the relations developed above specify how all these predicted temperatures depend upon the assumed effective temperature of the sun, and, to a much less degree, on the radiometric albedo of the earth. Reversing these relations, an immediate implication of the temperatures telemetered from actual satellites of which heating characteristics are known would thus be improved estimates of the heating characteristics of earth and sun.

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5 March 1958

Difference in Response of Phosphatases in Chick Embryo to Injection of Substrate

Recent studies have shown that the administration of appropriate substrates, both in vivo and in culture, can bring about an increase in the activities of certain enzymes in tissues of the chick embryo (*1*). As part of our work on the functional differentiation of the small intestine (*2, 3*), we examined the influence of an injected phosphate ester on the alkaline phosphomonoesterase activity of the chick embryo duodenum. Obtaining positive results in this attempt, we have extended our investigations to other organs and to acid phosphomonoesterase in order to determine whether the response we obtained is peculiar to one phosphatase in one organ, or is more general. These experiments have led to the discovery that although alkaline phosphatase is always elevated under the conditions we have employed, acid phosphatase is in all cases unaffected or is decreased slightly.

In the tests reported here, 25 mg of disodium phenylphosphate in 0.1 ml of isotonic saline was injected into the chorioallantoic vesicle daily, beginning at 14 days of incubation. At 17 days the eggs were opened, the embryos were weighed, and samples of duodenum, liver, mesonephros, and metanephros were removed. Enzyme assays were run on total homogenates of these tissues, with phenylphosphate being used as substrate (*2*). Individual determinations involved from 0.5 to 1.5 mg of fresh tissue in 3.25 ml of fluid (approximately 2 to 10 μg of nitrogen per milliliter). The alkaline phosphatase was determined at *p*H 9.6 in the presence of 0.01*M* $MgCl_2$, the acid phosphatase at *p*H 5.4.

The results (Table 1) reveal that administration of substrate increases the alkaline phosphatase activity not only in the duodenum, in which the enzyme is normally accumulating rapidly during the test period, but also in the two kidneys, in which it is accumulating at a lower rate, and in the liver, in which it decreases somewhat. The effect on liver is also interesting because the enzyme is not associated with brush borders in this organ, as it is in the intestine and the kidneys. Acid phosphatase is not affected or is even lowered slightly in the same samples in which the alkaline phosphatase is increased. Total nitrogen content is not significantly altered by phosphate injection except in the metanephros, in which the nitrogen content of the experimentals is about 10 percent less than that of the controls.

Since duodenal phosphatase may be elevated in the chick embryo by adrenal stimulation (*4*), the possibility presents itself that the enzyme increases reported in this paper are secondary to a general stress effect resulting from the administration of an abnormal substrate, or from release of phenol from the substrate. This possibility may be eliminated, for three reasons. First, the treated embryos at 17 days are as heavy as the controls and are capable of developing normally beyond the test period; their mesonephric weights also are the same as those of the controls. Second, the administration of phenol and dibasic sodium phosphate in quantities equivalent to those contained in the disodium phenylphosphate used in the principal experiments brought about no change in the alkaline or acid phosphatase content of the duodenum. Third, phenylphosphate (but not phenol) produced a significant increase in the alkaline phosphatase content of isolated duodenal fragments cultured in Earle's saline solution or Eagle's nutrient medium, as compared with fragments cultured without substrate. Thus one may infer that the effects observed in vivo are not dependent on the intermediation of organs other than the affected organ. These experiments will be reported in detail later.

Other experiments now in progress are concerned with the effectiveness of other phosphate esters in inducing increase of alkaline phosphatase. Beta-glycerophosphate (25 mg/day) has thus far given only slight and inconsistent results. Phenolphthalein phosphate (25 mg/day) is ineffective. Beta-naphthyl phosphate (25 mg in 0.5 ml of fluid per day) produces no change after 2 days. Since both the acid and alkaline enzymes have strong affinity for all these substrates, it may be that in embryonic tissues, as in microorganisms (*5*), a suitable substrate is not necessarily an effective inducer. This point is being further examined in in vitro experiments.

The uniformity of the difference of response of acid and alkaline phosphatase in all organs studied suggests that the explanation for the difference is not to be sought in terms of the intracellular associations of the enzymes, which vary considerably among the tissues we have examined, but rather in the nature of the enzymes themselves, or in the enzyme-forming systems. Before this question can be profitably approached, it is necessary to consider whether the positive results we have obtained are due to

Table 1. Phosphatase in tissue of chick embryos injected with disodium phenylphosphate (+ Php) or saline (− Php) between 14 and 17 days of incubation. Phosphatase activity is given in micrograms of phenol liberated per 10 μg of nitrogen in 30 minutes. Each value is the average of 8 to 22 determinations and is followed by the standard error of the mean.

Tissue	Phosphatase activity				
	14 days	17 days			
	− Php	− Php	+ Php	% difference	P
Duodenum					
alkaline	3.3 ± 0.17	8.7 ± 0.42	14.8 ± 0.85	+ 70.1	< 0.01
acid	1.9 ± 0.16	2.6 ± 0.15	2.8 ± 0.10	− 7.7	< 0.3
Liver					
alkaline	5.3 ± 0.36	4.7 ± 0.24	7.9 ± 0.43	+ 68.1	< 0.01
acid	4.9 ± 0.41	6.5 ± 0.32	6.3 ± 0.37	− 3.2	> 0.5
Mesonephros					
alkaline	18.4 ± 1.58	29.4 ± 3.13	48.4 ± 3.13	+ 94.1	< 0.01
acid	8.7 ± 0.32	11.5 ± 0.64	11.3 ± 0.33	− 1.7	> 0.5
Metanephros					
alkaline	9.8 ± 0.71	14.8 ± 1.12	42.5 ± 2.09	+ 187.1	< 0.01
acid	5.3 ± 0.29	7.6 ± 0.21	6.8 ± 0.23	− 10.5	< 0.02

the increase of a single alkaline phosphomonoesterase, or of a complex of phosphomonoesterases, or of one enzyme in a complex having many characteristics in common. This is among other aspects of the problem now being more fully investigated (*6*).

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29 November 1957

Synergistic Action of Ethylenediaminetetraacetate and Radiation on Yeast

When radioisotopes are used as radiation sources in studies with growing cultures of microorganisms, it is essential that the isotope be kept evenly distributed throughout the suspension. This is particularly true with isotopes emitting alpha particles whose ranges are only a few microns. The use of plutonium and polonium as sources of alpha radiation in growing cultures is complicated by their tendency to be adsorbed on or taken up by the cells. The chelating agent ethylenediaminetetraacetate (EDTA) is known to depress the deposition and promote the excretion of plutonium in animals (*1, 2*). The present investigation was initiated to determine whether EDTA would also be effective in decreasing or preventing the uptake of plutonium in growing cells and permit the use of plutonium as an alpha radiation source in cultures of microorganisms.

Growth studies were conducted with a diploid strain of *Saccharomyces cerevisiae* cultured at 30°C in an autoturbidimeter which automatically recorded changes in the optical density of the suspension (*3*). Plutonium in 0.2*N* nitric acid was added directly to a sterile, chemically defined medium. Sodium EDTA was used at a concentration of 3×10^{-4} *M* in all tests except those designed to evaluate the effects of varying concentrations of this compound.

The growth of yeast in the presence of plutonium and EDTA is shown in Fig. 1(*A*). The increase in optical density of the cultures is indicated by a plot of autoturbidimeter readings against time. Growth was not altered by EDTA, but it was delayed approximately 3 hours by 0.5 μc of plutonium per milliliter. With EDTA and plutonium present, a synergistic effect was observed and the inhibition of growth was much more pronounced.

Because other work in our laboratories had shown that EDTA was metabolized by yeast, it appeared that this additional inhibition possibly resulted from EDTA carrying chelated plutonium into and concentrating it in the cells. To test this supposition the concentration of plutonium associated with the cells was tested in both growing and nongrowing cultures. Under both conditions less plutonium was associated with the cells when EDTA was present than when it was absent from the medium. From this it appeared that the effects from EDTA were not due to an increased radiation dose in cells exposed to EDTA and plutonium.

Since the EDTA appeared to augment the radiation effects from plutonium, it was necessary to determine whether this was specific for plutonium or was a general synergistic effect with any radiation. Beta radiation from tritium was used because the tritium would not be chelated by the EDTA and would be uniformly distributed through the culture. Tritium oxide was added to the sterile medium, and growth curves were determined as before. Figure 1(*B*) shows growth curves for yeast grown in the presence of tritium and EDTA. As before, EDTA had no effect on growth in the control tubes. Tritium, at 90 mc/ml of growth medium, produced a marked inhibition of growth. This inhibition was doubled by the presence of EDTA with this and lower concentrations of tritium. The effect of EDTA thus appeared to be that of a general synergistic action with radiation.

By employing higher concentrations of sodium EDTA it was possible to obtain greater synergistic effects of EDTA with radiation. However, higher concentrations of EDTA also inhibited growth in unirradiated control cultures.

These results suggested either that radiation increased the sensitivity of yeast to EDTA or that EDTA increased the sensitivity of yeast to radiation. If radiation increased the sensitivity of yeast to EDTA, then exposure of yeast to x-radiation with subsequent growth in EDTA should result in a decreased rate of growth. However, EDTA in the growth medium did not affect the inhibition of growth produced by a single exposure of the inoculum to 300,000 r delivered either at the rate of 2000 or 13,000 r/min. Also, incubation of yeast in EDTA for 2 hours prior to x-radiation did not

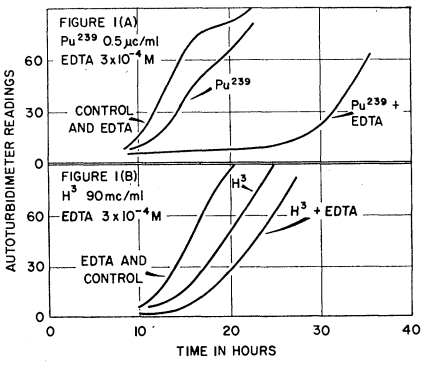

Fig. 1. Growth of yeast in the presence of (*A*) plutonium and EDTA and (*B*) tritium and EDTA.

alter its ability to grow either in liquid culture or on nutrient agar.

It is not possible at the moment to specify the mode of action by which EDTA amplifies radiation sensitivity. It appears probable, however, that the effect is produced by a general change in the electrolyte balance of the cell rather than by a specific deficiency of calcium since the addition of calcium to some of the cultures did not alter the effect of the EDTA. An effect of electrolyte concentration on the radiosensitivity of the respiratory system of yeast has been observed by Bair and Stannard (*4*). The ability of EDTA to produce chromosome aberrations and to increase the rate at which aberrations are produced by radiation administered at low dose rates has been reported by Wolff and Luippold (*5*), as have also more generalized effects on ionic balance which in turn affects chromosome behavior (*6, 7*).

Because EDTA increases the apparent radiosensitivity of yeast, its use in radiation studies may be limited to those in which this property is of interest (*8, 9*).

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10 October 1957