lead
to other pathways for the metabolism of physiologically active amines.
Adrenaline or serotonin, for example, could be oxidized to hallucinogens.
Woolley and Shaw, in 1954, first postulated that schizophrenia could be
the result of an error in the metabolism of serotonin. Likewise, Hoffer,
Osmond and Smythies (1954) postulated the presence of an abnormal adrenal-
line metabolite - adrenochrome - in schizophrenia. Later research has,
however, been unable to prove the validity of these theories. It should
also be remembered that MAO inhibitors can have more generalized systemic
effects such as connective tissue fibroplasia, peripheral ganglionic
inhibition and hypotensives.

Little has been done to correlate abnormal amine metabolism with
the pineal, but it is interesting to note that Giarman et al. (1960)
found the highest pineal gland serotonin in psychotic patients. Furthermore, Altschule and Giancola (1960) reported that extracts of pineal cause biochemical changes in schizophrenics. There is, however, insufficient knowledge in this area to warrant any conclusions as yet.

In spite of the limitations of present knowledge, the fact remains that MAO has extreme biological importance, and that inhibition of this enzyme can produce anti-depressant effects with concomitant increase in brain amine levels. Thus, from the many studies on the metabolism and pharmacology of these amines, some investigators (Brodie and Shaw, 1957; Marrazzi and Hart, 1957) have suggested that they might function as neurotransmitters. MAO could thus serve to detoxify the transmitter, in an analogous fashion to AChE. It cannot now be, however, categorically stated that serotonin and the catecholamines are acting as transmitter substances, although there is strong possibility of such a function.
PART II. HISTOCHEMICAL INVESTIGATION OF MAO


Since Oster and Schlossman's attempt in 1942 to demonstrate MAO in tissues by means of tyramine incubation, many investigators have explored the possibilities of more specific methods. The method of Oster and Schlossman depended on the ability of fresh frozen sections of guinea pig kidney to produce aldehyde when incubated with tyramine and phosphate buffer. Formation of the aldehyde was confirmed by Schiff's reagent. Gomori (1950) and Pearse (1961) severely criticized the specificity of localization given by this method, since the reaction product was soluble in water and diffused freely in the incubating medium. Pearse therefore suggested that a simultaneous coupling method be used to trap the aldehyde formed at the moment of its release, to form an insoluble precipitate in situ, but attempts to do this were unsuccessful.

Koelle and Valk (1954) then developed a method using naphthoic acid hydrazide as an aldehyde capture reagent and 0.01M hydrazine as a blocking agent. In place of tyramine, tryptamine was used as substrate, and 20 per cent sodium sulphate was added to the incubating medium. This method, however, although satisfactory in the majority of mammalian tissues, is not suitable for use on brain because of its high content of oxidizable lipid causing a pseudoplasmal reaction with the hydrazide, and resulting in deeply stained control sections.

A method based on pigment formation was attempted by Blaschko and Hellman (1953) by incubating sections with tryptamine hydrochloride at pH 7.4. Arioka and Tanimukai (1957) used the same method, with serotonin
as substrate, but found results inconsistent. Takamatsu (1958) also developed another method using tyramine and potassium tellurite, but because of the low redox potential of the latter, the method has limited value. Pearse (1961) believes that methods relying on pigment formation cannot be expected to give accurate localization of MAO, since pigment may be produced from aromatic amines by other enzyme systems in the absence of MAO.

The first method to be based on reduction of tetrazolium salts was reported by Francis (1953) who incubated tissues with tyramine and neotetrazolium chloride, a method unsuccessfully attempted by other workers. In 1957, Glenner, Burtner and Brown attempted a modification of the Francis technique, and by substituting tryptamine for tyramine, and by using a more easily reducible tetrazolium salt, obtained excellent results. Formazan pigment was produced under histochemical conditions from iodonitrotetrazolium (INT) and from nitro-blue tetrazolium (NBT), but not from neotetrazolium or blue tetrazolium because of their lower redox potentials giving difficulty of reduction. The following table shows the half wave potentials of some of the better known tetrazolium salts and tellurite (from Pearse, 1961):

<table>
<thead>
<tr>
<th>Oxidant</th>
<th>$E^{\frac{1}{2}}$, 22°, pH 7.2, volts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tellurite</td>
<td>- 0.95</td>
</tr>
<tr>
<td>TTC</td>
<td>- 0.49</td>
</tr>
<tr>
<td>NT</td>
<td>- 0.17</td>
</tr>
<tr>
<td>BT</td>
<td>- 0.16</td>
</tr>
<tr>
<td>MTT</td>
<td>- 0.11</td>
</tr>
<tr>
<td>INT</td>
<td>- 0.09</td>
</tr>
<tr>
<td>NBT</td>
<td>- 0.05</td>
</tr>
</tbody>
</table>

Results of the Glenner method for MAO localization corresponded in all respects to the Koelle-Valk technique, one method thus lending support to the other. This method has been used in the present investiga-
2. **Comparisons of Other Work on Brain MAO.**

With regard to investigation of MAO in brain by similar methods, Shimizu, Morikawa and Okada (1959) have used INT to examine the brain of rodents, Glenner (1957) has made a limited survey using NBT, and Smith (1962) has recently completed a survey of rat brain using NBT. The details of these investigations are tabulated and compared in the following table. Bogdanski's (1955) results, using biochemical methods of evaluation, are included for comparison, but unfortunately do not outline the same areas.

<table>
<thead>
<tr>
<th>Area</th>
<th>Rat, mouse, rabbit, guinea pig Shimizu et al. (1959)</th>
<th>Rat, rabbit, guinea pig Smith (1962)</th>
<th>Rabbit, cat This Investigation</th>
<th>Rat guinea pig, rabbit Glenner (1957)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locus coeruleus</td>
<td>+++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Habenula</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Midline nuclear gp. of thalamus</td>
<td>+++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periventricular grey matter</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Med. nucl. of hypothalamus</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Fasciculus retroflexus</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Interpeduncular nucl.</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Nucl. of brachium conjunctivum</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Dorsal nucl. of vagus n.</td>
<td>+++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleus ambiguous</td>
<td>+++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inf. olivary nucleus</td>
<td>+++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area postrema</td>
<td>+++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oculo-motor nuclei</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neocortex</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampus - neocortex</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- dentate gyrus</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Ammon's pyramids</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amygdala</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Regional Comparisons of MAO in Brain, etc., cont'd.

<table>
<thead>
<tr>
<th>Area</th>
<th>Rat, mouse, rabbit, guinea pig Shimizu et al. (1959)</th>
<th>Rat, mouse, rabbit, guinea pig Smith, et al. (1962)</th>
<th>Rabbit, cat Glenner (1957)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal ganglia - caudate</td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>- putamen</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>- globus pallidus</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Dorsal tegmental nucl.</td>
<td>±</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Mesenceph. nucl. of N. V</td>
<td>±</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Other thalamic nuclei</td>
<td>±</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Mammillary body</td>
<td>±</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Red nucleus</td>
<td>±</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Pineal</td>
<td>±</td>
<td>+++(pig) (ox)</td>
<td></td>
</tr>
<tr>
<td>Choroid plexus</td>
<td>+</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Pituitary - anterior</td>
<td>-</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>- posterior</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Distribution of MAO in Dog Brain by Biochemical Assay
(Bogdanski, 1955)
(in ng/5-HT destroyed per g/hr.)

<table>
<thead>
<tr>
<th>Area</th>
<th>MAO Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amygdala</td>
<td>968</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>1624</td>
</tr>
<tr>
<td>Septal region</td>
<td>1212</td>
</tr>
<tr>
<td>Midbrain</td>
<td>842</td>
</tr>
<tr>
<td>Piriform cortex</td>
<td>926</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>935</td>
</tr>
<tr>
<td>Medulla</td>
<td>1117</td>
</tr>
<tr>
<td>Thalamus</td>
<td>940</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>1176</td>
</tr>
<tr>
<td>Pons</td>
<td>936</td>
</tr>
<tr>
<td>Olfactory bulb</td>
<td>573</td>
</tr>
<tr>
<td>Cortical grey matter</td>
<td>819</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>930</td>
</tr>
<tr>
<td>Corpus callosum</td>
<td>466</td>
</tr>
<tr>
<td>+ internal capsule</td>
<td></td>
</tr>
<tr>
<td>Lateral geniculate</td>
<td>844</td>
</tr>
<tr>
<td>Optic tract</td>
<td>701</td>
</tr>
<tr>
<td>Fornix</td>
<td>707</td>
</tr>
</tbody>
</table>
3. Chemistry of Tetrazolium Salts.

In 1894, von Pechmann and Rune prepared the first tetrazolium salt, triphenyl tetrazolium chloride (TTC), but it was not until 1941 that Kuhn and Jerchel discovered their use in enzyme chemistry and found that a number of colourless tetrazolium salts were reduced to coloured compounds by plant tissues. Synthesis of new compounds was then made and it is now clear that the ditetrazolium salts give better histochemical results.

The formazan from INT is macrocrystalline, but that of NBT is microcrystalline. The reaction taking place is as follows:

\[
\text{Ditetrazolium salt} \quad \xrightarrow{+4H} \quad \text{Diformazan} \quad \xleftarrow{-4H} \quad +HCl
\]

\[
\begin{align*}
\text{Ditetrazolium salt} & \quad (\text{colourless}) \\
\text{Diformazan} & \quad (\text{blue purple})
\end{align*}
\]

The above reaction indicates complete reduction, but partial reduction can occur at one end of the molecule only, giving a monoformazan which is reddish. Nitro-blue tetrazolium is 2,2'-di-p-nitro-phenyl-5,5'-diphenyl-3,3'-((3,3'-dimethoxy-4,4'biphenylene) ditetrazolium chloride. With the use of suitable substrate, these compounds can also be used in dehydrogenase chemistry, and are the current choice for localization of
sucinic dehydrogenase, TPN and DPN (Friede, 1961).

4. Chemistry of the MAO-Tetrazolium Reaction.

The mechanism by which tetrazolium salts are reduced in the MAO reaction has been investigated by Weissbach et al. (1957) who demonstrated that in vitro, INT was reduced only when an indole amine was oxidized by the MAO of rat liver mitochondria. Other substrates for MAO did not give rise to products capable of reducing tetrazolium salts.

Further elucidation of the mechanism of tetrazole reduction was made possible in 1959 by the synthesis of indole-3-acetaldehyde, the process being described by Glenner et al. (1959) as follows:

\[
\text{Tryptamine} \xrightarrow{\text{mao}} \text{IAc} \xrightarrow{\text{aldehyde dehydrogenase}} \text{IAA} \xrightarrow{\text{oxidation}} \text{pigment}
\]

The implication of an aldehyde-intermediate in the formation of this pigment was first postulated by Blaschko (1953) on the basis of carbonyl reagent inhibition of pigment formation.

Thus, the product of MAO activity on tryptamine (IAc) appears to be an intermediate in this particular pigment formation. In the histochemical system, IAc directly reduces the tetrazole and forms, not IAA, but an unknown compound which may then go on to pigment formation. This is a somewhat unique mechanism, in that it stems from a non-enzymatic reduction of an indicator, by the product of an enzyme catalysed reaction. It should be added that no evidence of a diaphorase-like system in this
reduction process has been found (Glenner, 1959).

5. **Evaluation of Reaction Mechanisms and Specificity.**

It is recognized that the histochemical method has many limitations, not the least of which is the lack of any completely reliable method of quantitative determination. Friede (1960), working with succinic dehydrogenase (SDA), attempted a laborious and careful densitometric evaluation showing reasonable correspondence with microanalytical methods. Others (Farkas et al., 1962) have recently attempted quantitative measurement by chromatography. The reacted tissue section is adhered to chromatographic paper, and chromatography carried out. During this process the formazans provided by SDA shift off the section and separate from each other. This method would, however, be of limited value in sections containing several centres of marked activity.

By biochemical analytical methods of MAO measurement, diffusion of enzyme occurs after differential centrifugation. Diffusion artefacts are also a hazard of the histochemical method, but these may be minimized by careful handling, and consistent clear-cut results obtained. The general consensus of opinion is that "regional" localization only is possible by the histochemical method for MAO, but in this investigation it has been possible to obtain repeated patterns of intracellular detail in identical areas, i.e., choroid plexus, pituitary and neuropil. Although undoubted weaknesses exist in the method, the actual specificity for the reaction is well established (Weissbach, 1957), and in regions of such complex anatomy as the brain-stem, the advantages of a histochemical approach are still considerable.
PART III. EXPERIMENTAL PROCEDURE AND TECHNICAL DATA


A wide initial survey of brain and spinal cord was made on 34 animals of different ages and sexes, including mouse, rat, rabbit, cat, dog, pig and ox. Tests were performed using different inhibitors, substrates, methods of killing, freezing, fixing and durations of enzyme activity after death. The results of these experiments are summarized at the end of this section.

Finally, 6 healthy adult rabbits (mixed sexes) and one adult cat, were selected for brain-stem mapping as outlined in this study.

2. Methods of Treatment.

Animals in the initial survey were killed either by decapitation or by a blow on the head. It was noted, however, that no visible differences in enzyme localization and concentration could be detected after this method of killing, or after administration of ether or pentobarbital. Hence, the final animals used for brain mapping were given pentobarbital 30 mg/kilo body weight, followed by perfusion with normal saline, of the still beating heart. This method also clarified the picture, in that no confusion could be caused by the presence of blood-borne MAO. MAO has been found in the blood of some species, although generally absent from erythrocytes and plasma.

Initial experiments "quick-freezing" tissue with iso-pentane (-140°C) in liquid nitrogen (-180°C) appeared to cause more cell disruption and diffusion of enzyme in brain tissue than slower freezing methods.
Hence, the final tissues were placed on ice as soon as removed, then mounted on blocks and frozen at -15°C before cutting as soon as possible thereafter, at -5°C, cryostat temperature. Friede (1958) found that artefacts, loss of enzyme activity (SDA) and diffusion of stain, are minimized by cutting at a temperature of -3°C to -2°C for 60μ sections. In this case, -5°C was found to be best for these sections cut at 30μ. Cutting was performed in a cryostat, and the sections were mounted on slides, then briefly dried at room temperature before applying solution.

3. **Solutions (according to Glenner, 1957).**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptamine hydrochloride</td>
<td>25 mg (0.005M)</td>
</tr>
<tr>
<td>Sodium sulfate</td>
<td>4 g</td>
</tr>
<tr>
<td>Nitro-blue tetrazolium</td>
<td>5 mg</td>
</tr>
<tr>
<td>0.1M phosphate buffer, pH 7.6</td>
<td>5 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>15 ml</td>
</tr>
<tr>
<td>N.N. dimethylformamide (solvent for tetrazolium salts)</td>
<td>0.05 ml (own modification)</td>
</tr>
</tbody>
</table>

Following incubation for 2 hours at 25°C, the sections were washed for 2 minutes before being fixed overnight in formalin vapour and mounted in glycerin-gelatin.

4. **Controls.**

Controls used consisted of:

a) MAO inhibitors *in vivo* and *in vitro* (See Table III and IV, page 25 and 28).

b) Destruction by heat (60°C for 20 minutes).

c) Incubation without tryptamine.*

Controls were run on each area of brain-stem investigated. During

* The pineal alone, still gave moderate reaction without substrate.
the cutting of the serial sections throughout the brain-stem, adjacent sections were taken for staining with toluidine blue, so that each enzyme-incubated section had its equivalent set of stained sections. By a technique of superimposition of the cell-stained slide, over the enzyme incubation, it was thus possible to locate nuclei with precision, using low power optics. Sections were cut in a transverse plane.

5. Experimental Results.

TABLE III.

Results of In Vitro Tests of Various Drugs on MAO Inhibition in Rabbit Brain

<table>
<thead>
<tr>
<th>MAO Inhibition graded visible reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Hydrazines - MAO inhibitors</td>
</tr>
<tr>
<td>Iproniazid (Marsilid) ++</td>
</tr>
<tr>
<td>Catron (beta-phenyl-isopropyl-hydrazine) +++</td>
</tr>
<tr>
<td>(2) Non-Hydrazines - MAO Inhibitors</td>
</tr>
<tr>
<td>Tranylcypromine (Parnate) ++</td>
</tr>
<tr>
<td>Amphetamine -</td>
</tr>
<tr>
<td>(3) Non-MAO Inhibitor Anti-Depressives</td>
</tr>
<tr>
<td>Imipramine (Tofranil) -</td>
</tr>
<tr>
<td>Amitryptaline (Elavil) ++</td>
</tr>
<tr>
<td>(4) Muscle Relaxants</td>
</tr>
<tr>
<td>Flaxedil (gallamine tri-ethiodide) (cat) ++</td>
</tr>
<tr>
<td>Tubocurarine +</td>
</tr>
<tr>
<td>Decamethonium bromide (syncurine) ++</td>
</tr>
<tr>
<td>Succinylcholine (Anectine) ++</td>
</tr>
<tr>
<td>Intocostrin -</td>
</tr>
<tr>
<td>(5) Miscellaneous</td>
</tr>
<tr>
<td>Aminoguanidine +</td>
</tr>
<tr>
<td>Pyrogallol -</td>
</tr>
<tr>
<td>Reserpine ++</td>
</tr>
<tr>
<td>Dilantin -</td>
</tr>
<tr>
<td>Phenobarbitone -</td>
</tr>
</tbody>
</table>
Each drug was incubated on fresh frozen tissue sections in 0.1M concentrations in standard tryptamine-tetrazolium solution (Glenner), buffered at pH 7.6, for 2 hours at 25°C. These tests were performed on one set of rabbit brain tissues only, using sections from the same brain as controls. The drug was dissolved in distilled water and added in equal quantities concomitantly with the standard incubating solution.

Discussion of Previous Experiment (Table III).

**Group 1.** All hydrazines, having either the enclosed or terminal \(-\text{NH-NH}\) group, i.e., Catron -

\[
\text{C - C - NH-NH}_2
\]

All have been shown to have MAO inhibiting properties, and results of this experiment, substantiate this.

**Group 2.** Certain non-hydrazine compounds have also been found to have MAO inhibiting properties, i.e., tranylcypromine and amphetamine. Only tranylcypromine was effective here.

**Group 3.** Both groups 1 and 2 have anti-depressive properties plus MAO-inhibitory effects. Group 3 has anti-depressive effects but do not inhibit MAO. Only amitryptaline here showed inhibition *in vitro*.

**Group 4.** This consists of a group of muscle relaxants most of which are quaternary ammonium compounds having muscle end plate effects. All exhibited MAO inhibition except Intocostrin.

**Group 5.** Aminoguanidine is a known DAO inhibitor but not an MAO inhibitor. Pyrogallol inhibits o-methyl-transferase, and reserpine liberates catecholamines and acts as a tranquillizer.
No explanation of these results is possible.

Results of this experiment are of interest in the light of similar pharmacological work, but have no actual validity in that one set of tissues only was tested and "pre-incubation" as well as "concomitant" incubation with drugs was not done. Glenner (1957) noted that dissimilar results were sometimes obtained by the two methods and believed that inhibition by reagents incorporated in the incubation mixture (concomitant) could be caused by:

a) an inhibition of the enzyme systems per se,

b) a reaction of the inhibitor with a substrate utilized by the enzyme system(s),

c) an electron-trap preventing tetrazolium reduction.

Semi-carbazide and cyanide, which do not inhibit MAO in the biochemical assays, caused, for instance, a marked concomitant inhibition but no inhibition by pre-incubation methods.

One of the weaknesses of the method of using MAO inhibitors in vivo or in vitro, is the difficulty of assessing dosage since some MAO inhibitors are more effective than others and require lower dosage. On the other hand, some species are more susceptible to these drugs than others, and response may vary in duration and magnitude.

The following in vivo tests of MAO inhibition have perhaps greater validity than those performed in vitro, but the same difficulties apply with regard to dosage and species. In this experiment (Table IV) similar dosage was used as in the histochemical demonstration in rat of MAO inhibition by Catron (Mustakallio et al., 1961), and corroboration of their results was obtained. A dose of $1 \times 10^{-4}$M/kg body weight was given,
and the rats sacrificed 6-18 hours after. No. 1 and 2 are hydrazine MAO inhibitors; 3, 4 and 5 are non-hydrazines, non-MAO inhibitors; and 6 is a non-hydrazine MAO inhibitor.

TABLE IV.

**In Vivo Tests of MAO Inhibition**

<table>
<thead>
<tr>
<th></th>
<th>Species</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Iproniazid</td>
<td>rat</td>
</tr>
<tr>
<td>2.</td>
<td>Catron</td>
<td>rat</td>
</tr>
<tr>
<td>3.</td>
<td>Flaxedil</td>
<td>cat</td>
</tr>
<tr>
<td>4.</td>
<td>Amitryptaline</td>
<td>cat</td>
</tr>
<tr>
<td>5.</td>
<td>Reserpine</td>
<td>rabbit</td>
</tr>
<tr>
<td>6.</td>
<td>Tranylcypromine</td>
<td>rabbit</td>
</tr>
</tbody>
</table>

The inhibition noted in these *in vivo* tests with the known MAO inhibitors iproniazid, Catron, and tranylcypromine, is considered to be the most conclusive and valid control of those used, since true biological inhibition correlated with patterns of behaviour observed in the experimental animal, especially in rats. In rabbit (No. 6) there was less behavioural agitation but it was noted that three times the amount of pentobarbital was required to produce anaesthesia.
### TABLE V.

**Substrate Test**

Results of MAO activity with various substrates (all substrates 0.025 mg)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Hydroxytryptamine</td>
<td>+ positive</td>
</tr>
<tr>
<td>DL-Normetanephrine</td>
<td>± very slight reaction</td>
</tr>
<tr>
<td>4-Methoxyphenylethylamine</td>
<td>- negative, no reaction</td>
</tr>
<tr>
<td>3,4-Dimethoxyphenylethylamine</td>
<td>- negative, no reaction</td>
</tr>
</tbody>
</table>

### TABLE VI.

**Uptake Test**

Pre-incubation (25 minutes) with the following was carried out to determine any evidence of uptake

<table>
<thead>
<tr>
<th></th>
<th>Without Tryptamine</th>
<th>With Tryptamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOPA</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5-Hydroxytryptophan</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Histidine</td>
<td>-</td>
<td>+</td>
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</tbody>
</table>
TABLE VII.

Extinction Test

Experiment to test duration of activity of MAO in hours after death

Method: Six adult white rats were sacrificed by a blow on the head, the bodies then allowed to remain with brain in situ until time of treatment. One hemisphere was then removed and quick frozen in iso-pentane in liquid nitrogen, cut and incubated for MAO.
TABLE VIII.
Fixation Tests

Fixatives used after incubation and washing included:

(i) 80% alcohol, 5 minutes
(ii) 10% neutral formalin, 24 hours
(iii) Formalin vapour (90%) overnight

Results:

(i) Tissue shrinkage was observed, although demonstration of fine detail often improved.
(ii) Tissue swelling, and some sections lost.
(iii) Least tissue changes.

No effect on the incubation colour reaction was observed in any of these fixatives.
6. **Photography.**

Friede (1961) has noted the difficulty of photographing large, thick sections because of the differences in refractive index of components of the tissue, and recommended the use of a thin opal glass immediately below the screen. Black and white photography in our case was performed using a ground glass screen with the following equipment:

- **Film:** Ilford 35 mm. F.P.3
- **Camera:** Leica with focus slide copy attachment and extension bellows
- **Lens:** F/3.5 50 mm. Elmar
- **Developer:** Kodak, Microdol X
PART IV. ATLAS OF MAO IN BRAIN-STEM


The atlas consists of reproductions of transverse sections taken through the brain-stem of rabbit (Plates I, II and III in back pocket) from the region of the first cervical nerve posteriorly, up to the optic chiasma anteriorly. These sections were cut at a thickness of 30 μ at intervals of approximately 350 μ throughout the brain-stem. Sections were then incubated for MAO, then representative series reproduced as in the following atlas. Adjacent sections were stained with toluidine blue in order to distinguish cell groups. In addition, hand-drawn maps were constructed as keys to the enzyme and cell-stained sections.

Thus, the series consists of:

a) Map of each section, as indicated in Plates I, II and III of brain-stem (in pocket at back).

b) Accompanying legend.

c) MAO section photographed in black and white, of representative areas.

d) Toluidine blue stained section photographed in black and white, of similar areas.

2. Key to Atlas Abbreviations.

α cell group α of Meesen and Olzewski
am ambiguous nucleus
ant anterior nucleus of thalamus
aq aqueduct
ar area postrema
arc arcuate or ventrolateral nucleus of thalamus
as acoustic stria
at acoustic tubercle or dorsal cochlear nucleus
bc  brachium conjunctivum
bon basal optic nucleus (supra-optic)
čj first cervical nerve
cb  cornu ammonis
cb  cerebellum
cen central nucleus of dorsal horn
cf  central tegmental fasciculus
cg  central grey matter
cic commissure of inferior colliculus
cil ciliary nucleus
cp  cerebral peduncle
cpl choroid plexus
cs  cerebrospinal (pyramidal) tracts
cvs crossed vestibulospinal tract
d  cell group d of Meesen and Olzewski
D  dorsal tegmental nucleus (of Gudden)
don dorsal olivary nucleus
don dorsal nucleus of thalamus
dt descending root of trigeminal nerve
dur descending vestibular root

EW Edinger-Westphal nucleus
fc  fasciculus cuneatus
fg  fasciculus gracilis
fn  facial nerve
fx  fornix
gf  genu of facial nerve
hb  habenular bodies
hp  habenulo-peduncular tract
hy  hypothalamus
hyn hypoglossal nucleus
ic  inferior colliculus
int internal capsule
lp  interpeduncular nucleus
it  intercalate nucleus
ion inferior olivary nucleus
lat lateral nucleus of thalamus
lg  lateral geniculate body
lc  locus coeruleus
ln  lateral nucleus of oblongata
mb  mammillary body
mc  Meynert’s supraoptic commissure
med medial nucleus of thalamus
mes mesencephalic nucleus of the trigeminus
mg  medial geniculate body
mi massa intermedia
ml medial lemniscus
mlf medial longitudinal fasciculus
mt mammillo-thalamic tract (Vicq d'Azyr)
mrs medial reticular formation
mv dorsal motor nucleus of the vagus
n nodulus
nbc nucleus of the brachium conjunctivum
nc nucleus cuneatus
ng nucleus gracilis
nll nucleus of lateral lemniscus
not nucleus of the optic tract (pre-tectal)
npc nucleus of the posterior commissure
oc optic chiasma
ocn oculo-motor nerve
oh olfacto-habenular tract (ansa peduncularis)
om oculomotor nucleus
ost olfactory striatum or peduncular nucleus
ot optic tract
pc posterior commissure
peri peripeduncular nucleus (of Jacobsohn)
pin pineal body
pp pontal protuberance
prn pontal raphe nuclei
pul pulvinar
Q cell group Q of Meesen and Olzewski
rb restiform body
rn red nucleus
rs rubro-spinal tract
rsx rubro-spinal decussation
rt reticular formation
sc superior colliculus
sf solitary fasciculus (or descending sensory root of the vagus and glossopharyngeal nerves)
sg substantia gelatinosa trigemini
sgs superficial grey stratum of the tectum
sn substantia nigra
sp spinal part of the spinal-accessory nerve
sr sensory radiations
st spinotectal tract
sth spino- and bulbo-thalamic tract including lower trigemino-thalamic fibres
teg lateral tegmental nucleus of midbrain
tn trigeminal nucleus
tro trochlear nucleus
36.


Anatomical names of tracts, nuclei and other structures are according to Papez' "Comparative Neurology", 1929 (Hafner, 1961). Since these did not cover all the details required for description of rabbit brain, reference has also been made to Meesen and Olzewski's "Atlas of the Rhombencephalon of the Rabbit" (1949), Monnier and Gangloff's "Stereotaxic Rabbit Brain Atlas" (1961), Friede's "Histochemical Atlas of the Cat Brain Stem", and Gillilan's "Atlas of the Mid-Brain of the Rabbit" (1943).

Maps. These are shaded to denote regions of MAO activity, i.e.,

\[\text{**\textcolor{black}{\text{////}}\text{**}}\] indicates strong activity

\[\text{**\textcolor{black}{\text{///}}\text{**}}\] indicates moderate activity

Regions of weak activity are unshaded. Magnification is 10 x.
4. **Atlas Serials.**

**Atlas of MAO Concentrations in Rabbit Brain-Stem**

<table>
<thead>
<tr>
<th>Sec.</th>
<th>Page</th>
</tr>
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<tbody>
<tr>
<td>03-2</td>
<td>Oblongata</td>
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<tr>
<td>03-8</td>
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<td>03-9</td>
<td>&quot;</td>
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<td>03-36</td>
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<tr>
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<td>Midbrain</td>
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<td>04-20M</td>
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<td>04-17M</td>
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<tr>
<td>04-13M</td>
<td>Diencephalon</td>
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<td>04-11M</td>
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<td>04-10M</td>
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<td>04-6M</td>
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<tr>
<td>04-4M</td>
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In the lower oblongata, the main concentrations of enzyme are seen surrounding the central canal in the region of the dorsal and ventral commissures, and in the greatly enlarged substantia gelatinosa which here becomes the terminal descending nucleus of the trigeminal. The overlying tract of Lissauer can be identified laterally by oval areas of unreactive tissue.

This region is unique in the histochemical brain-stem picture, in that a regular pattern of well-marked activity can be determined, in which parallel bands of neuropil lie on the periphery of the trigeminal nucleus, and embrace certain cells with a basket of fine ramifications. Under oil immersion, these dendrites and cell expansions can be seen to possess a double nature, studded with fine granules along their course. Only in the olivary nuclei can a rather similar situation be found.
Here there is little basic change in regions of MAO activity from the previous section. The dorsal commissure broadens with strong MAO, and equal concentration continues in the trigeminal nucleus.
Sec. 03-9: Oblongata

Level of Obex

The vagal commissure contains strong MAO, spreading laterally in the ala cinerea of the vagal nuclei. The inferior olive has now entered the picture, and shows also strong MAO and marked reaction of the neuropil.

The nucleus of the reticular formation shows moderate MAO, so that the MAO reactive areas show up roughly as a figure eight - laterally the trigeminal nuclei, medially the vagal commissure, dorsally the nuclei of the fasciculus cuneatus, and ventrally the reticular formation.
In this section, the area postrema appears lateral to the vagal commissure and dorsal to the sensory vagal nucleus. It has approximately the same intensity of reaction as the vagal nuclei and inferior olive. The trigeminal nucleus, however, is becoming gradually less reactive, so that, in the next section, the nucleus has only mild MAO comparable to the intervening grey matter of the oblongata.

An area of fairly strong MAO appears in the motor nucleus of the vagus, continuing down into the neuropil of the solitary fasciculus and ambiguous nucleus. Ventrally, the lateral nucleus carries moderate MAO.
PLATE V

MAO reaction

SEC. 03-15

Tol. blue
The most prominent reactive area continues to lie within the neuropil of the vagal nuclei. The hypoglossal nucleus shows only moderate MAO, as do the solitary fasciculus, trigeminal nucleus and reticular formation. Moderate MAO is present in the inferior and superior olive. As in the last section, both the central regions of crossed vestibulo-spinal tracts, and the medial lemniscus show minimal activity.

In this area of the 4th ventricle, the choroid plexus is immediately distinguishable under high power by its dense concentration of highly reactive MAO-containing granules. The subependymal tissue within this region of choroid plexus exhibits an extraordinarily intense granule reaction in the neuropil, almost as though active diffusion were taking place between the choroid plexus and brain stem tissue.
The strong MAO in the vagal nuclei continues well defined. In the vagal nuclei, certain small cells appear to have reacted strongly, although the criss-cross felt-work of neuropil is the most distinctive pattern. Mild to moderate MAO is shown in the descending vestibular root, reticular formation and ambiguous nuclei. Ventro-medially, cell group d of Meesen and Olzewski shows prominent MAO.

Ventral to the strongly reacting subependymal layer, the hypoglossal and intercalate nuclei show the same moderate reaction throughout the neuropil.
The subependymal layer above the descending vestibular nucleus shows the strongest MAO in this section. Moderate activity is present in all other areas of grey matter, most pronounced in the following nuclei: hypoglossal, vagal, intercalate, descending vestibular, ambiguous, olivary, trigeminal, and in cell group d, and the solitary fasciculus. The medial reticulo-spinal tract is weak in MAO.
From this level of the oblongata, through the midbrain and up to the diencephalon, the most conspicuous feature is the absence of strong MAO. Except for a few scattered areas - locus coeruleus, central grey matter, oculo-motor complex and interpeduncular nucleus, no strongly marked activity is present.

The vagal nuclei and roots have become embedded in the deep surface of the descending vestibular nuclei and are here seen with only moderate MAO, forming a line of activity to the facial nucleus, against the weakly reactive reticular formation. Along the dorsal surface, below the nodulus, the descending vestibular nuclei show mild to moderate MAO, while along the lateral aspects, the acoustic stria, and moderately reactive dorsal and ventral cochlear nuclei, border the restiform body.
In the brain-stem at this area, the MAO activity is confined to the central grey matter, locus coeruleus, mesencephalic root of the trigeminal, nucleus of the lateral leminiscus and of the brachium conjunctivum. This follows roughly a v-shaped band medio-laterally. In the central grey, the weakly reactive cell group Q of Meesen and Olzewski is medially located. Lateral to this is the locus coeruleus, distinctly marked, as is the mesencephalic nucleus of N. V. These two areas are strongest in MAO.

In the region of the mesencephalic nucleus of N. V, individual cells can be recognised. The MAO is here apparently located only within certain cells, and faintly or not at all in the neuropil. This is a direct reverse of the usual situation, and continues through into the midbrain areas.

A band of moderately MAO reactive tissue occurs in the central pontal raphe nuclei, but the medial reticular formation is almost devoid of MAO.
In this section, the most conspicuous feature is the absence of virtually any MAO in the inferior colliculi. On the other hand, the collicular commissure and central grey matter are conspicuously reactive, particularly in the area immediately ventral to the aqueduct. Ventral again to this are the strongly reactive oculo-motor complex (N. III, and the Edinger-Westphal nucleus), on the ventral borders of which are the pale unreactive tracts of the medial longitudinal fasciculus. The dorsal tegmental nucleus is conspicuously strong in MAO.

On the periphery of the ventral part of the central grey matter a few large, oval, deeply stained cells of the mesencephalic trigeminal nucleus lie in a neuropil of strong MAO. The red nucleus appears to contain a few large reactive cells, as in the substantia nigra.
The inferior colliculi continue conspicuously unreactive, but the superior colliculi, with moderately strong MAO, come to overlie them as the tectum of the midbrain. The central grey matter still contains strong MAO, especially in the dorsal tegmental nucleus, and is carried on into the oculomotor, trochlear and Edinger-Westphal nuclei. Again, a few cells of the mesencephalic trigeminal nucleus are outlined by the reaction.
The superior colliculus shows a moderate MAO reaction throughout most of its layers (N.B., optico-sensory reaction centre), excepting the optic stratum and tecto-spinal tract. Surrounding the aqueduct, the central grey matter continues to show a strong reaction, with the Edinger-Westphal nucleus showing strong MAO between the pale tracts of the medial longitudinal fasciculus. The lateral tegmental nucleus shows moderate MAO.

In this section, however, by far the most predominantly reactive, is the now large interpeduncular nucleus lying immediately dorsal to the pons. Lateral to this are the pale relatively unreactive tracts of the cerebral peduncles, dorsal to which are a few scattered stained cells of the substantia nigra.
The interpeduncular nucleus is here the strongest MAO area, with the unreactive mammillary body lying on its ventral surface. The central grey and oculomotor regions are diminished in activity, and the tracts of the oculomotor nerve fibres can be seen emerging medial to the cerebral peduncles.

Moderate MAO can be seen in the superficial grey substance, in the medial geniculate body and in the peripeduncular nucleus or substantia nigra.
Reference has been made to the unusual reactivity of the habenulo-peduncular tract (fasciculus retroflexus) since, in general, fibre tracts are unreactive. In this section the two arms of the tract can be seen dorsal to the mammillo-thalamic tract.

The strongest MAO is to be found in the tuber cinereum, but the fornix and mammillo-thalamic tract are unreactive. Moderate activity is to be found in the central grey, ciliary and oculomotor regions, and in the medial geniculate body.

The neuropil of the superior collicus, and peripeduncular nucleus still contain mild to moderate MAO.
A more complicated pattern of MAO distribution is found, in general, in the diencephalon.

Starting from the dorsal surface, the superficial grey substance (moderate MAO) shelters a strongly MAO reactive pineal.

The unreactive optic tract surrounds the lateral borders of this area, and enters below the superficial grey substance to connect with the nucleus of the optic tract above the nucleus of the posterior commissure. Both these nuclei contain moderate MAO. The aqueduct here is surrounded by a narrow zone of moderate MAO, with the reactive habenulo-peduncular tract approaching now its ventral limit.

Very strong MAO is seen in the hypothalamus, and moderate MAO in the medial geniculate and peripeduncular nuclei.
In this region of the posterior commissure, the highly reactive pineal organ may still be seen, but lies now between the lateral geniculate bodies which contain moderately strong MAO.

The pulvinar of the thalamus (weak MAO) enters the picture in this section, and more ventrally the sensory radiations can be distinguished by moderate MAO.

The midline nuclei and hypothalamus show conspicuously strong MAO.
The habenulo-peduncular tract can here be seen entering the habenular body, and the olfacto-habenular tract can still be traced. The medial and central nuclei contain moderate MAO, but little or no activity is apparent in the ventral or ventro-lateral nuclei of the thalamus.

The lateral geniculate bodies can no longer be seen in the dorsal region but have come to lie lateral to the pulvinar. Thus, the section is almost completely bordered by the pale staining fibres of the optic tract. The whole midline area takes on great significance in relationship to MAO, since from the habenular trigones ventromedially, through the midline nuclei of the thalamus to the nuclei of the hypothalamus, is a region of strong activity, particularly in the periventricular area.
The dorsal nucleus of the thalamus now lies lateral to the habenular trigone. Weak MAO only is apparent throughout the thalamus, except in the central midline area where moderate MAO is present; higher MAO is present in the habenular bodies and hypothalamus.
5. Description of Special Areas.

a) Medulla spinalis and spinal cord.

In sections taken from the level of the first cervical nerve, a very similar distribution of MAO activity is seen, as in the rest of the cord at lower levels. Throughout the cord, activity is usually most marked in the neuropil surrounding the central canal, and in the remaining neuropil of the grey substance. Strong concentrations are often seen in the substantia gelatinosa, and as an exception to neuronal activity, some staining of anterior motor neurons, particularly at lower levels of the cord. The fibre tracts appear under low power, to contain little or no MAO, but under high power very small reactive granules may be seen. It has not been possible to determine the exact nature of these granules; whether mitochondrial, or associated with other structures such as the glia or myelin sheath.

b) Blood vessels and meninges.

Arteries and veins outside the brain showed slight activity within their walls, but no MAO was found in intracerebral capillaries. This is in agreement with the findings of Shimizu et al. (1959), but contrary to those of Arioka and Tanimukai (1957), using a method involving pigment formation on oxidative degradation of tryptamine and serotonin.

c) Ventricular walls - ependyma and sub-ependyma.

The MAO content of the ependyma cells was variable throughout the brain-stem. In some areas there was almost no evidence of any MAO activity, whereas in others the ependyma and subependymal tissue were intensely strong in MAO. In general, however, the ependyma had only mild to moderate activity, with the subependymal areas showing moderate to strong activity.
One or two peculiarly strong MAO areas were noted. One was found in a triangular or wedge-shaped area of subependymal tissue, located between the central grey matter and the medial vestibular nucleus. Aggregations of small round cells could be seen under high power, but no clear-cut correlations with particular cell types could be found. Other similar areas were found in the lateral walls of the caudal end of the aqueduct, in the anterior medullary velum and near the area postrema. Friede (1961) notes that these areas are also rich in succinic dehydrogenase and suggests that they could represent a cell type of yet unknown functional significance.

d) Choroid plexus.

Shimizu et al. (1959) report that "the choroid plexuses and ependymal cells were weakly positive for MAO activity in each species." This is in contrast to the findings in this investigation. Under low power, however, Shimizu's statement appears to be true, since the MAO reactive granules are small, and the cytoplasm is not reactive. In this study, however, each choroid plexus was shown to contain a vast quantity of dense, strongly reactive granules. Large specific cell types, unidentified by routine histologic methods, occurred here and there, somewhat resembling fat cells, but laden with dense clusters of extremely small reactive granules, surrounded by pink cytoplasm. Oddly enough, where diffusion of cytoplasm, or cell-rupture, had occurred, "pink balloons" were formed within the plexus. This phenomenon was also observed at the cut ends, or along the border, of peripheral nerves.

In some areas of the 4th ventricle choroid plexus, these small MAO granules appear to migrate into the subependymal tissue. The granules
are approximately the same size and shape as those observed in liver, by the same reaction, and in lesser quantities, in lung.

e) **Subcommissural organ.**

This is a high epithelium lining the ventral surface of the posterior commissure at the rostral end of the aqueduct. Only weak activity for MAO was noted in rabbit brain.

f) **Subfornical organ.**

The subfornical organ, or intercolumnar tubercle, is located under the hippocampal commissure, and shows strong MAO, confirmed by Shimizu *et al.* (1959). In this connection, it should perhaps be mentioned that in recent studies on uptake of epinephrine in the brain (Wilson *et al.*, 1962) intravenously administered epinephrine-H$_3^3$ was taken up in the subfornical organ, pineal body and area postrema, in concentrations considerably in excess of those in parts of the brain lying within the blood-brain barrier.

g) **Pineal body.**

In these experiments the pineal body appeared to contain a consistently higher concentration of enzyme than any other area of the brain. However, Shimizu *et al.* (1959) state that "the pineal body ... showed little if any staining for MAO in animals ..." (rodents). Comparative studies here have substantiated a strongly positive MAO in human, ox, and pig pineal, as well as in the rabbit. The strongest activity appears to be in intercellular tissue which runs in rows between the cells. This is particularly interesting in that Kappers (1960) demonstrated a very extensive autonomic network in the pineal organ, by means of the Champy technique. In further studies on the pineal, Prop and Kappers (1961) established the presence of melatonin and serotonin by paper chromatography,
although being unable to demonstrate by histological means the presence of aromatic amines or indoles, using acid diazonium, ferric ferricyanide reduction or the chromaffin reaction. This is perhaps understandable in that high concentrations of catecholamines or indoles may be required for these reactions, and are perhaps protected by the high lipid content of the pineal (Quay, 1957); or it might be that, unlike other organs, serotonin in the pineal is linked to other chemical compounds in such a way that its histological demonstration is impossible by the known techniques for indoles.

h) Substantia alba.

In general the white matter exhibited a weak or negative activity, except for a few fibre tracts such as the habenulo-peduncular tract mentioned later. This statement must, however, be made with considerable caution since under low power a completely negative reaction can be assumed which under high power can often be shown to have fine reactive granules throughout the tissue, their presence masked under low power by the greater quantity of non-reactive tissue.

i) Substantia grisea.

The MAO reaction in grey matter is almost entirely within the neuropil, and seldom within the soma of any nerve cell. This is also substantiated by Shimizu (1959). Friede (1961) defines neuropil as the close juxtaposition of dendritic ramifications, processes of glial cells and, possibly, synapses. These heterogeneous elements form a complex tissue which shows specific histochemical properties differing from the properties of the perikarya. That MAO could play a part in synaptic transmission is borne out in this thesis, where, in many regions, well-marked circular knobs, many of which exhibit ring shapes resembling
boutons terminaux, can be seen in proximity to neurons. These can only be distinguished under high power or oil-immersion.

j) Hypophysis.

Examination of the pituitary was made in pig and ox, but not in rodents or in cat. It is included here, however, as a matter of general neurological interest in the study of MAO, bearing in mind that reactions may differ in different species.

In the pituitary of species studied (approximately one dozen glands) a characteristic reaction occurs, distinctly different in the anterior from the posterior part.

In the pars distalis, or anterior portion, there is a weak general reaction for MAO which under high power, however, appears as a profuse abundance of very fine, highly reactive granules within the cytoplasm of certain cells. These granules are finer than those of the choroid plexus, and are located in cells situated on the posterior lateral aspects of the pars distalis, so that in sections cut as illustrated, the following pattern of distribution can be seen:
In the pars nervosa, a moderately strong, diffuse reaction is seen, resembling the reaction in cortical neuropil. There are no distinguishable stained cells, but the reactive areas tend to follow the 'sinusoidal' pattern of the pineal, and lie in canals between cells. Three general areas may be distinguished, with the strongest reaction occurring most posteriorly.

k) Brain-stem of cat.

In general, cat exhibited a much weaker reaction and only poor regional localization was possible. No granules or fine detail could be picked up under high power by the usual methods used for MAO identification. No reference can be found to other histochemical identification of MAO in this species, so it is not possible to compare results.

Nevertheless, regional localization appeared to be the same as in rabbit, except for the following:

(i) No reaction in the habenulo-interpeduncular tract.
(ii) Very weak reaction in the area postrema and locus coeruleus.
(iii) Much weaker general reaction in all areas (strongest in choroid plexus and hypothalamus).
PART V. DISCUSSION

1. Review of Regional Concentrations of MAO.

Table IX on page 71 shows regional comparisons of MAO-rich areas in the brain-stem and from this it can be seen that the strongest areas of MAO concentration in rabbit brain are in the choroid plexuses, the pineal gland and the interpeduncular nucleus. Not much lower in activity are the areas of the vagal nuclei, spino-trigeminal nuclei, hypothalamus and periventricular nuclei, habenulae and olivary nuclei. Still rich in MAO, but not so pronounced in intensity of staining, are the dorsal tegmental nuclei, habenulopeduncular tract, pontal raphe nuclei, locus coeruleus and posterior pituitary.

The diagram of cranial nerve nuclei (Table X, page 72) shows clearly the importance of MAO in these areas. Most of the brain-stem cranial nerve nuclei appear to contain marked quantities of MAO, so that in sections of the medulla, these areas, and the nuclei of the inferior olive, stand out as centres of MAO activity. These cranial nerve nuclei are, however, irregular in the amount of MAO they contain, so that the spino-trigeminal, vagal and commissural nuclei stand predominantly high above the rest. All other nuclei represented in the diagram are comparable in intensity.

Higher in the brain-stem, other non-cranial nerve nuclei assume importance in MAO activity. The central grey matter, interpeduncular nucleus, habenular ganglion and hypothalamic areas become prominent, while the thalamic nuclei in general are weak in MAO.
TABLE IX
Regional Comparisons of MAO-Rich Areas in Rabbit Brain Stem

<table>
<thead>
<tr>
<th></th>
<th>Moderate</th>
<th>Strong</th>
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<tr>
<td><strong>MEDULLA</strong></td>
<td></td>
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<tr>
<td>Dorsal and ventral commissure - neuropil</td>
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<td>Descending root of trigeminal - neuropil</td>
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<td>Vagal nuclei - neuropil</td>
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<td>Area postrema - neuropil</td>
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<td>Olivary nuclei - neuropil</td>
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<td>Reticular formation - neuropil - throughout</td>
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<td>Choroid plexus - granules</td>
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<td><strong>pons</strong></td>
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<td>Facial nuclei - neuropil</td>
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<td>Midline raphe nuclei - neuropil</td>
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<td>Locus coeruleus - neuropil</td>
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<td>Mesencephalic nucl. N.V. - cell bodies</td>
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<td>Vestibular nuclei - neuropil</td>
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<td>Dorsal tegmental nuclei - neuropil</td>
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<td><strong>mesencephalon</strong></td>
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<td>Central grey matter - neuropil</td>
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<td>Pineal body</td>
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<td>Superior colliculi - neuropil</td>
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<tr>
<td>Oculo-motor complex - neuropil</td>
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<td>Medial geniculate - neuropil</td>
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<td>Inter-peduncular nucleus - neuropil</td>
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<td><strong>diencephalon</strong></td>
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<td>Habenulo-peduncular tract - fibres</td>
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<td>Habenular bodies - neuropil</td>
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<td>Periventricular nuclei - neuropil</td>
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<td>Hypothalamus - neuropil</td>
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<td>Posterior pituitary - neuropil</td>
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<td>Anterior pituitary - granules (very fine)</td>
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TABLE X

Diagram Showing Nuclei of Cranial Nerve

Concentrations of MAO

- Nucl. N. III Visceral
- Nucl. N. III Somatic
- Mesencephalic nucl. N. V.
- Nucl. N. IV
- N. V
- Nucl. N. V
- Locus Coeruleus
- Genu of N. VII
- Nucl. N. VI
- Nucl. VIII (vestibular)
- Nucl. N. VII
- N. IX
- N. X
- Area Postrema
- Tract of Spinal V
- Fasciculus Solitarius
- Nucl. N. XII
- Dorsal motor nucl. N. X
- Nucl. ambiguous
- Commissural nucl. N. X
- Nucl. XI
- Spinial nucleus of N. V.
Although not included in the detailed mapping of this study, the whole brain was examined, so that an overall picture could be obtained. It is perhaps sufficient to record that diffuse reaction in the cortical neuropil and hippocampus was consistently present, and that the molecular and granular areas of the cerebellum contained moderate to strong amounts of MAO. At no time was any intracellular reaction observed in these areas.

A special examination was also made of the septal areas of rabbit brain, but no conspicuous activity was detected. Likewise, the olfactory bulbs were examined with similar results. (See page 18 for activity in the amygdala, striatum and other regions.)

From the general regional concentrations shown in this study, a pattern of activity emerges, arbitrarily classified into the following components:

a) Cranio-visceral
b) Olfactory-limbic
c) Pineal-hypothalamic-pituitary
d) Choroid plexus
e) Neuropil

In order to examine the possible reasons for these regional concentrations, it is important to review the main functions of these areas. Why should certain areas possess particular chemical properties, such as have been shown by similar methods for succinic dehydrogenase, DPN and TPN diaphorase, and cytochrome oxidase? Are these areas rich in certain enzymes because of lack of specific inhibitors in those areas, or because of more available substrate? A brief outline of comparative histochemistry may help define the functional picture.
2. **Comparative Histochemistry and Capillarization.**

Zeller (1951) has postulated that cholinesterase is high where MAO is low, and this has not been contradicted by the findings of Hebb (1961) or Koelle (1954) who have found acetylcholinesterase in the supra-optic and paraventricular nuclei of the hypothalamus. Cavanagh (1961) found that in the hen, in contrast to mammals, ACh-esterase was present in the cytoplasm of all central neurons examined. In mammals, not all neurons contain ACh-esterase, where non-cholinergic and cholinergic cells are found. Thus, although localized regions of MAO and ACh-esterase may be found together, it would seem that MAO is almost always confined to the neuropil. Koelle (1954) reports high specific cholinesterase activity in the dorsal nucleus of N.X, interpeduncular nucleus (especially pars lateralis) and in the caudate and amygdala. MAO is also high in the first two areas, but not in the caudate or amygdala. Koelle further noted that high ChE activity occurred in nearly all motorneurons, and was concentrated at the perikaryon and along the axons and dendrites. Low activity only existed in the neocortex.

Friede (1961) has shown some concordance between capillarization and succinic dehydrogenase (SDA) but this is not always consistent. For instance, in the nucleus dorsalis vagi which contains low SDA, there is high capillarization, high DPN and high MAO. This applies also to the area postrema. Again, the inferior colliculi are almost devoid of MAO, yet are high in SDA and proportional in capillarization, and reversely, the interpeduncular nucleus shows only moderate SDA compared with its high MAO content, and moderate capillarization.

Thus, there does not appear to be any consistent correlation between the distribution of SDA (or cytochrome oxidase which parallels
3. **Relationship of Regional Concentrations of MAO to Functions of Related Area.**

   a) **Cranio-visceral components.**

   Certain of the cranial nerve components in the brain stem are conspicuous for their strong MAO. These are intimately connected, phylogenetically, with the branchial or visceral arches, which in the lower aquatic vertebrates carry the respiratory organs. They may be called "branchiomeric" nerves, and include the trigeminal (V), facial (VII), glossopharyngeal (IX) and vagus (X). Both afferent and efferent fibres are carried, which leave the brain together and are not separated into dorsal or ventral roots. Of the efferent branchiomers, the seventh, ninth and tenth nerves supply many general visceral fibres to the glands and visceral muscle of the pharynx, larynx and thoracic and abdominal organs.

   The afferent fibres of these nerves are related to visceral sensibility, conveying general impulses from the pharynx, larynx, thoracic and abdominal viscera. The sensory root of the trigeminal nerve mediates touch, pain and temperature from the face, forehead and ectodermal mucous membranes of the mouth and nose, and contains also terminations of somatic afferent vagal fibres. Anatomically these areas are closely connected, and some chemical interaction may occur between them, possibly connected with the phenomena of cardiac arrest. For instance, the area postrema, shown to be an emetic chemoreceptor trigger zone (Wang and Borison, 1952) is affected by many medullary centres. The vomiting centre itself is in close proximity to the responsive loci for spasmodic respiration, inspira-
tion and expiration, vaso-motor control, postural tone and the vestibular nuclei. Afferent impulses from the gastrointestinal tract reach this area mainly through the vagus. The vomiting centre is thus influenced, like other brain-stem areas, by tonic excitatory and inhibitory nervous and metabolic regulators, in which it would appear that MAO and biogenic amines play a significant part.

b) Olfactory-limbic components.

In Table XI, page 77, the areas of the olfactory system high in MAO, are shown in conjunction with those of weak MAO. Certain of these brain-stem areas high in MAO, receive connections from the limbic system. For instance, the habenular nucleus receives impulses not only from the basal olfactory nuclei of the septal area, but also via the fornix, from the hippocampal cortex. This suggests connections from basic emotional centres, to pathways leading thence to the cranial nuclei of the medulla and to the viscera (See Table XII). In the lamprey, the forebrain lobes send fibres via the cortico-habenular tract, to the habenular body. The habenulo-peduncular tract then passes impulses back to the peduncular region - a system that is common to all vertebrates throughout phylogeny.

c) Pineal-hypothalamic-pituitary components.

Because of the consistently high level of MAO demonstrable in the pineal, a new concept of amine interaction, involving these three components, is postulated on the basis of this study. However, with the limited amount of knowledge concerning these mechanisms, it is possible only to suggest interactions. The following is the evidence on which this concept is based.

Although it is well known that the hypothalamus is phylogenetically one of the oldest parts of the vertebrate prosencephalon, forming,
TABLE XI

Olfactory Connections Showing MAO Concentrations

Basal olfactory nuclei
Septal area
Olfactory area
Fornix
Stria medullaris
Habenular nucleus
Habenulopeduncular tract
Dorsal tegmental nucleus
Tuber
Hypophysis
Mammillary body
Interpeduncular nucleus
TABLE XII

Diagram Illustrating Concept of Pineal-Hypothalamic-Pituitary Interaction
together with the pituitary, a complex neuroglandular mechanism which influences most visceral activities, perhaps equally important but neglected in the literature of comparative neurology, is the pineal gland and its habenular connections. These exist as early as the hypothalamic structures. The cyclostomes are the most ancient aquatic forms with a true vertebrate organization of the head region and these possess a well-formed pineal. Between the tectum and habenular nuclei are inserted the stalks of two small pineal "eyes", the fibres of which enter the posterior commissure. They have presumably been termed "eyes" since they occupied a paired frontal position in the brain at this level of phylogeny. Thus, some have postulated the existence of a primitive thermo- or light-receptive mechanism, controlling endocrine and other functions connected with mating and migration. In addition, the idea of a chemo-receptor organ suggests itself, connected with the biosynthesis and catabolism of monoamines.

It is well-known, that melanocytes have their embryonic origin in nerve tissue, but it has only recently been discovered that the newly found hormone of the pineal, melatonin, can cause temporary dispersion of the melanocytes in frogs and tadpoles (Lerner, 1962).

Shortly after the discovery and isolation from the pituitary of the melanocyte-stimulating-hormone (MSH) which causes darkening of frog skin, it was found that ACTH from the anterior pituitary can also darken frog skin, though not as efficiently as MSH. This hormone is effective in man as well as in frogs.

Later, a successful attempt by Lerner and his co-workers to isolate the substance from the pineal that lightens frog skin, led to
the discovery of the hormone, melatonin. It has now been shown that some tranquilizers potentiate this effect of melanocyte dispersion in frogs, whereas MAO inhibitors tend to prevent it.

It is felt that this evidence lends itself to the suggestion of interaction between the pineal, hypothalamus and pituitary, and that MAO could thus be concerned with neurohormones which regulate the endocrine and nervous systems of the body.

d) **Choroid plexus.**

Although it is generally believed that the choroid plexus is concerned with the elaboration of cerebro-spinal fluid, it is also known to be capable of absorbing certain substances from the cerebro-spinal fluid. Regarding its high content of MAO, however, too little is known to warrant any assumptions in this connection.

e) **Neuropil.**

The fact has been stressed that MAO occurs mainly in the dendrites and cell processes surrounding the neurons, rather than within the actual cell bodies. For lack of a better term, we must designate this area as "neuropil"; and it is clear that this region is a highly active part of the functioning nervous system. Schade and Baxter (1960) state that dendrites provide about 95% of the receptive surface of the neuron, so that one of the major sources of electrocortical activity could be attributed to summation of post-synaptic potentials (Purpura, 1959). Certain aspects of behaviour may apparently be correlated with the structure and density of the neuropil, so that a depletion in the density of neuropil (and hence the probability of axo-dendritic interaction) is associated with poor learning ability (Eayrs, 1961). Flexner et al. (1950) have shown that the growth of cell-processes coincides with changes in electro-
lyte distribution, and Himwich and Petersen (1959) state that similar changes occur in concentrations of glutamic acid and glutamine. In hypothyroidism changes occur in the neuropil connected with anoxia (Eayrs, 1954) and with an apparently irreversible effect on the action of succinic dehydrogenase (Hamburgh and Flexner, 1957).

That neuropil is also a prominent feature of invertebrates is substantiated by Horridge (1961) who states that all invertebrates have nervous systems where all the interesting activity seems to be in the neuropil, and a similar neuropil occurs regionally in vertebrates.

These facts are, it is felt, of sufficient significance to suggest that the concentrations of MAO in the neuropil serve a fundamentally useful purpose connected with the behaviour of the individual, and that disturbances of MAO and amine metabolism would have related behavioural effects.
SUMMARY

1. An attempt has been made to give an integrated picture of MAO activity in rabbit and cat brain-stem. This has been presented in atlas form.

2. A histochemical method was used which depended on the incubation of fresh frozen tissue sections with tryptamine as substrate and nitro-blue tetrazolium salts as reduction agents in a pigment production system. Specificity for the reaction was established by the use of known MAO inhibitors in vitro and in vivo.

3. Highest concentrations of MAO were found in the visceral autonomic centres of the brain-stem, particularly in the interpeduncular nucleus, hypothalamus and sensory vagal nuclei.

4. In addition, the pineal gland, pituitary and choroid plexuses exhibited significant amounts of MAO, being present intracellularly, in granule form, in the anterior pituitary and choroid plexuses.

5. Further, MAO activity was found to be predominant in the neuropil of reactive areas, rather than within the perikaryon of neurons.

6. Physiological implications of these findings are discussed.
BIBLIOGRAPHY


Dorsal Aspect - Brain-Stem of Rabbit

Showing Levels of Section Cutting.
VENTRAL ASPECT - BRAIN-STEM OF RABBIT