EXTRACTION OF MELANOPHORE CONCENTRATING HORMONE (MCH) FROM THE PITUITARY OF FISHES

KATSUTOSHI IMAI

Department of Experimental Biology, Endocrinological Laboratories, Gunma University, Maebashi

While it has long been admitted that intermedine originating in the pituitary is responsible for pigment dispersion within the integumentary melanophores of lower vertebrates, the presence of another pituitary principle affecting the melanophores in reversed way was suggested by rather inconclusive data (Hogben, 1936; Hogben and Slome, 1931 and 1936), till the recent rise of concrete information. The authors cited here presumed the idea on the basis of observations indicating that pigment concentration within the melanophores was disturbed in the absence of pars tuberalis in the frogs or of pars distalis in the elasmobranchs, but failed to prove its actuality by injection or implantation experiments. On the other hand, among the bulk of studies dealing with the mechanism of control of the pigmentary effector system in fishes, there are several reports showing that, depending on occasional circumstances, some of the crude extracts prepared from the fish pituitary would induce pigment concentration, not pigment dispersion (Hewer, 1926; Healey, 1940 and 1948; Weisel, 1948; Kazanskii and Persov, 1948 and 1949).

Recently, Enami (1955) has disclosed that raw extract or alcohol fraction of the extract from the pituitary of Japanese species of the catfish induced significant pigment concentration in the dermal melanophores of the fish both in vivo and in vitro. The effective principle, which has been tentatively called melanophore concentrating hormone (MCH), is inferred as being produced by a kind of hypothalamic neurosecretory activity and transported for storage to the so-called "Übergangsteil" of the pituitary ("meso-adenohypophysis" by Pickford and Atz, 1957). According to the recent monograph by Pickford and Atz (1957), who adopted name "MCH" proposed by Enami, occurrence of this hormonal principle is demonstrated in the adenohypophysis of twenty-one species of teleosts, suggesting the possibility of wide distribution of MCH in the Order Teleostomi. Unlike the melanophore expanding hormone (MEH) which has recently been elucidated as being composed of several kinds of amino acid united in a polypeptide linkage with configuration similar to ACTH (Geschwind and Li, 1957; Geschwind, Li and Barnafi, 1957; Harris and Lerner, 1957), the melanophore concentrating hormone has been yet sufficiently explored its chemical properties. Present research aimed to access to the chemical or physico-chemical properties of MCH.

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Materials and Methods

**Materials** Extraction of MCH was carried out from the pituitaries of the carp, *Cyprinus carpio*, weighing in the range of 350-400 g, supplied by a local dealer. While most of the present experiments were done with a crude extract in which the activity of MCH was detected, some were made out with raw water extracts of the pituitaries and of the hypothalami of the catfish (or rather bullhead), *P. asotus*.

**Assay of MCH** The activity of MCH was assayed upon the dermal melanophores in isolated thin sculp of the catfish according to the method proposed by Enami (1955). Small pieces of the skin were put into physiological saline for freshwater teleosts recommended by Yamamoto (1949), and washed by three exchange of the saline. Then the skin pieces were exposed to a fluorescent lamp for about 30-60 mins. till the melanophores attained states of pigment dispersion beyond semi-dispersion. Melanophore responses were recorded in the conventional melanophore indices (M.I.) first introduced by Hogben and Slome (1931). In terms of M.I., pigmented states of the melanophores at the maximal response to bright light were generally designated as 3.5-4. Test for MCH activity was performed upon replacing the medium of such melanophores with extracts dissolved in 1 ml saline.

**EXPERIMENTALS**

**Preliminary extraction of MCH**

Two hundred freshly excised carp pituitaries were frozen, ground in a mortar, and dried *in vacuo*. Dried tissue powder was then washed thrice with petroleum ether, and petroleum ether-soluble fraction was discarded. The residue was added

<table>
<thead>
<tr>
<th>Procedure of extraction of MCH from carp pituitary</th>
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<tbody>
<tr>
<td>Frozen-dried pituitaries</td>
</tr>
<tr>
<td>Petroleum ether</td>
</tr>
<tr>
<td>Residue</td>
</tr>
<tr>
<td>Acidified water (pH 4)</td>
</tr>
<tr>
<td>Boiling</td>
</tr>
<tr>
<td>Supernatant (discarded)</td>
</tr>
<tr>
<td>Supernatant</td>
</tr>
<tr>
<td>Lyophilization</td>
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<tr>
<td>95% ethanol</td>
</tr>
<tr>
<td>Residue</td>
</tr>
<tr>
<td>(Fraction I)</td>
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<tr>
<td>Supernatant</td>
</tr>
<tr>
<td>Drying</td>
</tr>
<tr>
<td>Water + Ethyl ether</td>
</tr>
<tr>
<td>Residue</td>
</tr>
<tr>
<td>(Fraction II)</td>
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<tr>
<td>Water layer</td>
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<tr>
<td>Ethanol up to 60%</td>
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<tr>
<td>Ether layer</td>
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<tr>
<td>(Fraction III)</td>
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<tr>
<td>Precipitate</td>
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<tr>
<td>(Fraction V)</td>
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<tr>
<td>Supernatant</td>
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<tr>
<td>(Fraction IV)</td>
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with 5 ml of distilled water acidified with diluted HCl to pH 4, and boiled at 100°C for 10 mins. After cooling, it was centrifuged at 3,000 rpm for 10 mins. Resultant residue was dried (Fraction I). The supernatant was dried in vacuo and added with 10 ml of 95% ethanol to be kept in refrigerator. After 12 hrs., it was washed with cold 95% ethanol and dried (Fraction II). Ethanol-soluble fraction was evaporated, and the residue was dissolved in 5 ml of distilled water, to which 3 ml of ethyl ether was added, and was stirred vigorously for several mins., and ether layer was collected by centrifugation (Fraction III). Water layer was added with ethanol up to 60 per cent and centrifuged. The supernatant (Fraction IV) and the precipitate (Fraction V) were dried under reduced pressure. Thus five fractions were obtained in such a way, and were tested at their melanophore concentrating activities.

As shown by Fig. 1, occurrence of MCH was demonstrated in Fraction II, though some measure of the activity resided in Fraction I too, being absent in Fraction III, IV and V. This points out that MCH is present in the pituitary of the carp, and easily soluble in water, but insoluble in high concentration of ethanol, which were already mentioned with MCH in the pituitaries of the catfish (Enami, 1955). Following experiments were principally carried out with extracts corresponding to Fraction II in this extraction experiment.

Digestion experiment

Since the pituitary hormones in general are believed to be polypeptide or protein in nature (Li, 1954), it appeared worthwhile to investigate whether MCH might be digested by proteinases, in order to learn the properties of the hormone. Aliquot of Fraction II was dissolved in 1.5 ml of distilled water and equally divided into 3 test tubes. Then 3 ml of distilled water was added to each test tube.
One of the diluted extracts was then adjusted to pH 2 with diluted HCl and added with 10 mg of pepsin, and the second was adjusted to pH 8 with diluted NaOH and added with 10 mg of trypsin. The third one was kept untreated which served as control. The three solutions were incubated at 37°C, and after 30, 48 and 72 hrs. 1 ml was taken out from respective tubes and dried in vacuo to be tested.

Result of the assay showed that the active material in question was not inactivated by pepsin during 3 days' incubation. On the other hand, MCH activity was markedly impaired in trypsin solution and diminished even in distilled water by long term incubation. Though the activity was largely preserved in distilled water in 30 hours' incubation, it was lower as compared to the effectiveness of pepsin-treated extract. Such an observation indicates that MCH is stable in low pH range, but liable to be decomposed in higher one. In this connection it was not certain whether inactivation of MCH in trypsin solution was due to digestion by trypsin or to the effect of high pH. However, the observed stability in pepsin solution seems to suggest that the hormone is probably made of small molecule.

Solubility in organic solvents

One or two mg of Fraction II were dissolved respectively in 2 ml of absolute ethanol, absolute methanol, chloroform, ethyl ether, and acetone, all of which had been deprived of traces of water by anhydrous sodium sulfate. Soluble and insoluble fractions were separated by centrifugation, and the solvents were evapo-
rated under reduced pressure. Each residue was dissolved in 1 ml of Ringer solution to be tested.

As shown by Fig. 3, MCH activity was not found with either ethanol-, methanol-, chloroform-, and ether-soluble fractions, but was demonstrable in their insoluble fractions. Slight measure of pigment dispersion of the melanophores was observed in soluble fractions, which might be attributed to possible contamination of intermedine. It was of interest that MCH activity was proved in acetone-soluble fraction. Though acetone-dried pituitary powder has been frequently used for studies of the pituitary hormones including melanophore hormones (Kazanskii and Persov, 1948 and 1949; Pickford—unpublished data cited from Pickford and Atz, 1957), the present result shows that MCH activity would largely be lost from fresh glands by treatment with acetone.

**Paper chromatography**

Small amounts of Fraction II which were dissolved in water were chromatographically developed on the filter paper (Toyo Roshi No. 52) by butanol-acetic acid-water mixture (4:1:5) at 25°C for 15 hrs. Chromatograms were sectioned at regular length and respective strips were eluted with physiological saline to be assayed.

As the result, MCH activity was found at Rf. 0.13 and more pronouncedly
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at Rf. 0.29 on the chromatogram, while MEH activity was detected only around the starting line. It is to be mentioned that any of the spots developed by ninhydrine, BPB and potassium ferricyanide did not represent the sites of MCH activity on the chromatograms in question.

**Paper electrophoresis**

Paper electrophoretic analysis was carried out with Fraction II, and with water extracts of the pituitary and the hypothalamus of the catfish too. The experiment was made with an apparatus of horizontal model using sheets of Toyo Roshi No. 52 filter paper. Each separation was run for 4–15 hrs. at 6–12 mA (0.5–1 mA/cm) and 400–750 V with barbiturate buffer of pH 8.6, or at 6–12 mA (0.5–1 mA/cm) and 450–600 V with acetic acid-sodium acetate buffer of pH 4.8. Samples were put on the origin settled midway between the electrodes. After electrophoresis, paper sheets were cut at regular sequence of 1 cm length to be eluted with 1 ml of physiological saline, result of assay being checked against colored bands developed by ninhydrine, BPB, PAS, Sudan III and Sudan Black.

As shown in Fig. 4, melanophore expanding principles were separated in two fractions. In veronal buffer one moved toward cathode, and the other to the anode, while in acetate buffer, one remained at the origin and the other moved about 4 cm toward the anode. On the other hand, MCH moved for short distance to the cathode irrespective of pH and duration of electrophoresis. In comparison with tinctorially developed bands on electrograms, MCH activity was learned to reside in portions that were not connected specifically with any stainable substances. All that came out from this experiment are that the hormone in question behaves as an anion in pH range of 4.8–8.6, and that it does not
Effect of choline esterase

As has been reported by Enami (1940 and 1955), dermal melanophores of the catfish are brought to pigment concentration by acetylcholine, but not by adrenaline, which fact is apparently contradictory to the generalized concept that fish melanophores respond by pigment concentration by adrenaline and not by acetylcholine. Further Enami showed that, in contrast to the case with acetylcholine, MCH activity was not effectively blocked by atropine, suggesting that the hormone might be either a form of bound acetylcholine or any other substance differing from acetylcholine. Concerning this question, following experiment was made. Fresh catfish pituitaries were ground with 3ml of saline in a glass homogenizer and boiled for a few minutes to inactivate possible enzymatic interference with MCH. After cooling, homogenate was centrifuged and the supernatant was used as MCH solution, in which high MCH activity was proved. Acetylcholine chloride was dissolved in saline at a concentration of 10µg/ml, the concentration being sufficient to induce pigment concentration of the catfish’s melanophores. As the source of choline esterase, freshly collected human erythrocytes were used. Test solutions were prepared as shown in Table 2.

<table>
<thead>
<tr>
<th>No. of test solutions</th>
<th>MHC solution (ml)</th>
<th>Acetylcholine chloride at a conc. of 10µg/ml (mg)</th>
<th>Erythrocyte suspension in 0.9% NaCl (ml)</th>
<th>Fish saline (ml)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>0.1</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td></td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>0.1</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>0.1</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td>0.1</td>
<td>0.8</td>
</tr>
<tr>
<td>6*</td>
<td>0.5</td>
<td></td>
<td></td>
<td>0.9</td>
</tr>
<tr>
<td>7*</td>
<td></td>
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</table>

* Not incubated

The solutions were incubated at 37°C for 80 mins. and centrifuged to remove erythrocytes and assayed.

As shown by Fig. 5, the melanophore concentrating activity of acetylcholine was remarkable even after incubation, if choline esterase was not added to the solution (6), but it was wholly impaired under the presence of the enzyme in the erythrocytes (5). On the other hand, MCH was not significantly affected by choline esterase. Unlike acetylcholine solutions, those which contained MCH showed marked pigmentary activity irrespective of the addition of acetylcholine and/or choline esterase. The activity of solution 4 in which MCH together with acetylcholine were included was expected to be comparable to that of solution 6 in which acetylcholine alone was added, because choline esterase was not added to solution 4 and possible effect of choline esterase in MCH solution had been abolished by previous boiling. But result showed that the melanophore concentrating activity was in line with those of other four solutions 1, 2, 3 and 7, in the manner that the co-existence of acetylcholine did not interfere with MCH activity.
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Fig. 5. Effect of choline esterase in human blood erythrocytes (BL.) on MCH extracted from the pituitary of the catfish (MCH) and acetylcholine (ACH) demonstrated by curves showing time relations of melanophore responses of isolated skin of the catfish, Parasilurus asotus

Though any decisive saying is not possible with such a phenomenon, the result appears to be implicable that MCH would not be acetylcholine itself, or that another principle with melanophore concentrating activity which is different from acetylcholine, might be included in the fish pituitary.

Adsorption to aluminum oxide

Taking into account of that MCH might not be composed of amino acids, a trial for further purification of MCH was carried out by means of adsorption to aluminum oxide. Five mg of Fraction II were dissolved in 5 ml of distilled water. Approximately 0.5 g of aluminum oxide prewashed with N HCl and distilled water was added to the extract. The mixture was stirred gently for several minutes and stood for half an hour. Non-adsorbable fraction was filtered off and residual aluminum oxide was washed with water and dried in vacuo. Elution was made with stepwisely decreasing concentration of acetone, i.e., 100%, 80%, 60%, 40% and 20% acetone, and finally with water. Six fractions obtained together with the non-adsorbable fraction were dried to be tested.

The results of the assay are shown by Fig. 6, in which it is reproduced that melanophore concentrating principles and melanophore dispersing principle were clearly separated by this adsorption method. While dermal melanophores in the non-adsorbable fraction showed maximal dispersion in less than 15 mins., MCH activity was found in the first two eluates (100% and 80% acetone) and in the last eluate by water, but not in the other three eluates (60%, 40% and 20% acetone). Melanophore concentrating activity was relatively intense with the water fraction as compared with that of the first two higher acetone fractions, nearly
maximal pigment concentration of more than one hour duration as the response to the former, in contrast to slight measures of pigment concentration of shorter duration as the effect of the latter. Though it was not certain whether difference between melanophore responses to these fractions resulted either from qualitative difference or from quantitative one of a single hormonal substance in the fractions, procedures of adsorption and elution of these fractions suggest that in the pituitary gland of fishes there may be two principles with melanophore contracting activity.

Polarographic observation

In order to examine the concept just mentioned, polarographic observation was carried out. As the reference substance, acetylcholine was used. Eluates resulting from aluminum oxide adsorption were dissolved in distilled water and observed polarographically by means of a pen-recording apparatus. In the present investigation, buffer could not be used, because reduction potentials of the conventional buffers are higher than that of tetra-alkyl ammonium salt such as acetylcholine.

As shown in Fig. 7, pattern of reduction wave of water fraction (A) with high MCH activity was quite different from those of acetone fraction (B) and acetylcholine (C). The same was the case with reduction potential; reduction potential of the water fraction was approximately \(-1.8\) V and those of the acetone fraction and acetylcholine were approximately \(-2.45\) V respectively. The latter two simulated to each other with respect to both the pattern of reduction wave and reduction potential. The reduction wave of acetone fraction markedly conformed with that of acetylcholine, though slight deviation was found. Such a
Fig. 7. Polarographic patterns of MCH purified by adsorption to aluminum oxide, and acetylcholine. A, B, and C, are polarograms of water fraction, 100% acetone fraction and acetylcholine solution respectively. (T = 20°C, t = 3.181, W = 4.88, m = 1.593, Scale = 0.5, Drum = 2, Pool = 1.00, v = −1.6, No buffer was used.).

line of observation together with the results obtained in the adsorption experiment would provide an evidence for that there were two principles with melanophore concentrating activity; one would be so-called "MCH" and the other certain substance closely related to acetylcholine.

DISCUSSION

Occurrence of the hormonic principle with melanophore concentrating activity was at first suggested by Hogben and Slome (1931 and 1936), who pointed out significant difference in time relations of melanophore responses between hypophysectomized and normal clawed-frogs Xenopus laevis. According to the authors, injection of raw pituitary extract was followed by pigment dispersion in the dermal melanophores of hypophysectomized as well as normal frogs, but the recovery process in hypophysectomized animals proceeded slowly than that of normal animals kept under a white background, and if pars tuberalis was left intact in hypophysectomized frogs, the recovery process took place almost simultaneously with that in normal animals. On the basis of such findings, Hogben and Slome presumed that the time relations of melanophore responses in these experimental animals would represent possible antagonism between a certain pigment concentrating principle (W-substance) and the well-known pigment dispersing agent, intermedine (B-substance), and that pars tuberalis was responsible for
the production of W-substance. The observations were supported later by Steg-
gerta and Siderwall (1939) in such a way that cauterization of pars tuberalis of
the frog (*Rana pipiens*) induce a permanent pigment dispersion in the melanophores.
The existens of W-substance was suggested in elasmobranchs too (Hogben, 1936),
but direct proof was not successfully made out, with both amphibian and elasmo-
branches injection of extract of the portion of the pituitary in question being
reported to be without any observable melanophore concentration.

On the other hand, in a number of species of teleosts whose pigments
y effect or system has been thought to be dually controlled by nervous and hormonic
ancies (Parker, 1948), crude pituitary extracts prepared from the fishes were
reported to induce occasional pigment concentration in the dermal melanophores
*in vivo* (Hewer, 1926; Parker, 1934; Waring, 1940; Healey, 1940 and 1948; Kazanskii
and Persov, 1948 and 1949; Weisel, 1948; Enami, 1955; Burden, and Pickford, unpub-
ished data cited from Pickford and Atz, 1957), as well as *in vitro* (Matthew, 1933;
Enami, 1955), though such extracts had been admitted or rather expected to be
responsible for pigment dispersion. According to Parker (1948) the observed in-
consistency of melanophore responses to crude pitcitary extracts was attributed to
that the used extracts had sometimes no or little melanophore dispersing activity,
and to that the melanores of recipients were different from species to species in
their susceptility to the pigment dispersing hormone. Nevertheless, Parker's con-
sideration did not explain the actual concentration of the melanophore pigment
by pituitary extracts. Though occurrence of melanophore concentrating principle
was at times presumed (Healey, 1948 and 1949; Kazanskii and Persov, 1948 and
1949; Enami, 1951; etc.), it was not substantiated until Enami (1955) reported the
occurrence of what he called *melanophore contracting hormone* (MCH) in the pituitary
as well as in the hypothalamus of the catfish. According to Enami, the hormone
is produced by a kind of hypothalamic neurosecretion at the site of *Nucleus lateralis
tuberis* to be transported for storage to the so-called "Übergangsteil" of the fish
in question. (Structure of the fish pituitary being much unlike that of higher
vertebrates, anatomical designations have been confused. Very recently, Pickford
and Atz (1957) proposed a new system of terminology, which looks like most ap-
propriate. According to their designations, "Übergangsteil" is dealt with by the
name meso-adenohypophysis.) As mentioned already, Pickford and Atz (1957)
have stated wide distribution of MCH among teleosts, occurrence of the horm-
one being detectable even in such kinds of fishes whose melanophores do
not respond by pigment concentration to pituitary extracts under ordinary experi-
mental conditions. According to the authors, non-responsibility of certain fishes
to MCH activity of pituitary extracts is due to effective masking of the hormone by
co-existence of intermedine. However, there were several investigators, who were
of opinion that observed concentration of the fish melanophores was due to some
indirect effect of injection rather than to any specifically nominable hormonic
substance (Kazanskii and Persov, 1948 and 1949). In this connection, it is to be
remarkable that assays of melanophore hormones should be carried out upon the
melanophores which are free as far as possible from the effects of any kind of
humoral agents such as adrenaline and intermedine, from the effect of nervous
control too. Also it must be taken into consideration that the recipient melano-
phores of fishes employed for the assay should be sensitive to MCH. In this respect, the isolated sculp of the catfish, which was advocated by Enami (1955), seems most favorable.

According to Kazanskii and Persov (1948 and 1949), injection of the carp pituitary extract into the loach was followed by melanophore concentration, but the response became observable after 40-50 mins and lasted for 10 to 12 days. This result appears much peculiar, if the response was induced by MCH, since melanophore concentration induced by effective doses of MCH usually takes place in several minutes in injection experiments as well as in vitro tests. The in vivo response of the dermal melanophores of the loach reported by Kazanskii and Persov might be induced by rather indirect effect of injection than by the direct effect of MCH itself. Present study made with isolated skin of the catfish would show indisputable evidences with respect to the occurrence of MCH in the carp as well as in the catfish, and it might be permitted to mention that, if suitable experimental conditions are prepared, MCH would be proved in wide taxonomic range of teleosts.

Apart from the question concerning to the occurrence of MCH in the fish pituitary, a problem arises with respect to the reference substance of MCH. By repeated experiments by paper chromatography and paper electrophoresis, it was learned that the site of distribution of MCH activity was not specifically associated with any developed bands by ninhydrine, BPB, potassium ferricyanide, PAS, Sudan III and Sudan Black. Taking into account of the results of digestion experiment and solubility test, MCH is deduced to be neither of protein, peptide, carbohydrate nor of lipoid nature. One important question is whether MCH is identical with acetylcholine or with similar substance, because, as has been reported by Enami (1940 and 1955), the dermal melanophores of the catfish are concentrated by acetylcholine. Concerning the problem, Enami (1955) has already demonstrated that MCH activity in the pituitary of the catfish was not blocked by atropine, and that the effect was observed even upon the dermal melanophores of the telescope-eyed black goldfish (Carassius auratus) which were brought to pigment concentration by adrenaline, not by acetylcholine (Enami, unpublished data). According to Lanzing (1954), an active principle which contracts guinea-pig uterus was found in the lamprey's pituitary, but the activity was attributed to neither acetylcholine, nor histamine, since it was not abolished by atropine or diphenhydramine. In the present study too, it was demonstrated that MCH was not inactivated by choline esterase in the human erythrocytes, while melanophore concentrating activity of acetylcholine was abolished by the enzyme. Furthermore melanophore concentrating principle was found to be separable into two fractions by adsorption to aluminum oxide, and polarographic study with the two fractions revealed that one of the fractions was evidently different from acetylcholine, though the other much simulates acetylcholine with respect to the pattern of polarogram. Such a line of observation would be indicative that there is at least one melanophore concentrating principle (MCH) which is different from acetylcholine, and that a second MCH which is very similar to acetylcholine occurs in association with the former.

Nevertheless, it is known that bound acetylcholine is not affected by atropine
or choline esterase. Therefore, if MCH represents a form of bound acetylcholine, its activity is reasonably kept intact in treatments with these blocking agents. Recently Abdon and Kreisky (1957) reported a bound form of acetylcholine called acetylcholine precursor. According to the authors, the substance was made up of loose bound of acetylcholine to certain protein, and extractable with 96% ethanol from frozen tissues and precipitated by water-free acetone. The mother molecule was easily decomposed by boiling for only 7 mins. at pH 5.5 to split acetylcholine. On the other hand, MCH was extracted with acidified water, not extractable with 95% ethanol, and soluble in water-free acetone. Such a line of evidence together with the unpublished data by Enami that melanophores of the black telescope-eyed variety of the goldfish, Carassius auratus, were concentrated by crude pituitary extract of the catfish, despite that acetylcholine was wholly ineffective in this respect, indicate that MCH may be different from bound form of acetylcholine.

A little will be added to the consideration with respect to some of the substances that are pharmacologically similar to acetylcholine.

Euler and Gaddum (1931) extracted the so-called “substance P” from various mammalian organs, notably from the intestine and the brain. The substance contracted smooth muscles in much similar fashion as acetylcholine. According to the recent study by Pernow (1953), “substance P” was found in various parts of the brain, especially abundantly in the hypothalamus, and it was resistant against atropine, but digested by trypsin, though pharmacological properties of the substance were similar to those of acetylcholine. Accordingly, MCH acting like acetylcholine on the dermal melanophores of the catfish appears comparable to substance P in these respects. However, chemical properties of substance P were different from those of MCH; i.e., with respect to solubility to 95% ethanol (substance P is soluble; MCH not), RF value in paper chromatography (former, 0.37; latter, 0.29), reaction to ninhydrine (former, developed; latter, not), and finally, substance P was assumed to be a peptide, but as shown in the present investigation, MCH was not. Such differences suggest that two substances are different from each other.

It has been proved that an extract from gastrointestinal tracts contains a muscle contracting substance termed enteramine or serotonin (Vialli and Erspamer, 1937) which was recently identified to be 5-hydroxytryptamine (Erspamer and Aspero, 1952; Dalgliesh, Toh and Work, 1952). Both biological and chemical properties are similar to those of acetylcholine and substance P (Pernow, 1953). But unlike acetylcholine, the activity is not abolished by atropine (Dalgliesh, Toh and Work, 1952), and resists against high concentration of hot acid and alkali (Pernow, 1953). On the other hand, notwithstanding the existence of several aspects of similarities, MCH was learned to be insoluble in high concentration of ethanol, and not so stable in alkaline solution. Though comparative study was not made with respect to chemical and biological properties of enteramine and MCH, it appears probable that the substances are different from each other.
SUMMARY

Melanophore concentrating hormone (MCH) extractable from the pituitary as well as from the hypothalamus of the carp and the catfish was examined at its gross chemical properties with the following results:

1. MCH activity of extracts is largely impaired by trypsin, but remains unaffected in pepsin digestion.
2. MCH is soluble in acetone, but insoluble in ethanol, methanol, ethyl ether and chloroform.
3. In paper chromatography (butanol : acetic acid : water = 4 : 1 : 5) MCH activity is found around the site of Rf. 0.13 and more significantly near Rf. 0.29.
4. Experiments with paper electrophoresis at pH 8.6 and 4.9 showed that MCH moved for a short distance toward the cathode irrespective of pH.
5. In both paper chromatographic and paper electrophoretic analyses, MCH activity resided at the site, where no specific tinctorial response was detected with ninhydrine, BPB, potassium ferricyanide, PAS, Sudan III and Sudan Black.
6. MCH was not inactivated by choline esterase in human erythrocytes.
7. MCH was separable from MEH by adsorption to aluminum oxide, and eluted by stepwisely decreasing concentration of acetone. The melanophore concentrating activity was found in 100% and 80% acetone fractions, and in water fraction too. Polarographic study of these fractions showed that acetone fractions contained acetylcholine-like substance, and water fraction contained MCH which appeared different from acetylcholine.
8. Observed chemical properties of MCH were brought to comparison with those of other substances with similar melanophore activity or with related pharmacological and chemical properties.

REFERENCES


