values for glycine, lysine, phenylalanine and serine do not show a significant difference.

Discussion. It is generally conceded that plasma values for various amino acids are relatively constant(7). It seems, therefore, that any significant differences are worthy of consideration. Although the changes we have demonstrated may be small in gamma per ml, they are nevertheless valid as shown by statistical analysis. The normal values established in this study compare favorably with those found in the literature(6,7,10).

Summary. Microbiological determinations of plasma values for arginine, glycine, histidine, lysine, phenylalanine, serine, and threonine have been determined in a group of normal individuals and compared with the values obtained from patients with rheumatoid arthritis.

1. The values obtained for arginine, histidine, and threonine in patients with rheumatoid arthritis were lower than those obtained from the normal group. These differences were highly significant(11). 2. The values for glycine, lysine, phenylalanine and serine were not significantly different in the group with rheumatoid arthritis from those found in the group of normal individuals.

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Free Amino Acids in Sea-Urchin Eggs and Embryos.* (18088)

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Free amino nitrogen has been determined in embryos of various animals; however, as yet there has been little attempt to analyze the sources of this qualitatively. Li and Roberts(1), using the paper chromatographic method, found no free amino acids in eggs and embryos of the frog. Drilhon and Busnel (2), also using chromatographic methods, found free glutamic acid, serine, alanine, and valine in the eggs of *Bombyx mori.* In later stages of development other amino acids appeared.

The present work was undertaken to investigate the occurrence of free amino acids in sea-urchin eggs, and to determine whether qualitative changes take place during development. Unfertilized sea-urchin *(Strongylocentrotus purpuratus)* eggs were extracted with 75% ethanol at 3-5°C for 2 days; 1.5 cc of ethanol were used per 50 mm^3 of eggs packed by centrifugation. The supernatant was analyzed for amino acids and peptides by means of 2-dimensional paper chromatography as described by Consden *et al.*(3). Whatman filter paper No. 1 was used with water saturated phenol as the first solvent and a n-butanol-acetic acid-water mixture (approximately 11:3:4) as the second. Amino acids and peptides were revealed by spraying the papers with 0.2% ninhydrin and heating to approximately 80°C for 10-20 minutes. Amino acid spots were tentatively identified by position and checked by adding pure amino acids to the unknown.

Free amino acids identified in the unfertilized sea-urchin eggs were glycine, glutamic acid, lysine, threonine, alanine, valine, leucine (or isoleucine). A ninhydrin-positive spot with R_f values slightly less than alanine was found to be glutamine. Addition of pure glutamine to the extract caused intensification of this spot. Acid hydrolysis of the material

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2. Drilhon, A., and Busnel, R., *Comp. Rend. Acad. Sci.,* Paris, 1950, v230, 1114.

3. Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.,* 1944, v38, 224.

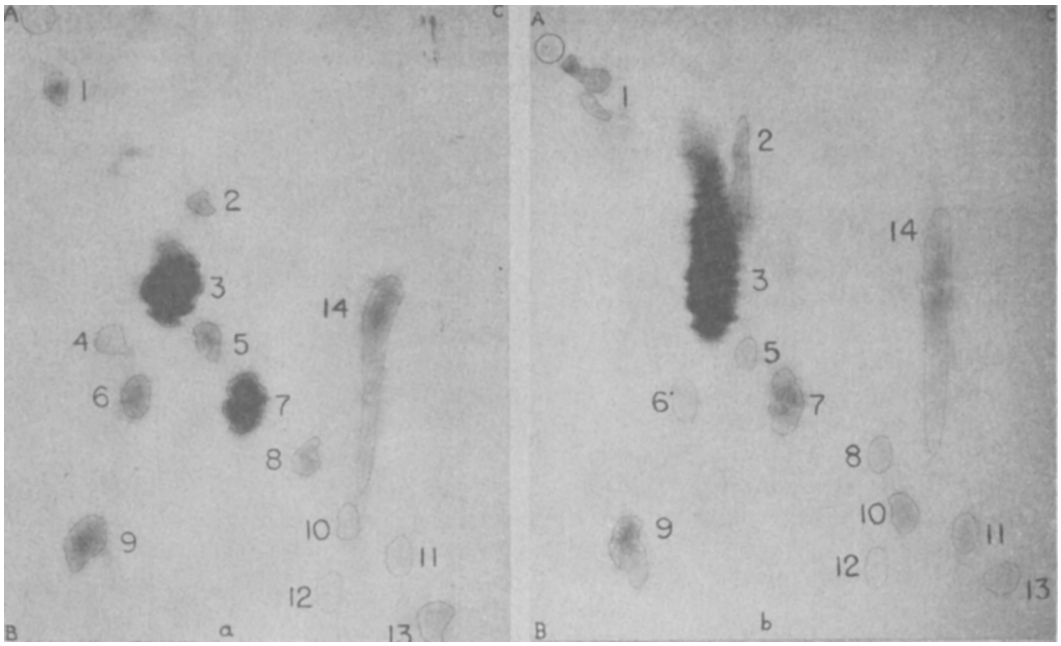

FIG. 1.

Paper partition chromatograms of extracts of sea-urchin eggs and embryos. The ethanol extracts of 25,000 eggs or embryos were placed in the circles in the upper left hand corners. *a*, unfertilized eggs; *b*, prism larvae. A-B, water saturated phenol; A-C, n-butanol-acetic acid-water. Spots (circled with pencil): 1, peptides; 2, glutamic acid; 3, glycine; 4, lysine; 5, threonine; 6, glutamine; 7, alanine; 8, peptide; 10, valine; 11, leucine (or isoleucine); 13, peptide; 9, 12, 14, unhydrolizable ninhydrin-positive substances.

eluted from this area resulted in the disappearance of glutamine and the appearance of glutamic acid.

Several peptides were found with widely different R_f values. These disappeared after acid hydrolysis (refluxed with 6 N HCl for 20 hours) of the extracts and new amino acids appeared and the color of pre-existing marks was intensified. The probable peptide nature of these spots was further investigated by eluting them from a one dimensional phenol chromatogram. The eluate was hydrolyzed with acid and the hydrolyzate chromatogrammed to check the presence of amino acids.

A typical 2-dimensional chromatogram of the free amino acids and peptides obtained from unfertilized sea-urchin eggs is shown in Fig. 1a. Glycine is present in a relatively large amount as indicated by the size and dark color of its spot. Alanine is less concentrated and the remaining amino acids appear as faint spots indicating a comparatively low concentration.

Since spot No. 1 often separates into two areas, it may represent more than one peptide. The amino acids in hydrolyzates of the material eluted from this area have been tentatively identified as glutamic acid, cysteine or cystine, glycine or serine, and possibly aspartic acid. Pure glutathione when added to an extract did not coincide with this spot.

Addition of α-amino butyric acid caused intensification of spot No. 8, which, however, did not appear in acid hydrolyzates of the extracted spot. Instead, a number of amino acids were obtained. The disappearance of spot No. 13 after acid hydrolysis indicates that it is also a peptide. Glycine, serine, alanine and possibly other amino acids are obtained after elution and hydrolysis of this peptide.

Spot No. 14 is an unidentified substance which initially appears as a yellow streak after spraying with ninhydrin, but which becomes dark purple within a few hours. It

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 *Bombyx 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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