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# EFFECT OF IONISING RADIATIONS ON THE HAEMOGLOBIN OF A MARINE BIVALVE *SCAPHARCA DEYROLLEI* SUB SP. *CRISPI* PATEL AND PATEL*

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ABSTRACT

Spectrophotometric studies on the iron linked respiratory protein haemoglobin in arcid clam *Scapharca* was discussed in earlier communication (Patel and Patel, 1968). The present paper discusses further investigations on the effect of ionizing radiations on the haemoglobin of *Scapharca deyrollei* sub. sp. *crispi*.

Oxyhaemoglobin solutions when exposed to Cobalt-60 source delivering 4,600 $\gamma$ per minute showed decrease in absorption maxima at 280, 415, 540 and 576-78 m$\mu$ and an increase around 510 and 630 m$\mu$. No difference in spectral behabiour was observed when various Hb-fractions obtained by calcium phosphate gel were irradiated separately. Exposure to dose exceeding 1150 $\gamma$ resulted in precipitation of the pigment. Changes occuring during irradiation were similar to those produced when oxyhaemoglobin was treated with hydrogen peroxide, suggesting that $H_2O_2$ produced during radiolysis of aqueous solutions may be the species responsible for the damage. Haemoglobin of *Scapharca* was found to be extremely radiosensitive as it could not be exposed to dose exceeding 1150 $\gamma$

## INTRODUCTION

A great deal of work in radiation chemistry has been aimed at obtaining an understanding of the effects of ionizing radiations on living cells. Large complicated protein molecules such as enzymes and nucleic acids are of critical importance to the functioning of the living cells. A knowledge of the changes brought about in the physico-chemical and biological properties of these proteins is essential in understanding these effects. Much of the work reported to-date on the effects of ionizing radiations relates to the large protein molecules derived from higher vertebrates (Barron *et al.*, 1949, 1955, 1956; Collinson *et al.*, 1950; McDonald, 1955; Lasser, 1955; Mee and Stein, 1956; Rothschild *et al.*, 1958; Moroson and Alexander, 1961; Kubota and Watanabe, 1967 a, b). Apart from the work of Savedberg and Brohult (1938) on splitting of haemocyanin molecule by ultraviolet light practically little is known of the effects of ionizing radiations on the invertebrate proteins, especially those of aquatic forms. These studies assume significance, since aquatic organisms are known to accumulate radioactivity from its environment significantly. Studies were therefore, initiated to understand the mechanism of the effects of ionizing radiations on haemoproteins of marine Lamellibranchs (*Anadara granosa* and *Cardita antiquata* (Patel and Patel, 1971). The present paper in continuation with the previous work reports the effect of ionizing radiations on the erythrocytic haemoglobin of *Scapharca deyrollei* sub sp. *crispi* Patel and Patel from Bombay waters.

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## EXPERIMENTAL TECHNIQUE

*Scapharca deyrollei* sub. sp. *crispi* were collected from Mahim and Alibag Creek. The technique of haemoglobin extraction and its purification was same as described earlier (Patel and Patel, 1964, 1968). The haemolyzed blood was purified by absorption on calcium phosphate gel, and eluted in M/15 phosphate buffer pH 7.0. A series of fractions thus obtained were irradiated separately. The whole blood and purified fractions (5-7 ml) in glass weighing bottles were exposed to cobalt-60 source delivering a dose of 4,600 $\gamma$ per minute. Radiation damage or degradation was measured spectrophotometrically by measuring the changes in the absorption maxima, and expressed as % increase or decrease with reference to unirradiated control.

### *Irradiation of* Scapharca $O_2Hb$

*Scapharca* haemoglobin on exposure to 1150 $\gamma$ showed general decrease in the absorption peaks at 280 (protein peak), 412 (Soret peak), 540 ($\beta$) and 570 ($\alpha$) m$\mu$ (Fig. 1a). On exposure to still higher dose (2300 $\gamma$) the pigment precipitated, suggesting the total destruction of the haemoglobin molecule. This at once confirmed our earlier observations on the extreme radiosensitivity of the intracellular haemoglobin of *Anadara granosa* (Patel and Patel, 1971).

TABLE 1. *Effect of ionizing radiation* (1150$\gamma$) *on* Scapharca *whole blood and its purified fractions expressed as % increase* (+) *or decrease* (—) *with reference to unirradiated control*

| Wavelength (m$\mu$) | Whole blood | Fractions I | II | III | IV | V | VI |
|---|---|---|---|---|---|---|---|
| 278—80 | - 0.81 | - 4.48 | - 1.42 | - 3.15 | - 4.39 | - 4.02 | - 6.07 |
| 412—15 | - 3.60 | - 3.77 | - 3.58 | - 7.19 | - 5.92 | - 9.07 | - 2.53 |
| 510 | - 1.03 | + 3.40 | - 3.26 | + 6.90 | - 1.37 | - | - 4.41 |
| 540—42 | - 7.96 | - 9.18 | -12.98 | -12.78 | -22.80 | -23.21 | -22.22 |
| 576—78 | -10.65 | -15.18 | -15.90 | -20.00 | -27.36 | -33.33 | -26.31 |

It will be seen from Table 1, which records the effect of ionizing radiations (1150 $\gamma$) that $\alpha$ peak was relatively more affected than the $\beta$ peak, in the sense that $\alpha$ peak almost disappeared. These effects could still be better understood by calculating relative changes in each of these peaks. Table 2 therefore, records the effect of ionizing radiations on the initial purification and browning indices of the whole blood and each of its six fractions. The purification index represents the ratio of optical density per 1 cm at 540 to that at 280 m$\mu$. The browning index, which represents the oxidation of the pigment was calculated taking the ratio of O. D. at 510 to that at 540 m$\mu$ (Patel and Spencer, 1963). It will be evident from the results shown in Table 2 that the purification indices in general, for the whole blood and its fractions six decreased on exposure to ionizing radiations, suggesting the degradation or changes in the protein molecule. The browning indices on the other hand, showed significant increase, indicating the oxidation of the Fe++ to Fe+++

Further it will be seen from Table 1, that there was no appreciable difference in spectral behaviour of the whole blood and each of its fractions, when exposed to ionizing radiations. The changes in the degree of effect reflect to the variation in the concentration of the pigment, which varied from fraction to fraction. To

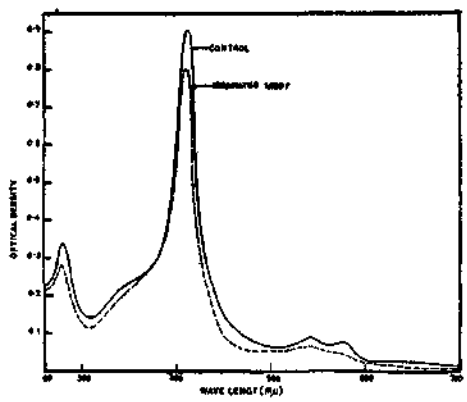

Fig. 1 a. Effect of ionizing radiation on $O_2$ Hb of *Scapharca.*

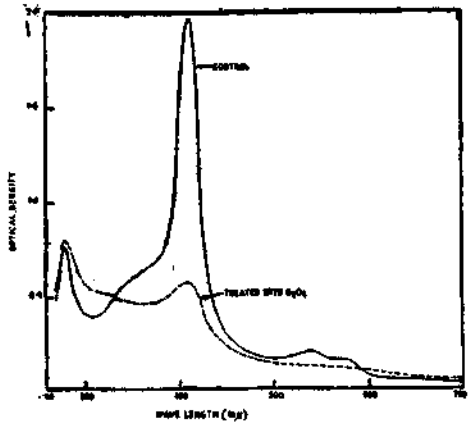

Fig. 1 b. effect of $H_2O_2$ on$O_2$ Hb of *Scapharca.*

confirm if the concentration of the pigment has any relation to the final effect on exposure to ionizing radiation, purified haemoglobin solutions of three different concentrations were irradiated (1150 $\mu$). It will be clearly seen from the results shown in Table 3, that dilute solutions were more affected than the concentrated ones.

TABLE 2. *Effect of Ionizing radiations* (1150 $\gamma$) *on purification and browning indices of* Scapharca deyrollei *sub sp.* crispi *haemoglobin and its fractions. (expressed as % increase* (+) *or decrease* (—) *with reference to control).*

| Index | Whole blood | Fractions I | II | III | IV | V | VI |
|---|---|---|---|---|---|---|---|
| Purification* | —7.0 | —5.0 | —11.5 | —9.9 | —19.2 | —20.1 | —17.2 |
| Browning** | +7.6 | +13.9 | +11.3 | +27.6 | +27.8 | +30.2 | +23.0 |

$$\text{* Purification Index} = \frac{\text{O.D. at 540 — 42 m}\mu}{\text{O.D. at 278-80 m}\mu}$$

$$\text{**Browning Index} = \frac{\text{O.D. at 510 m}\mu}{\text{O.D. at 540-42 m}\mu}$$

It is well known in radiation chemistry that the damages to large protein molecules on exposure to ionizing radiations is indirect and results from molecular decomposition products of water ($H_2O_2$ and $H_2$) or by short lived free radicals (OH and $O_2H$). The effect of direct addition of $H_2O_2$ was therefore, studied to understand the mechanism. On treatment of oxyhaemoglobin with $H_2O_2$ in contrast to exposure to ionizing radiation, the absorption at protein peak increased in dilute solution, whereas in concentrated solution it was not significantly affected. The other peaks were affected in a similar way as on irradiation (Table 3; Fig. 1b).

TABLE 3. *Effect of ionizing radiations* (1150 $\gamma$) *and hydrogen peroxide on* Scapharca *haemoglobin of different concentrations [expressed as % increase* (+) *or decrease* (—) *with reference to untreated control].*

| Wavelength (mμ) | Exposure to $\gamma$-rays | | | Addition of $H_2O_2$ | | |
|---|---|---|---|---|---|---|
| | X | 2 X | 4 X | X | 2 X | 4 X |
| 278—80 | —16.3 | —1.5 | —0.1 | +10.4 | +6.6 | —0.3 |
| 412—15 | —11.3 | —6.8 | —2.7 | —92.0 | —90.8 | —82.8 |
| 540—42 | —28.0 | —11.0 | —10.0 | —79.3 | —76.5 | —66.6 |
| 576—78 | —38.0 | —17.0 | —16.4 | —81.0 | —77.3 | —64.3 |

X — 50 μg per ml of $O_2$ Hb

The erythrocytic pigment of *Scapharca* was found to be extremely radiosensitive, since the pigment precipitated completely on exposure to dose exceeding 1150 $\gamma$, although the colour of the pigment did not change significantly. On addition of $H_2O_2$ however, the pigment has destroyed rendering the solution almost colourless without any precipitation, which invariably occurred on irradiation. The destruction of the protein on irradiation at dose exceeding 1150$\gamma$ was obviously therefore, not due to $H_2O_2$, although at a lower dose the effect on various absorption maxima may possibly be due to $H_2O_2$ produced during radiolysis. The precipitation of the pigment on exposure to doses exceeding 1150$\gamma$, which possibly involves denaturation of the protein could well be due to direct effect of ionizing radiations resulting in complete alteration of the protein structure.

It could be concluded from the results reported earlier (Patel and Patel, 1971) and discussed above that the intracellular haemoglobins in general, are more radiosensitive than are extracellular haemoglobins of clam (*cardita antiquata*, which could be exposed to dose as high as 300,000 $\gamma$ without any sign of the precipitation of the pigment. Further between the two intracellular haemoglobins the haemoglobin of *Scapharca* appeared to be more radiosensitive than that of *Anadara granosa* which could be exposed to about 18000 $\gamma$.

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