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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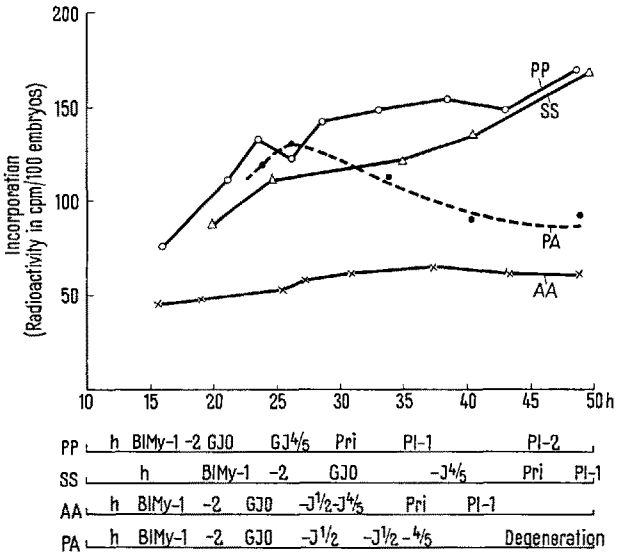
Station at Naples in March and April 1962. The hybridization of PA was performed according to the method of ELSTER[8] using 0.1 *N* NaOH and *Arbacia* egg water. All cultures were kept at 18°C. For *Paracentrotus*, *Sphaerechinus* and the hybrid PA 100, and for *Arbacia* 200 embryos were used in each analysis. The radioactive precursor was adenine-8-C^{14}-sulphate (28.3 mC/m*M*) from the Radiochemical Centre, Amersham (England).

Biochemical analysis (for details, see CHEN[9,10]): Embryos of desired developmental stages were counted and concentrated to a minimal volume of 30 µl in a siliconized glass tube. To this 50 µl sea water with labelled adenine (6 µC/ml) were added. The final isotope concentration in the incubation medium was 3.75 µC/ml. The embryos were incubated at room temperature (20–22°C) for exactly 40 min. At the end of incubation, 20 µl absolute ethanol were added to stop the uptake process. Procedures for extracting RNA were the same as those described by SCOTT, FRACCASTORO and TAFT[11] which have been also used in our previous studies[9,10]. In general, the acid- and fat-soluble substances were removed by treating the embryos first with 50 µl of 0.3 *n* perchloric acid and then with 100 µl of 80% ethanol. Finally they were washed three times in 100 µl of an ethanol–ether mixture (3 volumes 96% ethanol + 1 volume ethylether). All solutions were previously cooled, and during the treatments the tubes were kept in an ice-cold water-bath. RNA was extracted by treating the embryos with 50 µl of 1 *n* NaOH at 22°C for 60 min. After adding 10 µl of 6 *n* HCl and mixing the contents thoroughly by stirring, the reaction tubes were cooled down immediately in an ice-bath and then centrifuged.



Changes in the incorporation of ^{14}C-adenine into RNA during the embryonic development of PP (o), SS (Δ), AA (x) and PA (•). Ordinate: incorporation expressed as radioactivity in counts per min (cpm) per 100 embryos; abscissa: age in hours after fertilization at 18°C. Each point represents the average value of 2 to 6 determinations from 4 separate series (35, 38, 39, 40) for PP, 3 series (36, 39, 40) for AA, 3 series (38, 39, 40) for PA and one series (37) for SS. Symbols for the embryonic stages: h = hatching, BlMy-1 = early blastula with moving-in of the primary mesenchyme cells, BlMy-2 = late blastula with ring of mesenchyme cells, GJO = beginning of gastrulation, $J^1/_2$ = middle gastrula with gut half invaginated, $J^4/_5$ = late gastrula with gut fully invaginated, Pri = prisma stage, Pl-1 and Pl-2 = early and late pluteus stages before feeding.

The supernatant solution was carefully pipetted into a planchet and dried at 60°C for at least 24 h. The radioactivity of the RNA extract was measured in a windowless gas-flow counter from the Tracerlab.

It should be mentioned that almost no radioactivity could be detected in the residue after the treatment with NaOH. Our efforts to extract DNA by treating the residue with 1.6 *n* perchloric acid at 60°C also failed to detect any measurable amounts of radioactive substances in the extract. This indicates that under the present experimental conditions the labelled precursor was almost entirely incorporated into RNA.

As summarized in the Figure, the uptake of ^{14}C-adenine in normal development shows a species-specific pattern. For PP there is a rather rapid increase in incorporation from the early mesenchyme blastula (the earliest stage studied by us) to the beginning of gastrulation. During gastrulation there seems to be a slight drop. After that the increase continues, although more slowly, until the pluteus stage. The same results have been reported by MARKMAN[12] from his study on the radioactivity in whole embryos with the same labelled precursor. The increase in incorporation from the early mesenchyme blastula to the prisma stage is about double in both studies. For SS we found that its pattern of incorporation is very similar to that for PP, but apparently somewhat slower at all corresponding stages. This is obviously due to the fact that the development of this sea urchin species proceeds more slowly.

Compared to the above two species, the rate of isotope uptake is very low in AA. There is only a slight increase from the mesenchyme blastula to the early gastrula and thereafter it maintains a rather constant value until the pluteus stage. As can be seen in the Figure, the over-all relative values of radioactivity in AA amount to only 35 to 50% of those in PP. In this connection it should be mentioned that, according to our earlier data, the egg volume of AA is about 59% and its total N 64% of PP (see CHEN[6,13]).

The most interesting point is the remarkable similarity between the pattern of DNA synthesis and that of the ^{14}C-adenine incorporation into RNA for the three sea urchin species. In our previous studies we demonstrated that the DNA formation rises rapidly in PP and SS, with obviously a slight delay in the later species. In AA the DNA increase is much less and shows a plateau after gastrulation[3–5]. Such a similarity suggests the close metabolic relations between the two nucleic acids, although it provides us with no direct evidence for the DNA control of RNA.

Our data on the hybrid PA are less extensive. As shown in the Figure, its incorporation of ^{14}C-adenine is still close to that of the maternal controls up to about 26 h after fertilization. From the beginning of morphogenetic inhibition, the rate of uptake decreases continuously until the onset of degeneration. The overall values are however still higher than those of the paternal species. *Thus, in contrast to the total quantity of RNA which has been shown to be normal, that means equal to*

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that of PP, throughout the hybrid development, there is a definite reduction in its turnover. Since the synthesis of DNA in this hybrid is also reduced, this indicates again the metabolic correlation between the two nucleic acids[14].

Zusammenfassung. Inkubation der Seeigelkeime mit ^{14}C-Adenin ergab, dass bei reinen Arten der Einbau der markierten Substanz in die RNS von der Mesenchymblastula bis zum Pluteus eindeutig zunimmt. Parallel zur DNS-Synthese verläuft die Zunahme bei *Paracentrotus* und *Sphaerechinus* viel rascher als bei *Arbacia*. Beim Bastard PA nimmt dieser Einbauprozess mit Beginn der morphogenetischen Hemmung (nach ca. 26 h) rasch ab.

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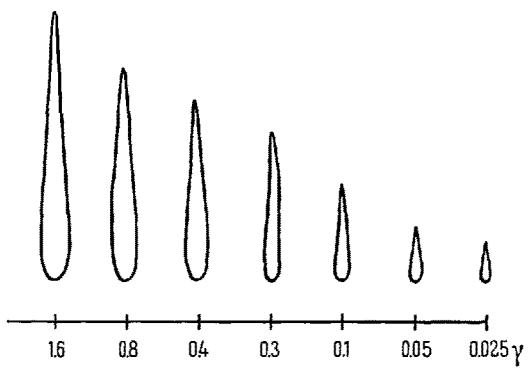
PRO EXPERIMENTIS

A Novel Quantitative Application of Thin Layer Chromatography: Assay of Tryptamine

Thin layer chromatography is a technique which is usually used for qualitative determinations. Previously recognized methods of quantitative analysis have been based on elution and subsequent UV analysis[1] and, more often, on the measurement of the area of the zone[2–4]. The method which we have used is based on the measurement of the distance between the spot of application and the front of the zone. (This may be proportional to the area but is considerably easier to measure.) It is not to be considered quantitative analysis of a chromatogram but rather as the use of a chromatogram for quantitative measurement.

A 20 cm square glass plate was covered with a layer (250 μ) of Kieselguhr-G (Merck, Germany), dried at 120°C for 1 to 3 h and stored at room temperature over $CaCl_2$ for 24 h or more. Tryptamine was spotted in 0.01 to 0.10 ml of chloroform, 1.5 cm from the bottom edge of the plate which was then developed with acetone (analytical reagent grade) and water (99 : 1, v/v) in a closed container. The solvent front reached the 10 cm end point in 10 to 15 min. The plate was then air-dried and sprayed with a freshly prepared 1 : 1 mixture of 1% aqueous ferric chloride and 1% aqueous potassium ferricyanide[5]. The tryptamine immediately turned a deep blue color, whereas the background developed a lighter blue color in about 2 to 3 h. The Figure is a photograph of such a plate (made after the blue streaks were outlined with a stylus) and shows the relationship between the quantity of compound and the distance of the movement of the front over the range of 0.025 μg to 1.6 μg. Greater quantities of tryptamine can be read, but as the gradient is steeper, accuracy is better at these lower levels. Since it is impractical to spot more than 0.1 ml of solution on a chromatoplate, the sensitivity of this method for tryptamine is 0.025 μg/0.1 ml. This sensitivity can be increased in application to analysis of biological tissues, if necessary, by the use of appropriate proportions of extractants or by concentration steps.



Relationship between quantity of tryptamine and the movement of its front on a thin layer chromatoplate. Kieselgur-G–Acetone-water 99:1 v/v.

The actual distance a given quantity of tryptamine moves from its point of application varies slightly from one plate to the next; thus, it is necessary to apply standard quantities to each assay plate. This variability is mainly the result of differences in the degree of hydration of the adsorbent[6]. Precision on a given plate, however, is good. One plate with 6 identical spots containing 0.1 μg tryptamine, gave streaks with a mean length of 29.4 $\pm$ 0.6 mm. Another plate with 6 identical spots containing 1.0 μg tryptamine gave a mean streak length of 49 $\pm$ 3.0 mm.

The application of this method to analysis involves standard preliminary procedures. For example, a 2.0 ml sample of canine plasma was made alkaline by the addition of 0.1 ml of 1 *N* NaOH and then extracted by shaking with 0.5 ml of chloroform. After centrifugation, the aqueous layer was aspirated and the remaining weak emulsion was broken by the addition of a small quantity of anhydrous sodium sulfate. A measured volume (0.05 or 0.10 ml) of the dried chloroform extract was then spotted on the thin layer chromatographic plate. Known amounts of tryptamine were added to plasma and yielded recoveries which were essentially quantitative. Thus, with this method, for each 0.10 μg of tryptamine per ml of plasma, each 0.1 ml of the chloroform extract will contain 0.04 μg, an amount easily measured on the plate.

The specificity of this method is not that usually expected with chromatography since streaks or trails

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[6] During the winter, the relative humidity in our laboratories was low, and satisfactory results could be obtained by airdrying the plates. However, with the advent of spring and rise in humidity, the tryptamine tended to follow the solvent front, so this procedure was no longer feasible. Therefore, all plates are now oven-dried.