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INHIBITION OF THE SECRETION OF INTERMEDINE BY D-LYSERGIC ACID DIETHYLAMIDE (LSD 25) IN THE TOAD, *XENOPUS LAEVIS*

By

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In the last ten years the psychic actions of the drug D-lysergic acid diethylamide [LSD-25[1], delysid (Sandoz Ltd, Basle)] have been extensively studied in man and other mammals. Little work, however, has been done on its action in lower Vertebrates. It was found that in fishes and amphibians the influence of LSD manifests itself in both behaviour- and colour-changes. Besides characteristic changes in behaviour a distinct darkening of the teleost fish *Betta splendens* was obtained with LSD by *Abramson & Evans* (1954) and *Evans et al.* (1956). Moreover, *Cerletti & Berde* (1955), and *Berde & Cerletti* (1956, 1957) demonstrated that LSD causes a marked pigment dispersion in the melanophores of the female guppy, *Lebistes* (= *Poecilia*) *reticulatus, in vivo* as well as *in vitro*. Hence, the last mentioned authors assumed that LSD acts directly on the dermal melanophores of this fish. *Kahr & Fischer* (1957) injected LSD into normal and hypophysectomized frogs, *Rana temporaria*. In normal frogs a distinct darkening was obtained; in hypophysectomized frogs, however, no colour-change could be observed. *Kahr & Fischer*, therefore, concluded that LSD acts indirectly, i. e. via the pituitary gland, on the dermal melanophores.

In previous investigations we have shown (*Burgers et al.*, 1953; *Burgers*, 1956; *Burgers & van Oordt*, 1956) that the melanophore reactions in *Xenopus*, induced by mechanical disturbance, e. g. by rough handling, and by certain drugs (adrenaline, nor-adrenaline and other phenylalkylamines) are, contrary

1. In the following only the initials LSD will be used.

to the melanophore reactions in other pale amphibians, caused by similar stimuli and substances.

It was therefore decided to investigate in *Xenopus laevis:*

1. the effect of LSD on the dermal melanophores, and
2. if such an effect could be established, to ascertain whether LSD acts directly or indirectly on these melanophores.

MATERIAL AND METHODS

Xenopus-specimens, reared in our laboratory and weighing from 9–20 gm., were adapted to different backgrounds by placing them in continuous light in white jars (white background) or in black jars (dark background) or in complete darkness. The dermal melanophores of the webs were studied microscopically at a magnification of 100 ×, and by means of *Hogben & Slome*'s (1931) melanophore-index (M. I.).

In preliminary investigations the toads were placed in solutions of LSD in tapwater in different concentrations; the controls were kept in tapwater only. Later, LSD dissolved in a saline solution of the following composition (*Burgers & van Oordt,* 1956) was injected into the dorsal lymph sac of the toads:

NaCl	8.30 gm.	$MgCl_2$	0.10 gm.
$CaCl_2$	0.12 „	$NaHCO_3$	0.10 „
KCl	0.33 „	aqua dest. ad.	1000 ml.

The quantity of fluid injected was always 1 ml.

The *in vitro*-experiments were carried out on the webs of *Xenopus*-specimens, adapted to a white background. These triangular web-pieces were excised between the first and second and between the second and third toes of both hindlegs. In this way 4 test objects were obtained from each animal. The webs were immersed in control- or in test-fluid in small glass-dishes and observed microscopically.

The M. I. of the melanophores of newly isolated webs is rather high (about 2.5); this is due to the excitement darkening reaction of the animal, induced by handling and excision (*Burgers,* 1956). To carry out our *in vitro*-experiments with melanophores possessing a minimal M. I., the webs were therefore first immersed in saline for about 10 minutes, during which time the M. I. decreased to about 1.6–1.8.

To induce pigment-dispersal in the melanophores a commercial corticotrophin-preparation (ACTH; cortrophine, Organon Ltd.), which possesses a high melanophoric activity, caused by the presence of intermedine, was used.

All experiments were performed at a temperature varying from about 19–23° C.

RESULTS

1. *Experiments in vivo*

To establish whether LSD has any effect on the melanophores of *Xenopus laevis*, the water in a white, and in a black jar, and in a jar placed in darkness, each containing 3 toads, was replaced by 250 ml. tapwater containing 5 μg. LSD/ml. It was found that after about 12 hours the M. I. of the toads, adapted to a dark background (B), had decreased from 4.9 to 2.2, the M. I. of the toads

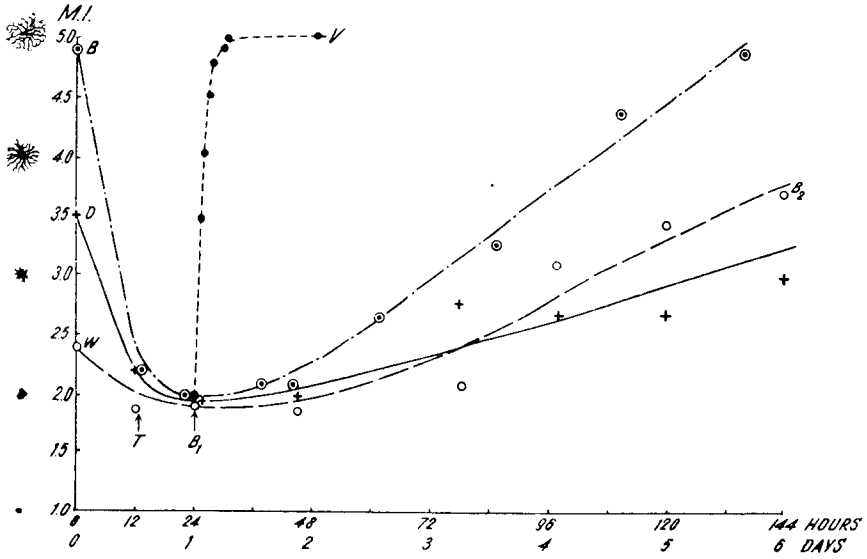

Fig. 1,
showing M. I. changes in *Xenopus*-specimens adapted to a dark (B), and to a white background (W), and to complete darkness (D), caused by placing the animals in solutions of LSD. T indicates the time that the LSD-solution was replaced by tap-water; B_1 the time when the LSD-treated, white adapted animals (small circles) were transferred from white into black jars, and the non-LSD-treated animals (black dots) were placed into a black jar (curve V).

kept in complete darkness (D) from 3.5 to 2.2, and of toads, adapted to a white background (W) from 2.4 to 1.8 (Fig. 1). After the animals had been kept for about 12 hours in this LSD-solution, the fluid was replaced by 1000 ml. tap-water (T). It was then found that the M. I. of the animals in the black jar (B) and the M. I. of the toads placed in darkness (D) increased slowly and reached their initial values after about 6 days.

Due to the fact that a pigment concentration in the melanophores of *Xenopus* is induced by a white background, the continuation of the LSD-effect in the toads, kept in white jars, could not be studied. Consequently after 24 hours these animals were transferred, with their water, from the white into a black jar (cf. the arrow at B_1 in Fig. 1); after more than 7 days their M. I. had increased to about 5.0 (cf. curve B_1 B_2 in Fig. 1).

The time required by the melanophores of LSD-treated *Xenopus*-specimens to attain a maximal M. I. was then compared with that of non-LSD-treated animals. The non-LSD-treated *Xenopus*-specimens, adapted to a white background, were thus placed in a black jar, simultaneously with the transfer of the white adapted LSD-treated animals into the black jar (Fig. 1: at B_1). As is shown by the almost vertical curve B_1V in Fig. 1, the M. I. of these non-LSD-

treated animals increased rapidly to about 5.0. On the other hand, the M. I. of all LSD-treated toads increased slowly. Hence, the time required for the melanin-dispersion in LSD-treated toads is much longer than that in non-LSD-treated animals.

The reactions of the toads treated with LSD are shown in the diagram, Fig. 2.

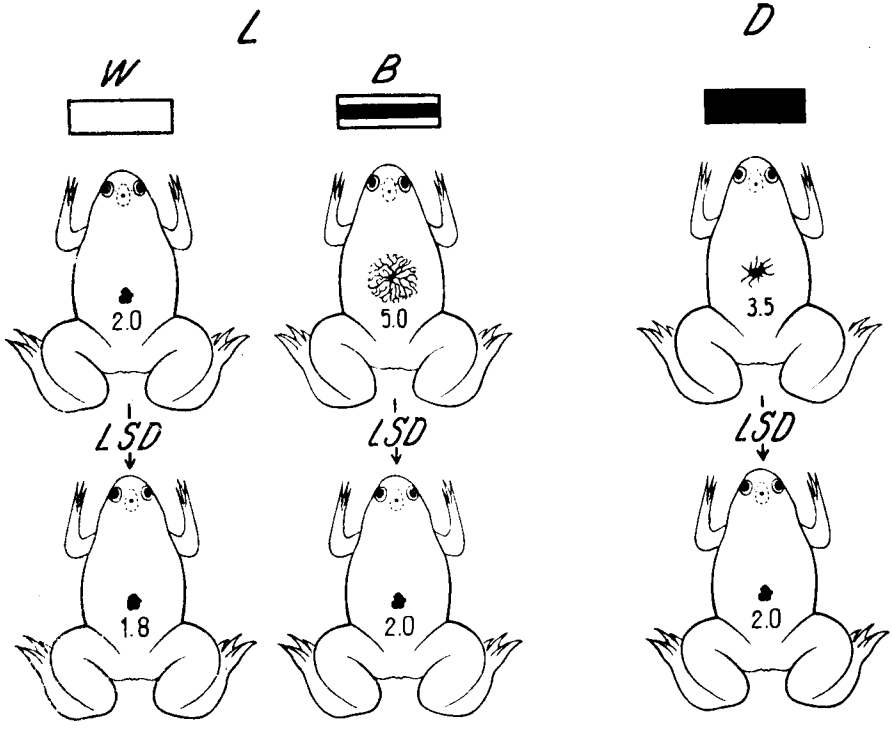

Fig. 2,
showing the effect of LSD on the melanophores of *Xenopus*-specimens, kept in light (L) and adapted to a white background (W) or to a dark background (B) and kept in complete darkness (D). The black dot between the eyes of each toad represents the pituitary gland; on their backs the pigment dispersion stage (M. I.) is shown.

The results of all these experiments show that LSD is absorbed by *Xenopus laevis* and that it has a marked pigment concentrating effect on the dermal melanophores of this species.

It is, however, difficult to estimate the quantity of LSD absorbed. Hence, in a following series of experiments LSD, dissolved in saline in various concentrations, was injected into the dorsal lymph sac of the toads.

In Fig. 3 the reactions of the melanophores of *Xenopus*-specimens, adapted to a dark background and injected with different solutions of LSD are shown.

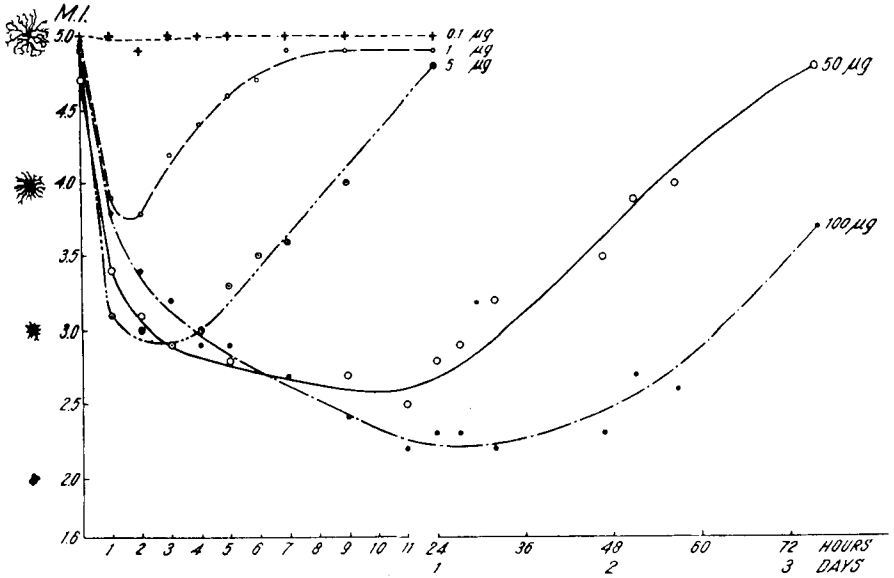

Fig. 3,
showing the M. I. changes in *Xenopus*-specimens, adapted to a dark background, after injection of LSD in various concentrations.

It can easily be seen that the potency and duration of the reaction are directly proportional to the dose of LSD administered.

Moreover, on comparing Fig. 1 with Fig. 3 it is clear that injected LSD acts on the melanophores of dark-adapted animals in the same way as when this drug has been absorbed by the toad.

To determine whether this is also the case in *Xenopus*-specimens adapted to a white background (M. I.: 2.0), animals kept in a white jar were injected with 50 µg. LSD, dissolved in 1 ml. saline. It was found that the M. I. of the melanophores of these animals decreased slightly (M. I.: 1.8).

Our next step was to determine whether LSD acts directly on the melanophores, or indirectly i. e. via the central nervous system and (or) the intermediate lobe of the pituitary gland.

If LSD acts directly on the melanophores then it is likely that the pigment concentration following LSD administration is caused by the inhibition of the pigment dispersing processes in the melanophores. If this were the case it would be expected that the melanophore reactions, following injections of a certain dose of intermedine in LSD-treated and non-LSD-treated animals would differ from each other. Hence the following experiments were carried out (cf. Fig. 4).

Six *Xenopus*-specimens, adapted to a dark background, were each injected with 1 ml. of a saline solution, containing 50 µg. LSD per ml., and divided into two groups of 3 animals (Fig. 4 a). After 3 hours it was found that the M. I. of

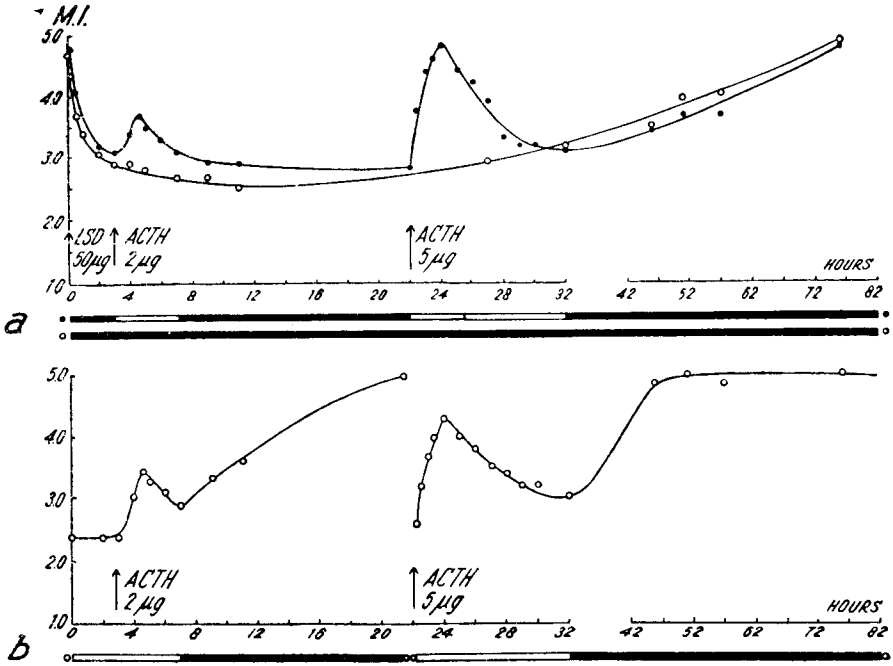

Fig. 4.

LSD- and ACTH [corticotrophin (intermedine)]-effects in *Xenopus*.

a: effect of LSD on the melanophores of two groups of *Xenopus*-specimens, both adapted to a dark background and the increase in M. I. caused by injection of corticotrophin in one group;

b: effect of corticotrophin injected into 2 groups of non-LSD-treated *Xenopus*-specimens, adapted to a white background. (The time spent by the animals in white or in black jars is indicated by the white or black stripes under the figures).

all the animals had decreased from about 4.8 to about 3.0. The toads of one group were then injected with 1 ml. of a solution, containing 2 µg./ml. commercial corticotrophin (intermedine). The M. I. of these animals increased in 1½ hour to a maximum of 3.7; 4 hours after the corticotrophin-injection their M. I. had dropped to 3.1 again. During this period the M. I. of the toads of the second group decreased further to 2.7.

Due to the large dose of LSD injected, the M. I. of these animals was still low on the following day, when a dose of 5 µg. corticotrophin was injected into the animals of the first group. As this corticotrophin-dose was larger, the M. I. also reached a higher maximum and the effect was more prolonged than during the previous day, when only 2 µg. corticotrophin were administered (cf. Fig. 4 a).

In order to compare the effect of corticotrophin in LSD-treated animals with that of corticotrophin in non-LSD-treated animals, 3 toads adapted to a white background (M. I.: 2.4) were injected with 2 µg. corticotrophin (Fig. 4 b)

at the same time as the LSD-treated animals (cf. Fig. 4 a). In the non-LSD-treated animals the M. I. reached a maximum of 3.4 (Fig. 4 b).

On the next day 3 other non-LSD-treated toads, adapted to a white background (M. I.: 2.6; Fig. 4 b) received 5 μg. corticotrophin at the same time as the LSD-treated animals shown in Fig. 4 a. In this case the M. I. of the non-LSD-treated toads reached a maximal value of 4.3 (Fig. 4 b).[2]

The results of these experiments appear to indicate that LSD does not affect the capacity of the melanophores to react to corticotrophin (intermedine).

In further experiments, carried out to establish whether LSD has an effect on the pigment migrations in melanophores, which are not affected by the central nervous system, by the pituitary gland or by the skin secretion, excised webs were used.

2. *Experiments with webs in vitro*

The melanophores of excised webs, immersed in saline, are excellent test objects to determine the sensitivity of these cells to certain substances (*Burgers*, 1956; *Burgers & van Oordt*, 1956).

Extirpated webs of *Xenopus*-specimens adapted to a white background were, after an interval of 10 minutes in saline, immersed in 1 ml. of the following solutions and mixtures:

1. LSD in concentrations of 100, 10 and 1 μg./ml.
2. 0.5 ml. LSD in a concentration of 200 μg./ml. and 0.5 ml. corticotrophin in a conc. of 10 μg./ml.
3. 0.5 ml. LSD in a conc. of 20 μg./ml. and 0.5 ml. corticotrophin in a conc. of 10μg./ml.
4. 0.5 ml. LSD in a conc. of 2 μg./ml. and 0.5 ml. corticotrophin in a conc. of 10 μg./ml.
5. corticotrophin in a conc. of 5 μg./ml.
6. saline only.

It was found that the pigment in the melanophores of the webs immersed in the solutions, mentioned under 2, 3, 4 and 5, dispersed in the same way, whereas the pigment in the melanophores of the webs immersed in the solutions 1 and 6 remained concentrated.

Further experiments were carried out to investigate whether LSD had an

2. For the demonstration of the effect of corticotrophin in non-LSD-treated animals. these must be kept on a white background; for the demonstration of the pigment concentrating effect of LSD, animals in black jars were used. In order to expose all animals injected with corticotrophin to the same background, the LSD-treated animals had to be transferred to a white jar; later when the pigment dispersing effect induced by corticotrophin decreased, all animals were simultaneously placed in black jars (see the black and white stripes under Figs. 4 a and 4 b).

effect on the melanophores in webs, pretreated with corticotrophin. Hence, isolated webs of *Xenopus*-specimens, adapted to a white background, were immersed in 0.5 ml. of a corticotrophin-solution having a concentration of 10 μg./ml.; 1½ hours later the M. I. had increased to 3.5 when 0.5 ml. of a LSD-solution in a concentration of 200 μg./ml. was added. After another 1½ hours the M. I. had reached a value of 4.0. Similar experiments with LSD in concentrations of 20 and 2 μg./ml. gave the same results.

We can conclude that LSD has no effect on the pigment dispersion of the melanophores *in vitro*, since 3 hours after the beginning of the experiments the M. I. of the melanophores in the webs in the corticotrophin (intermedine)-solution – to which LSD had been added – had reached the same value as that of the melanophores in webs which had been immersed during the same period in the corticotrophin-solution only.

A final experiment was performed to determine whether the pigment dispersing activity of corticotrophin was altered in the melanophores of webs, pretreated with LSD.

Webs were therefore immersed in 0.5 ml. LSD-solution of a concentration of 200 μg./ml.; no pigment-dispersion was observed. After 1½ hours 0.5 ml. of a solution containing 10 μg./ml. corticotrophin was added. 3 Hours after starting the experiment the M. I. of these webs was the same as that of webs immersed for 1½ hours in 1 ml. of a solution containing 5 μg./ml. corticotrophin only. The same results were obtained in similar experiments in which LSD in concentrations of 20 and 2 μg./ml. was administered.

The data obtained in all these *in vitro*-experiments indicate that LSD has no influence on the capacity of the melanophores to react to intermedine. This is supported by the results of our experiments *in vivo* (cf. p. 192).

The assumption that LSD acts directly on the melanophores of *Xenopus* (pag. 195) must therefore be abandoned. Consequently the results of our experiments indicate that LSD acts indirectly, and inhibits the production and/or the secretion of the melanophore hormone in *Xenopus laevis*.

DISCUSSION

Amongst the lower vertebrates, investigated so far, *Xenopus laevis* is the only species, which reacts with pigment concentration instead of pigment dispersion following the administration of LSD.

In the fishes *Betta splendens* (*Abramson & Evans*, 1954; *Evans et al.*, 1956) and *Lebistes reticulatus* (*Berde & Cerletti*, 1956) LSD, in doses of 1–2 μg./ml. per 100 ml. swimming water (*Betta*) or per 30 ml. swimming water (*Lebistes*), causes a marked pigment dispersion in the melanophores.

As far as we know *Rana temporaria* is the only amphibian, in which the

effect of LSD on the skin pigmentation has been studied: *Kahr & Fischer* (1957) found that the injection of 50 μg. LSD in a pale specimen of *Rana temporaria* causes a marked darkening. The effect of LSD on dark frogs, however, was not investigated.

As we found that

1. LSD, even in a dose of 1 μg., causes a pigment concentration in *Xenopus*-specimens, adapted to a dark background, and
2. an injection of the same dose of LSD as used by *Kahr & Fischer* - 50 μg. - in *Xenopus*-specimens, adapted to a white background, causes no pigment dispersion at all,

it must be concluded that there is a fundamental difference in the action of LSD on the colour change regulating system of *Rana temporaria* on the one hand, and of *Xenopus laevis* on the other.

The action of LSD on the colour pattern of the guppy, *Lebistes reticulatus*, is assumed to be a direct one, since *Berde & Cerletti* (1957) showed that this substance has the same effect on the melanophores of this fish *in vivo* as *in vitro*. In *Rana temporaria*, however, LSD seems to have an indirect action, for the melanophores of hypophysectomized frogs do not react after LSD administration (*Kahr & Fischer*, 1957).

The results of our experiments indicate that in *Xenopus* LSD also acts indirectly. The difference in reaction to LSD in *Lebistes* on one hand, and in *Rana* and *Xenopus* on the other, may be explained by the fact that in fishes the chromatophores are controlled by both nervous system and by hormones, whereas in amphibians the action of the melanophores is chiefly hormonal.

In previous investigations (*Burgers et al.*, 1953; *Burgers*, 1956; *Burgers & van Oordt*, 1956) it was shown that the melanophore reaction of *Xenopus* to rough handling and to certain drugs is contrary to that of most other amphibians. The discrepancy of the results obtained by *Kahr & Fischer* (1957) in *Rana temporaria*, and the present ones in *Xenopus laevis* may be due to a fundamental difference in the physiology of the pigment migration in the melanophores of these two amphibian species. It seems, therefore, worth-while to collect more data on the reactions of the melanophores, induced by LSD in *Rana temporaria* adapted to a dark background and in *Rana*-species in general.

SUMMARY

1. LSD causes a marked pigment concentration in the melanophores of *Xenopus laevis*. The reaction is similar whether the animals are kept in a LSD solution or whether the drug is injected.
2. The extent and duration of the pigment concentration reaction in the melanophores following LSD-administration is directly proportional to the concentration of the drug administered.

3. Intermedine causes pigment dispersion in the melanophores of *Xenopus*, which are adapted to a white background and in melanophores in which melanin-concentration has been induced by LSD.
4. The pigment dispersing reaction caused by corticotrophin (intermedine) in the melanophores of isolated *Xenopus*-webs is not influenced by LSD in different concentrations.
5. It is assumed that in *Xenopus* LSD does not act directly on the melanophores but indirectly i. e. that this drug inhibits the production and (or) the secretion of the melanophore hormone in the intermediate lobe of the pituitary gland.

ACKNOWLEDGMENTS

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