of values of oleic acid in the species investigated with those reported by Kapur *et al.*[4] shows a close relationship of *C. linifolia* with *C. agitiflora*. *C. waghtania* with *C. sericea* and *C. juncea* with *C. rubiginosa*. However, it may be noted that the percentage of this acid in *C. mucronata* as reported is only 7·5% which does not seem to fit into the general pattern of the major fatty acid component of leguminosae in general and *Crotalaria* in particular.

Wolff and Kwolek[1] and Gupta and Chakrabarti[6] observed that the compositional difference of fatty acids reflect heridity relationship; however, it may be pointed that it also shows a phylogenetic relationship at the levels of genus and species.

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## STUDIES ON OVALBUMIN AS A DILUENT IN VIROLOGICAL AND SEROLOGICAL TESTS

THE use of bovine albumin in buffered saline as a viral diluent and virus stabilizer in the field of arbovirology has been recommended by various workers[1,2]. The high cost and non-availability of good quality bovine albumin have compelled us to investigate the possibilities of finding a substitute. In the present studies we have used ovalbumin in virological and serological tests and the results are compared with those obtained with bovine albumin (fraction V from bovine plasma, Armour Pharmaceutical Company Limited, England).

Ovalbumin was prepared from white leghorn eggs obtained from Shreekant Poultry Farm, Narayanagaon, Poona, by sodium sulfate extraction method[3]. The purified albumin solution was dialysed against distilled water to remove excess of sodium sulfate. The total proteins were estimated by the biuret method[4]. Appropriate quantities of albumin solution were then added to phosphate buffered saline and borate buffered saline so as to make 1·5% ovalbumin in phosphate saline (OAPS), pH 7·2 and 0·8% ovalbumin in borate saline (OABS), pH 9·0 respectively.

*Virological Tests.*—The following arboviruses were employed in the study: TR 1751 strain of Dengue type 2, Kaisodi (VRC No. G 14132), African strain of Chikungunya (CHIK), Indian strain of West Nile (VRC No. G 22886), Japanese B Encephalitis (VRC No. P 20778), and Kyasanur Forest Disease (VRC No P. 9605). Three virus diluents were tested, *viz.*, OAPS, BAPS (0·75% bovine albumin in phosphate buffered saline, pH 7·2) and 1 : 1 mixture of OAPS and BAPS. All the diluents contained 1000 units/ml of penicillin and 2 mg/ml of stteptomycin.

Approximately 5 dex $LD_{50}$[5,6] of virus was incubated in each diluent for one hour at 37° C. Further ten-fold virus dilutions were made in the respective diluents. Three weeks old Swiss mice, maintained at VRC, were inoculated by intracerebral (IC) route. Each ten-fold dilution was inoculated into a group of six mice, inoculum being 0·03 ml for each mouse.

1·5% OAPS gave somewhat lower titres, maximum difference being 2·5 dex for P 20778 and minimum being 0·2 dex for P 9605, when compared to those obtained with standard diluent, BAPS. However, 1 : 1 mixture of OAPS and BAPS was found to be a satisfactory diluent as $LD_{50}$ titres for all the viruses tested were almost similar to those obtained with BAPS. When OAPS was employed in 2% concentration the difference in the titres compared to BAPS was considerably reduced.

*Serological tests*—Haemagglutination (HA) and haemagglutination inhibition (HI) tests were performed according to the methods of Clark and Casals[7]. The following arbovirus antigens were employed in the study: Indian strain of Chikungunya (VRC No. 634029), Sindbis (AR 339), Dengue-1 (Hawaii, DEN-1), Dengue-2 (VRC No. P 23085), Dengue-3 (VRC No. 633798), P 20778, G 22886 and P 9605.

All the antigens were diluted 1 : 10 in OABS and kept overnight before testing. Another set of antigens was diluted 1 : 10 in standard diluent—0·4% bovine albumin in borate saline (BABS), pH 9·0 and was also kept overnight before use.

At the optimum pH values, antigens diluted in both the diluents produced similar HA titres. Comparatively higher HA titres were obtained with OABS at higher pH values for P 20778, G 22886, P 23085, and P 9605 antigens. The slight variations

in HA titres obtained at the optimum pH values in the case of AR 339 and P 23085 antigens were within the range of experimental error and thus were not significant.

Homologous mouse hyper-immune sera and fifteen previously tested survey sera (containing 5 human, 5 monkey, 2 bullock, 1 camel and 2 bird) were employed in HI test against all eight antigens to determine the specificity of haemagglutination test with OABS. The tests were performed at optimum pH values of respective antigens for both the diluents. In addition to this, HI test was also performed using OABS at wider pH ranges where comparatively higher HA titres were obtained, *e.g.*, pH 7·0 for P 20778, G 22886 and P 9605, pH 6·0 for DEN-1, P 23085, and 633798. But at these pH values antigens did not remain stable and their titres dropped down. Comparable results were obtained at optimum pH values for both the diluents, *viz.*, OABS and BABS.

Similar results were obtained with different batches of ovalbumin. Ovalbumin being extremely susceptible to denaturation should be stored at 4–8° C and heating, stirring and frothing should be minimised. The precipitate obtained in our stock solution of OABS and OAPS was filtered through filter paper just before use. This, however, did not affect the results.

Our studies indicate that ovalbumin alone can be used for serological tests but needs supplementation with bovine albumin for virological studies.

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## AN ABNORMAL SPECIMEN OF *THRYSSA MALABARICUS* (BLOCH) (PISCES: ENGRAULIDAE) WITHOUT PELVIC FINS

While examining the Clupeoid fishes from Madras Coast, the author came across a specimen of *Thryssa malabaricus* (Bloch) without pelvic fins (Fig. 1). This is a rare case in Engraulids and hence is being reported here.

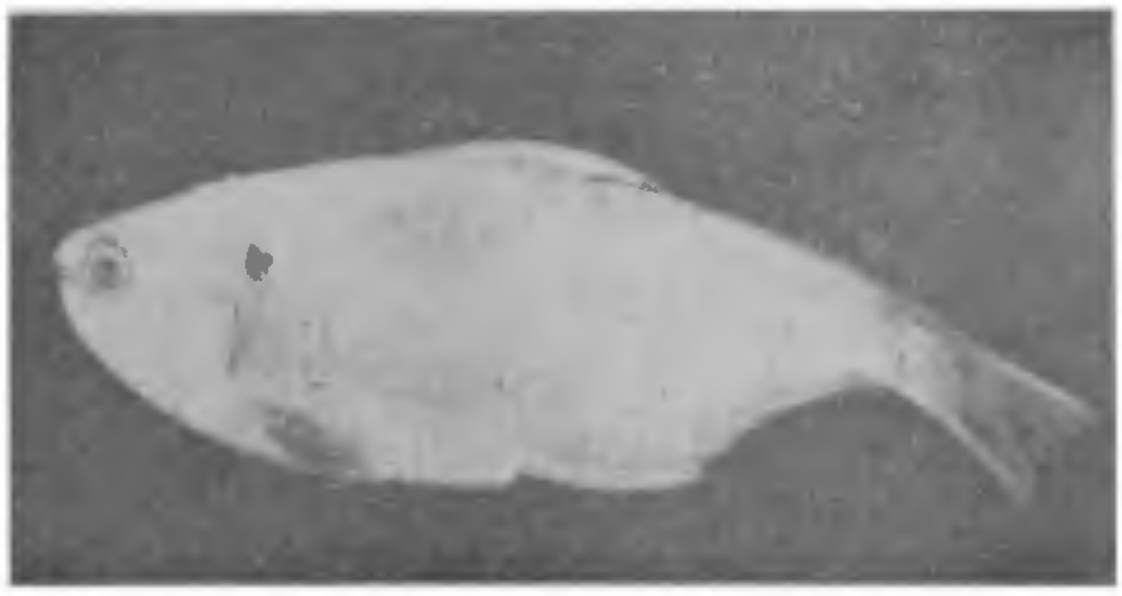

Fig. 1. *Thryssa malabaricus* without pelvic fins.

The meristic counts and the body measurements (in mm) of the specimen are as follows:

P. 13, D. I ii 12, A. ii 38, Scutes (total) 22, G.R. 14 + 17. Total length 139·0, standard length 115·0, Head length 27·3, Body depth 39·2, Eye diameter 7·5, Snout 5·7, Prepectoral distance 29·3, Pectoral fin length 22·5, Preanal distance 70·0, Anal fin base length 37·8, Predorsal distance 55·3, Dorsal fin base length 12·0.

It can be seen that the morphometric characters in general and meristic counts in particular are agreeing with the previous descriptions of this species (Dutt[1], Whitehead[2]) except for the number of scutes, which is somewhat less in this specimen, perhaps associated with the absence of pelvic fins.

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