number of adsorbents. All the thiaminases are adsorbed on fuller's earth, alumina C γ and bentonite. A number of solvents were tried, but none of them could selectively separate the three enzymes. Work on the separation of the three enzymes by paper chromatography is in progress.

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S. T. DEOLALKAR
KAMALA SOHONIE

Biochemistry Department,
Institute of Science,
Bombay.
Oct. 19.

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Free Amino-Acids in the Yolk during the Development of the Chick

THE progressive appearance of free amino-acids in the yolk during the development of the chick have been examined by paper chromatography, and we have observed that the density of the spots, from comparable aliquots, of yolks from fertilized hen's eggs increased from the fourth to the tenth day and decreased after this to the twelfth. To test these observations by an additional approach, similar samples were chromatographed on 'Dowex'¹ and the results are reported here.

Whole yolks of fertilized hen's eggs at the stages of embryonic development indicated were precipitated with a solution containing 2 ml. of 1.0 N hydrochloric acid per 100 ml. acetone. For each yolk approximately 150 ml. of acetone was added. The water in the yolk made this aqueous acetone approximately 80 per cent acetone. The extracts were evaporated at 25° C. under moving air. The dried residues were dissolved in known amounts of citrate buffer, pH 3.4, and, after centrifugation, aliquots representing approximately 10 gm. of yolk were chromatographed on 100 cm. and 15 cm. 'Dowex' columns². Proline was introduced into some extracts in order to relate other chromatographic positions with the increased absorption at

Table 1. FREE AMINO-ACIDS IN THE YOLK OF THE FERTILIZED HEN'S EGG AT SEVERAL STAGES OF EMBRYONIC GROWTH

Amino-acid	Days of incubation			
	3	6	9	12
		(μ M per aliquot*)		
Threonine	—	0.65	3.9	0.91
Serine	0.35	1.9	5.0	1.8
Glutamic	0.22	0.46	3.2	0.79
Proline	—	1.6	6.8	0.40
Glycine	—	0.41	4.4	—
Alanine	—	0.61	3.7	0.71
Cystine	—	0.56	0.99	0.75
Valine	—	0.51	1.3	1.1
Isoleucine	1.1	1.9	2.7	0.92
Leucine	2.2	2.0	4.0	1.0
Tyrosine phenylalanine	2.3	1.3	5.3	1.3
Lysine†	0.67	0.69	2	1.0
Arginine†	—	—	1.5	—
Methionine	0.10	0.20	0.20	—

* Each aliquot represents the free amino-acids from 10 gm. yolk.

† Released from 15-cm. 'Dowex' columns.

Fig. 1. Chromatographic separation of the amino-acids quantitatively released from 100-cm. columns of 'Dowex 50' with buffered solvents. A and B represent samples of yolks from fertilized eggs incubated three and six days respectively. Dotted lines indicate measurements at 420 m μ . 60 per cent of the peak shown for proline in B was due to introduced proline (see text). Methionine (unlabelled) is on left of isoleucine. The temperature on these columns was at all times less than 75°, and therefore the emergence of tyrosine and phenylalanine together in the effluent is not a limitation of the published procedure (ref. 1)

420 m μ that characterized the reaction product when this amino-acid is treated with ninhydrin. The paper chromatography followed the procedure of Levy and Chung³. Acids were identified by relating the chromatographic positions of known acids with those of the unknown acids, and by comparing the effluent acids from aliquots of the extracts examined on 'Dowex' columns with the distribution of the amino-acids on paper from the corresponding sample.

Typical chromatograms are shown in Fig. 1. The free amino-acids (Table 1) increase to the ninth day, but decrease on the twelfth day. Presumably these changes are related to the progressive proteolysis of the yolks, an observation not previously described for the yolk³ of the hen's egg, although it is known to contain three proteinases⁴. The absence of fixed relative quantities of amino-acids characterizing the intervals sampled is interesting. Similar observations were made on the basis of the density of the spots on paper chromatograms of samples of yolks from the fourth to the twelfth day. It is unlikely that the relative concentration of amino-acids appearing on the ninth, as contrasted with the twelfth, day are a function of proteolysis alone. Such changes are doubtless related to the demands in protein synthesis during the development of the chick.

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MATTHEW A. WILLIAMS
WILLIAM A. DACOSTA
LLOYD H. NEWMAN
LAWRENCE M. MARSHALL

Department of Biochemistry,
Howard University School of Medicine,
Washington, D.C.
Nov. 16.

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