

tent, by the red maple-tamarack swamp forest which occupies the shore zone. The littoral area out to a depth of about 12' is covered with a dense growth of *Chara* including some *Potamogeton* and white and yellow water lilies. Beyond the 12' contour there is some *Nitella* and bottom-growing moss of the genus *Fontinalis*. In this area there are very few benthic animals and in the greater depths, rich in hydrogen sulfide, practically no signs of animal life.

Throughout all months of the year there is a slight increase in temperature from the 30' level, near the upper part of the hypolimnion, to the bottom. In winter and early spring the increase is from a range of 39.2–39.7° F at 30' to one of 39.9–41.1° F at the bottom level. By midsummer the water at the 30' level has warmed up slightly to, on the average, about 40.4° F, and there is possibly a slight increase in the temperature of the water immediately above the bottom to about 40.5–41.1° F. The amount of thermal change from the top to the bottom of the hypolimnion varies at any one time from a few tenths of a degree to 1.6° F. Any seasonal variation in the temperature of the waters below 35' is at best only a matter of tenths of a degree Fahrenheit and difficult to define with certainty, using a Foxboro thermometer. The slight thermal gradient increasing toward the bottom, and the relatively great stability of the bottom temperatures is illustrated in Fig. 1. The fall and spring over-

surface during a prolonged period. The lower limit of the thermocline dropped from a depth of about 16' in late May to around 25' in early September. The decrease in temperature from the top to the bottom of the thermocline was approximately 19° F in late May and from 36 to 42° F during August and early September. The magnitude of the average drop in temperature per foot of increase in depth throughout the thermocline ranged from 1.2 to 2.2° F during the period May 27–September 22, 1947. Below 30' the temperature range was from 39.4° F, the lowest temperature at the 30' level, to about 41° F, which was the highest bottom-water temperature.

Two other comparable instances of temperature inversion, known as dichothermy, have been reported in American lakes, one being Fayetteville Green Lake near Syracuse, New York, studied by Eggleton (1), and the other, Lake Mary, Wisconsin, observed by Juday, Birge, and Meloche (2). In Europe and Asia the temperature inversion phenomenon has been reported for a number of lakes in Austria, Germany, and Japan (3).

References

1. EGGLETON, FRANK E. *Ecol. Monogr.*, 1931, 1, 231–352.
2. JUDAY, C., BIRGE, E. A., and MELOCHE, V. W. *Trans. Wisc. Acad. Sci. Arts Let.*, 1935, 29, 1–82.
3. YOSHIMURA, SHINKICHI. *Proc. Imp. Acad. Tokyo*, 1937, 13, 316–319.

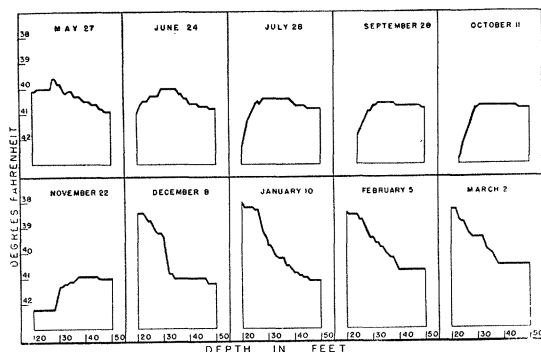


FIG. 1. Monthly variations in the extent of the temperature inversion in Sodon Lake, Oakland County, Michigan, during 1947–48.

turns in 1947–48 did not penetrate below 30–35'. Depths of around 30' represent ecotone levels that are seemingly the meeting points of shallow and deep-water thermal influences. The particular fall season will determine the distance below the 30' depth that cold, surface water conditions may penetrate. Likewise, factors operating in the hypolimnion will probably vary from year to year with respect to their influence on the temperature of the waters near the 30' level. The upper limit of the hypolimnion ranged from about 16' in late May 1947 to 29' on November 22, just preceding the complete disappearance of the thermocline.

The third unusual condition found in Sodon Lake is the existence of the thermocline within 1' or 2' of the

On the Origin of Virus Phosphorus¹

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Knowledge of the biological precursors of virus nucleic acid and protein is essential to the understanding of the mode of virus reproduction. Bacteriophages infect an autonomous host cell of well-elucidated metabolic pattern and viable on synthetic medium and thus offer very suitable systems for isotope tracer studies of the origin of virus constituents and of the extent to which these are directly derived from the host.

The isolation from broth lysates of purified *Escherichia coli* bacteriophage T₆⁺ with normal infectivity but containing radioactive phosphorus has already been reported (3). Isotope studies described in this paper demonstrate that when phage is propagated in bacteria maintained in a chemically defined medium, the medium itself can be the ultimate source of 70% of virus phosphorus. The remaining virus phosphorus is derived directly from the bacterial host, chiefly from some P fraction other than low-molecular-weight, acid-soluble compounds.

For these experiments the phage was harvested in the

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supercentrifuge and isolated by repeated differential centrifugation. The details of the procedure will be published separately. The purified phage had an average infectivity of $10^{15.89}$ gm N/infectious unit when measured by plaque count assay. For practical reasons physical-chemical characterization was limited to unlabeled virus. In the electron microscope the phage exhibited typical tadpole-shaped particles with crescent-shaped markings in the "head." Absorption photographs in the ultracentrifuge revealed two principal sedimenting boundaries with sedimentation constants (S_{20} = 1,034, and 787 Svedberg units) similar to those reported for ultracentrifugally isolated T_2 bacteriophage (2). On electrophoresis in the Tiselius apparatus the purified virus migrated with a single boundary over the pH range studied, pH 5.1-7.6. The stability range for infectivity was pH 5-8.6. Partition of the P of purified dialyzed T_6 phage by the methods of Schneider (5) and of Schmidt and Thannhauser (4) revealed that desoxyribonucleic acid (DNA) is the chief P constituent.

sion is in accord with recent observations of Cohen (1) on bacteriophages T_2^+ and T_4^+ grown in synthetic medium. In Experiment 1 the relative radioactivity of virus to medium demonstrates that some 70% of the virus P ultimately came from the medium by a path, presumably bacterial, not yet fully elucidated. Conversely, in Experiment 2 the data indicate that bacterial P was the precursor of only about 23% of virus P. In Experiment 3 with differentially labeled bacteria it is significant that, although the relative radioactivity of bacterial acid-soluble P (compared to total bacterial P) was reduced by one-half, the relative radioactivity of phage to bacteria was undiminished.

Cohen has reported that ribonucleic acid did not turn over in bacteriophage-infected cells (1). Our experiments indicate that gross changes in the specific radioactivity of bacterial acid-soluble phosphorus are not reflected in the radioactivity of the virus. It seems unlikely that phospholipids would materially be involved in virus synthesis. The possibility remains that bac-

TABLE 1
RADIOACTIVITY OF BACTERIOPHAGE T_6^+ GROWN ON *E. coli*
WITH CELLS OR MEDIUM CONTAINING P^{32}

Exp. No.	Bacteria	Medium	Specific radioactivity*					Relative radioactivity†		
			Bacteria		Virus			Virus Medium	Virus DNA Bact. DNA	Virus Total P Bact. Total P
			Total P	DNA‡	Acid-soluble	Total P	DNA			
1	Unlabeled	17.1	0	0	0	11.9	12.9	69.5
2	Labeled	0	80.6	75.8	80.6	18.2	19.6	...	25.8	22.6
3	Differentially labeled	0	348	415	195	108	128	...	30.8	31.0

* Counts/min/μg of P.

† Ratio of specific activities × 100.

‡ DNA = desoxyribonucleic acid.

However, significant amounts of acid-soluble P and ribose, and of ribonucleic acid, have been found in preparations that yield a single boundary in electrophoresis.

Virus containing P^{32} was isolated by the above procedure after multiple infection of labeled or of unlabeled bacteria. Three types of experiments were performed in synthetic (lactate) medium using (1) unlabeled cells in medium containing P^{32} , (2) washed labeled cells in unlabeled medium, or (3) differentially labeled cells in unlabeled medium. In each instance the bacteria were multiply infected with three phage particles per bacterial cell to produce a single virus generation without further bacterial growth. Labeled cells were produced by growing bacteria in medium containing about 1 μg of P^{32} /ml. Bacteria differentially labeled in the several phosphorus fractions were obtained by allowing labeled cells to metabolize lactate for 5 hrs in nitrogen-free, nonradioactive medium. The results are given in Table 1.

From the three experiments it can be seen that under these conditions the P of the virus was chiefly derived from inorganic phosphate of the medium. This conclu-

terial desoxyribonucleic acid is the major source of the bacterial contribution to virus phosphorus. The fact that the bulk of virus phosphorus is derived from the medium is in accord with our calculations, which indicate that at the time of infection bacterial DNA content is sufficient to account for only a third of the DNA contained in the liberated phage. Although these experiments indicate that the total bacterial acid-soluble P appears to make a negligible contribution to phage P, the data do not preclude the participation of an active low-molecular-weight phosphorus intermediate in virus reproduction. Rather, it appears probable that virus infection potentiates P absorption and metabolism by the host, directing it exclusively toward the synthesis of virus.

References

1. COHEN, S. S. *J. biol. Chem.*, 1948, **174**, 295.
2. HOOK, A. E., BEARD, D., TAYLOR, A. R., SHARP, D. G., and BEARD, J. W. *J. biol. Chem.*, 1946, **165**, 241.
3. PUTNAM, F. W., KOZLOFF, L. M., and EVANS, E. A., JR. *Fed. Proc.*, 1948, **7**, 179.
4. SCHMIDT, G., and THANNHAUSER, S. J. *J. biol. Chem.*, 1945, **161**, 83.
5. SCHNEIDER, W. C. *J. biol. Chem.*, 1945, **161**, 293.