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THE INFLUENCE OF AGE AND ADRENALECTOMY ON RAT HEART MONOAMINE OXIDASE

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Abstract—The influence of age and adrenalectomy of the animal on the activity of rat heart monoamine oxidase (MAO) was investigated. The model describing changes in tissue contents of an enzyme as a transition from one steady state to another was used to determine whether age and adrenalectomy act on the synthesis or degradation of MAO. Rats were treated with the irreversible MAO inhibitor pargyline to measure the rate of recovery of the enzyme activity following almost total inactivation in order to obtain values for the first order degradation rate constants. The sp. act. of the rat heart MAO increased with age due to a reduction in the degradation rate constant without any effect on the rate of synthesis. Adrenalectomy led to an increase in the sp. act. through an effect on the rate of synthesis unaccompanied by any change in the degradation rate constant. Treatment with pargyline at operation prevented the rise in MAO activity that normally followed adrenalectomy. The possible mechanisms involved and the presence of enzyme-degrading systems are discussed.

Over the past few years it has become apparent that the activity of the enzyme monoamine oxidase [Amine: oxygen oxidoreductase (deaminating, flavin containing), EC 1.4.3.4] (MAO) in a variety of tissues in different species can be affected by hormones. For instance, in the rat heart the sp. act. of MAO, i.e., the catalytic activity per mg of protein, increases following adrenalectomy of the animal [1–7]. It has been suggested that this rise in MAO is a consequence of a general alteration in protein synthesis following the fall in circulating glucocorticoid concentrations [7]. The increased MAO activity has been shown to have identical properties to that found in age-matched control animals with respect to heat inactivation, changes in pH, effects of dialysis, and inhibitor and substrate specificity [8]. This provides indirect evidence that the increase in MAO is not due to a change in the catalytic properties of the enzyme after adrenalectomy.

The picture is however complicated by the fact that the sp. act. of the MAO in the rat heart also increases with the age of the animal [9–16]. This is unusual, since most other heart enzymes such as succinic dehydrogenase, cytochrome *c* oxidase and malate dehydrogenase, only increase in activity as a result of the general increase in the weight of protein as growth continues [13, 15]. Horita [13], and de Champlain *et al.* [17], have suggested that this increase in MAO sp. act. may be closely involved with the growth process of the heart. Any change in MAO activity that occurs as a result of adrenalectomy will be superimposed upon the continuously increasing activity of the enzyme due to growth.

In the present investigation the model describing changes in the tissue content of enzyme in terms of a transition from one steady state to another [18, 19], has been applied with compensation for changes in MAO due to the growth of the heart. By this approach it was hoped to be able to separate the effects of age and adrenalectomy upon the MAO in the rat heart.

METHODS

Adrenalectomy. Male Wistar rats were anaesthetized with diethyl ether. The adrenal glands were removed through dorsal incisions as quickly as possible and the wounds closed with surgical clips. Sham operations were performed to provide appropriate paired age-matched control animals. The adrenalectomized rats were maintained on normal diet but with free access to 0.9% NaCl solution.

At appropriate times adrenalectomized and control animals were killed by a blow on the head or by cervical dislocation. The success of the adrenalectomy was established by inspection of the operation site at death.

Changes in body weight, heart weight and of cardiac MAO following adrenalectomy. The experiments were performed using adrenalectomized rats and their paired age-matched controls at various times between 0 and 68 days after operation. In all, 13 batches were used weighing from 100–125 g at the time of operation.

Pargyline treatment. Pargyline hydrochloride was dissolved in 0.9% NaCl solution and injected intraperitoneally. Preliminary experiments showed that a dose of 30 mg/kg of pargyline hydrochloride would reduce the cardiac MAO activity by at least 98 per cent after 2 hr without any significant amount of the drug persisting in the hearts of the animals to inhibit newly synthesized enzyme. This dose of pargyline was used in all experiments.

Two groups of rats were used: group A in which the animals weighed 100–125 g at adrenalectomy, and group B in which they weighed 220–250 g at adrenalectomy. Group A was subdivided into two further groups, A_1 to which pargyline was given on the same

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day as adrenalectomy was performed, the rats being killed, 0, 1, 2, 4, 8, 13 and 19 days later, and A_2 to which pargyline was given 14 days after adrenalectomy, and the rats killed 0, 5, 7, 9, 13, 16, 21 and 34 days later. When group B was used, pargyline was given 33 days after adrenalectomy and the rats killed 2, 4, 7, 10, 14, 17, 21 and 28 days later. At all the chosen times, appropriate untreated control animals were killed.

Preparation of heart homogenates. Crude heart homogenates were made by a modification of the method of Ernster and Nordenbrand [20] in which the MgCl was omitted and the EDTA replaced by EGTA. This modification reduced the activation of an extra-mitochondrial Mg^{2+} dependent ATPase present in contaminating structures such as myofibrils [22, 23], while protecting the mitochondrial membrane from significant loss of Mg^{2+} [24]. The tissue was first finely chopped, washed and then homogenized for 30 secs in 3–5 vol. of ice cold modified medium in a loose fitting Dounce homogenizer. The suspension was then transferred to a conical all-glass homogenizer and further homogenized for 2 min. The resulting suspension was diluted with medium to a final vol. of 1 g of tissue in 10 mls of medium. The suspension was centrifuged at 650 g for 10 min, decanted and the supernatant centrifuged again at the same speed to remove nuclei and debris. The resulting supernatants were decanted and provided the homogenates upon which all the experiments were performed. No significant loss of MAO activity was detected up to one week if the homogenates were kept at −20°.

MAO assay. MAO activity was measured by a method based on that described by McCaman, *et al.* [25] using tritium labelled tyramine as substrate. In most cases the modification described by Jarrott [26] was used, but in those experiments involving the use of pargyline, extraction of labelled product was made into an equal mixture of benzene and ethyl acetate without the acid backwash step [8]. The concentration of [^{3}H]tyramine used in the assays was 1 mM to ensure saturation of the enzyme without causing substrate inhibition. All radiochemical assays were performed in duplicate.

After extraction into organic solvent the radioactive metabolites were counted by liquid scintillation in 0.4% Butyl-PBD in toluene using a Packard 3000 series liquid scintillation spectrometer with external standardization. Counting efficiencies were calculated by computer program based on the quadratic fit to the calibration curve.

Protein assay. The total protein contents of the homogenates were determined by the micro-biuret method of Goa [27], using bovine serum albumin as standard.

Kinetic studies. The parameters controlling the change in MAO activity brought about by adrenalectomy were calculated from:

$$d[MAO]/dt = k_s - k_d[MAO]. \quad (1)$$

In this equation [28, 29] the rate of net formation or net degradation of the enzyme is a balance between a zero order rate of synthesis (k_s) and a first order rate of degradation k_d[MAO] where k_d is the first order rate constant. If k_{s0} and k_{d0} are the rate constants of the initial steady state, the activity before adrenalectomy is given by $MAO_0 = k_{s0}/k_{d0}$, while the new steady state will be a level of activity $MAO_\infty = k_s/k_d$. Integration of eq. (1) will give the MAO activity of adrenalectomized rats at time t after operation, i.e.,

$$MAO_t = MAO_\infty (1 - e^{-k_d t}) + MAO_0 e^{-k_d t}. \quad (2)$$

However, to determine the effect of adrenalectomy on the cardiac MAO, it is appropriate to compare the MAO activity in the hearts of the adrenalectomized animals with the activity in the hearts of controls on the day of operation, i.e., MAO_t/MAO_0.

Using this comparison, there are two ways of fitting the experimental data to eq. (2). First is the linearizing transformation of an exponential, [30]:

$$\ln\left[\left(\frac{MAO_\infty}{MAO_0} - \frac{MAO_t}{MAO_0}\right)\Big/\left(\frac{MAO_\infty}{MAO_0} - 1\right)\right] = -k_d t. \quad (3)$$

The expression in square brackets is described as MAO 'activity ratio'. For convenience, we have called this solution of (2) 'method 1'.

When using method 1 correct estimates for the half-life depend upon the correct estimate of the final steady state. A second method involves a nonlinear regression which is largely independent of any approximation in the initial estimates of the experimental parameters. This method ('method 2'), which involves successive iterations, is based on making a guess at the value of the parameters and then applying a method for successively correcting the guesses to bring estimates of the parameters to as near as possible to the correct solution. The nonlinear form of the equation is:

$$\frac{MAO_t}{MAO_0} = \frac{MAO_\infty}{MAO_0} - \left(\frac{MAO_\infty}{MAO_0} - 1\right)e^{-k_d t}. \quad (4)$$

The calculations using (4) were performed by computer program.

Chemicals. Pargyline hydrochloride was a gift from Abbott Laboratories, Queenborough, Kent. Tyramine-[G-^{3}H]hydrochloride was obtained from New England Nuclear GMBH, Dreieichain, Germany.

All other chemicals were standard laboratory reagents of analytical grade where possible.

RESULTS

Changes in body weight and in wet weight of the heart following adrenalectomy. At all times from one day onwards following adrenalectomy the operated animals had, as expected, lower body and heart weights than their corresponding age-matched controls. The percentage falls in body and heart weight in the adrenalectomized rats were not significantly different from each other at any time, while the greatest differences between adrenalectomized and control rats were seen between 14 and 18 days after operation.

In the rat heart growth appeared to stop for about 14 days after the operation when it then began again at the same rate as in the control animals (Fig. 1).

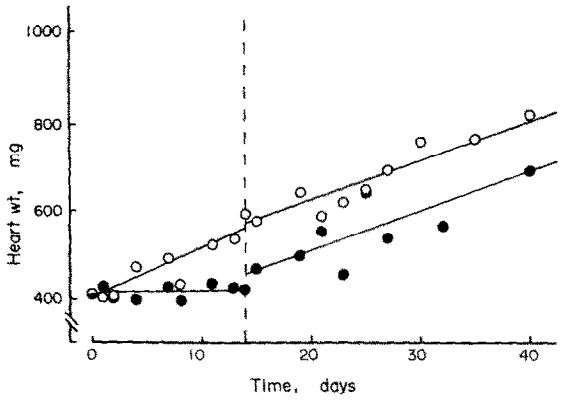

Fig. 1. Time course of the growth of the hearts of adrenalectomized and control rats. Wet weight of the hearts of adrenalectomized rats (●), wet weight of hearts of age-matched controls (○). Values are plotted against time in days after operation. Each point represents a mean value from 2–12 hearts. Each regression line was found by applying the method of least squares to the individual values. The vertical broken line separates regression lines calculated before and after resumption of growth in the hearts of the adrenalectomized rats.

The body weight responded in a similar manner. Thus the reduction in size of the hearts in the adrenalectomized animals would be wholly accounted for by the 14 days in which no apparent growth was observed.

Changes in the cardiac MAO activity following adrenalectomy: time course of the changes in relation to the growth process. In agreement with previous reports [13, 14] the heart MAO activity of control animals showed a significant correlation with both heart and body weights, thus appearing to be directly related to the rate of growth of the animal. After a lag of about one day, the sp. act. of the MAO of the hearts of adrenalectomized animals was increased at all times following operation in spite of the fact that the operated animals had smaller hearts than their age-matched controls (Fig. 2). The reduction in the weights of the hearts of adrenalectomized rats might be expected to have produced a reduction in the growth related rise in MAO activity. But if this does occur it is not sufficient to prevent the increase due to adrenalectomy, although it may well reduce the size of the apparent response. If experiments could be done in which changes in body and heart weight were prevented, the change in MAO activity induced by adrenalectomy could be expressed in terms of a transition from one steady state to another. The time course for this increase would be given by $d[MAO]/dt = k_s - k_d[MAO]$ (i.e. (1)), where k_s is the zero order rate for synthesis and k_d is the first order rate constant for degradation [28, 29]. In this situation the time course of the transition from one steady state to another induced by adrenalectomy is solely determined by the degradation rate constant k_d. However, in the present experiments this transition to the new steady state is superimposed upon the changes in MAO activity as a result of growth.

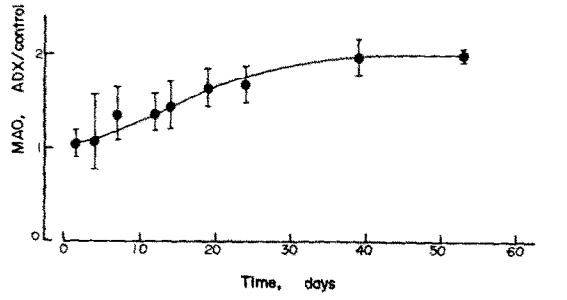

Fig. 2. Time course of the increase in the sp. act. of the MAO in the hearts of adrenalectomized rats. Values are expressed as ratios between adrenalectomized rats and their age-matched controls, and are plotted against time in days after operation.

Assuming that the effects of adrenalectomy and of growth on the cardiac MAO are two simultaneous but independent processes, it is possible to correct the values of MAO activity for the changes due to growth when applying eq. (2) to the effects of adrenalectomy.

If the sp. act. of the MAO in the hearts of both control and adrenalectomized rats in terms of nmoles (mg protein)$^{-1}$h^{-1} are divided by the appropriate heart weight in g (i.e. "transformed" units), a compensation for the rise in MAO sp. act. with age is achieved and an apparent steady state results. If it is assumed that this correction is appropriate for the adrenalectomized animals i.e., that only the effect of heart weight is removed, the increase in MAO activity following operation should fit an exponential curve between the two steady states as expected from eq. (2).

The experimental data can be fitted to this model by method 1. Thus in Fig. 3 the new steady state was estimated to be approximately twice the control steady state by taking the mean values of the compensated activities expressed as percentages of their appropriate controls from rats killed 19 days and later after adrenalectomy, when the increase in activity seemed to have reached its maximum. Using the method of least squares a value for k_d of 0.11/day was found, corresponding to a half-life of MAO in

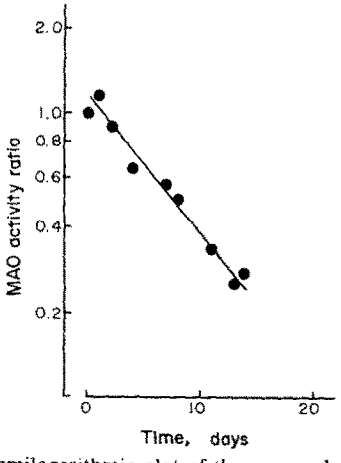

Fig. 3. Semilogarithmic plot of the approach towards the new steady state of the MAO activity in the hearts of adrenalectomized rats. The values of 'MAO activity ratio', as defined in the text under method 1, and calculated from activity in transformed units, are plotted against time in days after operation. Each point represents the mean from 2–5 animals. The regression line was fitted by 'method 1' using single values. (See Table 1 for parameter values.)

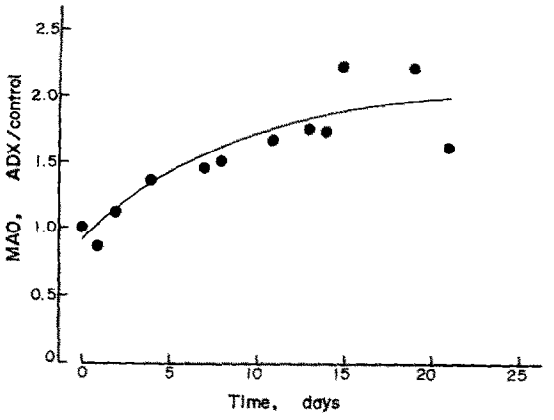

Fig. 4. Exponential plot of the approach towards the new steady state of the MAO activity in the hearts of adrenalectomized rats. Values of the ratio between the compensated MAO activities (in transformed units, see text) adrenalectomized and control rats are plotted against time in days after operation. Each point represents the mean from 2–5 animals. The exponential curve was fitted by 'method 2' using single values. (See Table 1 for parameter values).

these animals of 6.2 days. An intercept of 1.17 suggests that there is a lag period of about a day before the induction process starts. Using this method, correct estimates of the half-life of the MAO depend upon the correct estimate of the final steady state. In order to assess the accuracy of the estimated value of the new steady state the results were also fitted by method 2 (see Fig. 4). The estimate of the final steady state value, the k_d and the intercept are close to those values obtained by Method 1 (see Table 1).

Table 1. Parameters for the exponential increase of MAO following adrenalectomy, obtained by method 1 and method 2

Parameters	Method 1 Linear regression from semilog data	Method 2 Non linear regression
k_d (day^{-1})	0.11 (±0.06)	0.10 (±0.06)
half-life (day)	6.24	6.76
r	0.98	
MAO_∞/MAO_0	2.00 (assumed value)	2.10 ± 0.33
$(MAO_\infty/MAO_0) - 1$	1.17	1.22 ± 0.33

r = linear correlation coefficient, values in brackets represent S.E.M.

Effect of age and adrenelectomy on the recovery of the MAO following irreversible inhibition. The return of enzyme activity after the administration of the irreversible inhibitor pargyline has commonly been used to estimate the rate of degradation of MAO [31].

The time course of the reappearance of MAO activity following pargyline treatment was studied in heart homogenates from both adrenalectomized and age-matched control rats over a wide range of ages and weights. The nature of each group is described in Table 2. The fitting of experimental data to eq. (2), was performed by methods 1 and 2.

Following the conversion of the observed MAO activities from units to units divided by the total heart weight in g (transformed units), the complication due to changes in MAO activity following the increase in heart weight was abolished without affecting the increase in activity following adrenalectomy. This is particularly important in experiments where pargyline was given to adrenalectomized rats on the same day as the operation (see Discussion). Thus in control rats and in those adrenalectomized rats where the increase in MAO activity had reached a steady state, MAO activity could be taken as a constant over the period in which these experiments were conducted. This constant activity could then be used as the appropriate control value for comparison with the MAO activities following pargyline treatment in adrenalectomized and normal rats. In one case (Group A_1) where no steady state existed in the adrenalectomized rats, each group was compared with its appropriate control killed at the same time. In these experiments where pargyline caused almost complete inhibition of the MAO activity (2) and (3) become respectively:

$$MAO_t = MAO_\infty (1 - e^{-k_d t}) \quad (5)$$

and

$$\ln(1 - MAO_t/MAO_\infty) = -k_d t. \quad (6)$$

The semilog plots obtained using the non-linear method of fitting (method 2) are shown in Fig. 5. The goodness of fit of the experimental results by both methods 1 and 2 is shown in Tables 3 and 4 respectively. No significant difference was found between the values calculated using the two methods. Since, however, method 2 appeared to give a more precise estimate of curve parameters, comparisons between groups were carried out on the results

Table 2. Nature of the experimental groups used during pargyline recovery experiments

Experimental Group		Age (day) a	Age (day) b	Adrenalectomy (day) a	Adrenalectomy (day) b	Body Weight (g) a	Body Weight (g) b	Heart Weight (mg) a	Heart Weight (mg) b
A_1	Control	33	52	0	19	108 ± 3	206 ± 6	392 ± 13	602 ± 27
	Adx						160 ± 9		437 ± 27
A_2	Control	46	80	14	30	169 ± 4	304 ± 7	525 ± 16	823 ± 21
	Adx		62			145 ± 6	107 ± 16	435 ± 18	589 ± 57
B	Control	93	121	33	61	337 ± 6	381 ± 12	905 ± 33	930 ± 46
	Adx					302 ± 6	337 ± 20	893 ± 55	912 ± 53

a = Values at beginning of pargyline experiments, b = Values at the end of pargyline experiments. Body wts and heart weights are expressed as mean values ± S.E.M. Adx, adrenalectomized.

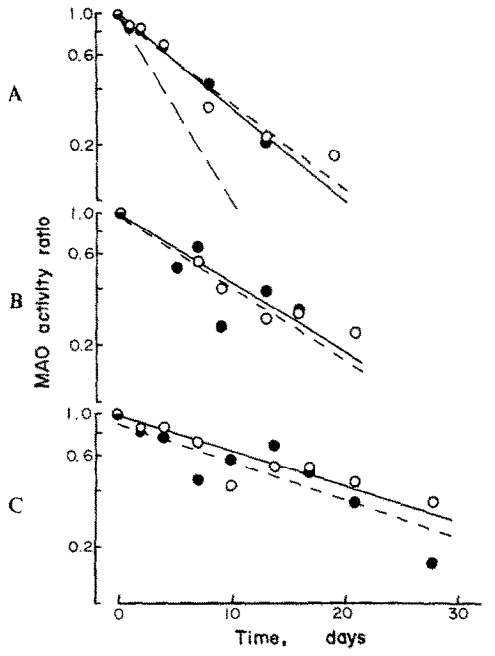

Fig. 5. Semilogarithmic plots of the recovery of rat heart MAO activity in adrenalectomized and control rats of various ages following irreversible inhibition by pargyline. (A), experimental group A_1, age 33 days; (B), experimental group A_2, age 46 days; (C), experimental group B, age 93 days. Values of 'MAO activity ratio', as defined under method 1 in the text and calculated from activity in transformed units, are plotted against time in days after treatment with pargyline hydrochloride (30 mg kg^{-1} i.p.). Control (O——O), adrenalectomized (●— —●), expected ratio for adrenalectomized rats of group A_1 (— —). The regression lines were derived from the exponential curve parameters obtained by fitting single values by 'method 2'. (See Table 4 for parameter values.)

obtained by this non-linear method of fitting. No difference was found between recovery rate constants for adrenalectomized animals and their controls in each age group, thus suggesting that adrenalectomy, although producing changes in MAO activity, does not affect the rate constant of degradation of the enzyme. However, the degradation rate constant of the cardiac MAO was reduced i.e., the half-life of the enzyme increased, with increasing age of the animals. Comparisons of 95 per cent confidence limits of k_d show a significant difference between group A_1 and group B for both control and adrenalectomized rats, while no significant difference was found in all three age groups, A_1, A_2 and B when adrenalectomized animals and controls were compared. Table 5 summarises the data for the half-lives of the MAO in the hearts of the animals used in these experiments, and in the experiments where the half-life was measured from the time course of MAO increase following adrenalectomy. The half-life of cardiac MAO appears to be correlated with the growth processes of the animal, whether the growth is expressed in terms of increase in body weight, heart weight or age.

Table 3. Mathematical fitting of pargyline recovery experimental curves by method 1

	Experimental group	Curve parameters: Intercept	$K_d \pm$ S.E.M. (day^{-1})	Half-life (day)
A_1	Control (n = 7)	1.00	0.10 ± 0.03	6.69
	Adx (n = 6)	1.00	0.11 ± 0.04	6.24
A_2	Control (n = 6)	0.87	0.07 ± 0.06	10.2
	Adx (n = 6)	0.89	0.07 ± 0.08	10.1
B	Control (n = 9)	0.96	0.04 ± 0.01	17.7
	Adx (n = 9)	0.93	0.05 ± 0.02	15.4

n = number of time points studied on the recovery curve. The values used at each time point were the means of 3–4 animals. Adx, adrenalectomized.

Table 4. Mathematical fitting of pargyline recovery experimental curves by method 2

	Experimental group	Curve parameters: Intercept value ± S.E.M.	k_d (day^{-1}): Value ± S.E.M.	k_d (day^{-1}): 95% Confidence limits	Half-life (day): Value (S.E. interval)	Half-life (day): 95% Confidence limits
A_1	Control (n = 24)	1.01 ±0.04	0.11 ±0.01	0.14 0.09	6.21 (5.59-6.99)	5.05 8.07
	Adx (n = 21)	1.00 ±0.02	0.11 ±0.01	0.12 0.09	6.38 (5.97-6.86)	5.58 7.47
A_2	Control (n = 18)	0.98 ±0.07	0.08 ±0.01	0.10 0.06	8.60 (7.65 9.83)	6.81 11.7
	Adx (n = 16)	0.96 ±0.02	0.08 ±0.03	0.14 0.02	8.36 (6.30-12.6)	4.85 30.1
B	Control (n = 22)	0.96 ±0.07	0.04 ±0.01	0.06 0.03	16.8 (14.1-20.7)	12.1 27.6
	Adx (n = 25)	0.92 ±0.09	0.05 ±0.01	0.07 0.03	14.9 (12.4-18.8)	10.5 26.0

n = number of animals studied over 6–9 time points. Adx, adrenalectomized. 95 per cent confidence limits of k_d and half-life values were calculated by the method of Colquhoun [30].

Table 5. Effect of age and adrenalectomy upon the half-life of cardiac MAO; summary of results

Experimental source of data	Groups	Mean age (day)	Mean body wt (g)	Mean heart wt (mg)	MAO half-life (day)
MAO increase after adrenalectomy (Fig. 4)	Control	45 (35–54)	162 (119–221)	514 (411–643)	6.76
	Adx		142 (119–175)	439 (411–498)	
Recovery of MAO after pargyline	Control				
A_1		40 (33–52)	139 (108–206)	460 (392–602)	6.21
	Adx		123 (108–160)	406 (392–437)	6.38
	Control	62 (46–80)	236 (169–304)	663 (525–823)	8.60
A_2	Adx	55 (46–62)	178 (145–207)	504 (435–589)	8.36
	Control		353 (337–381)	913 (905–930)	16.8
B (Fig. 5)	Adx	104 (93–121)	330 (302–337)	895 (893–912)	14.9

Age, body wt and heart wt are means of values obtained during the whole time course of each experiment. Ranges of values shown in brackets represent values at the beginning and at the end of each experiment. Adx, adrenalectomized.

Table 6 shows the values for k_s for the MAO in the hearts of control and adrenalectomized rats. At the same time the content of MAO is given in terms of its sp. act. with and without the correction for the increase in the total weight of the heart. For the calculation of k_s from the expression $k_s = k_d MAO_\infty$ the uncompensated MAO activity was used to reveal the real increase in the activity of MAO with increase in the weight of the heart.

It is clear that in controls the increase in MAO activity from 210 units in rats with a mean heart weight of 460 mg up to 508 units in rats with a mean heart weight of 913 mg was brought about solely by a decrease in k_d without any significant change in k_s. The same is true for the adrenalectomized rats where the increase in MAO sp. act. with increasing age is also explained by a decrease in k_d. The adrenalectomized rats, however, have higher MAO levels and a higher rate of synthesis than their controls. It is interesting to observe that in the case of the oldest group of rats (Group B) where the increase in MAO activity due to adrenalectomy was much less, the k_s value was only slightly greater than the corresponding control value.

DISCUSSION

The major problem encountered in the study of adrenocortical regulation of cardiac MAO in the rat is the rise in activity of this enzyme that takes place as the animal grows older and the heart and body weight increase. In the present experiments where the effect of adrenalectomy upon cardiac MAO was studied, it was necessary to show that adrenalectomy did not specifically affect heart growth before kinetic studies of the changes in MAO activity in this organ could be considered. In *ad lib.* fed adrenalectomized rats maintained on saline, deficient growth has been described [32]. Retardation in the rate of growth was confirmed in the present experiments, and the heart was found to follow the slowing down in the growth of the whole animal, thus excluding any specific atrophic effect upon this organ. It appeared that the hearts had not grown at all for about 14 days after operation. Growth then began again and progressed at about the normal rate. Consequently there was always, at all times, after the first two days a significant difference between adrenalectomized and control rats which began to disappear only after 40 days, due to the slowing down of the growth rate of controls when they reached adult weight. The adrenalectomized rats reached adult weight after a further few days. This growth pattern could possibly be explained by an initial very large reduction in food intake which then is corrected. The observation that the deficient growth due to adrenalectomy can be reversed by force feeding the operated animals [33, 34] supports this view. The reduction of food intake may be related to a delay in the response of the animal to drinking saline or to nausea. After adrenalectomy abnormalities of intestinal function, secondary to fluid and electrolyte disturbances, which are reversed by saline treatment [35], can also be mimicked by starving the animal [36].

Although a large reduction in cardiac performance after chronic adrenalectomy has been described, there is no evidence for an anatomical impairment of the myocardium [37]. Several authors have reported impairment in the mechanical performance of the myocardium [38–42]. However, in agreement with the present findings, the heart weight in terms of its ratio to body wt, has been found to be unchanged [43–45] or slightly decreased [46, 47], thus indicating that cardiac atrophy is not a major factor contributing to the impairment of the function of the heart.

The possibility of studying the time course of the increase of MAO activity following adrenalectomy, in the experimental conditions described here, is based on various assumptions. One of the assumptions is that the relationship between growth and cardiac MAO shown by normal rats still holds under conditions of adrenal insufficiency. Although it is not possible to exclude an interference with the relationship between growth and MAO activity in the heart by adrenalectomy, the apparent absence of an effect of adrenalectomy upon the normal development of

Table 6. Estimation of synthesis rate (k_s) of cardiac MAO in control and adrenalectomized rats of different ages

Experimental group	Mean heart wt (mg)	k_d (day^{-1})	MAO_∞ [units (total heart weight in g)$^{-1}$]	MAO_∞ (units)	k_s (units.day^{-1})
Control					
A_1	460	0.11	457	210	23
A_2	663	0.08	457	303	24
B	913	0.04	581	509	21
Adx					
A_1	406	0.11	914	371	40
A_2	504	0.08	914	461	38
B	895	0.04	700	627	27

MAO units = nmoles (mg prot)$^{-1}$ hr^{-1}. k_s was estimated from the expression $k_s = k_d\ MAO_\infty$ as explained in the text. Adx, adrenalectomized.

this organ supports the view that this assumption is valid.

The validity of assuming eq. (1) as a suitable model to describe the time course of the increase in MAO activity induced by adrenalectomy depends upon the possibility of maintaining steady state levels of MAO during the entire course of the experiment. To meet this condition the continuous increase of MAO activity due to the significant growth occurring during the course of the experiment must be eliminated. Furthermore the under-estimation of the effect of adrenalectomy due to the use of paired age-matched controls of heavier body and heart weight, which have a higher MAO content, should be avoided by considering weight-paired matched rats as appropriate controls.

The solution to these problems adopted in the present experiments was the use of a unit of activity which could compensate for changes in MAO due to the growth of the heart. This unit, obtained by dividing the MAO activity of both controls and adrenalectomized animals by their respective total heart weight, was an attempt to express the enzyme activity as though the experiment had been done on rats of constant heart weight during the entire course of the experiment. The correlation between MAO activity and heart weight shown by normal rats was abolished when the activity was expressed in these transformed units and an apparent steady state was obtained within the time course of the experiment. The model can then be used making the assumption that the effects of adrenalectomy and growth upon the cardiac MAO activity are two independent processes.

The results show that adrenalectomy is characterized by a new steady state level of MAO corresponding to a 2-fold increase of activity. This new steady state is defined by a rate constant of degradation k_d of 0.1/day. Values obtained by the use of the two different methods of fitting are in good agreement.

The higher steady state found after adrenalectomy could be either a consequence of an increased rate of synthesis, k_s, or of a decrease in the rate of degradation, k_d from the initial value. When the rate constant of degradation k_d, of both control and adrenalectomized rats was measured by following the time course of reappearance of MAO activity after pargyline treatment, it was found that adrenalectomy, as opposed to the alteration in the rate constant of degradation, k_d, produced by age, affects the cardiac MAO activity by changing, at least apparently, the rate of synthesis of this enzyme.

When the recovery of MAO activity after pargyline treatment was studied, using transformed units, the cardiac MAO activity of control rats of all groups used, and of those groups of adrenalectomized rats (group A_2 and B) where the increase in activity due to adrenalectomy had reached a steady state, was found to remain constant over the period in which the experiments were conducted. Under these experimental conditions the recovery of the MAO activity after pargyline treatment could be described by eq. (1) as a return to the steady state level of the untreated animals, MAO_0 and MAO_∞ for controls and adrenalectomized rats respectively. However, in the one case, where pargyline was given to adrenalectomized rats on the same day as operation (group A_1) the values of [MAO] in the untreated animals does not correspond to a steady state level, since it represents the exponential increase of [MAO] following adrenalectomy. In this case, assuming that the change in [MAO] with time, which occurs in the adrenalectomized animals, is not affected by pargyline treatment, both groups of adrenalectomized and adrenalectomized-pargyline treated rats are characterised by the same constants k_s and k_d, and eq. (1) applies to both. The only difference is in the MAO level at the beginning of the experiment, i.e.

$$MAO_{adx} = MAO_0 \text{ (at } t = 0),$$

$$MAO^*_{adx} = 0 \quad (\text{at } t = 0),$$

where MAO_{adx} and MAO^*_{adx} are MAO activities in the hearts of adrenalectomized and adrenalectomized-pargyline treated rats respectively, at a certain time t. Solution of (1) with these initial conditions gives:

$$MAO_{adx} = MAO_\infty (1 - e^{-k_d t}) + MAO_0\, e^{-k_d t}, \quad (7)$$

$$MAO^*_{adx} = MAO_\infty (1 - e^{-k_d t}), \quad (8)$$

where $MAO_\infty \equiv k_s/k_d$, i.e., the asymptotic value.

Consider the ratio:

$$R = (MAO_{adx} - MAO^*_{adx})/MAO_{adx}, \quad (9)$$

corresponding to the factor $(1 - MAO_t/MAO_\infty)$ of (6) used in the fitting by method 1, substituting eq. (7) and (8) into eq. (9) and taking the natural log of R gives:

$$\ln R = -k_d t - \ln\left[\frac{MAO_\infty}{MAO_0} + \left(1 - \frac{MAO_\infty}{MAO_0}\right) e^{-k_d t}\right], \quad (10)$$

which compares with the value $-k_d t$ obtained in eq. (6). If $t \ll 1/k_d$, a first order Taylor expansion of (10) gives:

$$\ln R \simeq -k_d \frac{MAO_\infty}{MAO_0} t = -\frac{k_s}{MAO_0} t \qquad (11)$$

which shows that in this case the slope of the semilog plot differs from k_d by a factor, MAO_∞/MAO_0. Since with the assumptions previously made MAO_∞/MAO_0 has a value of 2, the fitting of the data obtained using adrenalectomized rats of group A_1, would be expected to give a semilog plot with slope $2k_d$ as shown in Fig. 5. Surprisingly enough, not only the fitting of the experimental data gives a plot different from the expected one, but precisely reproduces the semilog plot obtained using control rats of group A_1, as if pargyline treatment had totally prevented the increase in MAO activity by adrenalectomy. This might have happened if pargyline either had prevented the two-fold increase in k_s produced by adrenalectomy, or alternatively had induced a parallel two-fold increase in k_d. The results obtained here strongly support the possibility that pargyline prevents the increase in k_s in group A_1. The evidence is against a change in k_d following pargyline treatment. It appears that in eq. (11) MAO_0 (k_{s0}/k_{d0}) represents the steady state value of MAO activity in control conditions, where k_d is known from the experiment where the recovery of MAO activity after pargyline treatment was studied in the paired age-matched controls (Fig. 5). Plotting the data obtained from the adrenalectomized rats of group A_1, the slope of the semilog regression ($-k_s/MAO_0$) is found to be identical to the slope (k_{d0}) of the semilog plot of the data from age-matched controls. This eliminates the possibility that, in this particular experimental condition, k_{d0} has been changed by pargyline treatment. Thus it would appear that the absence of enzyme activity impairs the ability of adrenalectomy to alter the rate of synthesis of MAO, although it has no effect upon the rate of synthesis k_s, once it has been altered and the corresponding higher steady state of MAO, due to adrenalectomy, has been reached.

The increase in rat heart MAO activity with age that has been found by several workers [9–14], has been shown to be brought about solely by a decrease in k_d without any significant change in k_s. Moreover, adrenalectomy of the animals has no detectable effect on k_d.

So far it has been assumed that k_d is a measure of the rate of degradation of the MAO by proteolytic enzymes. It is, however, not possible at present to demonstrate any specific system capable of degrading the MAO. Two general mechanisms have been proposed by Schimke [48]. One is that the protein molecules are individually available to a degradation process, which is present at all times. In the case where such a mechanism is operating, an increase in MAO activity with age could be produced by changes in the conformation of the enzyme protein, making it a less suitable substrate for the degradation process. This effect of age could be brought about by a change in the lipid or protein environment in which the MAO is bound to the mitochondrial membrane. An alternative mechanism is a change in the activity of the degrading system itself. A general intracellular degrading system, e.g., the lysosomal enzymatic activities, could become progressively less active with increasing age of the animals. However, the finding that, in the heart, the lysosomal hydrolase, cathepsin, increases with age [49], does not support the suggestion that the mechanism operating in the slowing down of the rate of degradation of MAO with age is a change in this general degrading system. Schimke [28] has proposed that the lysosomes are important when gross changes in protein degradation are involved, whereas the degradation that occurs in normal steady state conditions involves a different system at present unknown. Proteolytic enzymes that are selective for pyridoxal dependent enzymes have been found [50–52]. These enzymes are the first to attack the pyridoxal dependent enzymes to make a product that is susceptible to degradation by less selective enzymes such as trypsin. These enzymes may be responsible for the breakdown of the clorgyline resistant MAO that is also found in the rat heart, and which appears to be pyridoxal dependent [53], but are less likely to be involved in the breakdown of the FAD dependent MAO that is responsible for the metabolism of tyramine.

It remains to be seen whether changes in the binding or in the proteolytic enzymes are responsible for the increase in the specific activity of the rat heart MAO with age. However, the effect of ageing is quite distinct from that of adrenalectomy, which causes an increase in the rate of synthesis without changing the degradation in any way.

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REFERENCES

1. V. M. Avakian and B. A. Callingham, *Br. J. Pharmac.* **33**, 211 (1968).
2. B. Bhagat, *Br. J. Pharmac.* **37**, 34 (1969).
3. T. C. Westfall and H. Osada, *J. Pharmac. exp. Ther.* **167**, 300 (1969).
4. P. M. Caesar, G. G. S. Collins and M. Sandler, *Biochem. Pharmac.* **19**, 921 (1970).
5. M. Holzbauer and M. B. H. Youdim, *Br. J. Pharmac.* **44**, 355 (1972).
6. S. S. Sampath, T. M. Shih and D. E. Clarke, *Fedn. Proc.* **31**, 544 (1972).
7. H. Parvez and S. Parvez, *Acta endocr., Copenh.* **73**, 509 (1973).
8. B. A. Callingham and R. Laverty, *J. Pharm. Pharmac.* **25**, 940 (1973).
9. W. J. Novick, *Endocrinology* **69**, 55 (1961).
10. A. Stüder, *Histochemie* **6**, 274 (1966).
11. A. Ho-van-Hap, L. Babineau and L. Berlinguet, *Can. J. Biochem.* **45**, 355 (1967).
12. A. J. Prange, J. E. White, M. A. Lipton and A. M. Kinkead, *Life Sci.* **6**, 581 (1967).
13. A. Horita, *Nature, Lond.* **215**, 411 (1967).
14. A. Horita, *Biochem. Pharmac.* **17**, 2091 (1968).
15. B. A. Callingham and L. Della Corte, *Br. J. Pharmac.* **41**, 392 (1971).
16. M. C. Lowe, D. D. Reichenbach and A. Horita, *J. Pharmac. exp. Ther.* **194**, 522 (1975).

17. J. de Champlain, L. R. Krakoff and J. Axelrod, *Circulation Res.* **23,** 361 (1968).
18. C. M. Berlin and R. T. Schimke, *Molec. Pharmac.* **1,** 149 (1965).
19. H. L. Segal and Y. S. Kim, *J. Cell comp. Physiol.* **66,** 11 (1965).
20. L. Ernster and K. Nordenbrand, in *Methods in Enzymology* (Eds. R. W. Estabrook and M. E. Pullman), vol. 10, pp. 86–94. Academic Press, New York (1967).
21. J. B. Chappell and S. V. Perry, *Nature, Lond.* **173,** 1094 (1954).
22. G. F. Azzone, O. Eeg-Olofsson, L. Ernster, R. Luft and G. Szabolcsi, *Expl. Cell Res.* **22,** 415 (1961).
23. R. Hedman, *Expl. Cell Res.* **38,** 1 (1965).
24. J. B. Chappell and R. G. Hansford, in *Subcellular Components* (Ed. G. D. Birnie), pp. 77–91. Butterworths, London (1972).
25. R. E. McCaman, M. W. McCaman, J. M. Hunt and M. S. Smith, *J. Neurochem.* **12,** 15–23 (1965).
26. B. Jarrott, *J. Neurochem.* **18,** 7 (1971).
27. J. Goa, *Scand. J. clin. Lab. Invest.* **5,** 218 (1953).
28. R. T. Schimke, in *Mammalian Protein Metabolism* (Eds. H. N. Munro and J. B. Allison), vol. 4, pp. 177–228. Academic Press, New York (1970).
29. R. T. Schimke and D. Doyle, *A. Rev. Biochem.* **39,** 929 (1970).
30. D. Colquhoun, *Lectures on Biostatistics*, pp. 234–243. Clarendon Press, Oxford (1971).
31. N. H. Neff and C. Goridis, *Adv. biochem. Psychopharmac.* **5,** 307 (1972).
32. J. H. Leatham, in *Mammalian Protein Metabolism* (Eds. H. N. Munro and J. B. Allison), Vol. 1, pp. 343–380 Academic Press, New York (1964).
33. D. J. Ingle and M. C. Prestrud, *Endocrinology*, **45,** 143 (1949).
34. C. Cohn, E. Shrago and D. Joseph, *Am. J. Physiol.* **180,** 503 (1955).
35. R. J. Levin, *J. Endocr.* **45,** 315 (1969).
36. R. J. Levin, H. Newey and D. M. Smyth, *J. Physiol., Lond.* **177,** 58 (1965).
37. A. M. Lefer, in *Handbook of Physiology, Section 7: Endocrinology* (Eds. H. Blaschko, G. Sayers and A. D. Smith), vol. 6, pp. 191–207. American Physiological Society, Washington (1975).
38. N. Solomon, R. H. Travis and G. Sayers, *Endocrinology*, **64,** 535 (1959).
39. A. M. Lefer and D. C. Sutfin, *Am. J. Physiol.* **206,** 1151 (1964).
40. W. R. Webb, I. U. Degerli, J. D. Hardy and M. Unal, *Surgery*, **58,** 273 (1965).
41. A. Gerlach and P. A. Van Zweiten, *Pflügers Arch. ges. Physiol.* **311,** 96 (1969).
42. R. L. Verrier, M. J. Rovetto and A. M. Lefer, *Am. J. Physiol.* **217,** 1559 (1969).
43. W. C. Ullrick, B. B. Brennan and W. V. Whitehorn, *Am. J. Physiol.* **200,** 117 (1961).
44. M. J. Rovetto, R. A. Murphy and A. M. Lefer, *Circulation Res.* **26,** 419 (1970).
45. M. J. Rovetto, A. M. Lefer and R. A. Murphy, *Pflügers Arch. ges. Physiol.* **329,** 59 (1971).
46. B. Hoelscher, *Am. J. Physiol.* **179,** 171 (1954).
47. A. Aschkenasy, *Annls. Endocr.* **23,** 145 (1962).
48. R. T. Schimke, *Adv. Enzymol.* **37,** 135 (1973).
49. C. H. Barrows and L. M. Roeder, *J. Geront.* **16,** 321 (1961).
50. E. Kominami, K. Kobayashi, S. Kominami and N. Katunuma, *J. biol. Chem.* **247,** 6848 (1972).
51. N. Katunuma, T. Katsunuma, R. Kominami, K. Suzuki, Y. Hamaguchi, K. Chichibu, K. Kobayashi and T. Shiotani, *Adv. Enzyme. Reg.* **11,** 37 (1973).
52. N. Katunuma, *Rev. Physiol. Biochem. Pharmac.* **72,** 83 (1975).
53. G. A. Lyles and B. A. Callingham, *J. Pharm. Pharmac.* **27,** 682 (1975).