Experimental Oxidation of Hæmoglobin Effects of Irradiation in Rats

THE sensitivity of human intracellular hæmoglobin to oxidation by nitrite ions is raised during growth, pregnancy in malignant disease and in some other conditions¹. It has also been shown to be increased in similar circumstances in the rat^{2,3} and in this animal the changes appear to be related to riboflavin metabolism²⁻⁴. Both riboflavin deficiency⁵ and X-rays interfere with growth and are teratogenic. The effect of radiation on the stability of hæmoglobin against oxidation by nitrites has now been studied in the rat. A dose of 500 r. was chosen ($< \text{MLD}_{25}$) and was administered at approximately 20 r./min.

The age of the rat at irradiation had a definite effect on the reaction of the animal to this dose (cf. ref. 6). The growth-rate of suckling rats was only slightly affected, weaned but immature rats suffered a temporary pause of about one week in their growth, while mature rats lost weight for the week following irradiation.

Fig. 1. Change in the reaction time (the time taken to convert the hæmoglobin of a standard saline red cell suspension to methæmoglobin using 0.1 per cent sodium nitrite *in vitro*) with age and the effects of 500 r. cobalt radiation at various ages. (The S.D. of any point is approximately ± 5 sec.)

While the ultimate effect on the hæmoglobin oxidation sensitivity—marked increase in the hæmoglobin oxidation sensitivity for at least three months—is identical whatever the age at irradiation, the initial changes did vary with age (Fig. 1). Suckling rats merely retain the increased sensitivity of the rapidly growing animal into adult life. Mature rats, from puberty to old age, respond to irradiation with an immediate increase in their hæmoglobin oxidation sensitivity to levels found normally in the young or pregnant. Weaned, but still immature, rats, however, exhibit the ultimate increased sensitivity of their hæmoglobin only after an initial period of 3–4 days decreased sensitivity.

The increased hæmoglobin oxidation sensitivity of starving, young or pregnant rats can be altered towards the normal by 2 mgm. of riboflavin given subcutaneously^{3,4}. That riboflavin or flavin mononucleotide is without effect in the increased sensitivity following radiation but is effective when given in conjunction with adenosine triphosphate (Table 1) supports the suggestion⁷ that some at least

Table 1. THE EFFECT, IN IRRADIATED ADULT RATS, OF THE INJECTION OF VARIOUS SUBSTANCES ON THE TIME (SEC.) TAKEN TO OXIDIZE THEIR INTRACELLULAR HÆMOGLOBIN TO METHÆMOGLOBIN BY A 0.1 PER CENT SOLUTION OF SODIUM NITRITE *in vitro*. (S.D. given, samples of five animals.)

Substance injected	Reaction-time at intervals after injection			
	Before	1 hr. after	2 hr. after	4 hr. after
2 mgm. Riboflavin	35 $\pm$ 3	36 $\pm$ 6	36 $\pm$ 2	37 $\pm$ 5
2 mgm. Flavin mononucleotide	39 $\pm$ 7	—	37 $\pm$ 2	36 $\pm$ 3
25 mgm. Flavin mononucleotide	35 $\pm$ 4	35 $\pm$ 5	35 $\pm$ 5	35 $\pm$ 5
2 mgm. Riboflavin	32 $\pm$ 4	31 $\pm$ 6	38 $\pm$ 3	31 $\pm$ 5
2 mgm. Adenosine triphosphate	29 $\pm$ 2	30 $\pm$ 2	47 $\pm$ 5	34 $\pm$ 3
2 mgm. Riboflavin				
5 mgm. Adenosine triphosphate				

of the effects of radiation are due to interference with oxidative phosphorylation.

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Dose Effect Curve of Somatic Mutations in *Ephestia kühniella* Z. for Low Dose-range (0–200 r.).

Ephestia kühniella Z., the meal moth, proved to be a good object for investigation of quantitative relations between somatic mutations and ionizing radiations^{1,2}. These former investigations were concerned with relative high doses (100–1,000 r.). It seemed interesting to extend the experiments to the lower dose-range, since there is a complete lack of knowledge of this part of the dose effect curves of somatic mutations in animals.

On each of the hind-wings of *Ephestia* there are about 6,250 scales of uniform shape and colour. After irradiation at an early stage of development clusters of scales of different appearance are found, which have been described as somatic mutations³. After irradiation of 10–16 hr. old pupæ these mutations only affect single scales, so that the mutation-rate can be measured directly from the proportion of mutated scales to the total number of scales. This calculation is valid only on the assumption that the irradiation has no lethal effect on the mother-cells of the scales, no matter how high the dose. In order to ascertain this fact the number of scales on 6 wings of individuals irradiated with 10 resp. 200 r. was determined and the mean values proved to be not significantly different (6,270/wing and 6,233/wing, $P = 0.8$).

Groups of at least 40 pupæ were exposed to one of the different doses (10, 20, 40, 80, 200 r., and control), and both wings of 30 randomly chosen individuals were examined for mutations. This experiment was repeated twice, so that for each dose $3 \times 30 \times 2 \times 6,250 = 1,125,000$ scales were classified. For the irradiations an RT 100 X-ray apparatus (C.H.F., Hamburg) was used, running at 100 kV., with a 1.7 aluminium filter and a dose-rate of 133 r./min.