

Within the limits of our investigation we have failed to correlate the reduced erythrocyte count with the liver fat. The differences in observations of various investigators can possibly be explained by the fact that irradiation-induced change in the liver fat depends on more than one factor, viz., dose, resistance of species, time lapse after irradiation and degree of adrenocortical activation.

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INVESTIGATIONS ON FORMALDEHYDE AS A FIXATIVE AND PRESERVATIVE FOR ZOOPLANKTON

THE Scientific Committee on Oceanic Research of UNESCO constituted a Working Group-23 in 1968 to investigate into the production of better fixatives and preservatives of zooplankton for taxonomic studies. Since 1969 a series of experiments were conducted at the Indian Ocean Biological Centre, Cochin, with a view to studying the effect of climatic variations on tropical zooplankton preserved for a period of ten years to study the effect of time on them. The results obtained so far were presented before the Symposium on "Biology of Indian Ocean" held in Kiel, W. Germany, in 1971 and SCOR/UNESCO/WG 23 Symposium held at Bath, England, from 13th to 18th July, 1972.

Taking all precautions necessary in the collection and handling of zooplankton (Balachandran, 1972) fixation can be delayed upto two hours on arrival on deck as long as they can be kept alive. Experi-

ments on the ratio of the volume of fixative to that of plankton showed 9:1 to be reasonable when 2% formaldehyde was used. Formaldehyde (Walker, 1964) produced by the oxidation of methanol and sold in glass bottles at a storage temperature around 30°C and 35 to 40% in strength with a pH around 3.5, was found suitable for fixation and preservation. To ensure good fixation a gentle shaking was necessary to prevent formation of layers in the beginning. Experiments on fixation indicated that an average of ten days was required for complete fixation of zooplankton. The final penetration of formaldehyde by plankton depended on the strength of formaldehyde used rather than on the time given for fixation and preservation. The reactive group in fixation is the protonated derivative +CH₂ OH which is an electrophile reacting at areas of high electron density, thus providing best fixation at a pH 6 to 7. Acidity of the formaldehyde was caused mainly by cannizzaro reaction, oxidation to formic acid and its reaction with animal protein. This acidity may be neutralised with sodium acetate, sodium tetraborate (borax) or sodium glycerophosphate which can maintain a pH between 6.0 and 7.5. Their reaction with animal protein causing damage to zooplankton was found to be the minimum compared to that of hexamine, rochelle salt, sodium bicarbonate, calcium carbonate, sodium hydroxide, etc. In the tropics a pH value higher than 8 was found harmful as well as that lower than 5. The neutralising agents can be used either with concentrated HCHO (40%) or dilute formaldehyde (1-2%). Sea water itself being a weak buffer gives a pH of 7, and was found to be a better diluent for formaldehyde for mixed plankton.

After fixation is complete it was found better to pour off the fixative and replace it by 1% formaldehyde as a preservative. The less harmful dilute formaldehyde can be equated in properties to that of strong formaldehyde by the addition of 10% propylene glycol and 1% propylene phenoxetol which can maintain protein flexibility and prevent the specimens from becoming brittle. They also could reduce shrinkage due to protein contraction during fixation in spite of increasing the osmotic pressure of the solutions and increase the bactericidal, fungicidal and clarity effects of the solution. Since long duration storage leads to evaporation of diluent, increasing formaldehyde strength and reducing pH, annual replacement of preservative will be of prime importance. To prevent formation of layers and the resultant difference in the strength of formaldehyde at the top and the bottom, an occasional shaking was found necessary. Experi-

ments on plankton volume to 1% formaldehyde preservative have indicated a good ratio of 1 : 5 and above. Due to gelatinisation of protein at high temperatures, long time storage can be recommended in air-conditioned rooms preferably darkened to reduce the effect of light on loss of pigmentation. From the results obtained so far it can be concluded that 2% formaldehyde as a fixative and 1% formaldehyde as a preservative can do well as a fixative-cum-preservative for mixed zooplankton, provided (a) sea water is used as a diluent; (b) 2% sodium glycerophosphate is added to concentrated formaldehyde as a neutralising agent and (c) 10% propylene glycol and 1% propylene phenoxetol are added as additives.

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FATTY ACIDS OF THE BODY FAT OF TOAD (*BUFO MELANOSTICTUS*)

It is well known that the fatty acid composition of fats obtained from aquatic animals differs significantly from that of land animals. The former contains significant proportions of unsaturated fatty acids with 16, 18, 20 and 22 carbon atoms, while the composition of the latter is much simpler consisting of predominantly oleic and palmitic acids and does not contain the unsaturated acids of 20 and 22 carbon atoms. It should be a natural assumption that the fatty acid composition of amphibian fat will be of intermediate nature. A few but significant investigations^{1a} on such amphibia as frog, toad, tortoise, crocodile, etc., prove this to be true. Most of the earlier work was done before the introduction of gas liquid chromatography (GLC) for the determination of fatty acid compositions. Amongst the few investigations made with GLC mention may be made of Ackman's² studies on fats of Atlantic Leather-back turtle and Light and Waschek's³ work on liver fat of frog. This communication reports

the fatty acid composition of the body fat of toad determined by GLC.

Experiments were carried out with pooled tissues from mature male toads (average body wt. 50 gm). Bodies of eight toads were separated from the region between body and head (apparently neck region) and heads were discarded. The resulting tissues were homogenised and extracted with a mixture of chloroform and methanol (1 : 2) according to Bligh and Dyer⁴. The extracted lipid was washed by the method of Folch *et al.*⁵. The resulting lipid was saponified by the method of Hilditch^{1b} the unsaponifiable matter was removed by ether and the fatty acids were liberated with dilute hydrochloric acid in an atmosphere of nitrogen. The fatty acids were extracted with peroxide free ether and the combined extracts were freed from mineral acids by washing with distilled water, dried over anhydrous sodium sulphate, and ether was removed under vacuum. The mixed fatty acids were converted to methyl esters by refluxing with dry methanol and 1% conc. sulphuric acid. The mixed methyl esters obtained were purified on silica gel G preparative thin layer plates⁶. The fatty acid composition was determined by gas-liquid-chromatography⁷.

The composition calculated from chromatograms of methyl esters are presented in Table I. The

TABLE I
 Component acids of whole body fat of toad

Component acids	Percentage by weight
C ₁₂	0.66
C _{12:1}	Trace
C ₁₃	0.13
C ₁₄	0.78
C _{14:1}	Trace
C ₁₅	0.18
C _{16:1}	0.66
C ₁₆	21.69
C _{16:1}	3.00
C ₁₇	Trace
C _{17:1}	0.15
C ₁₈	10.54
C _{18:1}	37.86
C _{18:2}	21.57
C _{18:3}	1.78
C ₂₀	Trace
C ₂₂ unsaturated	Trace

results show that like the fats of land animals the whole body fat of toad contains only traces of C₂₀ and C₂₂ unsaturated fatty acids, which are found in appreciable amounts in the fats of aquatic animals. In one respect the fat of toad differs from the fats of land animals; it contains large proportions of C₁₆ acids which is also a characteristic of the fat of aquatic animals. However, in toad