Chemical Physiology of the Compound Eye, II. Demonstration of Adrenaline from the Eye[1)2)]

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(With 1 Text-figure)

Apparent evidence of sympathomimetic catechol amines was first adduced from insects by Östlund (1953). This work formed a valuable contribution in dispelling some doubtful points in the past concerning the existence of the amines in insects. However, the detailed localization of such biogenic amines within insect bodies has remained an unsolved problem. In his previous papers (1962a, b), the present writer suggested the presence of adrenaline in rhabdom of the compound eyes of the house fly, *Musca domestica vicina* Macquart from its histochemical features.

This paper deals with paper chromatographic and biologic demonstrations of adrenaline from the insect compound eye.

Material and methods

Material: Females of the false stable fly, *Muscina stabulans* Fallén were mainly used in this study. They were reared in the laboratory on the same medium as in *Musca domestica vicina*, namely, a mixture consisting of sawdust, bean refuse (Okara) and entrails of fish. Sucrose and tap water were supplied to emerged adults.

Procedure for dark and light adaptation: Prior to experiments for dark adaptation, adult specimens were overnighted in darkness without food. Light adapted specimens were obtained by irradiating overnight adults for 2–4 hours. In order to avoid the influence of their body movements in experiments for light adaptation, adult flies were irradiated after their wings and legs were firmly plastered with cellotape. Irradiation was made by means of a mercury vapour lamp (Toshiba Electric Inc., SLH-100 UV), and a water vat (14.5 cm in thickness) was interposed between specimens and the lamp to absorb excess heat. Specimens subjected to irradiation were placed just behind the vat: where the brightness was ca. 7600 lux and the rise of temperature was ca. 4°C after 4 hour irradiation.

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2) This paper is dedicated to Professor Atsuhiko Ichikawa, Zoological Institute, Hokkaido University, Sapporo, in honor of his sixtieth birthday, May 20, 1964.

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Preparation of samples: Heads of dark adapted specimens were detached from their bodies and divided into two along their clypeus. Each half of head was opened approximately at the occipital foramen with ophthalmic scissors, and optic lobes, brain and its accesories were carefully taken out from retina with forceps. Next, antenna, mouth parts and ocelli were successively removed, and only the compound eye was offered for various experiments. It took 5 minutes to complete the operation of a single specimen. These operations were all executed under a binocular dissecting microscope in a dark room. Irradiation for the operations was made with an insensitive yellow light for photographic use. The operation of light adapted eyes was performed in a light room after irradiation. Eyes of five specimens were ground down, together with small amounts of deionized water in a glassmortor, and the water extract of eyes was centrifuged for 10 minutes with the velocity of 2000 r.p.m. The supernatant, coloured light yellowish brown, was used as the unit sample of eyes in the lot of each experiment: its amount was ca. 0.1 ml. Immediately after centrifugation, the supernatant was applied to paper chromatographic and biologic tests.

Paper chromatographic procedure: Filter paper: Chemically untreated Toyo Roshi No. 51. Solvent: n-Butanol: acetic acid:deionized water=2: 1: 1 in volume. One dimensional ascending method was adopted. Samples of eyes and catechol amines were spotted at intervals of 2 cm on the paper sheet. In order to keep the spot size to a minimum diameter, microsyringes were employed in size ranging from 0.01 to 0.1 ml, and the solution was dried from behind the paper sheet with a hair dryer as soon as it flowed on the paper. Spotted paper sheet was accomodated in a chromatographic cabinet (E-1P sold by Toyo Roshi Inc., internal dimension: $30\times50\times45$ cm^3), and immersed in the solvent. Spots were carried on the paper at room temperature till the top level of the solvent reached the height of 26 to 28 cm over the original line on which the spots were placed before development. Within 24 hours, after the development was ended, the paper was air-dried at 60°C and sprayed for revelation of spots with a 0.5% solution of potassium ferricyanide in phosphate buffer, PH 7.8.

Results

1. *Chromatographic detection of adrenaline*: From preliminary paper chromatographic experiments in which *l*-adrenaline, *l*-noradrenaline (*l*-artenol), 3-hydroxytyramine hydrochloride and 5-hydroxytryptamine creatinine sulfate (serotonin) were used, it was supposed that water extracts of compound eyes contain adrenaline. In order to examine this supposition, further refined experiments were undertaken. The following nine spots were simultaneously applied to a sheet of filter paper: sample of light adapted eyes plus hydroxytyramine, sample plus adrenaline, sample plus noradrenaline, intact samples of dark and light adapted eyes without adding the three reagents referred to just above, each reagent alone, and a mixture of all three reagents. Spraying of 0.5% potassium ferricyanide produced a bright red spot with adrenaline and noradrenaline, and a grayish purple one with hydroxytyramine. These chromatograms changed within 48 hours to a dark grayish brown almost indistinguishable in colour from each other. A typical result is shown in Figure 1. Rf values of the reagents and the sample are listed in Table 1.

Table 1

Reagent	R_f
Hydroxytyramine	0.82
Adrenaline	0.80
Noradrenaline	0.61
Sample of eye	0.80

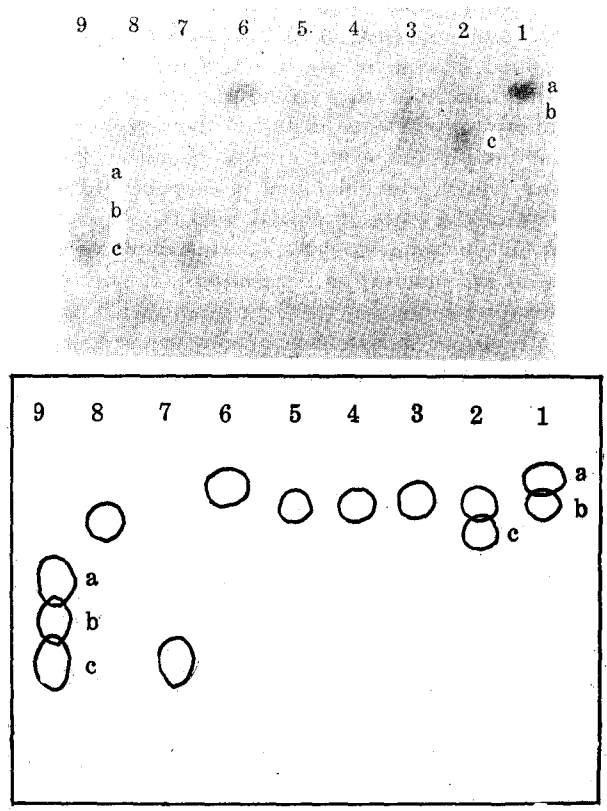

Fig. 1. Upper; Photo showing chromatograms of nine spots simultaneously developed. In this photo, a round trace extending over from 6 to 7 is an artifact caused by exudation of water. Lower; The same indicating the exact location of the chromatograms: 1. Sample of light adapted eyes plus hydroxytyramine, 2. sample plus noradrenaline, 3. sample plus adrenaline, 4 and 5. intact samples of light and dark adapted eyes, 6, 7, and 8. only hydroxytyramine, noradrenaline, and adrenaline respectively, 9. a mixture of these three reagents. Small letters, a, b, and c show each chromatogram of hydroxytyramine, adrenaline and noradrenaline. The accordance of appearing sites of chromatograms (b) in 1, 2, 3, 4, 5, and 8 is noticeable. More swift rising of noradrenaline in the mixed solution with sample of eyes (2) than in the single solution (7) is also seen.

Although absolute values of Rf showed some fluctuation by experimental conditions, chromatogram of adrenaline always accorded with that of samples prepared from eyes both in colour and site of appearance. Moreover, interestingly enough, the spot of adrenaline which originated from a mixed solution with eye sample was more intense in colour than from a single solution of adrenaline. This may mean the resulting increase in amount of adrenaline in the mixed solution by adding the sample. In other words, this is considered to give an indirect evidence to the hypothesis that the compound eyes contain a fairly large amount of adrenaline. Addition of eye sample has a serious influence upon Rf value of some amines: it was remarkable in noradrenaline but not in adrenaline or hydroxytyramine. When sample of eyes was added, the migration of noradrenaline became faster than in its single solution. In this case, noradrenaline was probably carried by the rapid ascending migration of retinal pigments present in the sample. This can be pointed out from the fact that noradrenaline was always visualized just above the top of the ascending pigments. Such was not found in cases of adrenaline and hydroxytyramine. The location of the spots developed from the single solution was almost unchanged from that in the solution mixed with sample of eyes.

2. *Calculation of the amount of adrenaline*: It was confirmed from the results of preliminary experiments that the log concentration of adrenaline behaved rectilinearly towards the size of spots within a scope below 10μg. The relation was thus expressed as a formula, $y=0.256591x-0.094985$, where y is log concentration and x size of spot in cm^2. By using this formula, amount of adrenaline was calculated as follows: Sizes of spots generally fluctuated according to experimental conditions. Therefore, in order to obtain the exact value of x, before application to the above formula, the size of spot was calculated from a simple proportion in which a known quantity of adrenaline was adopted as a standard of comparison: A: K=x: B, where K is a mean size of spots of the standard solution of adrenaline. For convenience of procedure, 4 μg of adrenaline was used as the standard. Based upon the results of 10 developing experiments, the mean value of spots was determined as 2.78 cm^2. B expresses the size of spot originated from the sample of eyes for test, and A is the size of spot from 4 μg of adrenaline developed for comparison together with the sample on a sheet of filter paper. All sizes of spots were measured with planimeter. By applying x obtained in this way to the formula mentioned above, the amount

Table 2

	Light adpated eye	Dark adpated eye	T-test
Adrenaline μg/head	0.335 ± 0.122	0.154 ± 0.07	$p<0.02$
Body weight without head mg	22.97 ± 1.36	22.45 ± 1.67	$0.3<p<0.2$

The figure indicates the mean with the standard error.

of adrenaline in the sample was determined. The results of 6 lots of experiments are summarized in Table 2. No difference was found in body weights between the light and dark adapted specimens. But the amount of adrenaline in the light adapted eyes was more than twice as large as that in the dark adapted eyes, and the difference was statistically significant.

3. *Biological demonstration of adrenaline*: Fish melanophores usually elaborate cells having branches well responsive to the action of catechol amines and electlytic ions. When immersed in the solution of catechol amines, pigment granules of the cells rapidly concentrate at the center, and the branches completely disappear. Adults of the wild fish, *Carassius carassius* Linné were employed as material for the experiment. Dispersed pigment granules of intact scales taken from the dorsal part of the fish also concentrated gradually in deionized water. Experiments for comparison of effects were therefore executed by measuring time spent for the complete concentration of most melanophores (melanophore index 1; Jenkin, 1962) on the scale being put into the examined solution. Samples of eyes were prepared by the same procedure as in the paper chromatographic test. Scales once used were discarded. The results of 10 repeated experiments are shown in Table 3. The difference of time between the sample and deionized water as

Table 3.
Time (min.) needed for the melanophore concentration in scales of *Carassius*. The concentration of the reagents was equal to 4 μg. The figure is shown with the mean accompanied by a fiducial range in 5% level of the significance.

Noradrenaline	Hydroxytyramin	Adrenaline	Smaple of light adapted eye	Deionized water
1.47 ± 0.34	1.76 ± 0.52	2.33 ± 0.41	7.62 ± 0.84	12.25 ± 0.75

control was statistically significant. It may be said therefore that the sample of eyes has some effects upon the concentration of melanophores. The effect of the sample seemed to be fairly inferior to that of the catechol amines applied to the experiments. This will be ascribed to the amount of effective component in the sample. It is estimated from the result of paper chromatography that the total amount of adrenaline in the sample were about 1.6μg and equal to four-tenths of that in the single solution of adrenaline used here.

Discussion

In his previous papers, the writer pointed out that rhabdom of compound eyes is positive for periodic acid-Schiff (PAS) and chromoargentaffin reactions. Among these reactions, both chromaffin and PAS reactions are well known to be closely related with the chemical structure of adrenaline. In this case, chromaffinity is induced from the presence of catechol radical, the main framework of

adrenaline, and PAS reaction is based upon the oxidation of hydroxyalkylamino radical, the side chain of adrenaline. Consequently, there seemed to exist sufficient ground from the histochemical inspection to surmise the presence of adrenaline in the compound eyes. According to Östlund, catechol amines present within insect bodies are adrenaline, noradrenaline and hydroxytyramine. In the experiments of the present writer in which three amines mentioned above were used, chromatogram of the sample of compound eyes was similar both in colour and site of appearance to that of adrenaline. Moreover, it was found in biological tests that water extracts of compound eyes produce a passable effect upon the melanophore concentration. Judging synthetically from the results of histochemical, paper chromatographic and biologic tests, it may be concluded that adrenaline is itself existent within the compound eyes, especially in its rhabdom.

The present knowledge concerning the function of adrenaline within the body of invertebrates seems to be a mere analogy of information obtained in vertebrates. Nagano (1950) observed in the dark adapted eyes of the shrimp, *Paratya compressa* de Haan that proximal pigments were moved proximally by injecting adrenaline into the abdomen as if the movement of the pigment cells was caused by irradiation of light. As already mentioned, the amount of adrenaline in light adatped eyes increased more remarkably than in dark adapted eyes. When taking Nagao's observation into consideration, the meaning of this result becomes fairly evident. That is, the irradiation of light accelerates adrenaline secretion in the compound eyes and consequently, some kinds of pigments such as the proximal pigments of *Paratya* are moved to take the arrangment for light adaptation. The pigments of *Paratya* have been regarded as the nervous stimulant. Also in moth eyes, there are many examples suggestive of pigment migration under nervous control (Day, 1941; Yagi and Koyama, 1963). Considering the fact that the rhabdom of compound eyes is morphologically connected with the nerve ending of optic lobes and contains a large amount of adrenaline, a possibility of participation of adrenaline may be pointed out in the migration of pigment cells excitable by the nervous stimulation.

Summary

Using the paper chromatographic method, it was verified that chromatogram of sample prepared from the compound eyes of the false stable fly, *Muscina stabulans* Fallén was always consistent in colour and appearing site with that of adrenaline among catechol amines the existence of which has hitherto been confirmed by tangible evidences in insect bodies. In biological tests, it was furthermore detected that water extracts of the compound eyes are effective for the melanophore concentration of fish scales. The conclusion was deduced from these results that probably adrenaline is itself contained in the compound eyes, particularly, in the rhabdom. In relation to the fact that the amount of adrenaline in light adapted eyes was more abundant that in dark adpated ones, the function

of adrenaline in the compound eyes was discussed.

Finally, the writer expresses his hearty thanks to Prof. Mayumi Yamada for his kindness shown in the course of the experiments.

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