min, and glucose concentration was determined in the supernatant by the *o*-toluidine photometric technique (Boehringer-Mannheim GmbH, Blutzucker Farbtest). Under the given conditions of experiment, addition of 50 mg/100 ml final glucose concentration at 10 min proved to be optimal. 10 replica experiments under such conditions had unequivocal results.

The results are shown in the Figure. It can be seen from the curve that the glucose intake of the unicellular organisms was markedly increased by the presence of insulin. The decrease of glucose content in the medium reached 20% at a concentration of 2×10^{-2} mg/ml and almost as much at 2×10^{-3} mg/ml. At the subsequent lower concentrations, the curve began to ascend slowly, until reaching the control level at 2×10^{-7}. The glucose consumption of the unicellular organisms differed significantly ($0.1 > p > 0.05$) between the insulin concentrations 2×10^{-2} and 2×10^{-4}.

The effect of insulin on *Tetrahymena* was formerly studied by HILL[6] and WAITHE[7] with negative results, to which HILL[6] referred in his book as unpublished data, so that no explanation of the failure is possible. WAITHE[7] used too high concentrations of insulin for a too long reaction time; we did not find any effect ourselves under such conditions. The present results however unequivocally show the influence of insulin on *Tetrahymena*, which accords well with our previous observation[3,4] that Protozoa are responsive to certain hormones of higher animals. The phenomenon cannot be explained unless it is postulated that insulin receptors are present in *Tetrahymena*. This does not seem possible unless the receptor corresponds with a given pattern of cell membrane which is already present at the lowest levels of phylogenesis, viz. also in cells not normally related to hormonal activity[8]. The structure and function of the membrane pattern are naturally not regarded as, or referred to as, a receptor until an interaction with a given hormone takes place. This seemed to be true ab ovo for animo acid type hormones, with the reservation that such hormones, being generally present in living biengs, could as well occur in unicellular organisms or their surroundings. The response of *Tetrahymena* to insulin, a polypeptide hormone occurring exclusively in higher animals, is unequivocally in favour of the above hypothesis.

Summary. Insulin stimulates the glucose uptake of *Tetrahymena pyriformis*. This shows the presence of insulin receptors in *Tetrahymena*, consequently receptors may be present in a level of phylogenesis, where the natural contact between the given hormone and the cell is unnecessary and impossible.

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Effects of Hypophysectomy, Bilateral Adrenalectomy and Hormone Replacement Therapy upon Organ Monoamine Oxidase Activity

The in vivo activity of monoamine oxidase (MAO) results from a delicately balanced input of a variety of physiological stimuli, including hormonal secretions[1-4].

Rat heart MAO has been reported to decrease after hypophysectomy (HX)[5], but no change was found by LANDSBERG and AXELROD[6]. Adrenal enzyme activity has been found to decline[7,8]. The in situ removal of pituitary function in the rabbit fetus by decapitation increased MAO activity in several tissues, including the adrenal[9]. The authors suggested that these increases might be related to the ensuing lack of glucocorticoids, since bilateral adrenalectomy (AX) enhances MAO activity[9-11]. However, further studies seem pertinent, since ontogenetic development affects MAO[12] and decapitation is a traumatic procedure.

In the present study, the effects of HX and AX on organ MAO are compared, and the effects of replacement doses of adrenocorticotropic hormone (ACTH) in HX rats and dexamethasone in AX rats were determined.

Materials and methods. Male albino Sprague-Dawley rats, weighing between 160 and 200 g were used. AX, bilateral adrenal demedullation[13] and sham-operations were carried out under pentobarbital anesthesia. The animals were given 0.9% W/V NaCl to drink and were sacrificed 10 days after surgery. Rats were checked visually for remaining adrenal or medullary tissue. Some animals received dexamethasone sodium phosphate (Decadron, Merck Sharp and Dohme, 2×30 µg/rat/day, i.p.) for 10 days.

HX and sham-operated rats were obtained from Zivic Miller Laboratories, Inc., Pittsburgh, Pennsylvania. The animals were kept at 26–27 °C on a normal laboratory diet plus pears and were given water containing 0.9% W/V NaCl and 5% W/V sucrose. The rats were sacrificed 10 days after surgery. Since HX retards growth, the sham-operated rats consisted of 2 groups. Group I was of equivalent age to the HX rats, whereas the group II was younger, so as to be of an equivalent mean weight to the HX rats at sacrifice time. In some experiments, purified ACTH in gelatin (Cortrophin Gel, Organon) was administered (4 units/rat/day, s.c.) for 10 days. The adequacy

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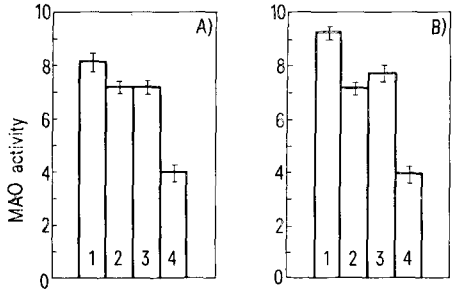
Table I. Mean percentage change in monoamine oxidase (MAO) activity 10 days after hypophysectomy (HX) and bilateral adrenalectomy (AX)

Organ or tissue	Change from control (%)	
	HX (6)	AX (6)
Kidney	−48[b]	− 3
Spleen	−38[a]	+ 47[c]
Adrenal	−38[b]	
Heart	−24[a]	+131[c]
Hypothalamus	−10	+ 47[b]
Rest of the brain	− 4	+ 6
Vas deferens	− 1	+ 34[b]
Liver	+ 2	+ 2
Superior cervical ganglion	+ 9	+ 61[b]

Difference from control: [a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$. Control is expressed as 100% (age matched, sham-operated rats). The number in the brackets denotes the number of animals used. Absolute values for MAO activity may be obtained from the subsequent Tables and the Figure.



Monoamine oxidase activity (μM indolacetic acid/g/h, ± SEM.) in the hearts of hypophysectomized (HX) (10 days) and ACTH (4 units/rat/day, s.c., 10 days) treated rats. A) weight matched, sham-operated controls; B) age matched, sham-operated controls. 6 rats were used in each group. Group 1 = sham-operated; group 2 = HX; group 3 = sham-operated + ACTH; group 4 = HX + ACTH. Significance of differences: A) 4 vs. 1, 2 and 3, $p < 0.001$. B) 1 vs. 2 and 3, $p < 0.05$; 4 vs. 1, 2 and 3, $p < 0.001$.

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of this treatment and the surgery was assessed by changes in adrenal weight. HX rats showed a mean percentage decrease of approximately 70% vs. age matched shams, and a 56% decrease vs. weight matched shams. ACTH increased ($p < 0.001$) adrenal weight in both sham-operated and HX rats.

MAO activity was assayed, on whole tissue homogenates, by determining the indoleacetic acid (IAA) formed from tryptamine in the presence of excess aldehyde dehydrogenase, as reported previously[14]. All values quoted are corrected for recovery.

Results. Table I compares the effects of HX and AX upon the MAO activity of various tissues 10 days after surgery. The results are expressed as a percentage change from the respective sham-operated, age matched controls (*100%*). Whereas HX selectively decreased the activity of MAO, AX selectively increased activity; but the spectrum of affected tissues differed.

With the exception of the hypothalamus, Table II shows that increases in organ MAO following AX are effectively prevented by dexamethasone and that dexamethasone itself, and bilateral adrenal demedullation do not alter MAO activity.

Dexamethasone could have exerted its effects indirectly, by reducing ACTH secretion. Thus, an experiment was made to study ACTH in both intact and HX rats. The results for the heart are shown in the Figure. Panel A shows that neither HX nor ACTH, by themselves, caused any significant change in MAO, but the combined procedure induced a marked drop (about 50%). Compared to the age matched shams (Panel B), ACTH (alone) and HX (alone) produced a small decrease. In other organs, the MAO activity of the sham-operated groups was not significantly different. Thus, the appropriate groups were pooled and the results are shown in Table III. The spleen exhibited a similar change to the heart, but kidney MAO declined by about 58% due to HX. This effect was not reversed by ACTH treatment. A small change in hypothalamic MAO occurred with ACTH alone but no procedure affected the MAO activity of the other organs examined.

Discussion. The effects of AX appear to be due to a deficiency of adrenal glucocorticoids and not to a lack of adrenal catecholamines or resulting elevated levels[15, 16] of ACTH. These findings support and extend previous observations[11]. However, the increase in hypothalamic MAO following AX was found to be unresponsive to dexamethasone therapy. Furthermore, this elevation also seems unrelated to ACTH (Table III).

The effects of HX upon MAO activity appear not to result through adrenal insufficiency, since AX produced a

Table II. Effect of bilateral adrenalectomy (AX), bilateral demedullation (DEMED) and dexamethasone (DEX) (2 × 30 µg/day/rat, i.p.) for 10 days on the monoamine oxidase activity of various rat tissues

Tissues	Sham + V	Sham + DEX	AX + V	AX + DEX	DEMED + V	DEMED + DEX
Heart	7.5 ± 1.1	8.1 ± 1.5	17.3[a] ± 0.9	7.2[b] ± 2.2	9.3 ± 1.7	8.2 ± 1.9
Spleen	4.3 ± 0.8	4.1 ± 0.9	6.3[a] ± 0.9	4.0[b] ± 0.8	4.0 ± 0.7	4.3 ± 0.6
Vas deferens	6.2 ± 0.5	6.4 ± 0.2	8.3[a] ± 0.4	6.4[b] ± 0.4	6.6 ± 0.3	6.2 ± 0.3
SCG	0.9 ± 0.1	0.9 ± 0.1	1.4[a] ± 0.1	0.8[b] ± 0.1	0.9 ± 0.1	0.8 ± 0.1
HT	5.1 ± 0.5	5.4 ± 0.4	7.5[a] ± 0.2	7.3[b] ± 0.6	4.6 ± 0.4	4.3 ± 0.3
Liver	25.5 ± 3.1	24.0 ± 2.9	26.0 ± 4.2	25.5 ± 3.1	26.3 ± 4.5	23.2 ± 2.5
Kidney	3.3 ± 0.5	3.1 ± 0.4	3.2 ± 0.4	3.4 ± 0.7	3.6 ± 0.5	3.4 ± 0.3

SCG, Superior cervical ganglion, μM indoleacetic acid/pair/h (×10). HT, Hypothalamus. Significance of differences: [a]different from Sham + V, $p < 0.01$; [b]different from AX + V, $p < 0.01$. Sham, age matched, sham – operated rats; V, vehicle. MAO activity is expressed as μM indolacetic acid/g/h ± SEM. 6 rats were used in each group.

Table III. Effect of hypophysectomy (HX) and ACTH (4 units/rat/day, s.c.) for 10 days on the monoamine oxidase (MAO) activity of various rat tissues

Organ or tissue	Sham + V	HX + V	Sham + ACTH	HX + ACTH
Spleen	4.2 ± 0.1	3.2 ± 0.1	3.8 ± 0.1	2.1 ± 0.2
Kidney	3.3 ± 0.1	1.4 ± 0.1	3.2 ± 0.2	1.4 ± 0.1
Hypothalamus	5.8 ± 0.2	5.6 ± 0.9	4.9 ± 0.3	5.3 ± 0.1
SCG	0.9 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Vas deferens	6.9 ± 0.4	6.4 ± 0.3	6.9 ± 0.3	6.6 ± 0.3

SCG, superior cervical ganglion, μM indoleacetic acid/pair/h ($\times 10$).
Significance of differences: HX + V vs. Sham + V, spleen and kidney, $p < 0.001$. Sham + ACTH vs. HX + ACTH, spleen and kidney, $p < 0.001$. Sham + V vs. Sham + ACTH, spleen and hypothalamus, $p < 0.05$. HX + V vs. HX + ACTH, spleen, $p < 0.05$. Sham, pooled age matched and weight matched, sham-operated rats; V, vehicle. MAO activity is expressed as μM indolacetic acid/g/h ± SEM. 6 rats were used in each HX group and 12 rats in each sham group.

different spectrum of effects from HX (Table I). Thus, the decreased level of plasma steroids following HX[17] seem insufficient to mimic complete adrenal removal. Also, the total loss of pituitary endocrine control and continued aldosterone secretion are highly likely to be crucial influencing factors.

The kidney proved to be the most sensitive to HX, but the decrease in MAO activity was unresponsive to ACTH. This resistance is unlikely to be due to insufficient ACTH dosage, since adrenal weight was increased (Materials and methods) and MAO activity was affected in other organs. In contrast, HX produced only a minor decrease in heart MAO which seems related to growth retardation. However, ACTH replacement induced a dramatic decline. A speculative interpretation is that HX resulted in the loss of a hormone or hormones which function to enhance cardiac MAO (e.g., thyroid hormones[4]). Concomitantly, ACTH, and perhaps other hormones acting to decrease cardiac MAO were also lost. The net result would be little or no change. However, ACTH replacement would then reveal a marked depression. The failure of ACTH to produce vast changes in heart MAO activity of intact rats could be due to compensatory hormonal readjustments. Similar changes were found in the spleen and further experiments are required to elucidate their precise mechanism(s). The decreased adrenal MAO after HX may be related to atrophy, since most of the enzyme is cortically located[18].

Denervation studies in the heart and kidney show that the vast majority of MAO is extraneuronally located[19–21]. Thus, a marked fall in the MAO activity in these organs after HX and/or ACTH must be almost entirely representative of the extraneuronal enzyme(s). In contrast, studies in AX rats suggest that intraneuronal MAO is predominantly affected[14, 22, 23]. Thus, as well as gross target organ differences between pituitary influences and AX, and different directional changes in commonly affected organs, the locus of the affected MAO also seems to differ.

Summary. Changes in MAO activity after hypophysectomy (HX) are not due to adrenal insufficiency. ACTH failed to reverse the effects of HX and enhanced the depression of cardiac and spleenic MAO. The data suggests both facilitatory and inhibitory effects of pituitary hormones on MAO activity.

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Ist allein die Glandula ecdysalis die Häutungsdrüse von *Lithobius*?

Is only Glandula Ecdysalis the Ecdysial Gland of *Lithobius*?

Die Häutungsdrüsen von *Lithobius forficatus* L. sind im hinteren Kopfbereich und den beiden ersten Rumpfmetameren gelegen[1]. Elektronenmikroskopische Untersuchungen wiesen diese «Lymphstränge» nicht nur als endokrine Drüsen aus, sondern machten auch einen Zusammenhang mit dem Häutungsgeschehen deutlich[2]. Durch Ausschaltexperimente[1] konnte allerdings nicht mit völliger Sicherheit ausgeschlossen werden, dass neben den Gl.ecdysales auch andere Organe dieses Körperbereichs das Häutungshormon synthetisieren und abgeben. Als solche müssen z.B. die als Speicheldrüsen angesehenen Gl.mandibulares, denen sich die Gl.ecdysales anlagern, sowie die ebenfalls paarigen Gl.maxillares II, die im Kieferfussmetamer lateral von ersteren zu finden sind, in Betracht gezogen werden.

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