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W D Denckla

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Research Article

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Role of the Pituitary and Thyroid Glands in the Decline of Minimal O_2 Consumption with Age

W. DONNER DENCKLA

From the Roche Institute of Molecular Biology, Nutley, New Jersey 07110

ABSTRACT Resting O_2 consumption rate (BMR) or minimal O_2 consumption rate (MOC) declines with age. Data are presented that suggest that a newly described function of the pituitary may be responsible for a considerable part of the total 75% decline in the MOC with age. The new function appears to decrease the responsiveness of peripheral tissues to thyroid hormones. Response curves to injected thyroxine indicated that immature rats were three times more responsive to thyroxine than adult rats. All the major endocrine ablations were performed in this and earlier work, and only pituitary ablation (*a*) restored in adults part of the responsiveness to thyroxine found in immature rats and (*b*) arrested the normal age-associated decrease in responsiveness to thyroxine in immature rats. Bovine pituitary extracts were found that decreased the responsiveness of immature rats to thyroxine. Experiments with the new pituitary function suggested a possible endocrine mechanism to explain why partial starvation doubled the lifespan for rats only when started before puberty.

INTRODUCTION

Recently a new physiological parameter, minimal O_2 consumption (MOC),[1] was studied in rats (2). MOC, unlike the resting O_2 consumption rate (BMR), appeared to be specific for the determination of thyroid status among the nearly 70 endocrine and nonendocrine factors studied (3). However, the MOC, like the BMR (4, 5), declined with age. This finding was difficult to interpret since hypothyroidism is not, at present, considered to be associated with old age (6–8). Nonendocrine mechanisms have been suggested to explain part of the BMR decline with age. However, up to the present there does not appear to be any endocrine or nonendocrine explanation which can account for more than a part of the decline (6–8).

Experiments were conducted to determine if an endocrine system was responsible for the decline in the MOC. Since previous studies (3) indicated that only ablation of the thyroid or the pituitary altered the MOC, the present work sought to find if some previously unknown function of these glands could account for the decline.

The results from these experiments prompted additional work to determine if an endocrine mechanism could explain the discovery of McCay, Pope, and Lunsford (9) that the lifespan of the rat could only be prolonged markedly by partial starvation started before puberty.

METHODS

O_2 consumption was determined volumetrically as previously described (2, 3, 10, 11) in anesthetized rats by using a thermobarometer-compensated (10), closed-circuit respirometer (3). MOC is expressed in milliliters of O_2 at standard temperature and pressure per min per 100 g fat-free body weight. The methods for fat corrections have been described (3, 11). All rats were fed a low iodine diet except where noted otherwise, and all rats with normal thyroid function were given KI in their drinking water for reasons given earlier (3).

Operations, drugs, treatments. All rats were operated on by the supplier, Zivic-Miller, Inc., Allison Park, Pa. Postoperative treatments have been described (3). Compounds were injected subcutaneously in 10% gelatin unless noted otherwise. Doses of material injected are expressed in units mass/kilogram body weight per day. 3,5,3′,5′-L-tetraiodothyronine (T_4) was injected for 3 wk, except where noted otherwise. Rats that grew more than 10% above initial weight had drug doses adjusted weekly. The protocol of McCay et al. (9) was followed closely in the starvation experiments except that the low iodine diet was used. Because food spillage was negligible, the food consumption rates of control rats, which fed ad libitum, could be determined by daily weighing the food that remained in their dishes. Starved rats were fed at a rate of 30–35% of the controls.

Part of this work has been summarized earlier (1).

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[1] *Abbreviations used in this paper:* BMR, resting O_2 consumption; MOC, minimal O_2 consumption; T_4, 3,5,3′,5′-L-tetraiodothyronine.

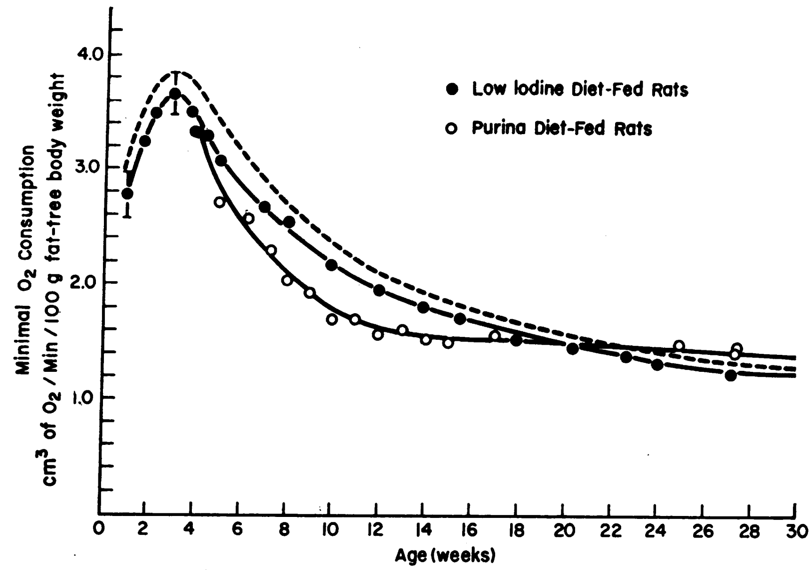

FIGURE 1 Change in MOC from 1 to 30 wk of age in the female rat. MOC values are given for female rats of the Zivic-Miller strain. After weaning, the rats were fed two different diets as shown. The standard deviations for low iodine diet-fed rats are indicated by the dashed line. Between 5 and 20 rats were used per point. Curve was fitted by eye.

Pituitary extracts. Fresh-frozen and lyophilized bovine pituitaries were extracted according to the method of Ellis (12) in the first series of experiment.[2] In the second series a modification of Ellis' B fraction was used. After homogenization in distilled water, the material was diluted to 1,500 ml/100 g tissue, the pH was adjusted to 4.0, and extraction was carried out at 5°C for 1 h. After centrifugation at 21,000 *g* the pellet was resuspended and re-extracted in one-half the original volume for 0.5 h. After centrifugation at the above speed, the supernate was discarded, and the pellet was extracted with 0.1 M · $(NH_4)_2SO_4$ at pH 4.0 for 1 h. After centrifugation as above, the supernate was retained, and it was subjected to isoelectric focusing in 0.2%, pH 3.5–10, carrier ampholytes (LKB Produkter, LKB Instruments, Inc., Rockville, Md.) on a 15,000-ml 10-compartment preparative instrument similar, in principle, to that of Valmet (13). The fractions were dialyzed (PM 10, Amicon Corp., Lexington, Mass.) and lyophilized.

Statistics. The significance of differences were calculated by Student's *t* test (14).

RESULTS

Changes in MOC with age. The MOC rose to a maximum at 3 wk of age and declined 77% thereafter (Figs. 1, 2). For comparison to the work of others, the same strain of rats was fed a conventional diet (Purnia Rat Chow). Compared to the low iodine diet-fed rats, rats fed this diet merely had a more rapid MOC decline during puberty (Fig. 1), and as noted earlier (11), the conventional diet spuriously elevated the MOC of adults.

Viscera. Some authors have claimed that 50% of the decline of the BMR with age could be accounted for by a change in the ratio of visceral mass to total body mass (15). A shift with age in the ratio of visceral mass[3] to fat-free body weight accounted for only approximately 10 of the total 71% decline in the MOC from three to 52 wk of age (Table I).

Changes in thyroidal and athyroidal components of MOC. The MOC decrease with age produced an apparent contradiction between two sets of data. Previous extensive work (3) suggested the MOC specifically measured changes in thyroid status. Yet a number of investigators (16–18) found that there was no significant change throughout life in plasma levels of thyroid hormones. However, the MOC could be shown to consist of two components. A residual O_2 consumption rate remains after either thyroidectomy or hypophysectomy which will be called the "athyroidal MOC." If the athyroidal MOC alone declined with age, then the decline in the total MOC despite the constant thyroidal hormone levels throughout life could be explained.

In experiments with several hundred rats of different ages, either hypophysectomized or thyroidectomized, it was first determined that the athyroidal MOC could be measured only after an 8-wk postoperative waiting period to permit the decay of the effects of endogenous thyroid hormones. The thyroidal MOC was then determined in rats of various ages (Table II) by subtracting the athyroidal MOC from the MOC of intact rats that were the same age as the operated rats had been at the time

[2] These extracts were kindly prepared by Dr. K. Gibson.

[3] The viscera used were those employed by others previously (15).

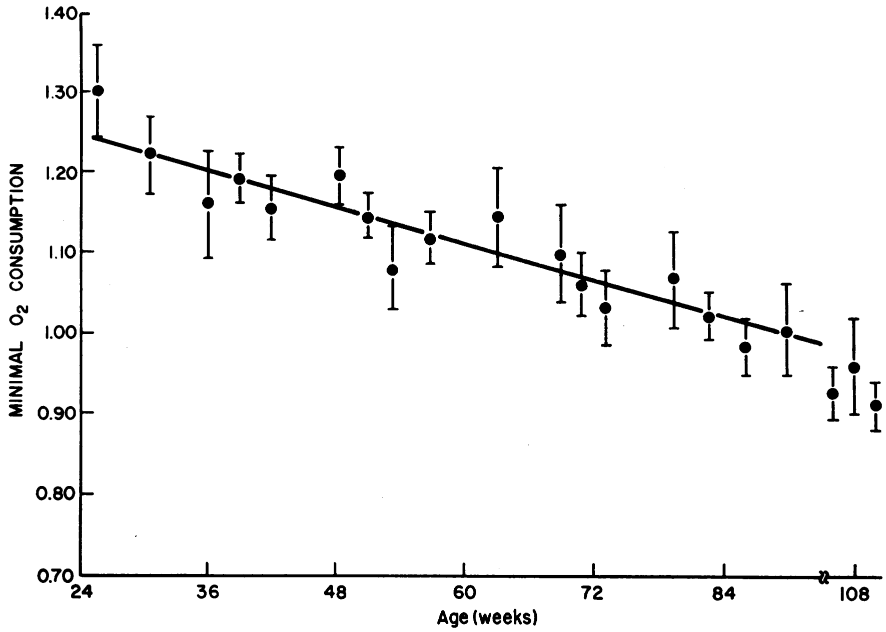

FIGURE 2 Decline in MOC between 24 and 108 wk of age in the female rat. MOC values are given for 8–20 female rats per point of the same strain as in Fig. 1. Standard deviations are indicated by vertical bars. All rats were fed a low iodine diet.

of operation. For example, the thyroidal MOC of a 3-wk-old rat was calculated by subtracting the MOC of a rat operated on at 3 wk of age from the MOC of an intact 3-wk-old rat. The results showed that both the athyroidal (−64%) and the thyroidal (−87%) MOC declined markedly with age (Table II).

Alternatively, the athyroidal MOC could have been subtracted from the MOC of intact rats of the same age at the time of measurement. However, this method, when used with young rats, produced meaningless data. After the 8-wk waiting period the athyroidal MOC of young, operated rats remained stable for the longest observation period of 24 wk. By contrast, the MOC of intact rats declined rapidly. These two effects resulted in values for the thyroidal MOC calculated in this manner that varied from a positive to a negative number. The value depended solely on the chronological age used for the calculation and not the age at operation. For example, if the athyroidal MOC of a 3-wk-old rat (Table II) was subtracted from the MOC's of intact 27-, 19-, or 11-wk-old rats (Fig. 1) these calculations produced values for the thyroidal MOC of, respectively, −0.37, 0.0, and +0.21 ml of O_2. By contrast, the calculation used in Table II produced a single, positive value for the thyroidal MOC, regardless of the age after the postoperative waiting period at which the athyroidal MOC was measured.

TABLE I

MOC and Age Changes in Relative Weights of Viscera

Age	Viscera (*n*)
wk	*% body wt*
1	11.7±1.5 (5)
3	11.2±1.8 (8)
12	6.9±0.8 (8)
52	5.0±0.6 (8)

MOC decrease 3–52 wk: 71%
MOC decrease due to viscera: 10.7%

The mean (±SD) percentages of fat-free body weight contributed by the viscera (brain, kidney, liver, heart) of intact female rats are given at various ages. The percent decrease in the MOC is given as well as the percent which might be attributed to the relative decrease in the weight of the viscera with age.

Endocrine basis for the decline. In view of the preceding findings, it was postulated that age might nonspecifically decrease the responsiveness of rats to endogenous thyroid hormones. An attempt to measure T_4 dose-response curves of young and adult intact rats failed when it was discovered that 3-wk-old rats maintained elevated MOC values to moderate T_4 doses (10, 30 μg) only for the first 2 wk of injection. After four additional weeks of injection the T_4-treated rats had the same MOC values as the controls. By contrast, adult rats maintained stable responses to these doses for 12 wk, the longest period tested. These experiments suggested that T_4 administration to young rats stimulated in some man-

TABLE II

Effects of Age on Total, Athyroidal, and Thyroidal MOC

Age	Intact MOC (*n*) −	Athyroidal MOC =	Thyroidal MOC	Operation (*n*)
Wk				
0.14		1.86±0.21	—	TPX-^{131}I (11)
1	2.87±0.32 (30)	1.75±0.14	1.12	TPX-^{131}I (24)
2	3.30±0.21 (30)	1.65±0.17	1.65	TPX (28)
3	3.82±0.18 (50)	1.77±0.11	2.05	TPX-^{131}I (45) Hypox (70)
4	3.48±0.21 (30)	1.52±0.10	1.96	TPX (30)
6	3.05±0.18 (20)	1.23±0.10	1.82	TPX (20)
8	2.61±0.17 (18)	1.06±0.08	1.55	TPX (20)
10	2.03±0.14 (30)	0.94±0.07	1.09	TPX (20) Hypox (22)
32	1.26±0.06 (30)	0.80±0.05	0.46	TPX (15) Hypox (10)
40	1.13±0.04 (40)	0.70±0.06	0.43	TPX-^{131}I (18) Hypox (40)
52	1.11±0.04 (20)	0.71±0.06	0.40	TPX-^{131}I (10) Hypox (36)
78	1.05±0.05 (20)	0.68±0.04	0.37	TPX-^{131}I (15) Hypox (20)
100	0.94±0.05 (20)	0.67±0.05	0.27	TPX-^{131}I (12)

The MOC's (mean±SD) are given for female intact and operated rats at the ages shown. In order to determine the thyroidal MOC, the athyroidal MOC, measured 8 wk after operation, was subtracted from the MOC of intact rats of the same age as the operated rats had been at the time of operation; for example, the athyroidal MOC of a rat operated on at 3 wk of age was subtracted from the MOC of an intact 3-wk-old rat. The thyroid was surgically ablated (TPX) or ablated and followed by a single 10 mCi/kg injection of ^{131}I (TPX-^{131}I). Because the MOC's of hypophysectomized (Hypox) and TPX'd rats were not significantly different, their MOC's at a given age were averaged.

ner the release of a factor that decreased responsiveness to T_4.

Because the decline of the MOC with age was affected only by removal of the pituitary or the thyroid (3), it was postulated that T_4 injected into intact rats might have stimulated the production of a hormone from one of these glands that, in turn, decreased responsiveness of peripheral tissues to T_4. This possibility was tested by injecting T_4 into rats that had been either thyroidectomized or hypophysectomized at 3 wk of age. The injection of 75 μg of T_4 restored the MOC of the hypophysectomized rats to the normal value of a 3-wk-old intact rat (Table II, Fig. 3). The MOC did not change during the course of the T_4 injections, and the athyroidal MOC was unchanged by the injections (Fig. 3).

In marked contrast, T_4 injected into thyroidectomized rats never produced as great a response as it did in hypophysectomized rats (*P* value, 0.001), and the response to T_4 fell continuously during the course of the injections. The athyroidal MOC decreased with age in the thyroidectomized group given T_4 as compared to the value found at the start (*P* value, 0.001; Fig. 3).

Effect of pituitary extracts. The preceding experiments suggested that T_4 injection into immature intact or thyroidectomized rats could stimulate pituitary secretion of a hormone that decreased the responsiveness of rats to T_4. Bovine pituitary extracts, prepared as described in Methods, were found to lower the MOC of rats injected with T_4 which had been hypophysectomized at 3 wk of age. The most active crude fraction, Ellis' C, contained a considerable amount of bioassayable growth hormone (Table III). After electrofocusing the modified B fraction had the highest apparent specific activity but produced only modest growth (Table III).

Changes in MOC with starvation. Rats were partially starved by the method of McCay et al. (9) for 6 wk. Starvation started at 3 wk of age but not at 12 wk of age caused significant retardation in the normal decline of the MOC (Table IV). However, rats starved from three weeks of age and also injected with an active pituitary extract (Ellis' fraction C) had a normal decline in their MOC compared to the controls (Table IV). It should be noted that the controls weighed three times more than the starved, injected rats (Table IV).

Effect of age on the T_4 response. It has been previously shown that 3 wk of injection of T_4 are required to obtain a full response (3). Consequently, perpubertal intact or prepubertally thyroidectomized rats could not be used for age comparisons of responses to T_4; the normal decline in the MOC with age was accelerated by T_4 injection in both types of rats within 3 wk. The only suitable immature rats, prepubertally hypophysectomized

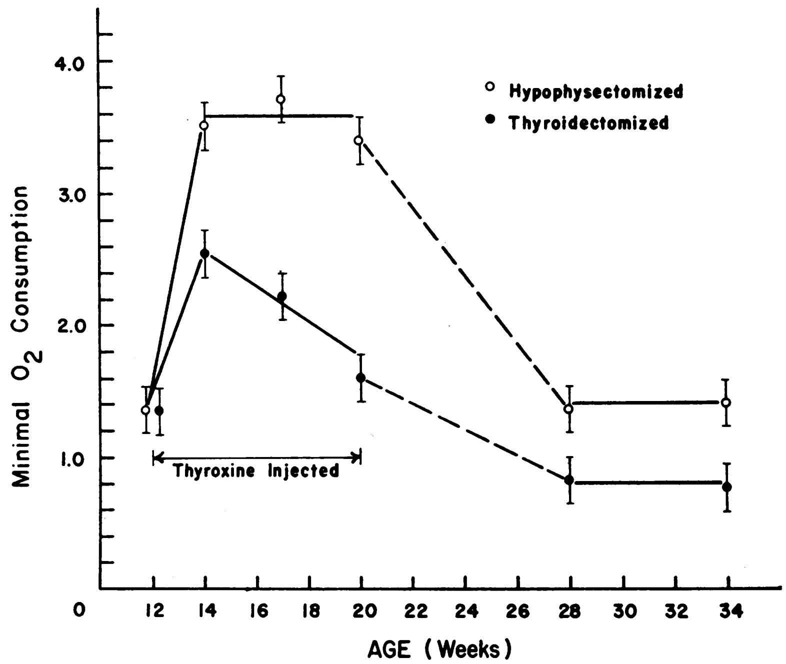

FIGURE 3 Effect of T_4 on the MOC of thyroidectomized and hypophysectomized rats. The MOC's (mean±SD) are given for female rats (6–12 rats per point) hypophysectomized or thyroidectomized at 3 wk of age. The rats were kept for 9 wk after the operations and then injected with 75 μg T_4 for 8 wk. The athyroidal MOC was measured 9 wk after the operation and then again 8 wk after the last T_4 injection. Curves were fitted by eye. T_4 produced significantly lower MOC values in thyroidectomized compared to hypophysectomized rats at all points tested (*P* value, 0.001). The response to T_4 of the thyroidectomized rats after 8 wk of injection was significantly lower than their response after only 2 wk of injection (*P* value, 0.001). The athyroidal MOC of the thyroidectomized rats was significantly lower after T_4 injection than either the preinjection value (*P* value, 0.001) or the athyroidal MOC of the hypophysectomized rats tested before or after T_4 injection (*P* value, 0.001).

rats, were found to have a threefold greater response to T_4 than adult intact or operated rats (Fig. 4).

Three facts emerged from the dose-response curves that required further elaboration or experimentation. (*a*) The adult intact rats appeared to be the least responsive to T_4 of all the rats tested. (*b*) Loss of the pituitary factor should have made the adult hypophysectomized rats more responsive to T_4 at all doses compared to the intact or thyroidectomized adult rats. (*c*) Adult hypophysectomized rats appeared to be more responsive to T_4 only at doses above 50 μg.

Response of intact adults to T_4. Earlier, Hsieh (19) concluded that hypothyroid rats were more responsive than intact rats to T_4 after plotting his response curves as in Fig. 4. However, valid comparisons between two groups can only be made when the responses are compared on the same part of the dose-response curve. The injected T_4 was considered to be equal to the total T_4 for all rats plotted in Fig. 4. For rats without functional thyroid glands this plot was appropriate. However, this plot did not take into consideration endogenous T_4 of intact rats. Endogenous T_4 was considered to be equal to the dose of T_4 (15 μg) that restored to normal the MOC of either hypophysectomized or thyroidectomized rats. When the response of intact rats was replotted as a function of total T_4 present (exogenous + 15 μg endogenous, Fig. 5), it was found to be the same as that of the thyroidectomized rats. No evidence was found by the present methods of bioassay for effective negative feedback control over T_4 secretion. If negative feedback had been operative, the MOC should have remained unchanged as long as the T_4 dose injected was equal to or less than the physiological replacement dose. However, doses of 5 and 15 μg were found to increase the MOC (Figs. 4, 5).

Restoration of responsiveness to T_4. It was disappointing to find that hypophysectomy of adult animals did not restore some youthful responsiveness to doses of T_4 in the range of the physiological replacement doses (5–50 μg). However, it was possible that the normal postoperative wait of 8 wk before testing responses to T_4 may have been too short, and the response to T_4 was therefore tested in thyroidectomized and hypophysectomized rats at various times after operation. The respon-

TABLE III

Depression of MOC by Pituitary Extract

Pituitary extract (mg/kg per day):	MOC (n) 10	30	100
1. Fraction B		3.15±0.37 (8)	2.64±0.22 (8)
Controls		3.38±0.25 (8)	3.55±0.35 (10)
P value		NS	0.001
2. Fraction C	3.02±0.35 (10)	2.58±0.18 (20) (body wt, 125±8 g)	
Controls	3.38±0.31 (10)	3.65±0.21 (18) (body wt, 75±6 g)	
P value	NS	0.001	
3. Focused B′	2.51±0.18 (10) (body wt, 81±9 g)		
Controls	3.10±0.2 (10) (body wt, 67±5 g)		
P value	0.001		

The MOC's (mean±SD) are given for pituitary extract-treated and control rats hypophysectomized at 3 wk of age. Injections were started 1–2 wk after the operation and lasted 4 wk. Pituitary material was injected with T_4 in oil; control rats received T_4 in oil only. Fractions B and C were made according to Ellis (12). Focused B′ was a modified version of Ellis' B fraction subjected to isoelectric focusing (see Methods). The doses of T_4 used were: 75 μg for Exps. 1 and 2 and 65 μg for Exp. 3. The drinking water contained 50 g sucrose, 5 g tetracycline, and 5 mg hydrocortisone succinate/liter and the chloride salts of Na, K, Mg, and Ca at a concentration ¼ that found in plasma. Body weights are given in two experiments to indicate the difference in bioassayable growth hormone present.

siveness of hypophysectomized rats to T_4 increased (Table V); the responsiveness to T_4 of thyroidectomized rats did not change. Thyroidectomized rats were used as controls to rule out any changes in responsiveness to T_4 which were secondary to prolonged hypothyroidism. Rats hypophysectomized as adults and kept for 28 wk after the operation showed nearly the same increase in MOC after 15 and 50 μg of T_4 (0.62 and 1.07 ml of O_2, Table V) as did rats hypophysectomized at 3 wk of age (0.60 and 1.37 ml of O_2, Fig. 4). These results suggested that the effect produced by the pituitary factor persisted for a long time after hypophysectomy.

T_4 pretreatment of operated rats. The preceding experiments suggested an explanation for the greater responsiveness of hypophysectomized rats to high doses of T_4 when they were tested 8 wk after operation, namely, that the high doses of T_4 might have accelerated the normally slow decay of inhibitory pituitary effect by a general stimulation of metabolism. Adult hypophysectomized and thyroidectomized rats were pretreated with high doses of T_4 (150 μg) for 4 wk immediately after operation. After a week they were injected for the usual 3 wk with a dose of T_4 (50 μg) that had previously produced equal responses after either operation. As postulated, T_4 pretreatment increased the responsiveness of hypophysectomized but not thyroidectomized rats (Table VI).

DISCUSSION

Evidence for a pituitary factor that inhibits the effects of T_4 on the MOC. In the past certain minimal criteria have been accepted as necessary in order to postulate the existence of a new endocrine function in a specific gland. (i) Ablation of a specific gland must arrest the function. (ii) In the case of a hormone that is involved in normal development, ablation might also reverse the function once established. (Clearly, the function of some developmental hormones such as growth hormone cannot be reversed.) (iii) Replacement of extracts from the gland in question should restore the function in an animal without the gland. (iv) Increasing (*a*) the rate of endogenous production of the hormone

TABLE IV

Effect of Starvation and Pituitary Extract on MOC

	MOC (n)	Wt (g)	Fat (%)
	A. Starvation, 3–9 wk of age		
Controls, ad libitum	1.62±0.13 (10)	245±24	18
Starved, 35%	2.01±0.12 (9)	78±13	5.5
Starved, 35% + C fraction	1.65±0.15 (9)	77±14	6.0
	B. Starvation, 12–18 wk of age		
Controls, ad libitum	1.33±0.12 (7)	302±28	17
Starved, 35%	1.21±0.10 (8)	175±12	3

Starting at 3 wk of age rats were fed 35% of the food consumed by the control rats, which fed ad libitum (A). The MOC's (mean±SD) of the starved rats were significantly elevated above that of the rats that fed ad libitum (*P* value, 0.001). The pituitary C fraction of Ellis (12), 30 mg/kg per day, was injected into partially starved rats. The MOC of extract-injected rats was significantly lower than the MOC of the uninjected, starved rats (*P* value, 0.001). Partial starvation (35%) when started at 12 wk of age did not significantly affect the MOC of the starved compared to the control rats (B).

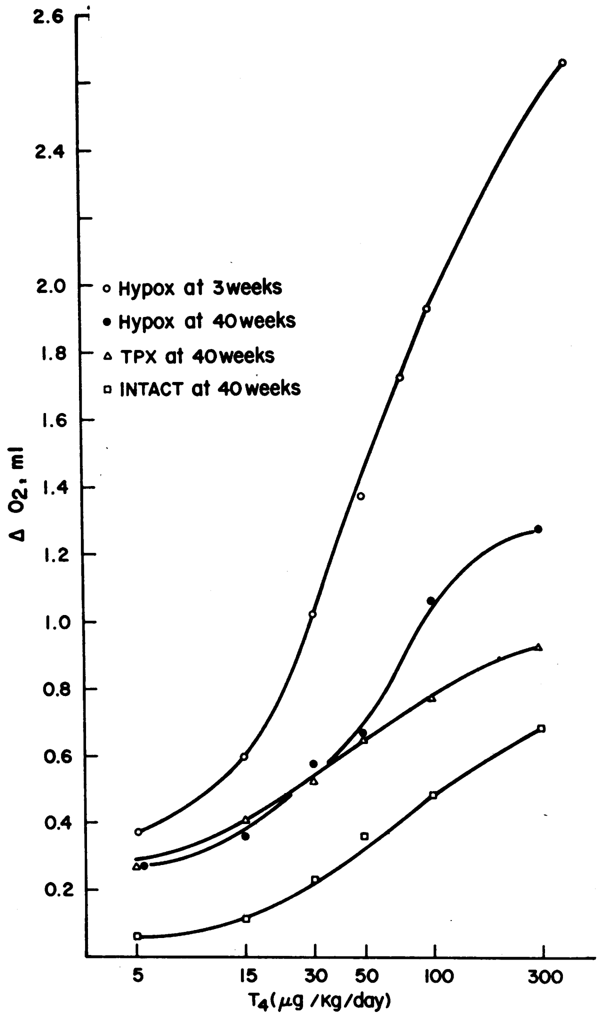

FIGURE 4 T_4 dose-response curves in young and old rats. The MOC's for rats hypophysectomized at 3 wk of age are compared to the MOC's of adult intact and operated rats treated with various doses of T_4 given for 3 wk. For the sake of clarity the standard deviations are not included. At least eight rats were used per data point and the average standard deviation of all points was 7.5±0.4%. Young rats appeared to be at least three times as responsive to T_4 as the adult rats. Curves were fitted by eye.

or (*b*) the amount present by injection of active material should accelerate the appearance of the function. Experiments with the new pituitary factors showed that all 4 criteria were met: (i) Prepubertal hypophysectomy arrests the decline of both the athyroidal and thyroidal MOC (Table II, Fig. 3). (ii) Hypophysectomy of adults restores responsiveness to T_4 (Tables V, VI). (iii) Injection of pituitary extracts decreased responsiveness to T_4 of immature hypophysectomized rats. (iv *a*) Injection of T_4 into intact immature rats accelerated the loss of responsiveness to T_4 (Results). Injection of T_4 into immature thyroidectomized rats caused a decrease in responsiveness to T_4 and in the athyroidal MOC (Fig. 3). (iv *b*) Partial starvation before puberty appeared to decrease the output of the factor and injection of a pituitary extract returned the rate of decline of the MOC to normal (Table IV).

Discussion of the possible mechanisms of action of this new pituitary function and whether its activity is associated with a new hormone will be deferred until purification is complete.

Response to T_4 and age. Most authors agree that the rate of degradation of T_4 decreases two- to fourfold with age (18, 20–24). Gregerman and Crowder (17) disagree with these findings. Consequently, it is possible that the plasma levels of T_4 after a given dose were higher in adult than in immature rats. Therefore, the actual loss of responsiveness to T_4 may be considerably greater than the threefold difference indicated by the dose-response curve. Precise quantitation of the degree of loss of responsiveness will require a knowledge of plasma levels of T_4 after various doses.

Nonuniformity of effects of pituitary factor. Theoretically, a sufficiently high dose of T_4 would make the MOC of adults equal to that of immature rats. However, when an attempt was made to raise the MOC of adults, the adults died before they reached more than ⅔ the MOC value for immature rats (unpublished observation). Apparently, the toxicity to T_4 is not lost even when the MOC response to T_4 is decreased.

Hypothyroidism and age. It was originally thought that hypothyroidism might contribute to the pathology of aging because (*a*) there was a strong clinical similarity between hypothyroidism observed in middle-aged persons and the appearance of "normal" old age (6–8) and (*b*) the supposedly specific test for thyroid status, the BMR, declined with age. This hypothesis was discredited for three reasons: thyroid therapy failed to rejuvenate older persons (7), the BMR was discredited as a specific thyroid assay (3), and plasma levels of thyroid hormones were found to be nearly constant throughout life (16, 17). However, it is evident that the postulated existence of a pituitary factor that decreases responsiveness to T_4 weakens two of the arguments against the original hypothesis. In the presence of this factor thyroid hormone treatment would be expected to fail, and the presence of normal plasma thyroid hormone levels throughout life cannot be used as an argument for euthyroidism. In view of the observations that the MOC appears to be a relatively specific test for thyroid status and that there is an 87% decline in the thyroidal MOC, it seems reasonable to assume that old rats, in some respects, may be severely hypothyroid compared to young rats. However, additional experiments will be required to demonstrate that the systems controlled by the thyroid

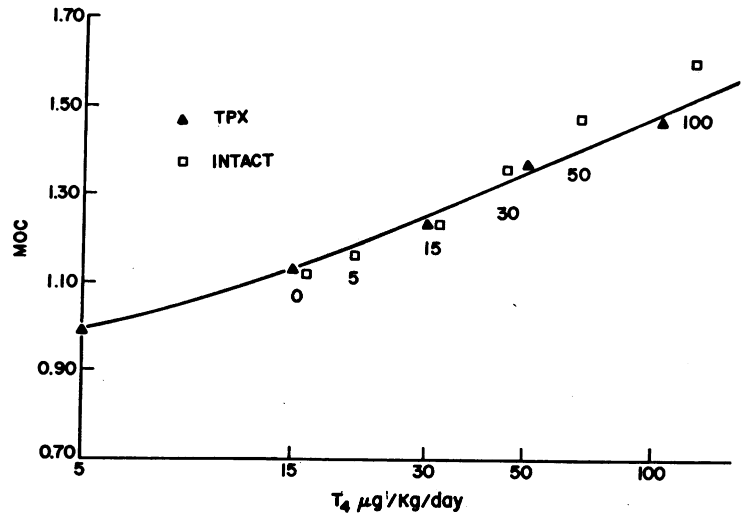

FIGURE 5 Dose-response curve of adult rats plotted as a function of total T_4. The data was replotted from that in Fig. 4. The total T_4 present in thyroidectomized rats was assumed to be equal to the T_4 dose injected. The total T_4 present in the intact rats was assumed to be equal to the sum of the injected dose plus the endogenous T_4 (15 μg). The T_4 injected into the intact rats is indicated below each data point for the calculated total T_4 present. Curves were fitted by eye.

which are necessary for life are as much affected by the pituitary factor as is the MOC.

Endocrine model for McCay's data. The following model is admittedly speculative but it is also eminently susceptible to experimental verification. Prepubertal starvation was shown to retard the normal development of two endocrine-controlled events associated with puberty: (*a*) the onset of sexual maturation (9) and (*b*) the normal decline in the BMR (25) or MOC. Therefore, it may be postulated that prepubertal starvation also pro-

TABLE V

Restoration of T_4 Responsiveness in Adult Operated Rats

	MOC (*n*)		
T_4 Dose (μg)	0	15	50
		8 wk after operation	
Thyroidectomy	0.70±0.04 (22)	1.08±0.05 (20)	1.35±0.09 (24)
Hypophysectomy	0.65±0.06 (20)	1.06±0.08 (15)	1.31±0.09 (8)
P value	NS	NS	NS
		16 wk after operation	
Thyroidectomy	0.80±0.06 (10)	1.13±0.06 (8)	1.40±0.10 (8)
Hypophysectomy	0.78±0.05 (10)	1.40±0.10 (10)	1.67±0.10 (12)
P value	NS	0.001	0.01
		28 wk after operation	
Thyroidectomy	0.88±0.05 (10)	1.17±0.05 (8)	1.45±0.12 (8)
Hypophysectomy	0.85±0.06 (10)	1.47±0.07 (12)	1.92±0.11 (8)
P value	NS	0.001	0.001

Rats were hypophysectomized and thyroidectomized at 40 wk of age and the response to T_4 was measured at various times after operation. The MOC's (mean±SD) indicated a gradual return of youthful responsiveness to T_4 in the hypophysectomized but not in the thyroidectomized rats.

TABLE VI

Effect of T_4 Treatment on Response to T_4 in Adult Operated Rats

	MOC (*n*)	
	Without T_4 pretreatment	With T_4 pretreatment
Hypophysectomized	1.35±0.04 (24)	1.69±0.10 (10)
Thyroidectomized	1.31±0.09 (8)	1.37±0.11 (10)
P value	NS	0.001

The MOC's (mean±SD) are given for female rats operated on at 40 wk of age and tested 8 wk later. The T_4-pretreated rats were injected with 150 μg T_4 for 4 wk immediately after the operation. After 1 wk without injections, they were then injected with 50 μg T_4 for 3 wk. The rats without T_4 pretreatment were injected with 50 μg T_4 for the last 3 wk of the 8-wk period. The MOC of the T_4-pretreated hypophysectomized rats was significantly greater than the similarly treated thyroidectomized rats or the hypophysectomized rats which had not received T_4 pretreatment (*P* value, 0.001).

longs life by retarding the development of an endocrine program which normally limits the lifespan of rats to the 3 yr typical for this species. To be consistent with McCay's data such a hormone would have to meet three criteria; (*a*) the secretion of this hormone would have to be retarded by starvation started before puberty, (*b*) its secretion should not be affected by starvation started after puberty, and (*c*) the hormone would have to be able to shorten the lifespan. The present experiments suggest that the new pituitary factor can meet the first two criteria, and as noted above, there is the possibility that it might also adversely affect systems controlled by the thyroid necessary for life.

APPENDIX

Various nonendocrine factors have been suggested as causes of part or all of the BMR decline with age.

Age. It is possible that age might nonspecifically decrease tissue metabolic rates. The present experiments, summarized in the first part of the Discussion, suggest that the MOC decline is largely under endocrine control.

Body weight. It has been argued that there must be an inverse correlation between body weight and BMR due to unspecified causes. The present and earlier works (3) indicate that the MOC and body weight are probably controlled by separate hormones. The MOC could be decreased by the pituitary factor which blocked the response to T_4 with little change in body weight, and body weight could be nearly doubled by growth hormone without changing the MOC (3).

Loss of cells/unit mass. Neither direct histological measurements of muscle cells/unit volume (26, 27) nor estimates of decrease in active cell mass/per unit total body mass (28, 29) could account for the MOC decline.

Correction factors. Some authors have attempted to prove the BMR does not decline with age by the trial and error determination of mathematical correction factors which, when applied to the primary data, demonstrate that the BMR does not decline with age. The lack of validity of these corrections has been discussed (3).

Viscera. Conrad and Miller (15) estimated that 50% of the BMR decline could be attributed to changes in the ratio of visceral mass/unit body mass. This estimate was based on tissue O_2 consumption rates determined in man that were then extrapolated to the rat. In the present work the estimated O_2 consumption rates of the viscera are based on more recent work in the rat (30, 31). These studies indicate that the viscera used in Table I contribute approximately 25% to the total MOC of adult rats rather than the 60% contribution to the total used by Conrad and Miller as the basis for their calculations.

The organs selected for Table I were chosen because they were used by others (15), they receive over half the blood flow of the combined thoracic and abdominal viscera (31), and there are available both in vivo and (31) in vitro (30) data for their metabolic rates/unit mass. If one assumes (*a*) that the metabolic rate/unit mass of the remainder of the major viscera (stomach, lungs, intestines, spleen, pancreas) is the same as the average for the organs in Table I and (*b*) that the proportion of these viscera by weight decreases to the same extent, then as much as 30% of the decline in the MOC could be attributed to shifts in the weight of viscera with age. However, this calculation does not take into consideration the increase per unit total body mass in adults of tissues with relatively higher metabolic rates such as the primary and secondary sex organs; this increase requires a correction opposite to that in Table I.

For the calculations used by Conrad and Miller (15) and those used in Table I it was assumed without evidence, that the percent decrease with age of metabolic rates/unit mass is the same for each tissue. Clearly this type of calculation is an approximation. However, the data of Tables I and II indicate measurement of the respiration rates of individual tissues at different ages may not be necessary to establish one of the main points of this paper. A 12-wk-old rat is sexually mature and has an almost identical visceral mass to body mass ratio compared to a 52-wk-old rat (Table I). Yet from 12 to 52 wk the MOC decreases nearly by half from 1.95 to 1.11 ml of O_2. The athyroidal and thyroidal MOC declined, respectively, 24 and 76% during this period. It is not apparent how any significant change in body composition or organ weight could occur in this period or account for the marked differential decrease in the two components of the MOC.

In conclusion, perhaps as much as 30% of the total decline in the MOC might be caused by a shift in the relative mass of the viscera. Unknown factors may yet be discovered that can cause a further decrease in the MOC without changing the metabolism of individual tissues. However, it is difficult to conceive how such changes will ever account for altered responsiveness to thyroid hormones observed in rats of similar weights and degree of maturity.

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