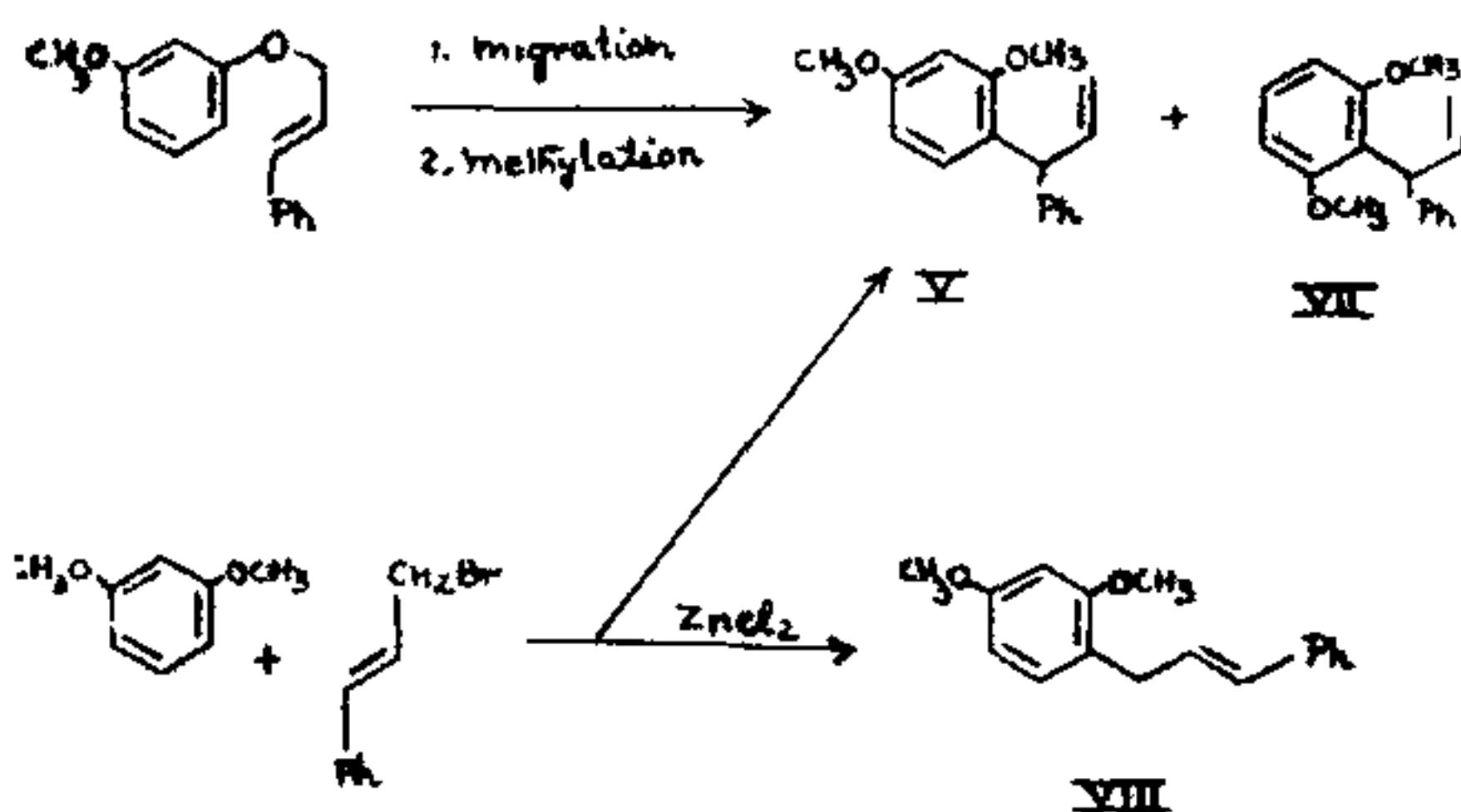


the composition of the product was essentially the same as when previously mixed reagents were employed. However, the use of zinc chloride leads to the formation of more of isomer (VI) (70%).

Authentic diphenyl allyl compound (V) was prepared by methylation of 3-(2-hydroxy-4-methoxyphenyl)-3-phenyl propene.³ It was also



obtained by coupling resorcinol dimethyl ether with cinnamyl bromide in the presence of zinc chloride. This reaction gave a complicated mixture of eight compounds; out of these two major and closely moving compounds were separated. They consisted of the allyl compound (V) and the isomeric benzyl styrene (VIII) and were used to identify the product obtained by the Claisen migration route which was contaminated with 3-(2, 6-dimethoxy-

phenyl)-3-phenyl propene (VII). The allyl compound (V) was a liquid; $\lambda_{\text{max}}^{\text{MeOH}}$ 285 μ (Found: C, 80.5; H, 7.6. C₁₇H₁₈O₂ requires C, 80.0; H, 7.1%).

Authentic propenyl isomer (VI) was prepared by Grignard reaction of 2, 4-dimethoxy benzo-phenone with ethyl magnesium bromide and dehydrating the product. It crystallised from methanol as colourless plates, m.p. 73-74° (Found: C, 79.5; H, 7.6. C₁₇H₁₈O₂ requires C, 80.0; H, 7.1%). $\lambda_{\text{max}}^{\text{MeOH}}$ 240 m μ with inflexion at 285 m μ .

Fuller scope of this reaction is under investigation.

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THIN LAYER CHROMATOGRAPHY OF INORGANIC IONS

Part I. Separation of Zinc, Copper, Cadmium, Cobalt and Nickel as their Thiocyanate Complexes

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THE technique of Thin Layer Chromatography (T.L.C.), on account of its speed and elegance, has been extensively employed for organic separations. Seiler¹ et al., in a series of papers, have attempted to apply this technique to inorganic ions but on repeating some of these experiments, no compact spots were obtained. Hranisavljevic—Jakovljevic² et al. also described separation of metals as their dithizonates. Apart from this no extended use appears to have been made of this technique for the separation of inorganic ions.

Following the suggestions of Pollard and McComie³ that the solubility of metallic thiocyanate in organic solvents should merit their examinations in chromatography, paper chromatographic methods were developed in this laboratory, for the separation from their mixture of (1) copper, cobalt and nickel⁴ and

(2) cadmium and zinc.⁵ It was considered desirable to extend these investigations to explore the possibility of the separation of all the above five ions from their mixture by using T.L.C.

It is well known that of the five above mentioned ions, Cobalt and Zinc thiocyanates are fairly soluble in organic solvents. However, on addition of Pyridine, all the five of the metal ions form complexes of the type M Py_n (SCN)_m⁶, which are extractable with organic solvents. The combination of solvents, and (i) Thiocyanate or (ii) Pyridine and thiocyanate in the same solution, however, gives an eluent which effectively buffers the chromatogram and thus become more effective for the chromatographic separations.

In the present investigation several eluents based on thiocyanate with or without pyridine

were tried for studying the chromatographic behaviour of the aforementioned ions on a thin layer of Silica Gel 'G' (E merck), coated on a glass plate. The ions in turn were detected by spraying with suitable reagents.

EXPERIMENTAL

Following solutions were prepared :

1. Solution of Metal Ions

Separate solutions of Zn, Cu, Cd, Co and Ni of M/100 strength.

2. Fluents

A. Ammonium thiocyanate 4 gm.; Alcohol (methyl or ethyl or *n*-butyl or isopropyl or iso-amyl) 80 ml.; Base (liquid Ammonia 0.910 or Sodium hydroxide 10% or Tetramethyl ammonium hydroxide 10% solution) 10 ml.

B. Ammonium thiocyanate 4 gm.; Ethanol 60 ml.; Methyl ethyl ketone 40 ml.; Tetramethyl ammonium hydroxide 10 ml.

C. Ammonium thiocyanate 4 gm.; Alcohol (methyl or ethyl or *n*-butyl or isopropyl or iso-amyl) 80 ml.; Base (liquid Ammonia 0.910 or Sodium hydroxide 10% or Tetramethyl ammonium hydroxide 10% solution) 10 ml.; Pyridine 10 ml.

D. Ammonium thiocyanate 4 gm.; Ethanol 60 ml.; Benzene 35 ml.; Tetramethyl ammonium hydroxide 10 ml.; α -Picoline 10 ml.

3. Detection

1% solution of Rubeanic acid in alcohol was sprayed on the developed chromatogram to locate the spots of copper, cobalt, nickel which respectively gave slate-grey, orange and violet coloured spots.

This was followed by spraying with 0.5% solution in alcohol of 8-hydroxyquinoline. On exposure to ultra-violet light Zn and Cd spots gave yellow fluorescence.

A slurry of Silica Gel 'G' (E merck) was made and applied in the form of a thin layer on a glass plate with the help of an applicator. The plates were dried at 110° C. for 2 hours and stored in a desiccator. One drop each of solutions of the salt was applied separately on the base line, the same kept in a battery jar and the chromatogram run in the usual manner, the concentration of the constituents of the eluents which gave compact and well separated spots of respective metal ions was worked out. These described under (2) above gave the best results.

In addition to the nature of eluents, the extent of migration of the ions was also

affected by the ambient temperature. At lower temperatures (about 10° C.), although the movement was slow, well separated spots were obtained.

Some of the special features of the present investigations are as under :

- (i) All the eluents used are alkaline. In the initial stages ammonia was used, but on account of its volatility, reproducible results were not obtained. Best results were obtained by using tetramethyl ammonium hydroxide for adjusting the alkalinity.
- (ii) Compactness of the spots is improved by incorporating thiocyanate in the eluent itself.
- (iii) Use of higher alcohols led to the formation of more compact spots, but it took longer to develop the chromatogram.
- (iv) The position of some of the metal ions on the chromatogram was changed considerably by incorporating pyridine or α -picoline in the eluent. Especially, the position of cobalt and copper is reversed in the two sets of eluents, viz., those with or without pyridine.
- (v) Zinc shows practically no movement in any of the eluents except those in which a typical ketone like methyl ethyl ketone is incorporated, however, in the latter case, other ions do not show a very distinctive separation.

Unlike paper chromatography, no definite R_f values can be given. However, over a fairly wide range of temperature the order in which various ions are likely to be located remains the same. Thus it becomes possible to detect the presence of one or more of the above mentioned ions from a mixture.

In a typical case, spots of the mixture of the above ions were applied on a plate alongside with the various metal ions separately and chromatogram run. It was found that a period of 40-45 minutes was enough to separate various ions and these in turn could be detected by spraying with rubeanic acid and 8-hydroxyquinoline solution separately. Typical separations using two eluents are shown in Figs. 1 and 2.

Summary and Discussion of Results

The present investigation makes available a process that may be utilized for getting a clear separation between number of metal ions on thin layer chromatogram. The respective ions can be located by spraying the chromatogram with suitable reagents when characteristic coloured spots are obtained. The essential con-

stituents of the eluents used is alkaline thiocyanate with or without pyridine.

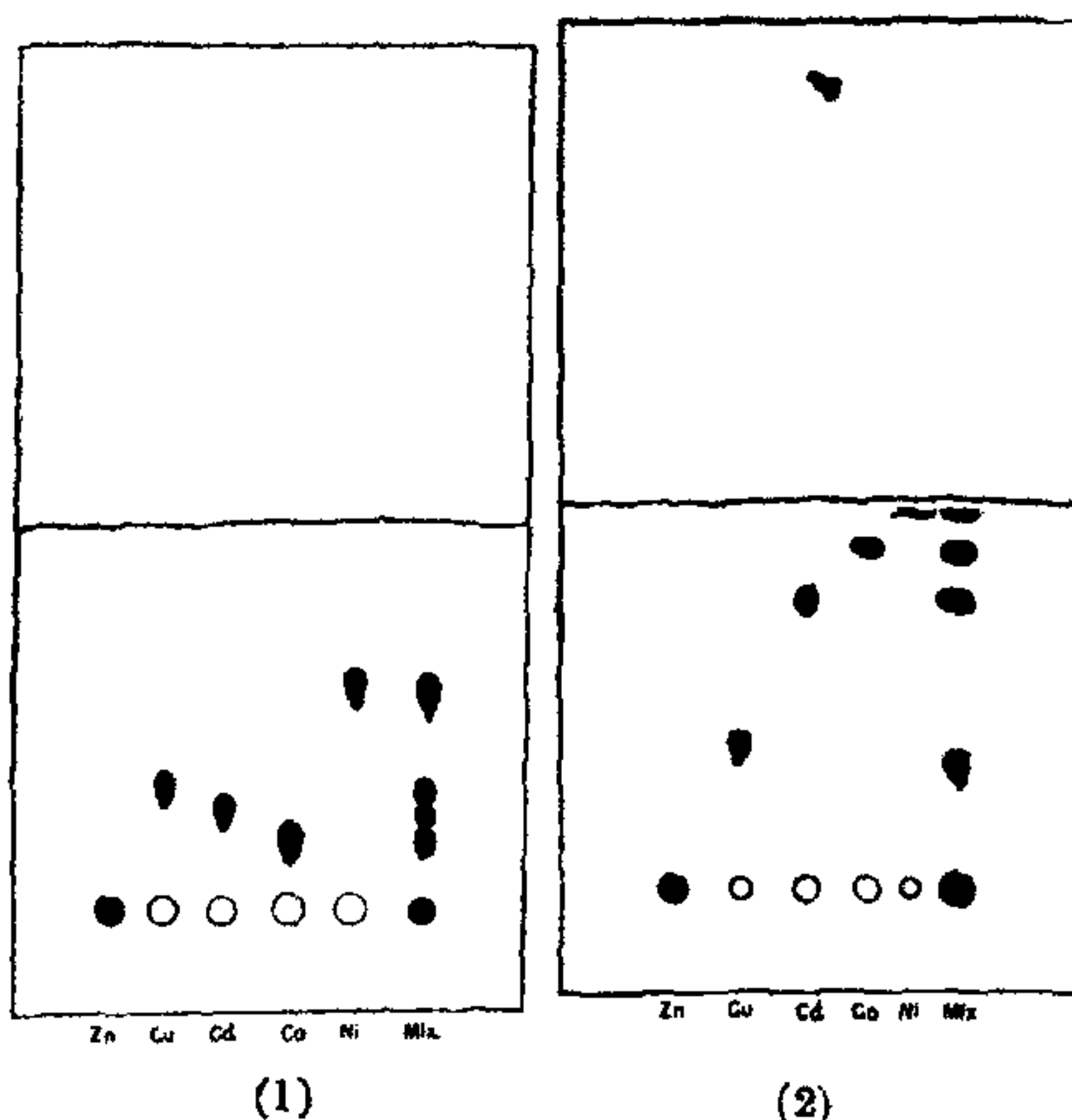


FIG. 1

Eluent: Ammonium thiocyanate 4 gm.
Methanol 80 ml.
Tetramethyl ammonium hydroxide (10%) 10 ml.

FIG. 2

Eluent: Ammonium thiocyanate 4 gm.
Iso-propyl alcohol 80 ml.
Tetramethyl ammonium hydroxide (10%) 10 ml.
Pyridine 10 ml.

The chromatographic separation of the metal ions treated is based on the formation of their thiocyanate complexes.

The procedure developed herewith is a one stage process and does not require a prior separation of the complexed metal ions by extraction. The medium, viz., silica gel does not have to be specifically freed of iron as the thiocyanate complex of the same is colourless in an alkaline medium.

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CARDIOVASCULAR RESPONSE TO THE ADRENERGIC AND CHOLINERGIC DRUGS IN EMBRYONIC AND LARVAL STAGES OF *GAMBUSIA AFFINIS* PATRUELLIS, BAIRD AND GIRARD

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ALTHOUGH it is generally accepted that fishes possess a sympathetic nervous system, there is no unequivocal evidence that the system has an effect on the piscine heart and circulation.¹ The existence of the cardio-accelerator sympathetic fibres has been reported by Mott,² but Lutz³ and Burger and Bradley⁴ have failed to find any evidence for this. Further, it is believed that there are two receptor sites, the α and β , available to the adrenergic drugs in the receptor cells. There is no evidence so far, as to which of the adrenergic receptors mediate in the action of adrenergic drugs on the piscine heart. It is for this reason that the present work was undertaken to throw some light on the problem. The embryonic and larval stages of the viviparous fish, *Gambusia affinis* were specially selected because of the considerable variation in the heart rate of this fish at different stages of the development suggesting a variation in the autonomous cardiac control in them.

The embryos of *G. affinis patruellis* were obtained from gravid females by dissecting out the uterus and removing the egg membrane. The larvae treated were about 30 hours of age and measured 6-7 mm. in length. To study myocardial activity, the embryo or larva was placed on wet cotton in the prone position under the dissecting microscope. The normal cardiac rate was first noted by counting the heart-beats per 10 seconds. Two drops of drug were then added to the surrounding medium in varying concentrations ranging from 0.01 μ g. to 1 μ g./c.c., observing constantly the rate of myocardial contraction. At least 10 readings for each response were taken.

The normal heart rate of the embryo is higher (102) than that of larva (72) per minute at 26° C., and it is in agreement with the report of Grodzinski^{6,7} on the piscine heart.

Adrenaline first increased and then decreased the cardiac rate of the embryo. In the larva, however, the increase was not followed by