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Control of Rat Testicular Monoamine Oxidase Activity: Age, Seasonality, Melatonin, HCG, FSH, Starvation and Irradiation

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CONTROL OF RAT TESTICULAR MONOAMINE OXIDASE ACTIVITY: AGE, SEASONALITY, MELATONIN, HCG, FSH, STARVATION AND IRRADIATION

by

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Ronald L. Urry

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ABSTRACT

Control of Rat Testicular Monoamine Oxidase Activity:

Age, Seasonality, Melatonin, HCG, FSH,

Starvation and Irradiation

by

Ronald L. Urry, Doctor of Philosophy

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Major Professor: Dr. LeGrande C. Ellis
Department: Physiology

A number of investigations were undertaken to determine what factors control testicular MAO activity. Activity was high in neonatal rats (19 g - body weight), showed a reduced activity at 53 g, was increased at 145-235 g and showed a progressively reduced activity at 415 g, 482 g and 650 g. Hypophysectomy of immature male rats at 3 weeks of age, or mature rats at 10 weeks of age, significantly reduced testicular weight and total MAO activity as calculated on a per animal basis. The specific activity of the enzyme, as expressed on a per mg of tissue basis, was increased by hypophysectomy. Treatment of hypophysectomized rats with FSH increased testicular weight and total MAO activity. Additions of 2 IU or 5 IU of FSH to seminiferous tubules in tissue culture also increased testicular MAO activity. Treatment of hypophysectomized rats with a combination of FSH, LH and prolactin or treatment with hCG increased testicular

weights but decreased MAO activity. This suggests that androgens may decrease MAO in vivo.

Injections of 1 or 2 mg of melatonin into mature hypophysectomized rats increased testicular MAO activity. Additions of melatonin in vitro at concentrations of 10^{-5} through 10^{-7} M also increased MAO activity. Treatment of male rats with either 10 or 100 µg of estradiol benzoate had no effect on testicular MAO activity. The activity was also not altered by adrenalectomy indicating that glucocorticosteroids may not play a role in regulating testicular MAO.

Restriction of feed intake by 47% reduced testicular MAO activity and this was attributed to a decrease in FSH. Restriction of feed by 67% increased pineal weight, hydroxyindole-O-methyltransferase activity, N-Acetyltransferase activity and testicular MAO activity; this increase was attributed to an increase in melatonin. Treatment of animals with 250 R whole-body x-irradiation increased MAO activity from 7-16 days after treatment. The increase in MAO activity 1 week after treatment corresponded with a previously noted increase in melatonin synthesis for the same period. Treatment of animals with 450 R of whole-body or testes only x-irradiation significantly decreased testicular weights and increased testicular MAO activity as expressed on a per mg of tissue basis. Irradiation with 450 R to the head-only lowered testicular MAO activity one-hour after treatment.

Pinealectomy increased testicular MAO activity, probably due to an increase in FSH levels. Testicular MAO did not appear to have a circadian rhythm in its activity.

Injections of pargyline, an MAO inhibitor, significantly inhibited testicular MAO while increasing endogenous serotonin concentrations.

Testicular MAO activity in the English house sparrow was observed to increase from November to April and to decrease from April through November. An increase in MAO activity from November to January preceded an increase in testicular weight. MAO activity and testicular weights of Uinta ground squirrels decreased dramatically from April to May as the animals went out of the breeding season.

Testicular MAO was observed to be localized mainly in the walls of the seminiferous tubules where it fluctuated with age. The ratio of activity in the seminiferous tubules to whole tissue was high in the young animal and low in older animals. Activity appeared to be lost in the seminiferous tubules during senescence faster than it was in the interstitial cells, thus shifting the ratio.

Treatment of animals with pargyline prevented return of testicular MAO activity in seminiferous tubules grown in tissue culture. Control seminiferous tubule MAO activity increased from 6 to 9 days after placing in tissue culture while those treated with pargyline did not have this increase.

(128 pages)

INTRODUCTION

Biogenic amines or "natural bases" are compounds synthesized in the body which have had a long and intimate involvement with the evolutionary process. Compounds commonly classified as biogenic amines are: acetylcholine, epinephrine, norepinephrine, dopamine, serotonin and γ-aminobutyric acid. Frequently, norepinephrine and epinephrine are classified as catecholamines, and acetylcholine and γ-aminobutyric acid are listed separately from the other biogenic amines. Most of the biogenic amines are metabolized by the enzyme monoamine oxidase [MAO, Monoamine: O_2 oxidoreductase (deaminating) E. C. 1.4.3.4] which converts the parent molecule to the corresponding indole acetaldehyde derivative by removal of the amine group. This enzyme has been demonstrated in many vertebrate and invertebrate tissues (Hare, 1928; Bhagvat, Blaschko, and Richter, 1939; Blaschko, Richter and Schlossman, 1937; Blaschko, 1952).

Serotonin is a biogenic amine that is normally inactivated by MAO and is present in many tissues of an organism. Serotonin is present in small quantities (60-250 nanograms/gram of tissue) in the rat testes (Kormano and Pentilla, 1968; Ellis, 1970), and its concentration there is age dependent (Zieher et al., 1971). Injections of serotonin are antigonadal and cause testicular regression in rats (Boccabella, Salgado and Alger, 1962) and mice (O'Steen, 1963). Serotonin has been implicated in the alteration of spermatogenesis observed after postural stress

in primates (Zemjanis et al., 1970), and it inhibits androgen synthesis in vitro (Ellis, 1972).

Because of the detrimental effects of serotonin on testicular function, its effective concentration in the gonad may alter testicular function. Similarly, the metabolism of this and other biogenic amines in the testes by MAO may be an important determinant of the physiological state of the male gonad. In this respect, a paucity of information exists as to what regulates the activity of testicular monoamine oxidase and to what extent the activity of this enzyme is related to the reproductive capacity of the animal. In the present study, the objectives were to:

1. Ascertain if age influences testicular MAO activity in the rat.
2. Ascertain if testicular MAO activity in the house sparrow shows seasonal changes that correlate with the corresponding changes in reproductive capacity of this organ.
3. Ascertain the effect the following hormones have on testicular MAO activity:
    a. melatonin, hCG, FSH, LH, prolactin, or a combination of FSH, LH and prolactin in hypophysectomized animals, and
    b. estrogen injections in male rats.
4. Ascertain the effects of the following treatments on testicular MAO activity:
    a. Adrenalectomy,

b. Starvation, and

c. X-irradiation.

The following abbreviations and trivial names are used in this manuscript: serotonin = 5-hydroxytryptamine = 5-HT; 5-HIAA = 5-hydroxyindole acetic acid; estradiol-17β = 1, 3, 5(10)-estratriene-3, 17β-diole; melatonin = N-acetyl-5-methoxy-tryptamine; hCG = human chorionic gonadotrophin; FSH = follicle stimulating hormone; LH = luteinizing hormone; testosterone = 17β-hydroxyandrost-4-ene-3-one; ACTH = adrenocorticotrophin.

REVIEW OF LITERATURE

Monoamine Oxidase (MAO)

Introduction

A great deal of interest has been shown in MAO since it was discovered by Hare (1928). This enzyme catalyzes the oxidative deamination of a wide variety of amines to produce the corresponding aldehyde according to the following equation:

$$R\text{-}CH_2\text{-}NH_2 + O_2 + H_2O = R\text{-}CHO + NH_3 + H_2O_2$$

MAO has a wide specificity which overlaps other amine oxidases including diamine oxidase and plasma amine oxidase (especially benzylamine oxidase, see Blaschko, 1966). Blaschko (1966) has shown, however, that MAO differs from other mammalian amine oxidases by being insensitive to inhibition by carbonyl reagents such as cyanide and semicarbazide. This has been attributed to the various coenzymes involved with the different amine oxidases. Ghisla and Hemmerich (1971) and Walker et al. (1971) have reported that monoamine oxidase is a flavoprotein while Blaschko (1966) has reported that diamine oxidase and spermine oxidase are both pyridoxal-phosphate dependent. In this regard, Ghisla and Hemmerich (1971) and Walker et al. (1971) have recently isolated a cysteinyl flavin peptide from purified MAO preparations. An additional difference is that monoamine oxidase is active towards N-methylated amines, while the other mammalian amine oxidases will not oxidize these compounds (Blaschko,

1966). Substrate specificities for MAO have been reviewed in considerable detail by Blaschko (1952 and 1966). Substrates include, among others, tyramine, serotonin, tryptamine, epinephrine, norepinephrine, metanephrine, normetanephrine, dopamine, benzylamine and kynuramine. The enzyme can oxidize primary, secondary and tertiary monoamines of the general formula $R_1 - CH_2 - NR_2 - R_3$, where R_2 and R_3 are hydrogen atoms or methyl groups (Tipton, 1973). One requirement is that the amino group must be attached to an unsubstituted methylene group and in the case of aliphatic amines the optimum chain length is between five and six carbon atoms. MAO is not active toward histamine or short chain diamines. Normal endogenous substrates include derivatives of phenylethylamine and indolethylamine (Tipton, 1973). Purified preparations of monoamine oxidase appear to be absolutely specific for oxygen as the second substrate.

Properties

Oreland (1971) has recently reported partial purification of MAO, although relatively little is known about the nature of this enzyme. The molecular weights of the preparations vary from 100,000 to over one million with the minimum molecular weight of the enzyme, based on the flavin content, usually reported to be about 100,000 (Tipton, 1973). Many authors have speculated concerning the presence and involvement of a metal ion in the activity of the enzyme. Copper was at first suspected to be necessary for activity, but recent reports indicate that

copper-chelating agents do not inhibit the enzyme. Nagatsu et al. (1972) believe that copper is not needed for MAO activity. Oreland (1971) agrees with this and also reports the presence of iron and hexosamine in the MAO enzyme.

Distribution and localization

Investigation by Hare (1928), Bhagvat, Blaschko and Richter (1939), Blaschko, Richter and Schlossman (1937) and Blaschko (1952) have confirmed the presence of MAO in several vertebrate and invertebrate tissues, and in most organs. The enzyme is reportedly either absent or present in low amounts in skeletal muscle, blood plasma and erythrocytes, although it does occur in platelets (Collins and Sandler, 1971). Areas that appear to be rich in MAO are liver, kidney, intestine, stomach and aorta. Bhagvat, Blaschko and Richter (1939), Zeller and Joel (1941), Penttila and Kormano (1968) and Urry, Jaussi and Ellis (1972) have shown that MAO is also present in the male gonad. Within the cells of several organs, the enzyme is localized exclusively in the mitochondria and is associated with the outer membrane of the mitochondria (Ernster and Kuylenstierna, 1970). In this respect, MAO is often used as a marker for identifying the outer mitochondrial membrane. Relatively smaller amounts of MAO which had previously been reported to be associated with the microsomal fraction of the cell may have been derived from the mitochondria as a result of damage that occurs during the cell fractionation procedure (Tipton, 1973). Differences in properties between the mitochondrial and microsomal MAO may indicate that the properties of the

enzyme are modified when the mitochondrial membrane structure is disrupted.

Silberstein, Shein and Berv (1972) have shown that brain MAO is present in the glial cells while Rodriguez and De Robertis (1962) have found it in synaptisomes. The enzyme is present both intraneuronally and extraneuronally in a number of innervated tissues. De la Lande et al. (1970) have histochemically localized extraneuronal MAO in the artery of the rabbit ear. Denervation of the vas deferens decreases MAO activity by some 50%, and Jarrott and Iversen (1971) have interpreted this to mean that about half of the enzyme is located in the sympathetic nerves and half in the extraneuronal cells. Horita and Lowe (1972) have used similar techniques to demonstrate the presence of extraneuronal heart MAO. Klingman (1966) has also reported that most of the enzyme content in several adrenergically innervated organs is extraneuronal. Marsden, Broch, and Guldberg (1971) have shown that quantitative estimates of the amounts of intraneuronal and extraneuronal MAO, based on denervation experiments, may be open to doubt since a fall in the activity of an extraneuronal enzyme would be expected if it depended on an intact nerve supply for full activity. Jonason (1969) reported that in parenchymal tissue, such as the salivary gland and liver, it appears that mooamine oxidase is very largely extraneuronal. Recently, Jacabowitz (1972) showed that MAO activity was present in fibroblasts and he speculated that much of the extraneuronal MAO activity in connective tissue is located in the fibroblasts.

Multiple forms of MAO

There have been many suggestions that MAO exists in more than one form. It has been postulated that such multiple forms of MAO might have different physiological roles in the deamination of the various biogenic amines (for reviews see Gorkin, 1966; Eiduson, 1972b; Sandler and Youdim, 1972). It was suggested several years ago that MAO may be a mixture of enzymes with different substrate specificities, and also that various tissues might have different distributions of the constituent enzymes. Alles and Heegaard (1943) tested a number of substrates with enzyme preparations from liver extracts of different species and found marked differences with respect to oxidation rates. Since that time, additional evidence supporting multiple forms of MAO has accumulated, especially when studying the effects of inhibitors on enzyme activity. Johnston (1968) and Hall and Logan (1969) have shown the presence of two forms of MAO in the rat brain. One form was named "enzyme A" which was sensitive to clorgyline and oxidatively deaminated tyramine and 5-HT. "Enzyme B" was relatively insensitive to clorgyline, and it oxidized tyramine, but not 5-HT. Neff and Goridis (1972) have shown that the proportions of these forms differ in various locations. The superior cervical ganglion has about 90% type A and 10% type B. Other authors have confirmed the presence of two forms of MAO by inhibitor studies (Johnston, 1968; Squires, 1968; Hall and Logan, 1969; Jarrott, 1971; Fuller, 1972). Youdim and Sourkes (1965) and Jarrott (1971) have used thermal stability characteristics and Barbato and Abood (1963) and Youdim and Sourkes (1965) have used pH optima

of various mitochondrial MAO preparations to show variance with the particular substrate employed. Gorkin and Romanova (1959) and Gorkin (1966) have used the above criteria to suggest MAO multiplicity.

Until recently, the existence of multiple forms of MAO was based on rather indirect evidence. Youdim and Sandler (1967) reported separation of purified rat-liver MAO into a number of electrophoretic bands each of which possessed enzymatic activity. Kim and D'Iorio (1968) have since separated multiple forms of MAO by electrophoresis by using a number of different solubilization procedures to rule out possible methodology artifacts. Other investigators have subsequently published additional evidence that in solubilized mitochondrial preparations, MAO exists in multiple forms (see Youdim, 1973, for list).

Youdim, Collins and Sandler (1969) reported that rat brain and rat liver MAO preparations separate into at least four and five forms, respectively, which differ in their properties and all exhibit different substrate specificities and inhibition patterns. Sandler and Youdim (1972) have shown that the occurrence of multiple forms of mitochondrial MAO in various tissues of several species is a common, but not necessarily universal finding. On the other hand, Tipton (1973) with pig brain and Murali and Rodhakrishman (1970) with monkey small intestine have reported that the enzyme appears homogenous and contains only a single band of activity after electrophoresis.

Multiple forms of MAO appear to be different in their properties: i.e., pH optimas, substrate specificities, sensitivity to MAO inhibitors and heat denaturation properties all depend on the source of the enzyme. One isoenzyme

in particular migrates toward the cathode during electrophoresis and has been referred to as MAO_R. It has different properties from other forms. It also seems to have a net positive charge while the other forms have a negative charge, indicating that it may be a different protein. This enzyme preparation is also insensitive to many kinds of inhibitors, especially of the hydrazine type, and more resistant to denaturation, both by heat treatment and by agents such as urea and guanidine (Collins, Youdim and Sandler, 1972). Lastly, MAO_R has a much higher substrate specificity for dopamine than for other substrates such as tyramine, tryptamine, benzylamine and kynuramine. Consequently, this form of MAO has been called dopamine-monoamine oxidase (Youdim, 1973). In this regard, Kroon and Veldstra (1972) reported that dopamine and serotonin are degraded by the same type of MAO or by different types with the same localization, whereas norepinephrine and kynuramine are degraded by forms of MAO which are different from these types.

Despite the abundance of evidence reporting multiple forms of MAO, recent immunological data has accumulated to the contrary. Hartman, Yasunobu and Udenfriend (1971) and Hidaka, Hartman and Udenfriend (1971) have reported that all the separable forms of liver and the bulk of brain mitochondrial MAO are immunologically indistinguishable. They have also reported that the separable forms of MAO are composed of the same protein subunit assembled or aggregated into forms of different molecular weights. Hartman (1972) later, however, discovered and isolated a new MAO in the brain that was antigenically different from

liver MAO. This would further support the concept of at least some degree of in vivo multiplicity of MAO.

Control of MAO

Aging and development of MAO. In comparison to the amount of work published on the characterization of the properties and multiple forms of MAO, relatively little work has been published concerning the control of MAO. There have been a few studies concerned with changes in tissue MAO activity with age. There is now some data concerning age-related variations of MAO activity in brain, liver and heart tissue with little information concerning other tissues. Karki, Kuntzman and Brodie (1962) showed a marked increase of MAO activity in brain from the newborn period to adult life, while Prange et al. (1967) reported constant MAO activity throughout the life-span of both male and female rats. Horita (1967) confirmed the latter observation. Robinson (1967) reported that there is little or no histochemically detectable MAO activity before 10 days of age in rat brain while at 15 days some activity was present. Prange et al. (1967) reported that MAO activity of the rat liver appears to rise about the 9th week and to decline about the 35th week. Horita (1967) claimed that MAO activity of the rat liver, as in brain, reached a maximum at 6 weeks of age. Novick (1961) observed a gradual increase in heart MAO activity during development while Gey, Burkard and Pletscher (1964, 1965) reported that MAO activity of the rat heart increased several fold during the course of the whole life span (1-24 months of age)--a

finding that was supported by Prange et al. (1967) and Horita (1967). Vaccari et al. (1972) have shown that MAO activity is present immediately after birth in rat liver, heart, brain, fundus and spleen. MAO activity of the liver was especially high after birth in comparison to the other tissues studied. Full activity in all tissues was reached at about the 18th day after birth. Horita and Lowe (1972a) have reported increases in heart and liver MAO activity, with a smaller increase in brain activity, from 2 through 130 weeks of age. The heart had much larger amounts of the enzyme compared to the liver, and the liver had much higher amounts than did the brain.

Recently, Robinson et al. (1972) studied MAO activity in human brain, platelet and plasma from the age of 25 to ages greater than 70 years. They found that the activity increased with advancing years especially past the age of 45. They related this activity to increased brain 5-HIAA levels with advancing age as well as decreased concentrations of endogenous brain norepinephrine. They also speculated that the increase in MAO might be involved in the onset of depression or parkinsonism disease that sometimes occurs with advancing age.

Sex differences and hormones. Horita and Lowe (1972b) have reported sex differences in MAO activity in the heart. The activity was higher in the male rat heart than in the female heart from six weeks through 24 weeks of age. Holzbauer and Youdim (1972) also found MAO activity higher in male rats than female rats. They measured the activity in the septum of castrated male rats. It was about twice that observed for castrated female rats. There have been some reports concerning hormonal control of MAO in the female. Cohen et al. (1964) and

Cohen, Bitensky and Chayen (1965) reported marked changes in the histochemical staining pattern of human endometrial MAO during the menstrual cycle. They found the activity to be low and confined to a particulate fraction during the proliferative phase. Later, the staining is more diffuse and intense. They suggested that the non-particulate enzyme might represent an inactive form and that amine substrates of MAO might consequently accumulate in the endometrium during the latter part of the menstrual cycle, perhaps in sufficient concentration to initiate menstruation by causing spasm of the spiral arteries. Southgate et al. (1968) have confirmed the variations in staining patterns of human endometrial MAO in addition to measuring biochemical changes. In this respect, they found MAO activity to be low in the early non-secretory phase, but there was a sudden increase in activity at about the 19th to 21st day of the cycle. These authors have speculated that the control mechanism for the sudden increase in MAO activity might be related to enhanced progestational action since the "switch-on" of activity correlated with the peak in circulating progesterone levels. Grant and Pryse-Davies (1968) examined endometrial specimens from patients taking oral contraceptives and in those taking a strongly progestational type of birth control pill. MAO activity was detectably increased by the 12th day of the cycle, whereas, with strongly estrogenic or sequential regimes endometrial enzyme activity was low throughout the cycle.

A number of studies have shown fluctuations in MAO activity during the rat estrus cycle (see Southgate, 1972 for list). In general, uterine activity is low during estrus and high during met-estrus. This cyclic change in activity correlates

well with low ovarian progesterone secretion rates during estrus and high secretion rates during met-estrus (Hashimoto et al., 1968 and Fajer, Holzbauer and Newport, 1971). Holzbauer and Youdim (1972) have reported a rise in brain MAO activity during increased locomotor activity which was most pronounced on the day of met-estrus. In contrast, on the day of estrus monoamine oxidase activity was lowest when the rats became active. Klaiber et al. (1971) examined plasma MAO activity in the pre- and post-ovulatory phases of the menstrual cycle of regularly menstruating women, amenorrheic women, post-menopausal women and in amenorrheic and post-menopausal women treated with estrogens and a progestin. They found MAO activity to be greater in the post-ovulatory than in the pre-ovulatory phase of the cycle. The activity was also greater in amenorrheic and post-menopausal women than in the post-ovulatory phase of regularly menstruating women. Estrogen treatment reduced MAO activity in the amenorrheic and post-menopausal women, whereas, estrogen plus progestin increased the activity of the amenorrheic women over their estrogen treatment levels.

Kobayashi et al. (1966) and Kamberi and Kobayashi (1970) have reported variations in female rat hypothalamic MAO activity which also varied during the estrus cycle. MAO activity in the posterior hypothalamus became significantly elevated after ovariectomy, while the administration of estradiol benzoate returned the levels to those found in normal rats.

Klaiber et al. (1972) have recently reported that premenopausal depressed women with regular menstrual cycle have higher levels of plasma MAO activity

and greater EEG responses to photic stimulation than do nondepressed women. These abnormalities were interpreted as evidence of an adrenergic insufficiency which were both successively treated (brought back to normal levels) with oral conjugated estrogen therapy.

Eiduson (1972a) reported that testosterone can alter the nature of the MAO enzyme. Three-day-old mice were injected with 1 mg testosterone propionate, and their brains excised when the mice were 21 days of age. They then examined the brain MAO electrophoretic pattern and found that the slowest moving band in the MAO pattern was missing following testosterone administration. Moreover, this was observed only when serotonin was used as a substrate. Gonadotropins may also alter MAO activity. Anton-Tay, Pelham and Wurtman (1969) reported that castration or treatment with exogenous FSH increased the turnover of ^{3}H-Norepinephrine in the rat brain. This action of FSH was not shared by LH.

Adrenalectomy, other hormones and factors. Physical activity is another factor that appears to influence MAO activity. Holzbauer and Youdim (1972) reported that in male rats MAO activity was increased in four brain regions when the animals were physically active and that of the heart was nearly doubled. The enzyme activity in the liver, however, was only slightly raised.

Adrenalectomy has been reported to influence MAO activity in various tissues. Avakian and Collingham (1968) have shown an increase in rat heart MAO activity after adrenalectomy which was later confirmed by Holzbauer and Youdim (1972). Adrenalectomy at birth also increases MAO activity in just 10 days

(Parvez and Parvez, 1973a). Parvez and Parvez (1973a) have also reported that metopirone, a substance that blocks the synthesis of glucocorticoids, increased MAO activity in the hypophysis, hypothalamus and the rest of the brain. Complete ablation of the adrenal cortex resulted in a 167 per cent rise in MAO activity of the hypophysis. Both metopirone and hydrocortisone inhibited MAO activity *in vitro*. The authors suggested that glucocorticoids in the circulation of normal animals inhibited the activity of MAO. Parvez and Parvez (1973b) in another study deprived rabbit foetuses of their hypophysis by decapitation and measured MAO activity in adrenals, kidneys, paraganglia, lung, liver and heart. There was a progressive rise in MAO activity on the 30th day in the adrenals, kidneys and paraganglia following decapitation on the 20th day to the 25th day. The activity in the above three organs remained higher than controls even after decapitation on the 27th day. Lung, liver and heart demonstrated maximum activity after decapitation on the 23rd day. Administration of ACTH and hydrocortisone to the decapitated foetuses lowered MAO activity in adrenals, kidneys, heart and liver. It was speculated that corticoids limit MAO activity by interfering with protein synthesis through one of the regulatory steps. On the other hand, Bhagat, Bryan and Lee (1972) reported that adrenal MAO responds differently than MAO in other tissues. In this respect adrenal MAO was decreased by hypophysectomy and was increased by replacement treatment with ACTH.

Ho-Van-Hap, Babineau and Berlinguet (1967) reported that thyroxin increased heart MAO in young animals, but was decreased in adult animals. Liver MAO was decreased in adults while kidney MAO was slightly increased, and brain

MAO remained constant. In their studies hydrocortisone had no effect on MAO activity in the liver, brain and heart.

Biological function

Despite the abundance of information on MAO little is known about its physiological role. One likely function is the destruction of potentially toxic amines which are ingested, and the high levels of activity in the liver, stomach and intestine are particularly important in this regard. Support for this concept comes from the severe hypertensive reaction which can occur when patients who have been treated with MAO inhibitors eat food with a high tyramine content (e.g., Knoll and Magyar, 1972). The action of tyramine is thought to be due to its action in releasing noradrenaline from the nerve-endings.

The hormone, epinephrine, can be inactivated by methylation with catechol-O-methyltransferase (COMT) or by oxidation with MAO. Uptake of circulating epinephrine by nerve-terminals and their subsequent inactivation by MAO, may be an important mechanism for terminating the activity of this hormone (for review see Molinoff and Axelrod, 1971). In the liver and kidney, methylation of catecholamines could precede or follow oxidation by MAO. Zeller (1959) has suggested that both of the pathways may be used in the overall metabolism of this substrate. The Km value for rat liver MAO for the oxidation of metanephrine has been shown to be some tenfold lower than the corresponding value for epinephrine, indicating that this enzyme would be more efficient in oxidizing the methylated derivatives than the parent molecule (Tipton, 1972).

In neural tissues the function of presynaptic MAO is to metabolize released transmitter amines after re-uptake into the nerve-endings or after leakage from the intraneuronal storage vesicles. MAO inhibitors have been shown to result in an increase in the concentrations of the catecholamines and serotonin in the neurones (Tozer, Neff and Brodie, 1966; Spector et al., 1967). In addition to normal neurotransmitters, other amines accumulated in the synaptisomes when MAO was inhibited. Moreover, these amines, which are termed "false transmitters," can be released upon nerve stimulation. The equilibrium between free and bound norepinephrine in the nerve-terminal is affected by the presence of MAO. In this regard the presence of MAO in the nerve-terminals governs the relative concentrations of free neuronal and extraneuronal amines. Under conditions of MAO inhibition the capacity of the nerve-endings to take up amines becomes exhausted as the storage vesicles become saturated and the level of free intraneuronal amines rises.

The function of postsynaptic MAO is not as clear. Hendley, Taylor and Snyder (1970) reported an extraneuronal uptake mechanism for norepinephrine in the rat brain and MAO would likely deaminate norepinephrine taken up in this manner.

Serotonin is metabolized principally by MAO oxidation in rat brain (Neff and Tozer, 1968) and pineal gland (Feldstein and Williamson, 1968). Histochemical studies have indicated that MAO inhibitors have a much greater effect on serotonin-containing neurones than on those containing catecholamines.

In addition to its role in the degradation of biogenic amines, other functions have been suggested for MAO. Fischer, Schulz and Oliner (1966) have shown that beef-thyroid-gland MAO appears to be inactive towards epinephrine and norepinephrine, but tyramine is a good substrate. They have suggested that the thyroidal enzyme may function as a hydrogen peroxide generating system for iodothyronine synthesis. Gorkin and Orekhovitch (1967) have suggested that the metabolites produced by monoamine oxidase may function as regulators of cellular oxidation reactions and Jouvet (1969) has suggested that the aldehydes produced by the action of MAO may be involved in the mechanism of sleep. This would imply that the function of the enzyme may not be solely degradative.

In summary, relatively little is known about the function of MAO, but it now appears certain that it plays an important role in the regulation of the uptake and storage of biogenic monoamines and is important in determining functional levels of biogenic amines that in turn are involved in a variety of physiological and behavioral processes. It appears extremely likely that other areas will be found where MAO plays a key physiological role.

Serotonin

Introduction

Serotonin is a vasoactive biogenic monoamine synthesized from the amino acid tryptophan, which is first converted to 5-hydroxytryptophan by tryptophan 5-hydroxylase. This metabolite is then converted to serotonin by 5-hydroxy-

tryptophan decarboxylase. Serotonin is metabolized by MAO with formation of 5-hydroxytryptophan acetaldehyde which is in turn transformed into 5-hydroxyindoleacetic acid by aldehyde dehydrogenase.

Serotonin is widely distributed in both the vegetable and animal kingdoms. The biological importance in plants of an analogue of serotonin--the auxin, 3-indolylacetic acid--has been known for a long time. Recently, serotonin has also been found to be present in several edible vegetables (see Garattini and Valzelli, 1965, for review). Serotonin has been found to be ubiquitously distributed in the animal kingdom, being present in both invertebrates and vertebrates. Erspamer (1966) reported that most of the serotonin in mammals is localized in the enterochromaffin cells of the gastrointestinal tract where it plays an important role in the physiological functions of the alimentary canal. Serotonin is synthesized in the cytoplasm of the enterochromaffin cells (Lovenberg, Weissbach and Udenfriend, 1962) and actively accumulated and stored in their specific granules (Baker, 1958), from which it is liberated into the blood by various drugs (Pletscher, Shore and Brodie, 1955) and by certain physiological conditions (Bulbring and Crema, 1959). Serum serotonin determinations indicate the release of this amine into the venous blood (Resnick and Gray, 1961), and fluorescence studies (Penttila, 1968) also indicate that serotonin is secreted into the gut lumen.

The brain is another area where serotonin is present in large amounts and unevenly distributed. It is present in relatively large amounts in the hypothalamus and midbrain while low concentrations are found in the cortex and

cerebellum (Garattini and Valzelli, 1965). Piezzi, Larin and Wurtman (1970) have reported that large amounts of serotonin, 5-HIAA and MAO are present in the bovine pituitary and median eminence. Highest serotonin concentrations were found in the infundibular process, the infundibular stem and the pars intermedia. Myers et al. (1971) showed that micro-injections of ^{14}C-serotonin into the rat hypothalamus resulted in the amine remaining localized in the hypothalamus.

It is now generally accepted that serotonin acts as a neurotransmitter in the central nervous system. In this respect, serotonin is present at a very early age in the development of the organism. Deeb (1972) has found serotonin in early embryos of molluscs, polychetes and echinoderms. Serotonin is reported to be present in fertilized eggs of such vertebrates as teleosts, toads and frogs, and Buznikov, Chudokova and Zvezkina (1964) considered serotonin to be a universal participant in early embryogenesis. The concentration of these mediators is much greater in the zygote and blastomeres than in non-nervous tissues of adults, and is similar to that observed in definite nervous tissue. Buznikov et al. (1968), Deeb (1972) and Gustafson and Toneby (1970) have demonstrated temporal patterns of these mediators in early embryogenesis.

Burden and Lawrence (1973) demonstrated the presence of serotonin in rat ova, zygotes and 2 to 4 cell embryos. Baker and Hoff (1972) reported differences in serotonin maturation levels in various brain regions of the mouse from 1 day postpartum to adulthood. Serotonin levels increased in the cerebral hemispheres, mesencephalon-diencephalon and medulla-pons as well as the whole

brain. The largest increases developed between the 1st week and 2nd week of life.

Hole (1972) has reported that when rats were injected with D, L-p-chlorophenylalanine (pCPA--a drug that blocks serotonin synthesis) during the first weeks of life there was reduced brain growth and behavioral deficits later in life. Woolley and van der Hoeven (1965) have suggested that low brain serotonin may be a factor of importance for the brain damage that occurs in phenylketonuria. Hole (1972) also reported that rats injected with pCPA (200 mg/kg at 2-3 day intervals from the 7th through the 15th day of life) showed significantly reduced brain serotonin and brain growth. Injections of 5-hydroxytryptophan (40 mg/kg three times daily) during the same period restored brain serotonin concentrations and prevented the decrease in brain weight by pCPA. Buznikov, Zvezdina and Makeeva (1966) reported that a low level of serotonin may interfere with protein synthesis. Hole (1972) has speculated, therefore, that the low brain serotonin levels may be of particular importance for the harmful effects on the brain by interfering with protein synthesis.

Serotonin has also been shown to be present in the rat testes (Kormano and Penttila, 1968 and Assaykeen and Thomas, 1965). Zieher et al. (1971) have followed the concentration of serotonin in the testes of Wistar rats at 1, 5, 10, 20 and 120 days of age. Serotonin was present at a maximal level at day 1 of age and showed a considerable decrease through the different age periods studied with minimal levels at adulthood or about 1% that of the neonatal period. In this study, serotonin appeared to be located in the walls of the seminiferous tubules and

interstitial cells, respectively. This agrees with findings of Kormano (1967) and Kormano and Penttila (1968) who also found serotonin localized in the intertubular spaces and tubular walls.

In the rat, serotonin is found in large quantities in the mast cells. These cells increase in number during conditions of inflammation when serotonin is released. It acts as a vasodilator and increases arteriole and venule permeability. It also acts to stimulate fibroblast proliferation, thus aiding in the repair process after tissue injury. In most other species, serotonin is carried primarily in the platelets. Berneis, Da Prada and Pletscher (1969) have shown that blood platelets of rabbits contain relatively large amounts of serotonin and nucleotides, especially adenosine-5'-triphosphate (ATP). They reported that these constituents are stored in specific subcellular organelles probably in the main as serotonin-ATP-aggregates of high molecular weight. These organelles can then be isolated by density gradient centrifugation and distinguished by electron microscopy from other elements because of the high osmiophily of their content that is due to the presence of serotonin. Tranzer, Da Prada and Pletscher (1966) have shown that, in contrast to rabbit platelets, the blood platelets of guinea-pigs are like those of man and contain only small amounts of serotonin in contrast to a larger amount of ATP. Da Prada, Pletscher and Tranzer (1971) have subsequently shown that the presence of nucleotides, e.g., ATP in the organelles is essential for the storage of biogenic monoamines. They theorized that this establishes an equilibrium between the free, unbound serotonin, and that bound with the nucleotides. The function of the

serotonin found in the platelet is still not known. Helene, Dimicoli and Brun (1971) have shown that serotonin can also bind to nucleic acids and that nucleic acids may act as a serotonin receptor site in the brain.

Sheving et al. (1972a) have demonstrated a circadian rhythm of serotonin in the rat brain by the estimation at hourly intervals. Friedman and Walker (1968) reported a circadian rhythm of serotonin in rat mid-brain and caudate nucleus. Okada (1971) reported that the maturation of the circadian rhythm of brain serotonin was observed vaguely at 27-30 days of age and became apparent from day 35-37. Asano (1971) has verified this rhythm and has shown that serotonin levels undergo a circadian pattern, i.e., elevated in the dark and reduced in the light period. Halberg et al. (1967) reported a circadian-like frequency in the levels of serotonin found in human serum. Recently, Sheving et al. (1972a) measured serotonin levels in rat serum at 2-hour intervals for three separate 24-hour spans and found a circadian rhythm with serotonin being highest during the last part of the light span and the first part of the dark span.

Sheving et al. (1972b) has also reported daily variations in serotonin content in the rat spleen. These patterns were similar to those previously reported for other body areas.

Effects on behavior

Sedation is one of the most commonly observed behavioral traits after serotonin administration. Segawa and Fujisawa (1972) recently verified this after injections of the amine into rabbits. Data from several laboratories have

shown that serotonin, in cells of the raphe nuclei, plays an important role in the sleep mechanism. Loss of serotonin in the brain either by chronic ablation of the raphe neurons (Jouvet, 1969) or by treatment with pCPA (Delorme, Froment and Jouvet, 1966; Koella, Feldstein and Czicman, 1968) yields a marked decrease in slow-wave sleep-time. The latter is proportional to the extent of the lesion-induced fall in serotonin. In addition, after pCPA administration, the return of sleep correlates temporally with the return of serotonin levels.

Serotonin is also thought to be involved in the regulation of sexual behavior. Shillito (1969), Tagliamonte et al. (1969) and Sheard (1969) have reported facilitation of mounting behavior in male rats by pCPA. Salis and Dewsbury (1971) reported pCPA facilitation of copulatory behavior in male rats while Meyerson and Lewander (1970) observed pCPA induced estrous behavior in female rats. Benkert and Eversmann (1972) claim, however, that testosterone is necessary in addition to pCPA for the facilitation of mounting behavior. Mitler et al. (1972) reported on data from four studies and concluded that pCPA alone can increase the frequency of heterosexual and homosexual mounting as well as copulation in male rats. Recently, Gawienowski, Merker and Damon (1973) reported that pCPA can elicit homosexual mounting behavior but that this effect is enhanced by testosterone.

Serotonin has been implicated in a number of other behavioral disorders and alterations (see Aprison and Hingtgen, 1972, for review) but it is generally accepted that serotonergic pathways are involved in at least the sleep mechanism and some aspects of sexual behavior.

Effects on abortions

Among the causes leading to recurrent abortion are psychogenic factors and emotional stress. Sadovsky et al. (1970) have found that cases of habitual abortion of psychogenic origin are often associated with increased serotonin production which in turn causes uterine contractions and reduced placental blood flow. Sadovsky et al. (1972) have recently found that in women who habitually abort there is often increased levels of 5-HIAA in the urine. In these cases, they have been able to successfully prevent abortions with the use of cyproheptadine, a serotonin antagonist, that blocks serotonin induced contractions of the uterine muscle. Sadovsky et al. (1970) have also shown that trengestone, a progesterone derivative, has a fetus-saving effect due to the fact that it increases formation of serotonin-metabolizing enzymes, including MAO in the endometrium. whereas estrogens decrease it.

Effects on pancreatic function

Using fluorescence microscopy and autoradiography, Cegrell, Falck and Rosengren (1967) among others, have established the existence of tryptaminergic mechanisms in the pancreatic islets. The distribution of fluorescence in fine cytoplasmic granules suggested that serotonin might be stored in the insulin-containing secretory granules of the β-cells (Falck and Hellman, 1964). The idea was supported by Jaim-Etcheverry and Zieher (1968) who showed an electron microscopic demonstration of an intense reaction in the β-granules after treating the islets with glutaraldehyde and potassium dichromate. Serotonin has been found to modify secretion from the β-cells (Lernmark, 1971), and it seems reasonable to suspect

that tryptaminergic mechanisms may play a role in the physiological regulation of insulin release. In this regard, serotonin may be a potent inhibitor of insulin release (Tjälve, 1971; Feldman and Lebowitz, 1970). Findings of Lernmark (1971) support this view as they observed the glucose-stimulated insulin release in mice was blocked by serotonin.

Effect on thyroid function

Serotonin has an influence on thyroid activity and has been the subject of several investigations and reviews (Söderberg, Harrison, 1964; Garattini and Valzelli, 1965) but there is no uniform concept of the nature of this influence. Both stimulatory (e.g., Werner, Tierney and Tallberg, 1964) and inhibitory (e.g., Cavazzuti and Ghigi, 1969) effects have been reported, as well as the absence of any effects (e.g., Brown and Munro, 1967). It has also been reported that any effects of such amines on thyroid activity are due mainly to changes in thyroidal blood flow (Ahn, Athans and Rosenberg, 1969).

Recently, Melander and Sundler (1972) have shown that serotonin induces the secretion of thyroid hormones by direct actions on the thyroid follicle cells. They speculated that serotonin and TSH activate a common mechanism in the cell, although they may differ in their initial interactions with the cell. Serotonin is released from thyroid mast cells and in this way is involved in thyroid hormone secretion.

Clayton and Szego (1967) and Clayton and Masuoka (1968) reported that amine-containing mast cells occur in varying numbers in the thyroid of different

species, and observations by them imply that the amount of thyroid mast cells is related to the level of plasma TSH. TSH is known to induce a rapid release of serotonin from these cells, and it has been postulated that this explains the increased thyroid blood flow which follows TSH stimulation (Clayton and Szego, 1967; Clayton and Masuoka, 1968).

Effect on gonadotropins and brain sexual pathways

Kordon et al. (1968) have reported that increased serotonin levels in the brain inhibit the phenomenon of superovulation in immature rats. Kordon (1969) later showed that pharmacological treatment that selectively increased hypothalamic serotonin levels inhibited gonadotrophin-induced ovulation in immature rats. Lippman (1968) observed similar phenomenon in adult hamsters. Labhsetwar (1972) injected an MAO inhibitor alone or in combination with 5-hydroxytryptophan and found a selective rise in the hypothalamic serotonin level that blocked ovulation in a significant percentage of rats tested.

The action of serotonin on gonadal function is generally believed to be a consequence of its influence on the release of anterior pituitary hormones, especially the gonadotropins. Recently, it was observed by Kamberi, Mical and Porter (1970) that serotonin, when administered to male rats by way of the third ventricle, suppressed the release of LH. Schneider and McCann (1970) confirmed that intraventricular injections of serotonin decreases LH secretion. Kamberi, Mical and Porter (1971) have also reported that injections of serotonin in the third ventricle of male rats, stimulated the release of prolactin and inhibited the release

of FSH. They suggested that serotonin may have suppressed the discharge of PIF (prolactin-inhibiting factor), and FRF (FSH-releasing factor), and thereby indirectly affected the release of prolactin and FSH. Porter, Mical and Cramer (1972) have recently contradicted their previous results, however, and report that intraventricularly administered serotonin stimulated the release of LH in intact rats, but suppressed the release of LH in castrated rats. They reported that serotonin had little effect on FSH release, but it did stimulate prolactin release, thus corroborating earlier observations on prolactin release. Koe and Weissman (1966) observed that pCPA alone or with methysergide, a serotonin antagonist, prevented the rapid depletion of pituitary FSH in female rats shortly before they attain sexual maturity. On this basis, Brown (1971) has speculated that serotonin may play an excitatory role in FSH secretion. Brown and Fawke (1972) later verified this conclusion.

Campas and Lodosky (1972) have recently reported that serotonin decreases oxygen consumption of the hypothalamus in both intact and castrated rats, and have suggested that it may act on the hypothalamus to alter its metabolism.

Effects on the testes

Salgado and Green (1955) while investigating the effect of serotonin on renal morphology noted reductions in testicular weight. Boccabella, Salgado and Alger (1962) reported that serotonin produced a significant decrease in testicular weight by the 5th day; the maximal decrease being attained by the 15th day. The weights of the accessory reproductive organs were significantly reduced compared with

those of the controls by the 10th day of treatment. Seminiferous tubules in the early period contained giant multinucleated spermatids as well as pyknotic primary spermatocytes. Many of the tubules of animals treated 15 days or longer had only Sertoli cells and spermatogonia, while others completely lacked cells. Other tubules, however, showed orderly spermatogenic progression. Spermatozoa were always present in the epididymal suspensions, but they demonstrated either poor or no motility. These authors concluded that the lesions were probably the result of testicular ischemia since simultaneous administration of the vasodilator apresoline prevented the morphological changes. Concomitant administration of pregnant mares serum gonadotropin (PMS) did not counteract the deleterious effects of serotonin. O'Steen (1963) reported that a single serotonin injection into male mice caused weight increases in the prostate glands and histological changes indicative of edema or vascular defect. Pathological changes in testes of treated mice include degenerating seminiferous tubules that contained spermatogonial cells with pyknotic nuclei and giant multinucleate cells. Absence of all spermatogenic cells from tubules was characteristic of advanced degeneration. They concluded that the changes observed were not due to vascular changes.

Seibel and Bush (1970) on the other hand, reported that injections of serotonin failed to alter reproductive organ weights in the male hamster, while Kormano, Karhunen and Kahanpaa (1968) observed severe detrimental changes to the gametogenic elements of the rat testis. Recently, Kinson and Liu (1973) found that implantation of immature rats with pellets of serotonin caused no significant changes in body weights or in the weights of the accessory sex glands. Serotonin

initially suppressed the concentration of testosterone in testicular venous blood, but more consistently lowered hormone levels in mixed venous blood. Liu and Kinson (1973) have also reported that serotonin implants in mature rats caused a decrease in testicular and prostatic weights, mixed venous blood levels of testosterone as well as spermatogenesis.

Ellis (1972) has shown that serotonin inhibits rat testicular 17 ∝-hydroxylase, 17 ∝-hydroxypregnene-C_{17}-C_{20}-lyase, and 17-keto reductase activities. Serotonin increased 17 ∝-hydroxyprogesterone. Serotonin inhibited androgen synthesis through a noncompetitive mechanism. Cardinali and Rosner (1971) using duck testicular homogenates reported that serotonin had no effect on steroid biotransformations with doses below 200 mμmoles/ml.

Cockett et al. (1971) have observed spermatogenic arrest in the seminiferous tubules after fourteen days of immobilization. They also found histochemical alterations following immobilization that included changes in lactic dehydrogenase, succinic dehydrogenase and alkaline phosphatase. Histological alterations were also demonstrated. These authors have suggested on at least two separate occasions (Cockett et al. 1971; Cockett et al., 1970) that serotonin may be involved in the testicular degeneration observed after immobilization.

MATERIALS AND METHODS

Animal Handling and Care

All rats (Holtzman Co. Strain) were maintained in a small animal laboratory with a southern exposure under controlled conditions. Artificial lighting was used during the daylight hours only when the caretaker was caring for the animals. Temperature in the room was maintained at 72^{o}F with a relative humidity of 35%. All animals were housed in professional animal care units (4-6 animals/cage, 13.5" x 16" in size) and unless otherwise specified, feed (laboratory chow) and water were given ad libitum. Animals were weighed and then sacrificed by decapitation. The testes were quickly removed, chilled, trimmed, weighed and then decapsulated. Portions of one testes were weighed, homogenized and assayed for MAO activity as described below.

Aging

The effects of aging on testicular MAO activity were determined by using various groups of male rats with average body weights of 19, 53, 145, 236, 415, 482 and 650 grams (N = 5, 5, 3, 6, 5, 5 and 5 respectively) which were weighed, sacrificed and their testes removed for MAO assay.

Hypophysectomy

Rats hypophysectomized at 3 and 10 weeks of age along with appropriate control animals were purchased from Hormone Assay Laboratories. These included animals hypophysectomized at 3 weeks (N = 8) and sacrificed along with controls (N = 5) at 7 weeks after surgery. The hypophysectomized animals were divided into two groups of four animals per group. One group was injected, subcutaneously, with 50 I.U. of hCG (Sigma Chemical Co.) for 18 days prior to necropsy. Four hypophysectomized animals were injected with saline and used as hypophysectomized controls. Injections were administered daily at 4:00 p.m. Other animals hypophysectomized at 3 weeks of age (N = 6) were sacrificed along with controls (N = 6) at 15 weeks after surgery. Additional animals were hypophysectomized at 10 weeks of age and sacrificed at either 1,3,4 or 6 weeks after surgery (N = 5, 5, 6 and 6, respectively). Control animals were sacrificed at 1 and 4 weeks along with the hypophysectomized animals (N = 5 and 6, respectively).

In other experiment six control and 29 hypophysectomized animals (3 weeks old at the time of surgery) were sacrificed at 6 weeks of age. The hypophysectomized animals were randomly subdivided into five groups. The first group contained five animals that were injected subcutaneously with 20 μg of Ovine FSH NIH-FSH-59 (in 0.1 ml of 0.9% NaCl) twice daily (9:00 a.m. and 4:00 p.m.) for 4 days prior to sacrifice. The second group of six rats was injected twice daily with 2.5 μg of Ovine LH NIH-LH-517. The third group of six animals was injected twice daily with 100 μg of Ovine prolactin NIH-P-59, while the fourth group of six rats

was injected twice daily with a combination of 20 μg Ovine FSH NIH-FSH-59, 2.5 μg Ovine LH NIH-LH-517 and 100 μg Ovine prolactin NIH-P-59. The fifth group of six animals was injected with 0.9% NaCl.

Melatonin

The effects of subcutaneous melatonin injections on testicular MAO activity were determined using 16 previously hypophysectomized male rats (Hormone Assay Laboratories). All animals were injected daily, for 12 days. One group of animals received 1 mg of melatonin daily suspended in 0.1 ml corn oil. Five additional animals were injected with 2 mg of melatonin daily in 0.1 ml of corn oil. The five control animals received 0.1 ml of corn oil daily. The animals were 11 weeks old at the time of hypophysectomy and 13 weeks of age when sacrificed.

In vitro effects of melatonin on testicular MAO activity were determined by using rats from our small animal colony. Concentrations of melatonin (10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} M, dissolved in 95% ethanol--six samples each) were added to homogenates in vitro and MAO activity was determined. Aliquots of ethanol were added to all control samples.

Estrogen

Eighteen male rats were divided into three groups with six animals per group. One group of six animals was injected for 10 days with 10 μg per day

per animal of estradiol benzoate (nutritional biochemistry) in 0.1 ml corn oil. Another six animals were injected with 100 μg per day and six controls were injected with the vehicle.

Adrenalectomy

Seven control and eight additional male rats adrenalectomized at 62 days of age (Hormone Assay Laboratories) were maintained in the animal laboratory with feed, tap water and saline (0.9% NaCl) given ad libitum to all animals. The animals were sacrificed 10 days after surgery.

Starvation

In the first experiment, 10-week-old animals were divided into three groups. The control group (N = 5) was given feed (Purina Laboratory Chow) ad libitum during the entire experimental period. They consumed an average fo 23.5 grams of feed each, per day. Group II (N = 5) was restricted to between 12.2 and 12.7 grams of feed per animal per day for 21 days (feed was restricted by 47% or 53% of the control diet). Group III (N = 4) was given between 7.5 and 8.0 grams of feed for each animal per day for 21 days (a 67% restriction or 33% of the control diet). All animals were given water ad libitum.

At the end of the 21-day experimental period the adrenal, testes, prostate and seminal vesicles were quickly removed, chilled, trimmed and weighed. The cranial vaults of the animals were then opened and the pituitary and pineal

glands were removed with a pair of dissecting forceps and weighed using an automatic precision analytical balance (accuracy of 0.1 mg). Hydroxyindole-O-methyltransferase (HIOMT) activity was measured in the pineal glands as described below.

In the second experiment 28 animals were divided into four groups. The control group (N = 7) was given feed ad libitum. The first experimental group (N = 7) was given between 12.2 and 12.7 grams of feed per animal per day for 28 days, and the second group (N = 7) was given between 7.5 and 8.0 grams of feed per animal per day. The third experimental group (N = 7) was pinealectomized 1 week before the beginning of the experiment and during the experimental period they were given between 7.5 and 8.0 grams of feed per animal per day for 28 days. Two of the pinealectomized animals subsequently died during the experimental period leaving five animals in this group.

At the end of the 28-day experimental period the adrenal, testes, prostate and seminal vesicles were quickly removed, chilled, trimmed and weighed. Pineal glands and pituitaries were also removed and weighed. N-acetyl transferase activity was measured in the pineal glands as described below.

Radiation

250 R whole-body

Fifty six male rats were irradiated with 250 R of whole-body x-irradiation (12 weeks of age) using a 250 kvp instrument (Westinghouse Quadrocondex Therapy

Unit) fitted with a 0.5 mm Cu and 1 mm Al filter. An additional 56 comparable animals were sham irradiated to serve as controls. The average dose-rate in air was 44.2 R/min. The total dose was calculated using a thimble-type ionizing chamber (Victoreen Inst. Co.). Three animals were sacrificed from both the control and the irradiated groups according to the following schedule: 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 42, 52, 68, 80 and 153 days after treatment. At sacrifice the animals were killed with ether anesthesia, their testes removed and subsequently teased to separate the seminiferous tubules from the interstitial cells (Ellis and van Kampen, 1971) and aliquots of either teased tubules or homogenized testicular tissue preparations were assayed for MAO activity. A mean comparison analysis was used to test for statistical significance between groups of animals for a given time period.

450 R whole-body, No. 1

An experiment was designed to determine the effects of pinealectomy, whole-body irradiation and alterations in photoperiod on rat testicular MAO activity. Twenty-one animals were pinealectomized and these along with 19 additional sham operated animals were divided into six groups. Twenty-six of the animals were irradiated with 450 R of whole-body x-irradiation (Seifert 150 kvp x-ray machine) and the remaining 14 animals were sham-irradiated. A one mm Al filter was used to filter out the soft x-rays. Two groups of irradiated animals were then placed into constant darkness for 21 days. These consisted of six non-

pinealectomized irradiated animals and seven pinealectomized irradiated rats. The remaining four groups were placed in the small animal room with natural light conditions. These consisted of seven controls, seven pinealectomized non-irradiated animals, six non-pinealectomized irradiated animals and seven pinealectomized irradiated animals.

The animals were sacrificed at the end of the 21-day experimental period.

450 R whole-body or head only, No. 2

Eighteen animals were divided into three groups with six animals per group. Six animals were irradiated with 450 R of head-only x-irradiation (Seifert 150 kvp x-ray machine) and six were irradiated with 450 R of whole-body x-irradiation. The remaining six were sham irradiated. All animals were sacrificed one hour after irradiation.

450 R testes only, No. 3

An experiment was designed that was similar to No. 1 (above) except that the animals had only the testes x-irradiated. Sixteen animals were pinealectomized and 20 animals were sham-operated. After a two-week recovery period the animals were divided into six groups. Twenty-four animals were irradiated with 450 R of testes-only x-irradiation (Seifert 150 kvp x-ray machine) and the remaining 11 animals were sham irradiated. Two groups of the irradiated animals were then placed into constant darkness. These consisted of six non-

pinealectomized irradiated animals and six pinealectomized irradiated animals. The remaining groups in natural light consisted of seven controls, four pinealectomized non-irradiated rats, six pinealectomized irradiated rats and six non-pinealectomized irradiated animals.

The animals were sacrificed at the end of a 21-day experimental period.

Alterations in Photoperiod

Experiment No. 1

The effects of extreme changes in photoperiod were determined by placing animals into three different lighting schedules for 10 days. One group of eight animals was placed in constant light. One group of seven animals was placed in natural light and one group of eight animals was placed in constant darkness. The animals were sacrificed at the end of the 10 day experimental period. The experiment was conducted in early May.

Experiment No. 2

This experiment was similar to the one above except that younger 24-day-old animals (Spargue Dawley Derived Strain - Horton Lab.) were used and the experiment was conducted in late October. Each group had seven animals. One group was placed in 24 hours of constant light; the second group received 12 hours light followed by 12 hours darkness; the third group was in 24 hours of

constant darkness. The animals were sacrificed 10 days after they were places in their respective photoregimes.

Pinealectomy

To determine the effect of pinealectomy on testicular MAO activity six rats were pinealectomized using the technique of Hoffman and Reiter (1965) (Sprague Dawley strain - Horton Lab.) while the control group of six was sham-operated. Both groups were sacrificed 9 days after surgery.

Pargyline Injections

To determine the effects of pargyline, an MAO inhibitor, on testicular concentrations of serotonin and testicular MAO, pargyline was injected into five animals (15 mg/kg, injected i.p.). Five animals were injected with 0.9% saline solution to serve as controls. All animals were sacrificed 24 hours after the injections and the testes were removed, weighed, immediately placed in foil and frozen on dry ice. Portions were then assayed for MAO as described below while other aliquots were assayed for endogenous serotonin concentrations by the fluorometric method of Wise (1967).

Circadian Rhythm

To determine if testicular MAO fluctuates with a circadian rhythm six male rats were killed between 11:30 a.m. and 12:30 p.m. and six other male rats

were killed between 11:30 a.m. and 12:30 p.m. Tissues were frozen and later assayed for MAO activity.

Seasonality

Sparrow

English male house sparrows (Passer domesticus) were collected with a mist net from the Cache Valley area. Seven sparrows were collected in January, six in March, six in April, six in June, six in July, five in August, seven in September, five in November and six in December. All sparrows were collected between 1:00 p.m. and 4:30 p.m. the day before sacrificing and they were kept overnight in a wire cage with feed (pigeon feed) and water given ad libitum.

Squirrel

Six adult male uinta ground squirrels (Spermophilus armatus) were trapped in early April, soon after emergence, at a location in the Logan Canyon area approximately 30 miles from the Utah State University campus. Five other squirrels were trapped at the end of May. All animals were immediately weighed after trapping and then sacrificed by decapitation. The testes were immediately removed and frozen until later assayed for MAO activity.

Localization of Testicular MAO

Experiment No. 1

Two animals were used in this experiment. One had a body weight of 323.5 grams and the other a body weight of 514.8 grams. Four samples were used from each animal and assayed for MAO activity. Two of the samples were whole testicular homogenates and two were teased-seminiferous tubule homogenates. Two other samples were also used that were from teased-seminiferous tubules that had been frozen at -20°C for two months. The experiment was run in late August.

Experiment No. 2

One animal was used that had a body weight of 529.6 grams. Four whole testicular homogenates and four teased seminiferous tubule homogenates were assayed for MAO activity. The animal was killed in early September.

Experiment No. 3

Five animals were sacrificed in late October with an average body weight of 477 grams. One whole testicular homogenate sample and one teased seminiferous tubule homogenate sample from each animal were assayed for MAO activity.

Experiment No. 4

Five animals were sacrificed in early December and had an average body weight of 667 grams. One whole testicular homogenate sample and one teased seminiferous tubule homogenate sample from each animal was assayed for MAO activity.

Experiment No. 5

An experiment was designed to determine the relative amounts of MAO in the seminiferous tubules and interstitial cells of the rat testes. Tubules and Leydig cells were collected from a number of animals and were stored at -20^{o}C until assayed for MAO activity. Six teased-seminiferous tubule homogenate samples and six interstitial cell homogenate samples were assayed for MAO activity.

Experiment No. 6

Teased seminiferous tubules, interstitial cells, seminiferous tubule walls (without contents) and seminiferous tubule contents (without walls) were collected from a number of animals over a 3 month period, and stored at -20^{o}C. Five samples from each group were then assayed for MAO activity.

Tissue Culture

FSH effects

Seminiferous tubules were teased from the testes of one animal. Aliquots of the tubules were then clotted to the bottom of Wasserman tubes using chicken plasma and chick embryo extract. One ml of media was then added to each tube. Each 100 ml of media which was the same as described by Steinberger et al. (1967), consisted of 10 ml minimum essential media (Eagles 10X), 1 ml sodium pyruvate (100 X), 1 ml non-essential amino acids (100 X), 10 ml antibiotic (penicillin-25 U/ml and 25 mcg streptomycin/ml), 2 ml fungizone and 76 ml pyrogen free water. There were six control samples, six samples that had 2 IU FSH added per ml of media and six samples with 5 IU FSH added per ml of media. Fresh media was then added every 2 days and all samples were cultured at 37^{o}C for 6 days. At the end of the culture period the tubules were frozen at -20^{o}C until they were assayed for MAO activity.

Pargyline effects

One animal was injected with pargyline (10 mg/kg, i.p.) and a control animal was injected with 0.9% saline. Twenty-four hours after the injection the animals were sacrificed and the seminiferous tubules were teased apart from each animal. Two samples of seminiferous tubule homogenate from each animal were assayed for MAO activity at the time of sacrifice. Ten aliquots of tubules from each animal were then placed into tissue culture as described above. Two samples

from each animal were then removed from culture on 1, 2, 4, 6 and 9 days after the initiation of the tissue cultures. These were then stored at -20°C until later assayed for MAO activity. Media was the same as described above and was changed every 2 days.

MAO Activity

MAO activity was measured by the amount of radioactively labeled serotonin that was converted into 5-hydroxyindole acetic acid which was then extracted into ethyl acetate, isolated by thin layer chromatography and counted in a scintillation counter (see Urry, Jaussi and Ellis, 1972, for exact details).

HIOMT

HIOMT activity was assayed using the basic method of Axelrod and Weissbach (1961) with adaptions for thin-layer chromatography and individual pineal glands as described by Barfuss, Tait and Ellis (1969). Basically the technique utilized individual pineal glands homogenized in buffer containing N-acetyl serotonin and radioactively labeled S-adenosyl methionine. After an incubation period the resulting labeled melatonin was extracted in chloroform, isolated by thin-layer chromatography and counted in a scintillation counter.

N-Acetyltransferase

N-Acetyltransferase activity was measured by homogenizing individual pineal glands in buffer containing radioactively labeled serotonin and acetyl-coenzyme A. The resulting labeled N-acetyl serotonin and melatonin were isolated with thin-layer chromatography as described by Klein, Weller and Moore (1971) and counted in a scintillation counter.

Statistics

Statistical comparison of sample means was made with a standard t-test unless otherwise indicated.

RESULTS

Aging

Body weight and testicular weight changes associated with an increase in age (i.e., testicular/body weight ratios--Table 1) indicated that these two entities were directly related to each other up to a given point (482.2 g body wt) in the animal's growth period. Testicular weights then declined while body weights continued to increase (649.6 g body wt.).

MAO activity calculated on a per 100 g body weight basis (Figure 1) showed a high activity in neonatal animals (19.05 g) a reduced activity at 52.8 g ($P < 0.05$), an increased activity at 145-235 g ($P < 0.01$) and a reduced activity at 415 g ($P < 0.05$), 482 g ($P < 0.01$) and 650 g ($P < 0.001$).

Hypophysectomy

Hypophysectomy of immature male rats at 3 weeks of age significantly reduced testicular weight and total MAO activity (expressed on a per animal basis, Table 2). The specific activity of this enzyme (expressed on a per mg of tissues basis) was, however, markedly increased 7 weeks after surgery but had regressed to a level equal to that of the control animals by 15 weeks past treatment. Treatment of hypophysectomized animals with hCG resulted in an

Table 1. Body and testicular weights of animals used to study the effects of aging on testicular MAO activity. Values are expressed as mean ± standard error of mean

Animals	No. of animals	Body weight (g)	P value	Testicular weight (g)	P value	Testicular/ body weight ratio
Group 1	5	19.05 ± 1.04	<< 0.001	0.037 ± .002	<< 0.001	.002
Group 2	5	52.75 ± 0.45	0.001	0.290 ± .010	<< 0.001	.005
Group 3	3	145.2 ± 15.2	<< 0.001	1.70 ± .02	<< 0.001	.012
Group 4	6	235.6 ± 7.9	<< 0.001	2.63 ± .14	<< 0.001	.012
Group 5	5	415.0 ± 13.5	< 0.01	3.68 ± .06	<< 0.001	.008
Group 6	5	482.2 ± 5.5	<< 0.001	3.82 ± .06	< 0.05	.008
Group 7	5	649.6 ± 23.5		3.60 ± .08		.006

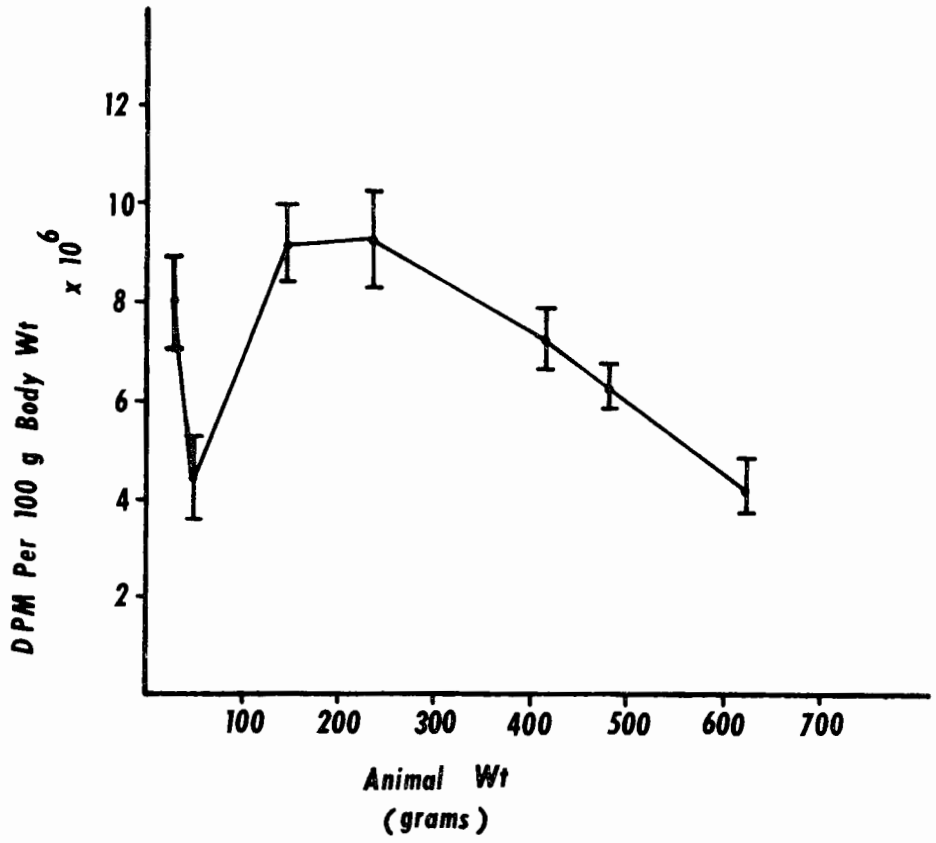

Figure 1. MAO activity of rat testicular preparations determined by the amount of 5-HIAA-2-^{14}C formed from 5-HT-2-^{14}C and expressed on a per 100 g body weight basis. The vertical lines for each observation denote the standard error of mean.

Table 2. Rat testicular MAO activity of control and hypophysectomized animals expressed on both a per mg of tissue and a per animal basis. Values are expressed as mean ± standard error of mean

Treatment	Time after hypophysectomy (weeks)	No. of animals	Testicular weight (g)	P value	MAO specific activity (per mg of tissue) CMP x 10^3	P value	Total MAO activity CPM x 10^3	P value
Immature Animals (3 weeks old at surgery)								
Control	7	5	3.33 ± .07		25.47 ± 1.99		84.68 ± 3.23	
Hypophysectomy	7	4	0.11 ± .01	<0.001	57.38 ± 4.88	<0.001	6.17 ± 0.53	<0.001
Hypophysectomy & HCG	7	4	0.29 ± .04	<0.001 <0.01*	27.01 ± 2.16	>0.50 <0.001*	7.50 ± 0.69	<0.001 >0.50*
Control	15	5	3.63 ± 0.12		15.83 ± 2.20		57.66 ± 8.54	
Hypophysectomy	15	6	0.19 ± 0.04	<0.001	15.46 ± 3.22	>0.50	2.73 ± 0.59	<0.001
Mature Animals (10 weeks old at surgery)								
Control	1	5	3.33 ± .04		21.14 ± 4.60		70.80 ± 15.80	
Hypophysectomy	1	5	2.62 ± .05	<0.001	25.23 ± 3.40	>0.50	66.24 ± 9.30	>0.50
Hypophysectomy	3	5	1.06 ± .06	<0.001 <0.001*	43.17 ± 9.40	<0.10 <0.10*	44.79 ± 9.20	<0.20 <0.20*
Control	4	6	3.50 ± 0.22		19.80 ± 0.90		69.78 ± 6.71	
Hypophysectomy	4	6	0.74 ± 0.03	<0.001	46.74 ± 2.68	<0.05	34.36 ± 1.94	<0.001
Hypophysectomy	6	6	0.54 ± 0.02	<0.001 <0.001*	96.86 ± 3.80	<0.001 <0.001*	52.71 ± 3.36	<0.001 <0.001*

*Statistical comparison of the two treated groups

increase in testicular weight. It increased total, but decreased specific activity of MAO.

Hypophysectomy of mature male rats at 10 weeks of age decreased testicular weights and total MAO activity. Total MAO activity was maximally depressed 4 weeks after surgery, but it was increased 2 weeks later. Specific activity of this enzyme increased for all groups of hypophysectomized animals with time and was maximal 6 weeks after surgery.

In another investigation, hypophysectomy of immature animals decreased total MAO activity, but increased the specific activity (Table 3). Treatment of hypophysectomized animals with LH, FSH or prolactin failed to increase the specific activity of MAO when compared with untreated hypophysectomized animals. FSH did, however, increase testicular weight and the total activity of MAO in the hypophysectomized animals. A combination of FSH, LH and prolactin increased testicular weight concomitant with a decrease in specific activity of MAO. Total activity, however, was increased in this group compared with the untreated hypophysectomized animals. The combination treated group had a lower specific activity and total activity when compared with the FSH treated rats. Testicular weight of the combination treated group was greater than all of the other hypophysectomized groups except the FSH treated animals.

Melatonin

Testicular MAO activity was significantly increased (Table 4), both on a per mg of tissue basis and on a per animal basis, by injections of either 1 or

Table 3. Rat testicular MAO activity after various treatments expressed on both a per mg tissue and per animal basis. Values are expressed as mean ± standard error of mean

Treatment	No. of animals	Testicular weight* (g)	MAO specific activity per mg tissue* (CPM x 10^3)	Total MAO activity per animal* (CPM x 10^6)
Control	6	1.30 ± .05	19.35 ± 1.79	25.06 ± 2.20
Hypophy.	6	0.14 ± .01	41.25 ± 7.72	6.92 ± 0.57
Hypophy. & LH	6	0.17 ± .01**	55.88 ± 7.75	9.30 ± 1.48
Hypophy. & FSH	5	0.24 ± .01#,##	50.93 ± 7.12	12.15 ± 1.58#
Hypophy. & Prolactin	6	0.15 ± .01	49.99 ± 5.04	7.47 ± 0.85
Hypophy. & FSH, LH & Prolactin	6	0.26 ± .01	37.17 ± 3.57@,+	9.66 ± 0.85#

* $P < 0.001$ when controls were compared with all other groups.
** $P < 0.10$ when compared with the hypophysectomized animals.
\# $P < 0.05$ when compared with the hypophysectomized animals.
$P < 0.001$ when compared with LH or Prolactin treated animals.
@ $P < 0.10$ when compared with either the LH or Prolactin treated animals.
\+ $P < 0.01$ when compared with FSH treated animals.

Table 4. MAO activity in rat testes after melatonin injections and additions of melatonin to in vitro determinations of MAO activity. Values expressed are mean activity ± standard error of mean

Treatment	No. of animals	Testicular weight (mg)	P value	Per mg tissue CPM x 10^3	P value	Per animal basis CPM x 10^6	P value
Melatonin Injections							
Hypophysectomized Control	5	1620 ± 40		66.79 ± 7.04		107.01 ± 9.15	
Hypophysectomized plus 1 mg Melatonin	6	1720 ± 50	<0.20*	86.41 ± 6.06	<0.10*	148.15 ± 10.88	<0.05
Hypophysectomized plus 2 mg Melatonin	5	1670 ± 100	>0.50*	92.99 ± 2.46	<0.01*	155.12 ± 9.76	<0.05
			>0.50**		>0.50**		>0.50
Melatonin In Vitro[t]							
Control				32.47 ± 1.47			
10^{-4} M Melatonin				30.23 ± 1.60	>0.50*		
10^{-5} M Melatonin				36.29 ± 1.18	<0.05*		
10^{-6} M Melatonin				37.32 ± 1.23	<0.01*		
10^{-7} M Melatonin				40.35 ± 1.76	<0.001*		

* P value when compared with controls.

** P value when compared with other treated group.

t N = 6 for each assay.

2 mg of melatonin when compared with the control animals. Additions of melatonin *in vitro* at concentrations of 10^{-5} through 10^{-7} M also increased testicular MAO activity.

Estrogen

Body and testicular weights were significantly reduced after treatment with either 10 or 100 μg estradiol benzoate (Table 5). Testicular MAO activity was not changed after estradiol administration when calculated on a per mg of tissue or a per animal basis.

Adrenalectomy

Adrenalectomy significantly reduced body weights of the treated vs. control animals (Table 6), but failed to alter testicular weights. Testicular MAO activity was not changed either on a per mg of tissue or on a per animal basis.

Starvation

The data show (Table 7) that restriction of feed intake by 47 or 67% of the amount normally consumed significantly reduced body, adrenal and pituitary weights in a dose-dependent manner. Testicular, prostate and seminal vesicle weights (Table 8) were also significantly reduced in a dose dependent manner by feed deprivation, except for testicular weights of the 67% deprived animals in

Table 5. Testicular MAO activity and testicular and body weights in animals injected with 10 or 100 µg of estradiol benzoate daily for 10 days. Values expressed are mean ± standard error of mean

Treatment	No. of animals	Body weight (g)	P value	Testicular weight (g)	P value	Per mg tissue CPM x 10^3	P value	Per animal basis CPM x 10^6	P value
Control	6	293.2 ± 6.9		3.42 ± 0.06		62.82 ± 4.03		215.54 ± 15.92	
10µg Estradiol	6	265.2 ± 3.4	<0.01*	3.04 ± 0.11	<0.05	66.71 ± 2.57	<0.50	202.22 ± 9.67	<0.50
100 µg Extradiol	6	248.2 ± 7.6	<0.01*	3.14 ± 0.07	<0.05	69.02 ± 4.65	<0.40	215.57 ± 11.97	>0.50
			<0.10[t]		>0.50		>0.50		>0.50

* P value when compared with controls.

t P value when compared with other treated group.

Table 6. Testicular and body weights and testicular MAO activity after adrenalectomy. Values are expressed as mean ± standard error of mean

Treatment	No. of animals	Body weight (g)	P value	Testicular weight (g)	P value	Per mg tissue CPM x 10^3	P value	Per animal basis CPM x 10^6	P value
Control	7	238.0 ± 3.7		2.98 ± .104		21.70 ± 0.96		64.305 ± 2.155	
Adrenalectomized	8	189.7 ± 5.4	<0.05	3.00 ± .104	>0.50	21.76 ± 0.54	>0.50	65.208 ± 1.942	>0.50

Table 7. Changes in body, adrenal and pituitary weights of rats subjected to two levels of feed deprivation. Weights are expressed as mean values ± standard error of mean

Treatment	No. of animals	Body weight (g)	P value	Adrenal weight (mg)	P value	Pituitary weight (mg)	P value
Experiment One							
Control	5	358.3 ± 10.7		54.1 ± 4.1		11.04 ± 0.78	
Group I (47% deprived)	5	210.4 ± 2.1	<0.001*	45.9 ± 0.9	<0.10	7.10 ± 0.54	<0.01
Group II (67% deprived)	4	171.4 ± 3.7	<0.001*	41.8 ± 2.3	<0.05	7.03 ± 0.20	<0.01
			<0.001**		>0.50		>0.50
Experiment Two							
Control	7	378.7 ± 10.9		47.2 ± 1.9		12.07 ± 0.52	
Group I (47% deprived)	7	260.8 ± 10.0	<0.001*	52.7 ± 1.4	<0.05	9.31 ± 0.94	<0.05
Group II (67% deprived)	7	196.0 ± 8.56	<0.001*	41.9 ± 1.8	<0.1	8.48 ± 0.55	<0.001
			<0.001**		<0.001		>0.50
Group III (67% deprived) (pinealectomized)	5	185.5 ± 9.47	<0.001*	37.8 ± 3.8	<0.05	7.32 ± 0.98	< .001
			<0.001**		<0.01		<0.20
			<0.50[t]		<0.40		>0.50

* P value when compared to controls.

** P value when compared to group I (47% deprived animals).

t P value when compared to group II (67% deprived animals).

Table 8. Changes in testicular, prostate and seminal vesicle weights of rats subjected to two levels of feed deprivation. Weights are expressed as mean values ± standard error of mean

Treatment	Testicular weight (g)	P value	Prostate weight (mg)	P value	Seminal vesicle weight (mg)	P value
Experiment One						
Control	3.48 ± .06		422.1 ± 50.7		1,222.5 ± 85.0	
Group I (47% deprived)	3.05 ± .09	<0.001*	231.3 ± 35.4	<0.001	508.5 ± 69.8	<0.001
Group II (67% deprived)	2.76 ± .04	<0.001* <0.01**	135.4 ± 19.6	<0.001 <0.001	272.6 ± 31.2	<0.001 <0.001
Experiment Two						
Control	3.19 ± .09		455.4 ± 13.4		804.1 ± 82.2	
Group I (47% deprived)	3.0 ± .23	<0.40*	331.4 ± 25.6	<0.01	558.6 ± 33.1	<0.02
Group II (67% deprived)	2.59 ± .12	<0.01* <0.02**	146.8 ± 22.9	<0.001 <0.001	170.0 ± 27.5	<0.001 <0.001
Group III (67% deprived) (pinealectomized)	2.67 ± .18	<0.02* <0.30** >0.50[t]	156.2 ± 48.0	<0.001 <0.01 >0.50	193.4 ± 44.0	<0.001 <0.001 >0.50

* P value when compared to controls.

** P value when compared to group I (47% deprived animals).

t P value when compared to group II (67% deprived animals).

experiment two, which did not differ from the control animals. Pineal weights of the animals in experiment one (Table 9) were reduced in the 47% feed deprived animals (Group I) but not different from controls in the 67% feed deprived animals (Group II). HIOMT activity was not changed by feed deprivation when calculated on a per incubation basis (Table 9), but was increased in the 67% feed deprived animals when calculated on a per 100 grams of body weight basis.

Pineal weights of the animals in experiment two were not significantly changed by feed deprivation (Table 10). N-acetyltransferase activity was reduced in the 47% feed deprived animals but back approaching control levels in the 67% feed deprived animals when calculated on a per mg of tissue basis. When calculated on a per 100 grams of body weight basis the activity was significantly increased in the 67% feed deprived animals when compared with controls.

Testicular MAO activity was significantly reduced (Table 11) in the 47% feed deprived animals of experiment one, and back to control levels in the 67% feed deprived animals when calculated on either a per mg tissue or on a per animal basis. Testicular MAO activity of the experimental groups in experiment two showed the same trend but values calculated on a per mg of tissue basis were not significantly different from controls. When calculated on a per animal basis the 47% feed deprived animals and the 67% feed deprived pinealectomized animals in experiment two were significantly lower than controls.

Table 9. HIOMT activity of pineal glands and pineal gland weights from controls and feed deprived animals of experiment no. 1. Values are expressed as mean ± standard error of mean

Treatment	Pineal gland weight (mg)	P value	Counts/ 10 min/incubation $X10^3$	P value	Counts/10 min/ 100 g body weight $X10^3$	P value
Control	1.4 ± 0.2		18.4 ± 5.0		.51 ± .12	
Group I (47% deprived)	1.1 ± .09	<0.01*	18.9 ± 6.0	>0.50	.91 ± .29	<0.20
Group II (67% deprived)	14. ± .06	>0.50*	24.7 ± 7.8	>0.50	1.44 ± .45	<0.05
		<0.01[t]		>0.50		<0.40

* P value when compared with controls.

t P value when compared with Group I (47% deprived animals).

Table 10. N-Acetyltransferase activity of pineal glands and pineal gland weights from controls and feed deprived animals of experiment no. 2. Values are expressed as mean ± standard error of mean

Treatment	Pineal gland weight		Counts 10 min/incubation		Counts/10 min/ 100 g body weight	
	(mg)	P value	$X10^3$	P value	$X10^3$	P value
Control	1.84 ± 2.0		7.02 ± .49		1.85 ± .12	
Group I (47% deprived)	2.21 ± .15	<0.20*	4.39 ± .40	<0.01	1.71 ± .15	<0.50
Group II (67% deprived)	1.77 ± .18	>0.50*	6.13 ± .37	<0.20	3.17 ± .26	<0.001
		<0.20[t]		<0.02		<0.001

* P value when compared with controls.

t P value when compared with Group I (47% deprived animals).

Table 11. Testicular MAO activity of control and feed deprived animals. Values are expressed as mean ± standard error of mean

Treatment	Per mg tissue		Per animal basis	
	CPM	P value	CPM x 10^3	P value
Experiment No. 1				
Control	19.41 ± 3.17		678.4 ± 116.7	
Group I (47% deprived)	11.47 ± 1.52	<0.10*	348.4 ± 46.0	<0.05
Group II (67% deprived)	21.23 ± 3.00	>0.50*	586.8 ± 84.1	>0.50
		<0.05**		<0.05
Experiment No. 2				
Control	23,037 ± 1,726		73,214 ± 4,782	
Group I (47% deprived)	19,092 ± 1,472	<0.20*	56,092 ± 3,796	<0.02
Group II (67% deprived)	23,307 ± 1,215	>0.50*	60,939 ± 5,514	<0.20
		<0.20**		>0.50
Group III (67% deprived) (pinealectomized)	20,506 ± 1,484	<0.40*	55,665 ± 1,190	<0.05
		>0.50**		>0.50
		<0.40^t		<0.50

* P value when compared to controls.

** P value when compared to Group I animals (47% deprived).

t P value when compared to Group II animals (67% deprived).

Radiation

250 R whole-body

Testicular MAO activity as measured using whole testicular homogenates (Table 12) was higher in the irradiated animals than in control animals from 7-16 days after treatment and lower from day 19 to 31. Testicular weights significantly declined ($P < 0.05$) from day-7 to 52 (Figure 2). MAO activity of the seminiferous tubules (Figure 3) was depressed from 42-68 days after treatment. A marked increase in MAO activity was noted 80 days after irradiation concomitant with a significant increase in testicular weight and regeneration of the seminiferous tubules. MAO activity at 153 days after treatment was not significantly different between the two groups of animals. Tubular MAO activity of the control animals declined from 42 through 153 days after irradiation. This decline in MAO activity preceded the noticeable decline in testicular weight observed in the control animals after 152 days of age (68 days after treatment).

450 R whole-body, No. 1

Body weights were higher in the non-irradiated non-pinealectomized rats than any other group of animals (Table 13). There was no difference in body weights between any of the other groups when compared with each other. Pituitary weights were significantly decreased in the irradiated non-pinealectomized animals in the dark when compared with the non-irradiated non-pinealectomized animals, but not significantly different between any other groups. Irradiated

Table 12. Changes in rat homogenized whole testicular MAO activity at various time intervals after 250 R x-irradiation. Values are expressed as mean ± standard error of mean

Treatment	Days after irradiation	Mean testicular MAO activity C/10 min X 10^6	P value
Control	1	57.20	P > 0.50[1]
Irradiated	1	69.00	
Control	4	175.70	P > 0.50
Irradiated	4	156.50	
Control	7	158.80	
Irradiated	7	259.19	P < 0.05
Control	10	55.70	
Irradiated	10	160.64	
Control	13	62.62	
Irradiated	13	89.64	P < 0.10
Control	16	20.24	
Irradiated	16	40.90	
Control	19	50.78	
Irradiated	19	19.53	
Control	22	28.06	
Irradiated	22	8.71	
Control	25	15.71	P < 0.05
Irradiated	25	9.58	
Control	28	22.45	
Irradiated	28	11.94	
Control	31	39.05	
Irradiated	31	11.80	
Control	42	20.07	P > 50.0
Irradiated	42	17.92	

[1]Group mean comparison of irradiated animals compared to controls.

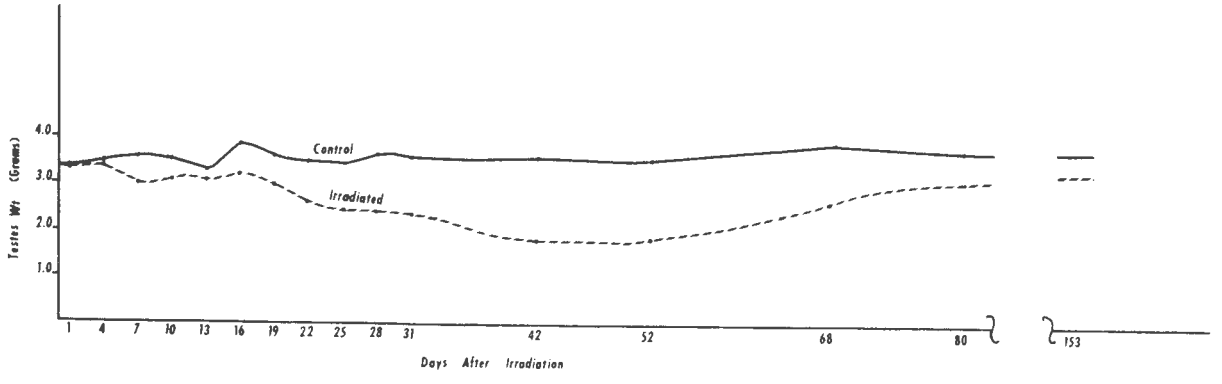

Figure 2. Testicular weights of animals irradiated with 250 R of whole-body x-irradiation and sham-irradiated controls. Each point represents the average of three samples. The solid line is the control values while the broken line is the irradiated animals.

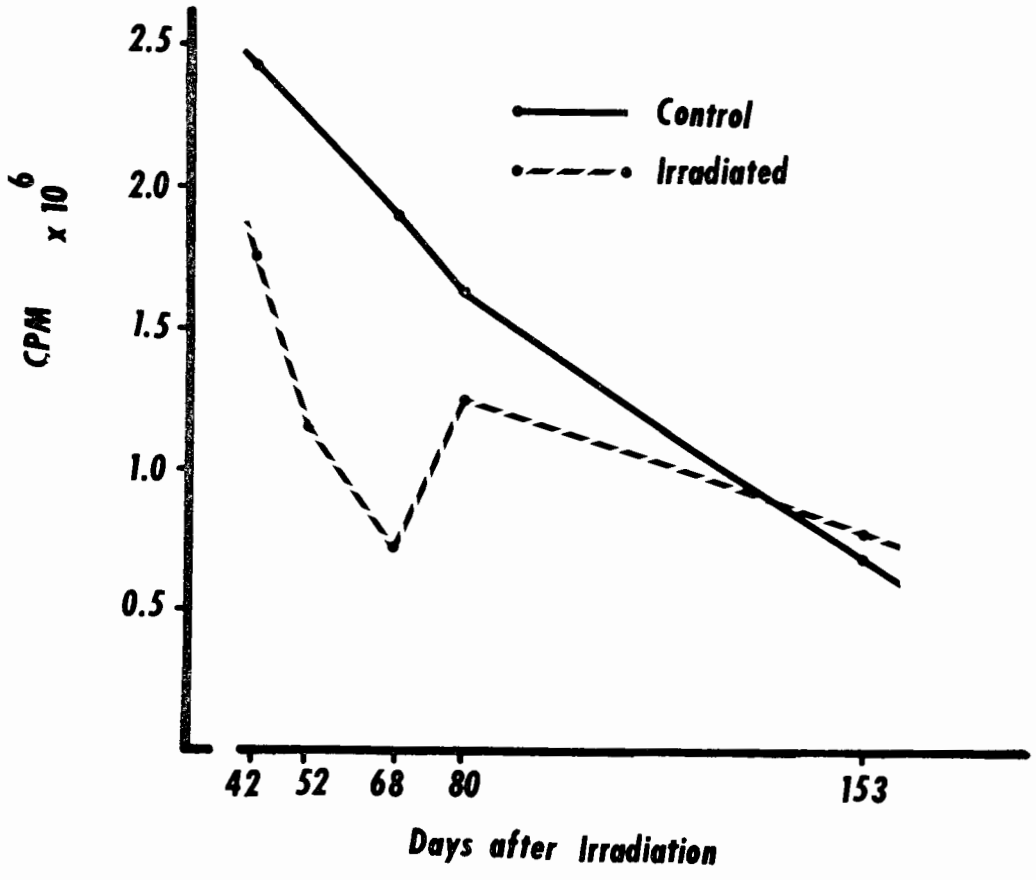

Figure 3. Testicular MAO activity of animals irradiated with 250 R of whole-body x-irradiation and sham-irradiated controls. Each point represents the average of three samples. The solid line is the control values while the broken line is the irradiated animals.

Table 13. Body, pituitary, adrenal, prostate and seminal vesicle weights of animals irradiated with 450 R of whole-body irradiation. Values are expressed as mean ± standard error of mean

Treatment	No. of animals	Body weight (g)	P value*	Pituitary (mg)	P value	Adrenal (mg)	P value	Prostate (mg)	P value	Seminal vesicle (mg)	*
Experiment No. 1											
Group I Non-irrad., non pinealectomized in light	7	408.6±4.3		11.1±0.6		49.5±3.5		503.0±37.4		1418±34	
Group II Non-irrad., pinealectomized in light	7	384.4±9.1	<0.05**	10.2±0.8		51.5±4.5		463.0±27.3		1352±67	
Group III Irrad., non-pinealectomized in light	6	388.5±7.9	<0.05**	9.9±1.3		51.1±3.7		404.0±35.0	<0.10t	1267±105	
Group IV Irrad., pinealectomized in light	7	386.5±8.9	<0.05**	9.0±1.5		43.1±1.7	<0.05tt	408.0±48.0		1276±73	
Group V Irrad., non-pinealectomized in dark	6	376.3±5.3	<0.01**	9.2±0.6	<0.05**	47.7±2.4		493.0±25.3		1393±68	
Group VI Irrad., pinealectomized in dark	7	370.4±8.5	<0.01**	9.4±0.9		49.3±1.8		471.0±53.6		1289±106	

* Only those P values that are < 0.10 are shown.

** P value when compared with Group I.

t P value when compared with Group I or Group V.

tt Value when compared with Group VI.

pinealectomized animals in the dark had significantly higher adrenal weights compared with irradiated pinealectomized animals in the light, but adrenal weights were not different between any other groups. Prostatic weights were lower in the irradiated non-pinealectomized animals in the light when when compared with either the non-irradiated non-pinealectomized animals in the light or irradiated pinealectomized animals in the dark. Prostatic weights did not differ between any of the other groups of animals. Seminal vesicle weights were not significantly different between any of the groups of animals. Testicular weights (Table 14) were significantly higher and testicular MAO activity significantly lower when calculated on a per mg of tissue basis, in the non-irradiated non-pinealectomized animals and the non-irradiated pinealectomized animals in the light when compared with all other groups of animals. Testicular MAO, when calculated on a per mg of tissue basis, was significantly higher than the irradiated non-pinealectomized animals in the dark when compared to the non-irradiated pinealectomized animals in the light. Testicular MAO activity, when calculated on a per animal basis was significantly lower in the irradiated non-pinealectomized, and the irradiated pinealectomized in the dark groups as compared with the non-irradiated pinealectomized animals in the light. Testicular MAO activity, on a per animal basis, was lower and approached significance in the irradiated non-pinealectomized animals in the light when compared to the irradiated pinealectomized, animals in the light.

Table 14. Testicular MAO activity and testicular weights of animals irradiated with 450 R of whole-body irradiation. Values are expressed as mean ± standard error of mean

Treatment	Testicular weight		Per mg tissue		Per animal basis	
	(g)	P value	CPM x 10^3	P value	CPM x 10^6	P value*
Experiment No. 1						
Group I Non-irrad. non-pinealectomized in light	3.67 ± .08		54.4 ± 2.4		200.5 ± 11.8	
Group II Non-irrad. pinealectomized in light	3.73 ± .04		56.7 ± 1.9		211.3 ± 7.0	
Group III Irrad., non-pinealectomized in light	2.77 ± .08	<0.001**	71.6 ± 1.6	<0.001**	198.3 ± 7.7	
Group IV Irrad. pinealectomized in light	2.65 ± .08	<0.001**	75.6 ± 2.3	<0.001**	200.0 ± 5.7	
Group V Irrad., non-pinealectomized in dark	2.62 ± .09	<0.001**	67.9 ± 2.0	<0.001** <0.05^{tt}	178.3 ± 10.5	<0.05^{t} <0.10^{tt}
Group VI Irrad. pinealectomized in dark	2.68 ± .05	<0.001**	69.8 ± 3.0	<0.001**	187.4 ± 9.5	<0.10^{t}

* Only those P values that are < 0.10 are shown.
** P value when compared with Group I or with Group II.
t P value when compared with Group II.
tt P value when compared with Group IV.

450 R whole-body or head only, No. 2

Treatment of animals with 450 R of whole-body irradiation, with subsequent sacrifice one-hour after irradiation, failed to alter MAO activity when compared to control animals (Table 15). Testicular MAO activity of the animals irradiated with head-only irradiation approached significance when compared with controls, but was not significantly different from the 450 R whole-body irradiated animals.

Table 15. Testicular MAO activity in animals sacrificed one-hour after 450 R of whole-body or head only irradiation. Values expressed are mean ± standard error of mean

Treatment	No. of animals	Body weight	Testicular weight	Per mg tissue basis	
				CPM x 10^3	P value
		(g)			
Control	6	299.37 ± 4.73	3.41 ± 0.20	58.18 ± 5.04	
Whole-body	6	302.37 ± 3.41	3.40 ± 0.11	53.68 ± 2.94	> 0.50*
Head-only	6	314.77 ± 2.29	3.41 ± 0.21	49.28 ± 2.32	< 0.10* > 0.50**

* P value when compared with controls
** P value when compared with whole-body group

450 R testes only, No. 3

Irradiation with 450 R to the testes (Table 16) significantly decreased body weights in the irradiated non-pinealectomized animals in the light, the irradiated

Table 16. Body, pituitary, adrenal, prostate, and seminal vesicle weights of animals irradiated with 450 R of testes only irradiation. Values are expressed as mean ± standard error of mean

Treatment	No. of animals	Body weight (g)	P value	Pituitary (mg)	P value	Adrenal (mg)	P value	Prostate (mg)	P value	Seminal vesicle (mg) *
Experiment No. 2										
Group I Non-irrad., non pinealectomized in light	7	419.3±6.8		8.7±2.3		47.7±2.6		450.7±27.2		1475.4± 104.0
Group II Non-irrad., pinealectomized in light	4	433.9±1.7		11.8±1.1		82.0±28.5		559.8±62.2		1512.8± 117.3
Group III Irrad., non-pinealectomized in light	6	416.1±5.5	<0.05**	9.7±1.9		50.3±2.6		578.8±63.2	<0.10t	1458.8± 28.8
Group IV Irrad., pinealectomized in light	6	414.0±6.6	<0.05**	6.3±2.0	<0.02**	50.3±4.3		554.8±58.1		1504.0± 93.9
Group V Irrad., non-pinealectomized in dark	6	410.7±11.9		10.2±1.9		46.7±6.6		511.5±48.4		1446.0± 107.8
Group VI Irrad., pinealectomized in dark	6	397.2±11.5	<0.05**	11.6±2.4		54.7±2.3	<0.01	523.2±38.6		1528.7± 65.0

* Only those P values that are < 0.10 are shown.

** P value when compared to Group II.

t P value when compared to Group I.

pinealectomized animals in the light and the irradiated pinealectomized animals in the dark when compared with the non-irradiated pinealectomized animals in the light. Pituitary weights in the irradiated pinealectomized animals in the light were significantly lower than the non-irradiated pinealectomized animals in the light. Adrenal weights of the irradiated pinealectomized animals in the dark were lower and approached significance when compared with the non-irradiated, non-pinealectomized animals in the light. Prostatic weights were higher and approached significance in the irradiated, non-pinealectomized animals in the light when compared to the non-irradiated, non-pinealectomized animals in the light. Seminal vesicles were not significantly different between any of the groups. Testicular weights (Table 17) were significantly reduced by irradiation in all irradiated groups when compared with non-irradiated, non-pinealectomized animals in the light and approached significance when compared with the non-irradiated, pinealectomized animals in the light. Testicular MAO activity when calculated on per mg of tissue basis was significantly higher in all irradiated groups when compared to the non-irradiated, non-pinealectomized animals in the light. Testicular MAO activity on a per mg of tissue basis, was significantly higher or approaching significance in the irradiated pinealectomized animals in the dark when compared with the other irradiated groups. When testicular MAO activity was calculated on a per animal basis the irradiated, pinealectomized animals in the dark had a significantly higher activity when compared with all other groups of animals.

Table 17. Testicular MAO activity and testicular weights of animals irradiated with 450 R of testes only irradiation. Values are expressed as mean ± standard error of mean

Treatment	Testicular weight (g)	P value	Per mg tissue CPM x 10^3	P value	Per animal basis CPM x 10^6	P value*
Experiment No. 2						
Group I Non-irrad. non-pinealectomized in light	3.70 ± 0.10		50.4 ± 3.8		188.3 ± 18.2	$<0.05^{tt}$
Group II Non-irrad., pinealectomized in light	3.57 ± 0.16		68.5 ±18.7		241.6 ± 62.9	
Group III Irrad., non-pinealectomized in light	2.69 ± 0.13	$<0.001^{**}$ 0.01^{t}	73.2 ± 3.5	$<0.01^{**}$ 0.10^{tt}	195.8 ± 9.7	$<0.05^{tt}$
Group IV Irrad., pinealectomized in light	2.85 ± 0.07	$<0.001^{**}$ 0.01^{t}	72.6 ± 4.5	$<0.01^{**}$ 0.10^{tt}	207.3 ± 16.4	$<0.10^{tt}$
Group V Irrad., non-pinealectomized in dark	2.75 ± 0.06	$<0.001^{**t}$	68.4 ± 5.8	$<0.05^{**tt}$	187.4 ± 14.1	$<0.05^{tt}$
Group VI Irrad., pinealectomized in dark	2.87 ± 0.05	$<0.001^{**}$ $<0.01^{t}$	93.7 ± 9.1	$<0.01^{**}$	271.5 ± 28.6	

* Only those P values that are < 0.10 are shown.
** P value compared with Group I.
t P value when compared with Group II.
tt P value when compared with Group VI.

Alterations in Photoperiod

Experiment No. 1

Ten days of constant light had no effect on body or testes weights when compared to animals in natural light (Table 18), but significantly increased testicular MAO activity, calculated on a per mg of tissue basis, and on a per animal basis. Constant darkness significantly decreased testicular MAO activity when compared with both the constant light and natural light animals and when calculated on a per mg of tissue or per animal basis, but failed to change body or testicular weights.

Experiment No. 2

In this experiment (Table 18), constant light increased body weight when compared with the animals in 12 hours light:12 hours dark. Constant light also increased testicular weights when compared with animals in 12 hours light:12 hours dark or animals in constant dark. Testicular MAO activity did not change on a per mg of tissue basis in any of the groups. It was, however, significantly different when calculated on a per animal basis and when the constant light animals were compared with the animals in constant dark. Testicular MAO activity approached significance when the constant light animals were compared with the animals in 12 hours light:12 hours dark.

Table 18. Testicular MAO activity and testicular weights of rats after alterations in photoperiod. Values expressed are mean activity ± standard error of mean

Treatment	No. of animals	Body weight (g)	P value	Testicular wgt. (g)	P value	Per mg tissue CPM x 10^3	P value	Per animal basis CPM x 10^6	P value
Experiment No. 1									
Constant light	8	316.3 ± 9.58	>0.50*	3.57 ± .07	>0.50	69.04 ± 2.62	<0.01	246.04 ± 7.72	<0.01
Natural light	7	325.21 ± 6.86		3.52 ± .08		54.34 ± 3.15		191.80 ± 14.84	
Constant darkness	8	320.16 ± 6.29	>0.50*	3.64 ± .07	<0.3	36.25 ± 1.83	<0.001	131.63 ± 12.66	<0.01
			>0.50**		>0.50		<0.001		<0.001
Experiment No. 2									
Constant light	7	159.0 ± 3.31	<0.01*	1.38 ± .04	<0.05	30.03 ± 2.33	>0.50	40.94 ± 2.20	<0.10
12 hrs light: 12 hrs dark	7	145.3 ± 3.77		1.16 ± .07		32.38 ± 2.33		34.72 ± 2.45	
Constant darkness	7	156.8 ± 5.58	<0.01*	1.11 ± .07	>0.50	30.47 ± 1.08	>0.50	31.42 ± 1.77	<0.40
			>0.50**		<0.01		>0.50		<0.01

* P value when compared with natural light (Experiment No. 1) or 12 hrs light: 12 hrs dark (Experiment No. 2) animals.

** P value when compared with the constant light animals.

Pinealectomy

Testicular MAO was significantly increased on a per animal basis by pinealectomy and approached significance on a per mg of tissue basis when compared with controls (Table 19). There was no difference in testicular weights between the control and pinealectomized animals.

Pargyline Injections

Injections of 15 mg/kg of pargyline significantly decreased MAO activity when compared with controls, and when calculated on a per mg of tissue basis (Table 20). Endogenous serotonin concentrations were significantly increased (Table 20), but there was no change in testicular weights.

Circadian Rhythm

There was no difference in testicular weight or testicular MAO activity with animals killed between 11:30 p.m. and 12:00 a.m. when compared with those killed between 11:30 a.m. and 12:00 p.m. (Table 21).

Seasonality

Sparrow

Testicular weights of the house sparrow (Figure 4) increased from December through April and decreased from July through November. Testicular

Table 19. Testicular weights and MAO activity of control and pinealectomized animals. Values are expressed as mean ± standard error of mean

Treatment	No. of animals	Testicular weight (g)	P value	Per mg tissue CPM x 10^3	P value	Per animal basis CPM x 10^6	P value
Control	6	980 ± 75		31.96 ± .99		30.45 ± 2.88	
Pinealectomized	7	1070 ± 48	< 0.40	35.57 ± 1.36	< 0.10	36.78 ± 1.68	< 0.001

Table 20. Changes in testicular MAO activity and endogenous serotonin concentrations after pargyline injections. Values are mean ± standard error of mean.

Treatment	No. of animals	Testicular weight (g)	P value	Per mg tissue CPM x 10^3	P value	Nanograms 5HT Per g tissue	P value
Control	5	3.28 ± .14		45.8 ± 2.3		255.8 ± 9.1	
Treated (15 mg/kg)	5	3.19 ± .14	>0.50*	12.4 ± 0.8	<0.001	425.2 ± 70.5	<0.05

* P value when compared with controls.

Table 21. Testicular MAO activity in animals sacrificed between 11:30 p.m. and 12:00 a.m. (Group I) and between 11:30 a.m. and 12:00 p.m. (Group II). Values are expressed as mean ± standard error of mean

Treatment	No. of animals	Body weight	Testicular weight	Per mg tissue basis	
				CPM x 10^3	P value
		(g)	(g)		
Group I	6	172.0 ± 3.8	1.80 ± .09	47.69 ± 1.63	
Group II	6	161.1 ± 3.5	1.92 ± .11	48.16 ± 2.82	> 0.50

MAO activity (Figure 4), calculated on a per animal basis, increased from December to April but decreased from April to November. Testicular MAO activity (Figure 4), when calculated on a per mg of tissue basis, increased from November to March, but decreased from April through November. Increases in MAO activity from November to January preceded increases in testicular weight.

Squirrel

Squirrels trapped in May (Table 22) had significantly higher body weights, but lower testicular weights and testicular MAO activity (expressed on a per mg of tissue basis) when compared to animals trapped in April.

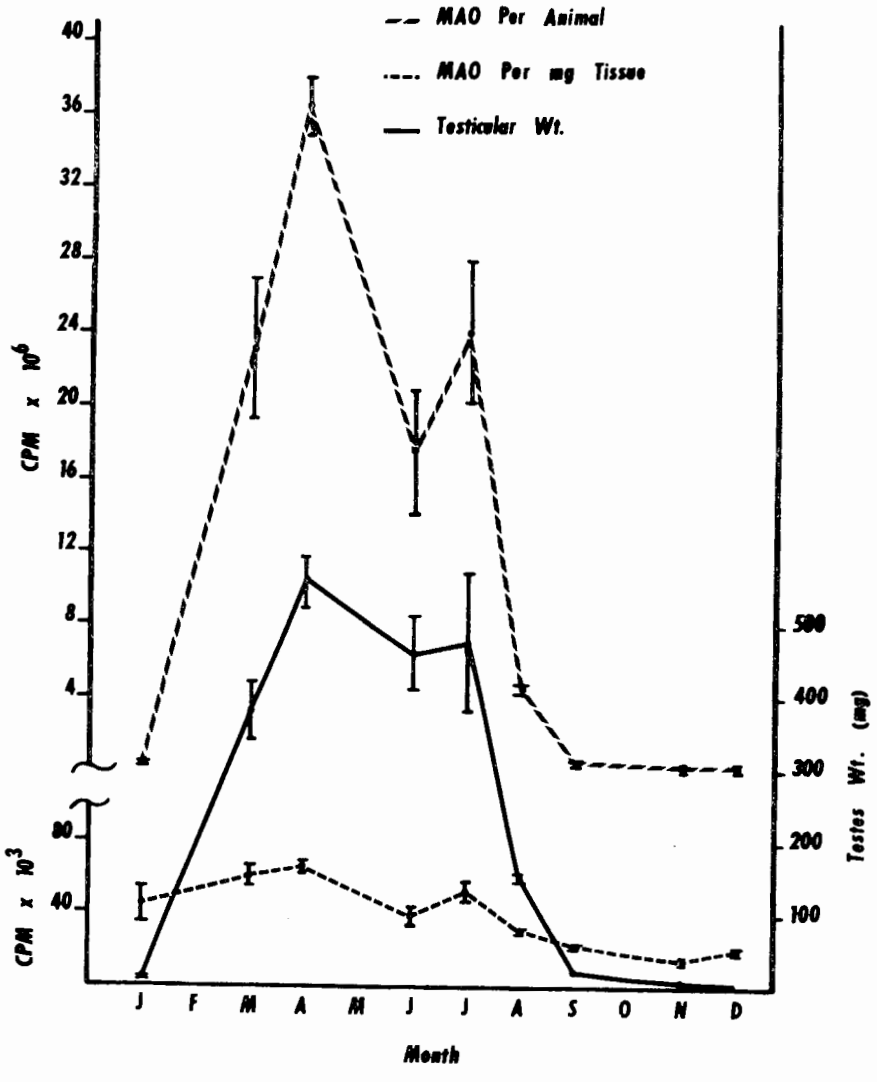

Figure 4. Testicular MAO activity and testicular weights of English house sparrows trapped from January 1971 through December 1972. Each point represents the mean. Vertical lines are the standard error of mean.

Table 22. Squirrel testicular MAO activity from animals trapped on April 19, 1972 (Group I) and animals trapped on May 25, 1972 (Group II). Values expressed are mean ± standard error of mean

Treatment	No. of animals	Body weight (g)	P value	Testicular weight (mg)	P value	Per mg tissue CPM x 10^3	P value
Group I	5	258.2 ± 13.4		968 ± 73		26.14 ± 1.51	
Group II	5	303.9 ± 5.7	<0.01	368 ± 25	<0.001	7.85 ± 0.36	<0.001

Localization of Testicular MAO

In the first animal MAO activity of the seminiferous tubules approached significance when compared with the whole tissue homogenates (Table 23). In the second animal MAO activity was not different between the seminiferous tubules or whole tissue homogenate. MAO activity of the frozen seminiferous tubules was lower than any of the other samples in animal one or animal two. In the second experiment (Table 23) MAO activity was significantly increased in the whole tissue homogenate when compared with the seminiferous tubule homogenate. In the third experiment (Table 23) there was no difference in MAO activity between whole tissue or seminiferous tubule homogenates. In experiment number four (Table 23) MAO activity was significantly higher in the whole tissue homogenate samples when compared to the seminiferous tubule homogenates.

Activity in experiment five (Table 23) was significantly lower in interstitial cells when compared to seminiferous tubules. In the sixth experiment (Table 23) activity was lower in the tubule walls, tubule interior or interstitial cell homogenates when compared to the seminiferous tubule homogenates. Activity was lower in the tubule interior than in the tubule-wall and lower in the interstitial cell homogenates as compared with the tubule interiors. Activity of the tubule-walls plus the tubule-interiors did not sum to the whole-tubule homogenates because of numerous freezing and rethawing during the collection procedure.

Table 23. Testicular MAO activity in various locations of testes in animals of different ages. Each group represents five samples. Values expressed are mean ± standard error of mean

Treatment	Body weight (g)	No. of animals	Testes weight (g)	Per mg tissue CPM x 10^3	P value
Experiment No. 1					
Animal One	323.5	1	3.26		
Whole tissue homogenate				27.87 ± 2.01	
Seminiferous tubule homogenate				33.82 ± 2.24	<0.1
Animal Two	514.8	1	3.50		
Whole tissue homogenate				30.83 ± 2.62	
Seminiferous tubule homogenate				32.43 ± 1.84	>0.50
Frozen seminiferous tubules				24.88 ± 1.01	<0.05*
Experiment No. 2	529.6	1	3.50		
Whole tissue homogenate				70.80 ± 2.02	
Seminiferous tubule homogenate				50.05 ± 1.32	<0.01
Experiment No. 3	477.3 ± 11.3	5	3.47 ± .09		
Whole tissue homogenate				26.11 ± 1.95	
Seminiferous tubule homogenate				27.69 ± 2.44	>0.50
Experiment No. 4	667.2 ± 52.5	5	3.63 ± 0.12		
Whole tissue homogenate				28.82 ± 0.60	
Seminiferous tubule homogenate				18.93 ± 0.39	<0.001
Experiment No. 5					
Seminiferous tubule homogenate				19.34 ± 0.61	
Interstitial-cell homogenate				16.42 ± 0.72	<0.02
Experiment No. 6					
Seminiferous tubule homogenate				9.17 ± 0.44	
Tubule-wall homogenate				0.77 ± 0.052	<0.001*
Tubule-interior homogenate				0.45 ± 0.022	<0.001*
Interstitial-cell homogenate				0.34 ± 0.017	<0.001*

*P value when compared with all other groups in the same experiment.

Tissue Culture

FSH effects

Addition of 2 IU or 5 IU of FSH (Table 24) to seminiferous tubules in tissue culture significantly increased MAO activity when compared to controls. There was no difference, however, between the 2 IU of FSH samples when compared with the 5 IU of FSH samples.

Pargyline effects

Pargyline injections decreased seminiferous tubule MAO activity 24 hours after injection (Figure 5), when compared with control animals. Activity remained the same in the controls after 1 day of tissue culture. Activity then sharply dropped between 1 and 2 days after initiation of the cultures and continued to decline through 6 days of culture. The activity in the controls increased from day-6 to day-9. Activity in pargyline treated tubules decreased through the first day of culture, remained the same from day-1 to day-2, and then declined through day-4. Activity was then constant through day-9. In each case MAO activity was lower in the pargyline treated animals than in the controls. The pargyline animals did not show the increase in activity from day-6 to day-9 as was noted in the controls.

Table 24. Testicular MAO activity in seminiferous tubules after addition of FSH to tissue cultures. Activities are mean ± standard error of mean

Treatment	No. of samples	Per mg tissue	
		CPM x 10^3	P value
Control	6	3.07 ± 0.29	
FSH, 2 IU	6	4.55 ± 0.11	< 0.05*
FSH, 5 IU	6	4.56 ± 0.06	< 0.05*

* P value when compared with controls

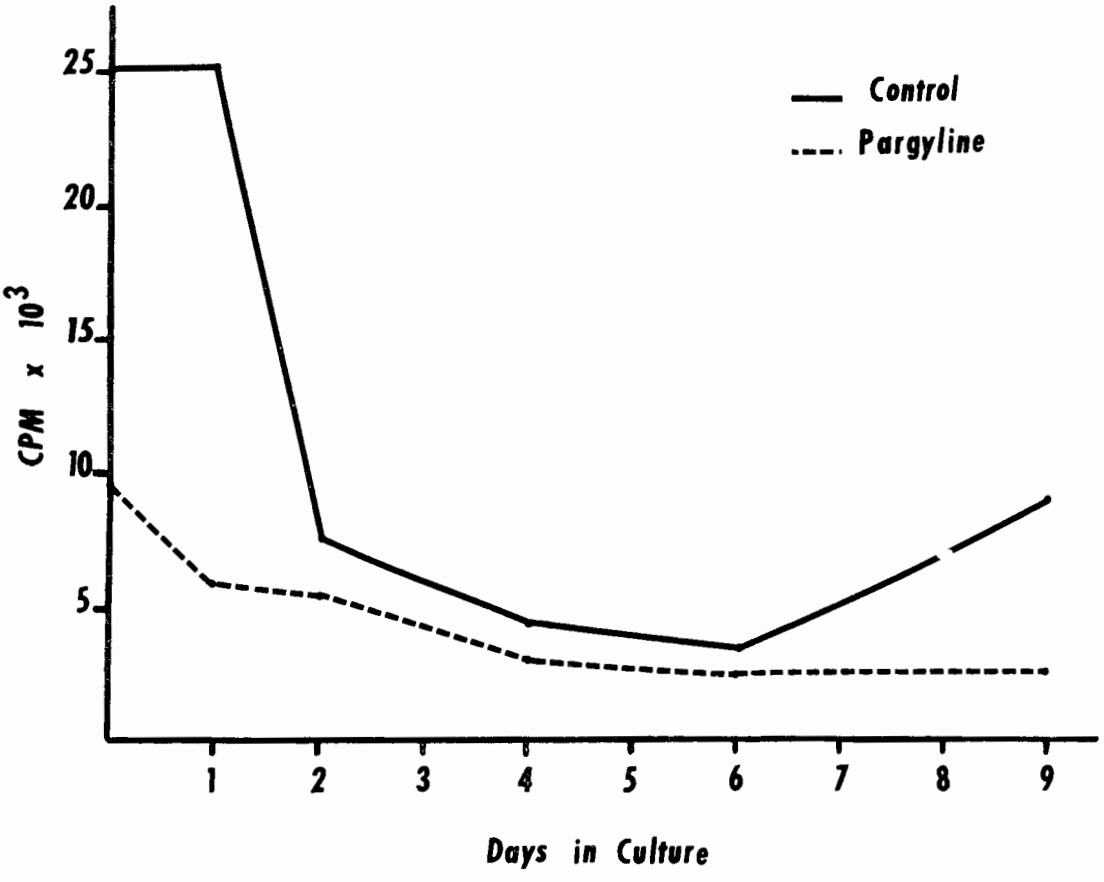

Figure 5. MAO activity of seminiferous tubules in tissue culture from an animal treated with pargyline and a control animal. Each point represents the average of two samples.

DISCUSSION

The increase in testicular MAO activity with age through sexual maturity agrees with findings by Karki, Kuntzman and Brodie (1962) who found that brain MAO activity also increased from the newborn period to the adult period. Horita and Lowe (1972a) have found increases in heart and liver MAO activity from 2 through 130 weeks of age. Robinson et al. (1972) reported that human brain MAO activity follows this same trend and gradually increases with age. Testicular MAO activity appears to be different from any other tissues studied in the fact that it decreases with advancing age. In this respect decreases in MAO activity preceded a decline in testicular weight in the animals with an average body weight of 649.6 grams. Ellis et al. (1972) have observed that changes in testicular MAO activity are also positively correlated with changes in androgen synthesis. Total androgen synthesis (testosterone plus androstenedione fractions) increased from 30 days of age through 90 days of age and then declined in 410 day old animals. Ellis (1970) has shown that serotonin inhibits androgen synthesis in vitro, and since the primary route of serotonin metabolism is through MAO, any change in testicular MAO with age could alter endogenous testicular serotonin concentrations. This, in turn, could change androgen synthesis patterns. In this respect, it was observed in these investigations that inhibition of MAO activity by pargyline increased endogenous serotonin concentrations. Thus the senescence occurring in the male gonad with respect to androgen synthesis and testicular weight could

be related to alterations in MAO activity and subsequent changes in the endogenous concentrations of serotonin. Histochemical studies by Penttila and Kormano (1968) suggest that the bulk of testicular MAO is located in both the interstitium and the walls of the seminiferous tubules. Radiometric studies in these investigations verify this conclusion as the bulk of the activity was found in the seminiferous tubule homogenates. Tubule-walls contained about twice the activity of tubule interiors and over twice the activity of the interstitial cells. This suggests that a protective mechanism may exist in the testis whereby the MAO localized in the tubule walls might prevent serotonin from normally penetrating into the lumen of the tubules where it could have detrimental effects on the germinal elements. The increase in MAO activity, per mg of tissue, noted in these investigations after 250 R of whole-body irradiation, and after hypophysectomy of 3 and 10 week animals, concomitant with a decrease in testicular weight and loss of germinal cells further indicates that the enzyme is not located primarily in the germinal cells. It was also noted in these investigations that MAO activity was not different in seminiferous tubule homogenates as compared to whole testes homogenates in younger animals weighing below 514 grams. In older animals over 530 grams, MAO activity was significantly higher in the whole testicular homogenates as compared to the seminiferous tubular homogenates. This indicates that the ratio of activity in the seminiferous tubules as compared to the interstitial cells changes with age and shifts to the interstitial cells with age as the total MAO activity is decreasing. This change in the MAO ratio, concomitant with an overall decrease in MAO with age, appears to be due

to a more rapid loss of activity in the seminiferous tubules than in the interstitial cells. This suggests that the tubules in older animals may be more susceptible to serotonin induced damage than those in younger animals. This conclusion is corroborated by the findings of Liu and Kinson (1973) who reported that serotinin implants caused histological alterations in the seminiferous tubules and a diminished rate of spermatogenesis in mature animals, but not in immature animals.

The overall decrease in total MAO activity noted after hypophysectomy shows that testicular MAO activity is dependent on hypophyseal hormones in both immature and mature animals. The increase in total MAO activity by FSH in hypophysectomized animals indicates that FSH may be the primary hypophyseal factor responsible for maintaining testicular MAO activity. The increase in activity after addition of FSH to tissue cultures supports this conclusion. Studies by Anton-Tay, Pelham, and Wurtman (1969) indicate that FSH administration increased the turnover of rat brain norepinephrine and they suggested that this effect was on the synthesis of norepinephrine rather than on the turnover. The data of these experiments suggest a direct effect of FSH on testicular MAO activity. Total testicular MAO activity was also somewhat increased after LH administration, while hCG, and a combination of FSH, LH and prolactin actually decreased MAO activity on a per mg of tissue basis. Urry, Frehn and Ellis (1973) found that hCG and a combination of FSH, LH and prolactin increased androgen synthesis and the data suggest that androgens may decrease testicular MAO activity.

Eiduson (1972a) has also reported that testosterone can alter the nature of the MAO enzyme. These data appear to verify this conclusion.

The increase in testicular MAO activity in the mature animals noted 6 weeks after hypophysectomy may indicate that some other factor in addition to the hypophyseal hormones could regulate testicular MAO activity.

The increases in testicular MAO activity after melatonin administration both in vivo and in vitro are opposite to the effects of melatonin on brain MAO. Urry and Ellis (1973) reported that melatonin decreased MAO activity in the hypothalamus, pituitary and pineal. This indicates that the enzyme in the testes differs from that in the brain and gives additional biochemical evidence for multiple forms of MAO. The rise in testicular MAO activity with melatonin, as noted in these studies, also is among some of the first evidence for a direct effect of melatonin on the testes. Pinealectomy increased testicular MAO activity in these investigations. Melatonin has been reported by Fraschini, Mess and Martini (1968) and by Kamberi, Mical and Porter (1970) to inhibit LH release. Debeljuk, Feder and Paulacci (1970) and Kamberi, Mical and Porter (1971) have reported that melatonin also inhibits FSH release. Moreover, Motta, Fraschini and Martini (1967) found that pinealectomy increases FSH synthesis and release. Thus the increase in testicular MAO activity after pinealectomy might best be attributed to an increase in FSH. The rise in MAO activity observed after placing animals in constant light and the decrease in activity with the animals in constant dark might also be attributed to alterations in melatonin

which in turn change gonadotropin concentrations and thus could alter testicular MAO activity.

The reduction in testicular MAO activity by partial feed deprivation (47% reduction in intake) indicates that moderate restrictions of feed intake reduces the capability of the testes to inactivate serotonin. In this respect, Mulins and Pomerantz (1941) and Mann and Rawson (1957) have observed degenerative changes in male gonads after underfeeding that could be corrected by exogenous gonadotropins. In this regard, Leathem (1963) has shown that feed restriction is the same as pseudohypophysectomy and serves to reduce gonadotropin levels. Since FSH serves to regulate testicular MAO, it is probable that the decrease in MAO activity with feed deprivation was due to a decrease in FSH secretion. Negro-Vilar, Dickerman and Meites (1971) have recently reported that starvation decreases pituitary weights, hypothalamic FSH-RF and pituitary FSH levels. The decrease in pituitary weights noted in the experiment corroborate the conclusion that gonadotropin secretion is diminished as a result of feed deprivation. The elevated testicular MAO activity observed for animals in the 67% feed deprived group of the first starvation experiment, when compared with the 47% feed deprived animals corresponded with an increase in HIOMT activity for these animals. Pineal weights were also increased by 67% feed deprivation when compared to 47% feed deprivation. The increase in HIOMT activity and pineal weights observed in the 67% feed deprived animals suggests that these animals had a higher rate of melatonin synthesis than did the 47% feed deprived animals. This is corroborated by an increase in N-acetyl transferase observed in the second starvation experiment (67% feed deprivation compared with the 47%

feed deprivation). Thus, it is likely that the decrease in MAO activity in the 47% feed deprived animals is due to a decrease in FSH synthesis while the increase in the 67% feed deprived animals can be attributed to the increase in melatonin synthesis.

The increase in testicular MAO activity, after 250 R or whole-body irratiation, noted from 7 through 16 days after irradiation agrees with findings of Renson and Fischer (1959) and Erschoff and Gal (1961) who have observed increased 5-HIAA in the urine of rats one week after irradiation. Ellis and van Kampen (1971) have reported similar increases in melatonin from 4-19 days after irradiation. Thus, it is possible that the increase in testicular MAO activity after irradiation is due to the increase in melatonin synthesis. This offers additional evidence supporting an in vivo physiological role of melatonin in altering enzyme activities in the male gonad.

The decline in MAO activity noted for the seminiferous tubules of the control animals from 4 through 153 days after treatment (250 R whole-body irradiation) is consistent with the decline in MAO activity of rat testes associated with senescence of this organ. The increase in MAO activity of the treated animals noted on day 80 and the return of this activity to control levels is thought to be due to the repair process and repopulation of the germinal epithelium that was evidenced by an increase in testicular weight.

Treatment with 450 R of head-only irradiation, with sacrifice one-hour later, resulted in a lower testicular MAO activity that approached significance when compared with controls. Its possible that this decrease is due to epinephrine release since Urry and Ellis (unpublished observations) have observed

that epinephrine injections also decrease testicular MAO activity and Frehn et al. (1973) have reported that crowding stress decreases testicular MAO activity in Uinta ground squirrels. It is possible, of course, that other factors are involved which decrease MAO activity after head-only irradiation.

Irradiation of animals with 450 R of whole-body irradiation, with sacrifice 21-days later, decreased testicular weights with concomitant increases in testicular MAO activity when calculated on a per mg of tissue basis. These results are similar to the increase in MAO activity noted after hypophysectomy and are due to a shrinkage of the tissue which concentrates the enzyme and gives higher activities when expressed on a per mg of tissue basis. This further indicates that the bulk of the activity does not reside in the germinal elements, which are subsequently lost after irradiation or hypophysectomy.

Treatment of the testes directly with 450 R irradiation also increased testicular MAO activity when calculated on a per mg of tissue basis. The higher activity noted in the irradiated pinealectomized animals in the dark when compared to all other groups could be attributed to an increase in FSH since, in addition to melatonin, it was the only compound observed to increase MAO activity in these investigations. It is possible, however, that other unknown factors are involved that serve to increase MAO activity; a conclusion which is supported by the increase in testicular MAO activity in mature hypophysectomized animals from 4 to 6 weeks after surgery. What this factor might be is not clear at this time. It is known that a lack of ACTH with a resulting decrease in

adrenalcortical hormones can increase MAO activity and this may explain the increase in MAO activity. In this respect, Avakian and Collingham (1968) have reported increases in rat heart MAO activity after adrenalectomy. In addition, Parvez and Parvez (1973a) have reported that hypophysectomy increases MAO activity in adrenals, kidneys, paraganglia, lungs, liver and heart. They found that administration of glucocorticoids blocked this increase in MAO activity. Adrenalectomy, in our investigations, did not alter testicular MAO activity ten days after surgery. This could indicate that glucocorticoids may not regulate testicular MAO activity although the data is still inconclusive on this point. Estrogen injections did not alter testicular MAO and Urry and Ellis (unpublished observations) have observed that estrogen does not alter hypothalamic MAO activity, although it does increase MAO activity in the pineal gland. Reports by Klaiber et al. (1972) indicated that estrogen reduces MAO activity in the plasma of women and Southgate et al. (1968) have found similar patterns in uterine tissue. Estrogen has also been observed by Kobayashi et al. (1966) and Kamberi and Kobayashi (1970) to decrease female hypothalamic MAO activity. It appears from these investigations that the male does not respond the same to estrogen as the female and that estrogen either has no effect or actually can increase MAO activity in the male.

Okada (1971) has demonstrated a circadian rhythm of serotonin in the rat brain. The onset of the circadian rhythm was at 35 to 37 days of age. In these investigations, there was no observable circadian rhythm in testicular MAO activity between animals killed at 11:30 a.m. to 12:00 p.m. and those killed at

11:30 p.m. and 12:00 a.m. Other studies by Urry and Ellis (unpublished observations) have also failed to find variations in hypothalamic or pituitary MAO activity in animals killed at noon or midnight. Its possible that, although the animals showed no difference in MAO activity at the two time intervals measured, there could still be a circadian rhythm that was not evident in these studies. Activities could have been the same at these two time intervals measured, but varied at other times. More rats sacrificed at shorter time intervals would be required to verify this point. It is also possible that MAO does not undergo a circadian rhythm and that the serotonin rhythm is caused by the enzymes responsible for its synthesis.

Although MAO did not show a circadian rhythm it did show a seasonal variation in two seasonal breeders; the house sparrow and Uinta ground squirrel. In the sparrow the MAO activity was highest at the peak of reproductive activity and testicular development (weights) and it declined sharply as the animals went out of the breeding season and testicular weight declined. Its important to note that a large increase in MAO activity from December to January preceded a large increase in testicular weight from January to February. The increase in MAO activity may be a necessary factor for the subsequent increase in testicular weight. It might also be a mere reflection of other factors such as gonadotropin release that serves to alter testicular MAO activity and testicular development. It is evident, however, that the seasonal factor involved probably regulates the synthesis and degradation of the enzyme. It is probable that FSH is important in regulating MAO activity in the sparrow as it also appears to be in the rat. The squirrel also

showed seasonal variations and a large decrease in testicular MAO activity in a short 1 month period. The rapid decline in activity in the squirrel probably indicates a decline in FSH levels as HIOMT activity has been shown to increase in this species during this time interval (Ellis and Balph--unpublished data). The low MAO levels may increase endogenous biogenic amine levels which could have a detrimental effect on the testes and serve to rapidly decrease testicular weights. Cockett et al. (1971) have shown dramatic decreases in primate testicular weights in as little as 9 days after orbital space flight. They have suggested that serotonin may be involved in this rapid decline and its evident from the above data that a similar mechanism might be involved in the house sparrow and ground squirrel. The factors involved could decrease testicular MAO thus increasing serotonin, thereby decreasing testicular weights.

Further detrimental effects of serotonin on testicular function are suggested by the experiment where pargyline decreased MAO activity and prevented its subsequent regeneration in tissue culture. The seminiferous tubules apparently were damaged in some way and not able to grow in culture whereas the control samples without pargyline grew satisfactorily in culture with the MAO regenerating after 6 days. The detrimental effects might best be attributed to serotonin and its effects on the testes. These studies have shown additional evidence that any factors which tend to decrease testicular MAO are detrimental to the testes. Those factors increasing MAO are usually, but not always, of benefit to testicular function.

CONCLUSIONS

1. It is evident from these studies that rat testicular MAO activity varied with age and was positively correlated to testicular development and function. It appeared to be different from all other tissues studied to date in the fact that it declined with senescence.

2. Testicular MAO appeared to be regulated by hypophyseal factors. The primary factor from the hypophysis was FSH which increased testicular MAO activity.

3. Androgens may decrease testicular MAO activity and could be another factor in vivo which regulates the enzyme in the testes.

4. The bulk of the testicular MAO activity was localized in the walls of the seminiferous tubules where it may serve as a protective barrier for the germinal cells against fluctuations in serotonin levels. The ratio of MAO activity in the seminiferous tubules and whole tissue with changes in age indicate that activity was lost faster in the tubules than in the interstitial cells. This could mean that spermatogenic elements in older animals were more susceptible to serotonin induced damage than were those in younger animals.

5. Melatonin increases testicular MAO activity both in vitro and in vivo. This is among the first evidence for direct in vivo effects of melatonin on testicular enzymes.

6. Irradiation increased testicular MAO activity on a per mg of tissue basis and decreased overall MAO activity as calculated on a per animal basis. Overall activity directly corresponded to testicular weight.

7. Adrenalectomy had no effect on testicular MAO activity which suggests that glucocorticoids do not regulate testicular MAO which differs from other tissues studied to date.

8. Estrogen does not effect testicular MAO activity.

9. Rat testicular MAO did not appear to undergo a circadian rhythm in its activity.

10. Seasonal breeders had dramatic fluctuations in testicular MAO activity which were directly correlated with testicular development and reproductive activity. These fluctuations may be important in promoting the large alterations in gonadal weight observed within a very short time period.

11. Pargyline treatment inhibited seminiferous tubule growth in tissue culture, possibly by raising inter-tubular serotonin concentrations high enough to permanently damage the tubules.

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