

is not affected by acid hydrolysis. Spot No. 9, also unidentified, often partially separates into 2 areas and may consist of several substances with similar R_f values.

Ethanol extractions of amino acids and peptides from various stages of development (unfertilized eggs, early cleavage, swimming blastulae, gastrulae, and prism larvae) with comparable amounts of material (250,000 eggs or embryos per cc of extract) indicate that there is no striking qualitative change during development.

A typical chromatogram of extracts of a late stage of development is shown in Fig. 1b. No new amino acids appear on the chromatograms of these later embryonic stages and a few (alanine, lysine and glutamine) apparently decrease in concentration. In chromatograms of gastrulae and prism larvae glycine and glutamic acid often spread out more in the direction of the phenol run (Fig. 1b) which suggests an increase in concentration of these amino acids. Aside from these

slight changes the patterns of amino acids and peptides on chromatograms of later embryonic stages are similar to those of unfertilized eggs.

Summary. The free amino acids of sea-urchin (*Strongylocentrotus purpuratus*) eggs and embryos were extracted with 75% ethanol and identified by means of paper chromatography. Extracts of unfertilized eggs contain relatively large amounts of glycine and small amounts of alanine, glutamic acid, valine, threonine, lysine, glutamine, and leucine (or isoleucine) as well as several peptides and unknown ninhydrin-positive substances. The patterns of amino acid and peptide spots on chromatograms of extracts of cleavage stages, blastulae, gastrulae and prism larvae are dissimilar to that of unfertilized eggs.

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Studies in Protein Metabolism of the Amphibian Embryo. II. Free Amino Acids. (18089)

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The method of paper chromatography(1) has only recently been used for the identification of free amino acids in eggs and embryos, namely, by Drilhon and Busnel(2) for *Bombus mori*, Agrell(3) for *Calliphora erythrocephala*, and Berg(4) for *Strongylocentrotus purpuratus*. Li and Roberts(5), however, were unable to demonstrate any free amino acids in the developmental stages of the frog [presumably *Rana pipiens*] ranging from

the unfertilized egg to the young non-feeding tadpole. They detected peptides, however, which upon hydrolysis yielded small quantities of aspartic acid, glutamic acid, glycine, and alanine.

We have examined two amphibians, *Rana pipiens* and *Hyla regilla*, for free amino acids in 4 stages of development: zygote (or, in case of *Hyla*, early cleavages), beginning gastrula, middle neurula, and young tailbud embryo. In terms of numbered stages described by Shumway(6) for *Rana pipiens* and by Eakin(7) for *Hyla regilla* the stages used were 2 (or, in case of *Hyla*, 2 to 7), 10, 14,

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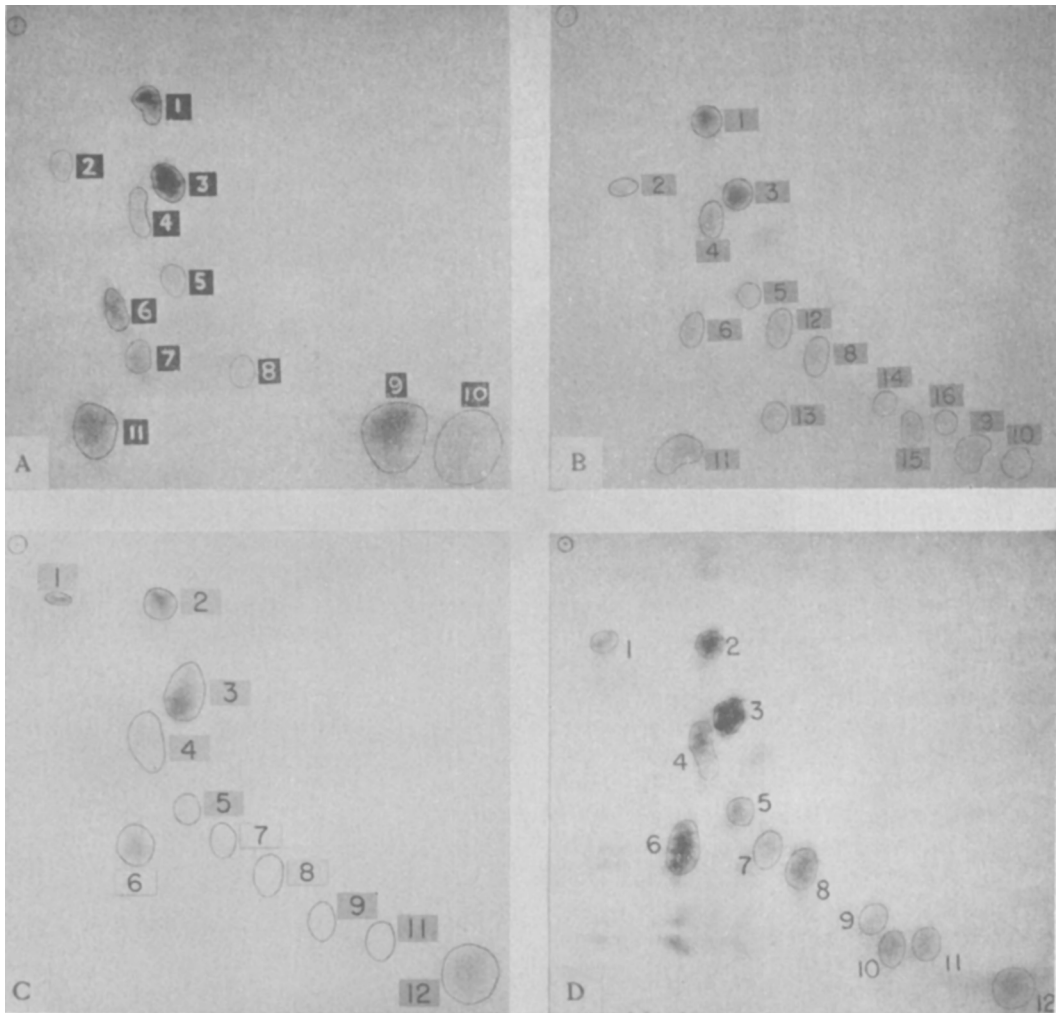


FIG. 1. Chromatograms of Ethanol Extracts.

A, *Rana pipiens* zygotes (stage 2); B, *R. pipiens* tailbud embryos (stage 18). Spots (circled with pencil): 3, glutamic acid; 4, serine; 5, threonine; 6, glutamine; 7, methionine sulfoxide (?); 8, tyrosine; 12, alanine; 14, valine; 15, phenylalanine; 16, leucine (or isoleucine); 1, 9, and 10, peptides; 2, 11, and 13, unidentified.

C, *Hyla regilla* early embryos (stages 2-7); D, *H. regilla* tailbud embryos (stage 18). Spots (circled with pencil): 3, glutamic acid; 4, serine; 5, threonine; 6, glutamine; 7, alanine; 8, tyrosine; 9, valine; 10, phenylalanine; 11, leucine (or isoleucine); 2 and 12, peptides; 1, unidentified.

Movement of solvents: phenol-water, vertical direction; n-butanol-acetic acid-water, horizontal direction. Spots in Fig. C not well shown because photograph was made with direct light; photographs A, B, and D taken with transmitted light. Photographs made by Victor G. Duran.

and 18. The embryos were first rendered free of most of the jelly; tailbud embryos were removed from the chorion. For each stage of both species 500-1300 embryos were homogenized in a final concentration of 75% ethyl alcohol. Extraction by the alcohol was continued at 2°C for 48 hours. Samples were

centrifuged. The supernatant from each was decanted off, evaporated to dryness, and brought by the addition of distilled water to a final concentration of 167 (*Rana*) or 200 (*Hyla*) embryos per ml. The paper chromatographic method(1,4) was employed. The solvents used were phenol saturated with

water and a mixture of n-butyl alcohol, acetic acid, and water (approximately 11:3:4).

The results are illustrated in Fig. 1. Spots 1, 9 and 10 in *Rana* (Fig. 1A-B) and marks 2 and 12 in *Hyla* (Fig. 1C-D) are assumed to be peptides since they disappeared upon acid hydrolysis (refluxed with 6N HCl for 20 hours). They will be analyzed in a later study.

Rana and *Hyla* chromatograms are remarkably similar. *Rana*, but not *Hyla*, shows a spot tentatively designated methionine sulfoxide (Fig. 1A, No. 7). Methionine added to the extract of *Rana* zygotes intensifies this spot, presumably by the oxidized form and a new spot (unoxidized methionine) appears to the right and in the position of valine. Valine and methionine have similar R_f values, but the mark designated valine on chromatograms of older material probably is not methionine. The methionine sulfoxide spot is absent or very faint in tailbud papers whereas the valine spot has by that stage become very definite. Alanine, leucine (or isoleucine), and valine present in *Rana* after the beginning of gastrulation, can be detected in the youngest *Hyla* material. Unfortunately because of the difficulty of procuring an adequate quantity of *Hyla* zygotes, early cleavage stages were also included so that the early *Rana* and *Hyla* extracts are not strictly comparable.

There is no marked change in the picture as development proceeds. In both species the large peptide spots decrease in intensity;

phenylalanine first appears in neurulae in *Rana* and in gastrulae in *Hyla*; and in *Rana* leucine (or isoleucine) and valine are first detectable in gastrular extracts. In addition, the spot on *Rana* papers tentatively identified as oxidized methionine is so faint on stage 18 chromatograms that it was not circled in the photograph (Fig. 1B).

Finally we report one trial experiment in which extracts of dorsal lip explants in *Hyla* were compared chromatographically with those of explants of ventral ectoderm. For this one run approximately 300 explants of each type were needed. The explants corresponded to those used by Friedberg and Eakin(8) in their study of glycine uptake. The two chromatograms were essentially alike in the number and intensity of the spots and agreed with the picture for the whole gastrula.

Summary. Several free amino acids and one amide were identified by paper chromatography in alcoholic extracts of eggs and early developmental stages of *Rana pipiens* and *Hyla regilla*. Other ninhydrin-positive substances, including several peptides, will be studied later. One experiment on *Hyla* revealed no major chromatographic difference between explants of dorsal lip and ventral ectoderm.

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The Effect of Cyclopropane, Ether, and Thiopental Sodium upon the Over-Digitalized Heart.* (18090)

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Since cyclopropane tends to shift upward

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the pacemaker which has been displaced by digitalis preparations(1), it seemed of interest to investigate the effect of certain other anesthetics. This report deals with a comparison of the effects of cyclopropane, ether

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