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Waltair, *January* 23, 1973.

1. Martin, A. L., *Proc. Zool. Soc. Lond.*, 1964, 143 (4), 525.
2. Schmitz, E., *Amer. Mid. Natur.*, 1967, 78 (1), 1.
3. Stainer, J. E., Woodhouse, M. A. and Griffin, R. L., *Crustaceana.*, 1968, 14, 56.
4. Hirsch, G. C. and Jacobs, W., *Z. Vergl. Physiol.*, 1928, 8, 102.
5. — and —, *Ibid.*, 1930, 12, 524.

ON ANEMIA AND TOTAL LIVER FAT IN THE X-IRRADIATED GARDEN LIZARDS

OBSERVATIONS on the total fat in the liver of different mammalian species after whole body X-irradiation with lethal doses lack in agreement. Increase in sudanophil fat in the mouse liver is reported by Ellinger[1] and in the liver of rats and mice by Skalka[2], while the findings of Bacq *et al.*[3] point out no fatty infiltration in the liver of irradiated rats not fed with fat-rich diet. The reported cases of accumulation of fat in the liver seem to be effected by an excess of glucocorticoids in the blood[4]. Further, a relationship seems to exist between the total liver fat and anemia in irradiated mammals[2]. The present communication deals with our findings on the erythrocyte count and estimation of total fat in the liver of a whole body X-irradiated reptile, the common garden lizard, *Calotes versicolor*.

Adult male lizards ($20 \pm 0{\cdot}5$ g), acclimated to laboratory conditions for 3 days, were whole body X-irradiated with a single dose of 990 R. Acclimation, irradiation as well as maintenance of corresponding controls were done in the manner described earlier[5]. 3 lizards from control and 3 from irradiated group were sacrificed both at 24 and 72 hr after irradiation for erythrocyte count and estimation of liver fat. Estimation of total fat[6] in the liver was done after extraction of fat in Soxhlet apparatus, and improved Neubauer-Haemocytometer was used following the procedure as described by Chatterjee[7] for erythrocyte count. The data were statistically analysed and Students *t* test was performed to assess the significance of difference, if any, between the data in control and irradiated groups.

The results, shown in Table I, reveal no significant alteration either in the weight of the liver or in the total fat therein in the 990 R irradiated lizards at 24 or 72 hr. In fact, the mean average weight of the liver and of the total fat in the liver ran parallel with those of the corresponding controls. The erythrocyte count, however, dropped significantly at 72 hr after irradiation. This drop in count, therefore, was not accompanied by a decrease in the weight of total liver fat. It is on record[8] that a total dose of 1700–2350 R, given to rat in daily dose of 50R increased the glyceride content in liver while the liver lost weight. Skalka[2] has pointed out the possibility of a relation between anemia and total liver fat in mice and rats in the second week after whole body X-irradiation with 500–640 R. In both the cases the erythrocyte count dropped significantly and the total liver fat increased.

TABLE I

Showing weight of liver, total liver fat, and erythrocyte count in control and 990 R X-irradiated lizards

Animal group and number		Weight (mg) of liver		Total lipid (mg) in liver		mg lipid/ 100 g liver		10^6 erythrocytes/ mm^3 blood	
		24 hr	72 hr	24 hr	72 hr	24 hr	72 hr	24 hr	72 hr
Control	1 ..	468·7	430·0	58·3	54·0	12·438	12·558	9·42	9·34
	2 ..	449·4	443·5	55·5	46·9	12·347	10·574	9·40	8·80
	3 ..	458·7	465·5	55·3	55·6	12·055	11·944	8·80	9·60
Mean average	..	458·9	446·3	..	..	12·280	11·692	9·20	9·24
±	..	±	±	..	..	±	±	±	±
SD	..	9·6	17·7	..	..	0·200	1·015	0·35	0·40
990 R	1 ..	469·0	477·1	40·5	55·5	8·771	11·632	8·10	7·25
	2 ..	452·5	427·7	57·0	51·8	12·596	12·111	9·30	6·20
	3 ..	556·1	454·0	62·8	59·5	12·292	13·105	8·70	5·60
Mean average	..	492·5	452·9	..	..	11·219	12·282	8·70	6·35*
±	..	±	±	..	..	±	±	±	±
SD	..	52·9	24·7	..	..	1·911	0·751	0·60	0·83

* $P < 0{\cdot}01$.

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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1. Ellinger, F., *Radiology*, 1945, 44, 241.
2. Skalka, M., In *Proc. Second U.N. Intern. Conf. on the Peaceful Uses of Atomic Energy, Geneva*, 1958, 22, 250 (U.N., 1959).
3. Bacq, Z. M., Burg, C., Chevallier, A. and Heusghem, C., *J. Physiol.* (*Paris*), 1951, 43, 640.
4. Bacq, Z. and Alexander, P., *Fundamentals of Radiobiology*, Pergamon Press, Oxford, London, N.Y., Paris, Chap. 13, p. 311.
5. Ghosh, (Miss) A., Maity, L. K. and Banerjee, S. K., *Ind. J. Exptl. Biol.* (In press).
6. Milton, R. F. and Walter, W. A., *Methods of Quantitative Microanalysis*, Edward Arnold Pub., London.
7. Chatterjee, C. C., *Human Physiology*, New Central Book Agency, Calcutta, 1969.
8. Richmond, J. E., Altman, K. I. and Salomon, K., *J. Biol. Chem.*, 1951, 190, 817.

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 Experiments 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 $+ CH_2$ 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-