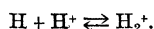


suspension under oxygen bubbling, whereas in alkaline suspension radiation effects are enhanced. Under hydrogen bubbling there is much less pH dependence (Fig. 1b). These facts would appear to support the hypothesis that O_2^- radical ions are inactivating agents for phage. The pH dependence under hydrogen bubbling may arise from the reaction proposed by Weiss (8),



The effects of pH variation on formation and decomposition of H_2O_2 were reported previously by Ebert and Boag (9). As Fig. 2 shows, the equilibrium

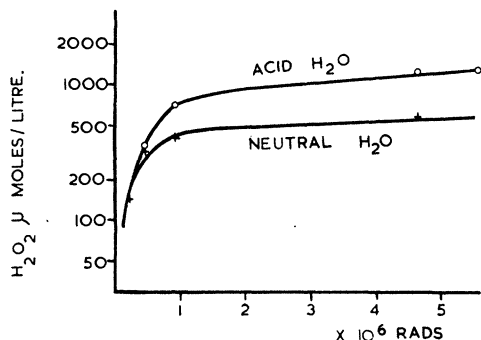


Fig. 2. Formation of H_2O_2 by electron irradiation under oxygen bubbling, at two hydrogen ion concentrations.

yield of H_2O_2 under oxygen bubbling was higher in more acid solution, indicating that the back reaction (decomposition of H_2O_2) became equal to the forward reaction (formation of H_2O_2) earlier, in more alkaline solution. The formation of H_2O_2 under nitrogen bubbling could not be followed by the methods used, because the yields were not measurable. The effect of varying the pH was therefore studied by means of decomposition reactions, and, as is shown by Fig. 3, these were very much slower in more acid solution.

Although the dependence of radiation effects on pH is well established in radiation chemistry and is to be predicted from theories of radical reactions (10), only a few biological systems appear to have been studied

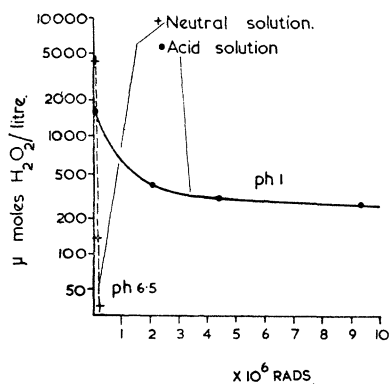


Fig. 3. Decomposition of H_2O_2 by electron irradiation under nitrogen bubbling, at two hydrogen ion concentrations.

from this point of view (11-13). The nonuniform distribution, within the cell, of metabolites, enzymes, and colloidal matter probably involves local and temporal variations in pH and in the concentration of dissolved substances. If radiation-produced radicals are responsible for some effects in the living cell, then a complicated pattern of radiosensitivity is to be expected on these grounds alone.

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Theoretical Rate of Fat Formation by Yeasts

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Synthesis of fat by microorganisms has interested a number of investigators since the early work of Lindner (1). We recently reported on some studies concerning the effect of several cultural conditions on fat formation by *Rhodotorula gracilis* (2). In this work the fat content of the yeast was expressed as a weight ratio of fat to nonfat dry yeast. When this fat ratio was plotted against time, the curve was a straight line after an initial lag period. This indicated that the cells produced an equal quantity of fat during like intervals of time. Thus the fat was produced at a constant rate which was represented by the slope of the linear portion of the curve. We have termed this slope the *fat rate*; it is expressed in grams of fat per 100 g nonfat dry yeast per hour. It was of interest to compare experimental fat rates with the maximum rate theoretically possible. Such a comparison would indicate whether a larger rate of fattening could be obtained as a result of further investigations on this problem.

The relationship between the energy required for growth of the yeast and that required for synthesis of fat was the basis used in deriving a theoretical fat rate. Thus, a given rate of growth is evidence of a definite rate of energy utilization. If this energy were applied solely to the formation of fat, then a corresponding rate of fattening would be obtained. This theoretical fat rate was derived as follows.

The weight of cellular material present in the logarithmic growth phase of a microbial fermentation may be expressed as

$$W = W_0 2^{(t/g)},$$

where: W is the weight of cells present at time t , W_0 is the weight of cells present at time $t = 0$, and g is the generation time. Converting to natural logarithms and differentiating,

$$\ln W - \ln W_0 = (t/g) \ln 2,$$

$$\frac{dW/dt}{W} = \frac{0.693}{g}.$$

Thus, the instantaneous rate of cell increase per unit weight of cells present is inversely proportional to the generation time. Taking the observed generation time for *Rh. gracilis*, namely, $g = 2.8$ hr, we find that the rate of dry weight increase in grams per hour per 100 g of dry yeast present is $100 \times 0.693/2.8 = 25$. The cellular material being synthesized at this rate is composed of carbohydrate and protein; that is, almost no fat is being produced.

If we assume that, on a weight basis, the energy content of fat is 2.5 times that of carbohydrate or protein (3), we find the maximum rate of fat production to be

$$\frac{25 \text{ g carb. + prot.}}{100 \text{ g yeast/hr}} \times \frac{1 \text{ cal/g carb. + prot.}}{2.5 \text{ cal/g fat}} = 10 \text{ g fat per 100 g nonfat dry yeast per hour.}$$

Therefore, assuming that the energy transport enzyme system of the cell is as efficient during fat production as when carbohydrate and protein are being formed, we may conclude that the maximum rate of fat formation by *Rh. gracilis* would be 10. Calculations on other microorganisms, for example, *Torulopsis utilis*, showing the same generation time, should give the same results.

The converse of this conclusion must also be examined. If the rate of fattening is below the theoretical value calculated in preceding paragraphs, then we may state that the slowest or rate-determining steps occur in the fat-forming process and not in the respiratory or energy-yielding system leading up to it.

We may now compare this theoretical maximum fat rate with results obtained under experimental conditions. Enebo *et al.* (4) presented data on the fattening of *Rh. gracilis* at two levels of inoculum under otherwise similar conditions. These data were recalculated in order to determine fat rates. At both levels of inoculum, the calculations showed the fat rate to be 2.0. Kleinzeller (5) reported that *T. lipofera* increased in fat content from 23 to 33 percent in 9 hr. If we assume that these data came from the linear portion of the fattening curve, the calculated fat rate would be 2.2. The maximum fat rate found in our work, using *Rh. gracilis*, was 3.1. It is to be noted that these several experimental fat rates are about one-fourth of that theoretically possible as derived in preceding paragraphs. This suggests that further investigations should reveal the conditions required for more rapid synthesis of fat by microorganisms.

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The Fertile Life of Mouse and Rat Eggs

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Recently, Runner and Palm (1, 2) have reported ingenious experiments on the transfer of unfertilized mouse eggs at various times after induced ovulation to previously mated recipient mice. The transplanted eggs thus underwent fertilization in the host, and the foetuses developing from these eggs were recognized by eye color. They recorded a mean of 1.1 living young at term from eggs transplanted at the time of ovulation and 0.2 and 0 young from eggs transplanted 4 and 8 hr, respectively, after ovulation. Runner and Palm interpreted their results to mean that a delay of 4 hr or more between the ovulation and the fertilization of mouse eggs would cause a large reduction in the number of eggs capable of normal development. They remarked that their findings were consistent with those previously reported for other species in indicating that fertilization must commence during a "precariously short" interval after ovulation if mammalian eggs are to develop normally to term.

Some of the observations that we have made also bear upon the problem of the length of the fertile life of mouse and rat eggs. Our findings, while confirming the reduction in fecundity after delayed fertilization, indicate that most of the eggs retain their capacity for normal development for a much longer period than that implied by Runner and Palm. It also appears that the losses observed after delayed fertilization can be partly imputed, in the rat but not in the mouse, to the occurrence of polyspermy.

Our evidence is as follows. In one experimental series, 115 adult female rats that had mated during the early evening were killed in groups of 10 to 30 animals at hourly intervals from midnight to 7 A.M. the following morning (3). Ovulation occurred between midnight and 4 A.M. The number of eggs ovulated and the proportion of these that had been penetrated by sperms were recorded. When the percentage of rats that yielded eggs and the percentage in which all eggs were penetrated are plotted against time, the mean interval between the two curves is found to be about 5½ hr. This is interpreted to mean that, in any one rat, the mean time between the beginning of ovulation and the penetration of all the eggs by sperms was about 5½ hr.

The procedure was repeated with 100 rats that had