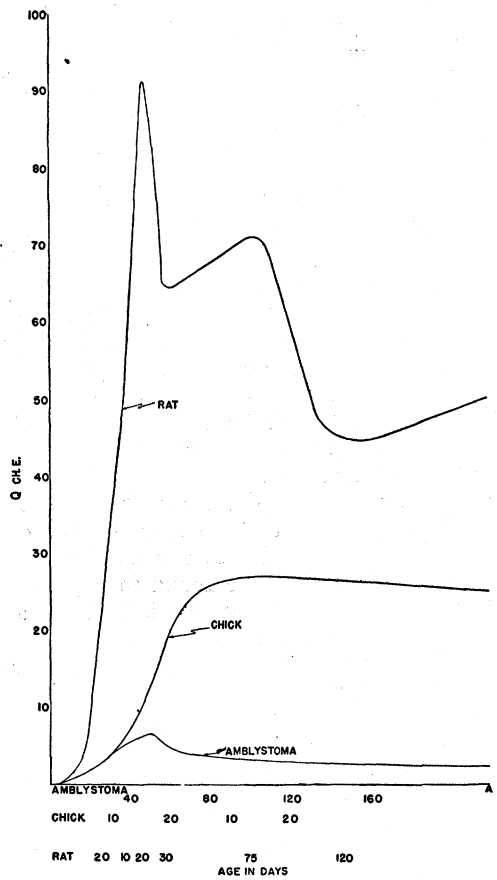

FIG. 2. Comparison of the *Q CH.E.* of rat, chick and salamander nervous tissue, during comparable stages of development.

crease in muscle coordination from the day of birth and continuing through the 22nd day, at which time the rat possesses virtually all the adult responses. These data correspond very well with the rise in the esterase content in the rat brain. In the early fetal stage, in which Angula found the animal to be nonmotile, the enzyme content was too low to be measured. The development of reflexes is accelerated in the days just following parturition. The rise in esterase activity during this period is probably responsible for the increased use by the organism of functional nervous pathways.

Nachmansohn (*1*), in his examination of the esterase content in the brain of the chicken, found that the activity of the enzyme increases sharply to the 8th day after hatching and subsequently fell only slightly in the later development. Nachmansohn made a series of determinations from early embryonic stages to the time of hatching. At the 6th day of incubation, the *Q CH.E.* was 1.38, increasing to 20.8 at 20 days' incubation, and reaching the highest value of 26.0 at 8 days after hatching. The value for the adult is 25.6. These data show that the curve in the chick begins to rise at an early developmental stage, probably as a result of the very rapid early development of the embryo within the egg. By the time of hatching the chick is well developed and has most of the adult reflexes. Determinations made by Nachmansohn (*7*) on a few isolated stages of the sheep embryo indicate merely that the enzyme activity increases from the 75th day to the 138th day of life.

The data relative to the development of cholinesterase in the nervous tissue of *Amblystoma* as described by Sawyer, in the brain of the chick as observed by Nachmansohn, and in the rat brain can be plotted for comparison on the same coordinates in a manner similar to the one employed by Sawyer (*3*) (Fig. 2). In obtaining our *Q CH.E.* values for the rat brain tissue, the fresh weight of the tissue was assumed to be four times the dry weight, this being sufficiently accurate to show the similarity of the curves for the three animals.

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The Effect of Radiations on Galactozymase Formation in Yeast

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Adaptive enzyme formation in microorganisms is reduced or prevented by such cell poisons as azide (*1*), arsenate (*2*), and 2,4,dinitrophenol (*1, 3*), which probably interfere with protein synthesis in general. Since various radiations readily prevent cell division, it has been thought that they interfere with protein syntheses (*4*). A study of the effects of radiations upon adaptive enzyme formation therefore seemed pertinent. Galactozymase formation in yeast after addition of galactose is easily followed manometrically and occurs in resting cell suspensions in the absence of exogenous nitrogen (*1*); it is therefore an excellent test material. In a strain of *Saccharomyces cerevisiae* used, the adaptation, after addition of galactose to the suspension of yeast, began in about 90 min at 27° C, as indicated by a rise in rate of oxygen consumption. It was complete in about 180 min, as indicated by the achievement of a maximal rate. In the experiments reported 20 mg of galactose were added to each Warburg vessel containing in the order of 10^7–10^8 cells/ml, as determined by a count with a hemocytometer.

Whereas ultraviolet dosages of approximately 18,000 ergs/mm² from a sterilamp striking the face of the vessel merely retard the appearance of adaptation, a dosage twice as large inhibits it entirely. Dosages of ultraviolet sufficient to prevent adaptation have little

effect on the respiratory rate of yeast utilizing glucose. A considerable degree of photoreactivation can be achieved by subsequent illumination with white light, as is discussed elsewhere (*5*).

When completely adapted to galactose, yeast was irradiated in the presence of an excess of galactose. The respiration was little affected by dosages which would have prevented adaptation had they been applied at the time galactose was first added to an unadapted culture. This bears out the original hypothesis that synthetic processes are sensitive to these radiations and, in fact, it emphasizes the nature of adaptation, since the enzymes directly concerned with galactose respiration, once formed, are not easily affected by ultraviolet radiations.

Ultraviolet radiations act upon proteins in the cell, as shown by the action spectra (*4*). The possibility that nucleoproteins are involved in the formation of adaptive enzymes has been tested by one of us (*12*) by a determination of the ultraviolet action spectrum of galactozymase inhibition.

Since x-rays are known to interfere with nucleic acid conversions (*6, 7*), the effects of x-rays were next tried. A dosage of 4,850 r of x-rays at 40 kv and 20 ma with a tungsten target prevents division of 90% of the cells exposed as a thick paste on the surface of agar. Tests were made by diluting the irradiated yeast and plating in the standard way, counts being made 24, 48, and 72 hr following incubation at 30° C. In spite of this effect on viability, no noticeable effect was observed on the adaptation of the yeast to galactose. The increased respiration, indicating use of the galactose, occurred simultaneously in both irradiated and control cultures and to the same extent in both. Not only is the adaptation normal, but response to glucose is about the same in irradiated and control cultures. X-rays, as used here, appear to have relatively little effect on total respiration of the cell and on adaptive enzyme formation. However, experiments in another laboratory (*8*) have demonstrated effects of x-rays on anaerobic activities of yeast. Anaerobic adaptation to galactose required several days in our strain of yeast; therefore the effect of x-rays on the adaptation process could not be effectively studied, since in this much time so many other factors might vitiate the study.

The failure of x-rays to prevent adaptation is most surprising in view of the effects of x-rays on nucleic acid conversions. If such conversions are involved in galactozymase formation, one would anticipate effects on adaptation. X-rays readily affect enzymes with SH groups (*9, 10*), the latter being oxidized by the peroxide formed by dissociation of water by the radiations. Such inactivation is reversed by an appropriate reducing agent. It may be that the enzymes involved in galactozymase formation do not depend for their activity upon such groups, and that therefore they are relatively insensitive to x-rays. Enzymes may be irreversibly inactivated by direct hits (target theory), but the probability is much smaller and the dosages required are much larger (*9, 10*). Perhaps for that reason no measurable change in aerobic adaptation was observed with the dosages used.

Since visible light in the presence of photodynamic dyes is thought to act superficially on the cell (*11*), one might anticipate that it would have little effect upon adaptive enzyme formation. However, parallel experiments using yeast photosensitized with rose bengal proved that in this case light interferes with adaptive enzyme formation, the degree of change increasing with exposure. In these experiments the cells were illuminated for 15 min in 1:40,000 rose bengal with a 100-w G-E CH4 Spotlamp at a distance of 70 cm from the yeast suspension, the light being first passed through a 15-ml water cell to remove the heat and through a #3389 Corning glass filter to remove the long ultraviolet radiations. The intensity of the lamp as determined with a thermopile was approximately 500 ergs/mm^2/sec.

In an attempt to interpret the results with photodynamic action, the effect of a similar exposure on the respiration of yeast in glucose and on viability was determined. The results were found to depend upon the time at which glucose was added. If it was added before exposure, the cells were little affected. If added after exposure, the exogenous respiration was markedly reduced and the cells were sterilized, as shown by their lack of ability to form buds in nutrient solutions. The tentative conclusion is drawn that the entry of materials is in some way reduced or prevented after photodynamic action.

The experiments indicate that each of the radiations tested acts in a different manner upon galactozymase formation. Photodynamic action of visible light seems to be confined to the surface, since the effects on respiratory systems depend upon whether the nutrient is added before or after exposure. Ultraviolet light appears to strike deeper and prevents galactozymase synthesis, since a dose which prevents adaptation has no measurable effect on respiration of glucose added after exposure, showing that entry of nutrients is not blocked by action of the radiations. The apparent correlation between adaptation and division of cells by photodynamic action and ultraviolet radiations suggests a close relation between processes governing the division of cells and adaptive enzyme formation. However, the observation that x-rays as tested prevent division but have no effect on galactozymase formation suggests that the two processes pass along separate pathways.

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