maxima between 0.01 and 0.30 *M* sucrose concentrations, the greatest swelling occurring at 0.04 *M*, which was the molarity used in the thiamine deficiency studies. Liver mitochondria, on the other hand, showed one unique maximum at 0.15 *M*. The variety of agents that induce or enhance the swelling of sarcosomes was found by us to be much more restricted [16] than for liver mitochondria [6, 8]. The sarcosomal receptors for swelling-inducer compounds appear to be sites in the pharmacological and enzyme kinetic sense. It was now found that the sarcosomal swelling reaction velocities *versus* concentration of inducer (DPN, PCP, or PCMB) follow the Michaelis-Menten equation (liver mitochondria, see [17]).

Résumé. La combinaison des substrats – acides pyruvique et fumarique – inhibent le gonflement de mitochondries isolées du coeur de rat, en accord avec le maintien de l'intégrité de la structure mitochondriale par l'adénosine triphosphate produite. On constate la diminution successive de cette inhibition dans des rats déficients en thiamine, par suite du blocage au niveau de la décarboxylation pyruvique. De plus cette carence vitaminique provoque une décroissance graduelle du nombre des mitochondries dans le tissu cardiaque.

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On the Action of β-Melanocyte Stimulating Hormone (β-MSH) on Spontaneous Electric Discharge of the Transparent Knife Fish, *G. eigenmannia*

In previous communications from these laboratories [1–3], it was reported that β-MSH facilitated submaximally induced spinal reflexes in the cat, although behavioral changes were not observed unless the animal had been depressed previously. Failure to have observed behavioral changes might be attributed to two factors: either β-MSH does not modify behavior except under unusual conditions; or β-MSH modifies behavior, but the change could not be detected.

The second supposition stimulated our interest in quantitation of behavioral changes in a species for which special experience with psychological techniques would not be required.

On the basis of EULER's observation that the nervous system of various fishes contain substance P [4], KRIVOY and LANE (in preparation) found that LSD modified the spontaneous electric discharge of the transparent knife fish. It was further observed that LSD and substance P combined produced a greater effect than LSD alone. The nature of the effect was a decrease in the number of alterations in amplitude of the spontaneous 300 cycle per second discharge which is continuously emitted by this fish. Since we had been able to demonstrate the actions of substance P on this species, it became of interest to determine the effects of β-MSH.

Methods and Materials. Fishes were placed in individual aquaria which had been divided into two unequal compartments by means of a plastic screen. One fish was kept in the smaller compartment and a pair of silver electrodes was placed in the larger. The electrodes were placed close together and at some distance from the fish. The placement of these electrodes was such that they recorded changes in the amplitude of the discharge from the fish-generator, as opposed to changes due to simple geometric manipulation associated with free movements of the fish. The potential was amplified, recorded on tape, and simultaneously monitored on a cathode ray oscilloscope. For analysis of this activity, the tape was played back through an analysis system designed to count the number of changes in amplitude of the 300 cycle per second (cps) signal. A band-pass filter was used to reject artefacts (60 cps etc.) that were recorded with the signal. The signal was then amplified, rectified and integrated, yielding the contour of the 300 cps signal. This signal was then capacitance coupled to a squaring amplifier in a manner which would provide a square wave corresponding to each change in amplitude of the 300 cps signal. These square waves were then counted by means of a decade counter to give the number of amplitude changes. Analysis of the record for changes in the fundamental 300 cps signal showed this not to be influenced by drug administration.

Prior to each experiment, the aquarium was enclosed to maintain the fish in relative darkness for a period of 2 h before any drug was administered. At the end of this time, the electrical activity was recorded on tape for a period of 1 h, and the drug to be tested was then pipetted into the aquarium.

The β-MSH was prepared by the method of SCHALLY et al. [5], by Dr. J. Fischer, of the Armour Pharmaceutical Co. It had a potency of 5×10^6 U/mg.

Results. Disturbances, such as modification of the conductivity of the environment of the fish, or the introduction of drugs (KRIVOY and LANE, in preparation), resulted in the production of a potential of constant strength relative to the control period. The frequency of discharge of each fish was found not to change, even during strong stimulation, or from day to day.

With β-MSH concentrations of 0.1–0.2 μg/ml the frequency of change of amplitude was reduced to from 19–65% of control values ($P = 0.1$ or less). This effect became maximum between 40 and 90 min after introduction of the β-MSH. The changes observed with concentrations below 0.05 μg/ml were not consistently significant ($P \geqq 0.1$).

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α-MSH, 2 μg/ml, was observed not to affect the normal frequency of amplitude changes.

Neither β-MSH nor α-MSH produced any change in the frequency of the basic 300 cps signal.

Discussion. The results presented clearly show that β-MSH, like LSD and provocative stimulation, decreases the number of times the transparent knife fish alters the amplitude of its spontaneous discharge. These data are in agreement with the suggestion that β-MSH enhances the excitability of the mammalian nervous system[1-3], and would imply that this may be part of its physiological role in the fish as well as in the mammal. However, from the observations described here, we cannot determine whether this action in the transparent knife fish is due to a direct action on the nervous system. It would appear to be relatively specific for β-MSH since α-MSH had no such action in the doses used.

The latent period required for activation of the knife fish was longer than that reported for the actions of β-MSH on the spinal cord[1-3] in mammals. This long latent period in the fish is of the order of that required for maximal dispersion by MSH of the melanin granules in the melanocytes of the frog and may simply be peculiar to actions of MSH in cold blooded animals. It is, however, reminiscent of the latent period required for β-MSH activation of the 'stretching crisis' described by FERRARI, GESSA, and VARGIN in the dog[6].

The electric fish appears to be an effective tool for the study of drug action. In addition, the observations derived from the present studies, as well as those published earlier[1-3], suggest that the possible clinical significance of β-MSH should be evaluated in a variety of neurological and psychiatric diseases[7].

Résumé. L'hormone mélanophorétique β-MSH, à des concentrations de 0.1 à 0.2 μg/ml dans l'eau de l'aquarium, produit une diminution de la fréquence des modifications spontanées de l'amplitude des décharges électriques du poisson *G. eigenmannia*. Ces résultats sont en accord avec les observations antérieures montrant divers effets de β-MSH sur l'activité électrique du système nerveux central chez les mammifères.

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Experiments Concerning the Incorporation of Labelled Adenine into Ribonucleic Acid in Normal Sea Urchin Embryos and in the Hybrid *Paracentrotus*♀ x *Arbacia*♂

In our previous studies[1-5], it was found that there is a distinct increase in the content of desoxyribonucleic acid (DNA) in normal sea urchin embryos from early blastula to the pluteus stage. The same conclusion has been reached by other authors (for references, see WHITELEY and BALTZER[1], CHEN[6]). The increase is rapid in *Paracentrotus lividus* (PP) and *Sphaerechinus granularis* (SS), but apparently much slower in *Arbacia lixula* (AA) (compare[5], Figure 3).

In the hybrids, the behaviour of DNA is different according to the parental species used. In the combination *P. lividus* ♀ × *A. lixula* ♂ (PA), the DNA synthesis becomes already reduced at the end of blastulation and the beginning of gastrulation. At a later period, the values are approximately intermediate between the parental species. In the reciprocal combination AP, the DNA content is at first the same as that in the maternal controls; after 25 h, it amounts to only 50% of AA. For the hybrid combinations of *P. lividus* × *S. granularis*, the DNA synthesis is almost normal in SP, but strongly inhibited in PS (about 50% of PP). In the last combination, cytological evidence indicates that most of the paternal chromosomes are eliminated at the early cleavages[3-7].

One significant result of these studies is that the total quantity of ribonucleic acid (RNA) remains constant during normal development of all three sea urchin species. Even in the hybrid embryos, the total RNA remains normal in spite of the reduction in DNA (see Figure 7 in BALTZER and CHEN[3]; Figure 4 in CHEN, BALTZER and ZELLER[4]). In other words, the inhibition of DNA synthesis has no effect on total RNA. This result is unexpected and appears inconsistent with the current hypothesis that DNA acts as a template for the synthesis of RNA which plays a key role in the biosynthesis of proteins. It is possible that in spite of the constancy of total RNA, changes in the turnover of this nucleic acid take place in the course of morphogenesis. In the hybrid embryos, the inhibition of DNA synthesis perhaps affects only the metabolic processes in RNA and has no influence on its total quantity. As a first step in our attempt to check this important point, investigations have been carried out to analyse the incorporation of radioactive adenine into RNA in normal embryos and in the hybrid PA. We found that the patterns of RNA turnover in the controls is species-specific, and that in the hybrid it differs distinctly from the parental species. Although our experiments are still of a preliminary character, they have yielded a new aspect on the morphogenetic role of RNA, especially for the hybrid development. In the following, some major points of our recent results will be summarized.

The sea urchin species *Paracentrotus lividus*, *Sphaerechinus granularis* and *Arbacia lixula* were used in the present study, which was carried out in the Zoological

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