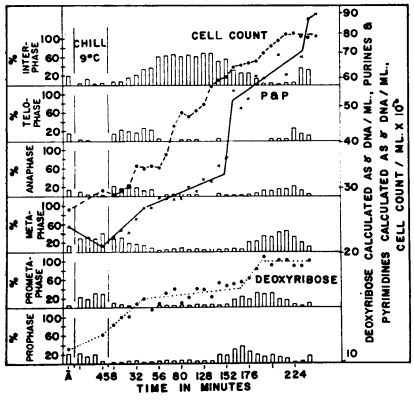

Fig. 2. *Bacillus megaterium* was grown in "M-9" salt-glucose medium at 34° C. The period of chilling was 45 minutes at 9°C. Two hundred nuclei were counted, and the phases were identified for each test interval. Deoxyribose was identified for each test interval with Dische reagent. Purines and pyrimidines (P&P) were determined by absorption at 260 mμ in a Beckman spectrophotometer. Cells were counted in a Petroff-Hausser counting chamber.

that may not be the same for *E. coli*. In both *E. coli* and *B. megaterium*, there is evidence suggesting that there is a fall in the ribose moiety at the same time that there is a rise in the deoxyribose moiety. This in turn suggests that there is a conversion of ribose to deoxyribose in the synthesis of deoxyribose nucleic acid (*2*).

Details of the methods, results, and possible interpretations of these findings will be presented in other reports (*3*).

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References and Notes

1. This study was supported in part by grants from the U.S. Atomic Energy Commission, contract #AT(30-1)-1341, and from the National Institutes of Health, U.S. Public Health Service (PHS#C-2189).
2. M. C. Lanning and S. S. Cohen, *J. Biol. Chem.* **216**, 413 (1955).
3. E. D. DeLamater, "Bacterial chromosomes and their mechanism of division," in *Bacterial Anatomy* (Cambridge Univ. Press, 1956), pp. 215–260; D. B. McNair Scott and E. C. Chu, in preparation.

5 December 1955

Purification and Crystallization of Coxsackie Virus

Coxsackie A-10 virus (Huebner strain 1816) was grown in suckling mice. Moribund mice were harvested; 1 vol of carcasses was homogenized in 2 vol of 8.6-percent sucrose and cleared (*1*) of subcellular particulates. The resulting supernatant, titering 10^9 LD_{50} per milliliter, was purified as follows.

The fluid was cleared by adjusting to *p*H 3.0 (normal HCl) and adding 10 g of ammonium sulfate to each 100 ml. After neutralization (with NaOH), virus was concentrated by precipitation through the further addition of 30 g of ammonium sulfate to each 100 ml of suspension. The virus in the precipitate was resuspended by dialysis against 5-percent sodium chloride, cleared, and again cleared at *p*H 3.0. After neutralization, further clarification was obtained by adding 30 ml of 95-percent ethanol to each 100 ml of suspension. The virus suspension was ultracentrifuged (*2*) and the virus in the pellet was resuspended in 40-percent ammonium acetate and cleared. The virus suspension was dialyzed against either 5-percent ammonium acetate or 1-percent sodium chloride and ultracentrifuged into a pellet. Finally, the pellet, layered with a small quantity of appropriate salt solution, was stored at about 4°C, as had been done by Schaffer and Schwerdt (*3*) in crystallizing poliomyelitis virus.

Five lots of from 1 to 1½ lit have been so treated. Three lots were ultracentrifuged and layered with 1-percent sodium chloride, and two with 5-percent ammonium acetate. In the sodium chloride preparations, small dodecahedral crystals with four hexagonal faces were observed in from 1 to 2 weeks of 4°C storage. On resolution, clarification, ultracentrifugation, and 4°C storage, similar dodecahedrons recrystallized, and on continued refrigerator storage, these crystals attained maximum dimensions of about 100 μ (Fig. 1).

Normal suckling mouse homogenate (775 ml) was treated similarly. Ultracentrifugation produced a minute pellet which revealed only a small quantity of amorphous material after prolonged refrigerator storage. Attempts to "recrystallize" resulted in removing almost all of this amorphous material.

The ammonium acetate preparations, on original crystallization, resulted in somewhat ill-defined rectangular and square plates, which on recrystallization appeared clearly as flat, square, or rectangular plates (Fig. 2). Assuming a crystal density of 1, it was estimated volumetrically that more than 1 mg of crystalline material was obtained from each lot.

Both forms of crystal are extremely unstable and have been maintained only in suspension. In this respect, and with respect to shape, they resemble two forms of unstable crystals seen in plants infected with tobacco mosaic virus (*4*).

The infectivity for suckling mice of the crystalline suspensions has varied

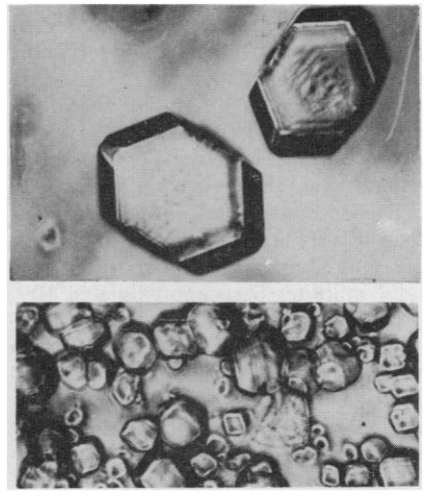

Fig. 1. Second crystallization of dodecahedral crystals in 1-percent sodium chloride. (Top) Late and (bottom) early crystallization seen under phase-contrast illumination.

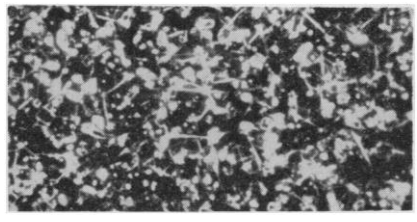

Fig. 2. Platelike crystals seen under dark-field illumination. Second crystallization in the presence of 5-percent ammonium acetate. The small whitish bodies are small plates seen on end and undergoing Brownian movement.

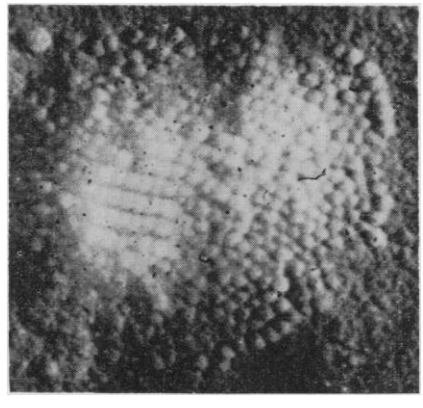

Fig. 3. Electron micrograph of pseudoreplica of dodecahedron residue dried on agar. Similar pictures have been obtained with the residue of platelike crystals. Several rows demonstrate the potentiality of these particles to form rectangular arrays. [The pseudoreplicas were made and the electron micrographs taken through the courtesy of Bolivar J. Lloyd, Jr., National Cancer Institute, National Institutes of Health]

from 10^{11} to 10^{12} LD_{50} per milliliter. When the crystals were sedimented by centrifugation at 1000 rev/min for 5 min, the supernatant fluids were found to contain from 0.1 (early in crystallization) to 0.01 (after maximum crystallization) of the infectivity of the supernatant plus the crystals. The crystal pellet accounted for considerably less than 0.1 of the total volume of the suspension. Thus, the calculated titer on a solid milliliter basis approximated 10^{13}.

Since a volume of 1 cm^3 can be calculated to contain about 4.6×10^{16} particles of 28-mμ diameter, and since the animal titration showed approximately 10^{13} infectious doses per cubic centimeter, it appeared that the ratio of the total number of particles to the number of infectious particles was apparently greater than 1000 to 1. That a similar discrepancy existed for the starting material is shown by the following.

On the basis of infectivity, the starting material contained 10^{12} particles per liter. Since more than half of the infectivity was lost in purification, at most, 10 μg of infective particles per liter should have been obtained. However, more than 100 times this quantity of homogeneous particles was crystallized. Apparently, in both the starting and crystalline materials, similar major discrepancies existed between the number of particles and the infectious doses. These discrepancies may be attributed to the inefficiency of the intraperitoneal titration system, the number of particles necessary to establish cellular infection, the degree of aggregation of virus particles, and the ratio of inactive to active virus particles.

When they were dried in air, crystals of either form disintegrated into an amorphous, hygroscopic residue. Therefore, no completely satisfactory electron micrographs showing crystals have been obtained from replicas. Figure 3 shows a pseudoreplica of crystal residue that has been dried on agar. This and other micrographs closely resemble those obtained by Breese and Briefs (*5*), which probably represented early crystal formation of the same virus. Almost complete aggregation of the virus has been seen throughout these preparations, with orderly alignment of particles 28 mμ in average arrayed diameter. Very little extraneous material has been seen in pseudoreplicas of these recrystallized preparations. The angles formed by hexagonal arrays of particles studied by electron microscopy were found to be the same as those of the hexagonal faces of the dodecahedrons.

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References and Notes

1. Clearing consisted of centrifugation at about 4000 rev/min for 20 to 60 min in an International refrigerated centrifuge (head No. 812).
2. Ultracentrifugation was performed in a Model L Spinco, applying a maximum force of 144,000 g for 60 min. Percentages indicate number of grams in 100 ml of solution. All procedures were carried out at 0° to 4°C. Partially purified preparations were stored at − 37°C for varying periods and were usually cleared after they had been thawed.
3. F. L. Schaffer and C. E. Schwerdt, *Proc. Natl. Acad. Sci. U.S.* 41, 1020 (1955).
4. F. C. Bawden, *Plant Viruses and Virus Disease* (Chronica Botanica, Leiden, Netherlands, 1939) chapt. 3.
5. S. S. Breese, Jr., and A. Briefs, *Proc. Soc. Exptl. Biol. Med.* 83, 119 (1953).

9 March 1956

New Synthesis of Oxalic Acid

Results indicate that oxalic acid is formed when bicarbonates in aqueous solutions are exposed to beta or gamma radiation. Although the procedure appears less complicated than Wöhler's synthesis of this acid from cyanogen, which was the first "organic" synthesis from "inorganic" materials (*1*), the mechanism of the reaction is complex, since it involves the presence of water and possibly also oxygen to effect the carbon to carbon bondage of the carbon dioxide moiety.

Solutions of ammonium, calcium, and sodium bicarbonates and sodium and ammonium carbonates were prepared in various concentrations and irradiated in polyethylene bags. In order to allow complete penetration of the ionizing radiation, a large enough bag was used so that the thickness of the filled bag (approximately 250 ml) was nowhere greater than 6 mm. The samples were exposed to beta radiation by means of a 2 Mev van de Graaff electron accelerator. The dose rate was 2.10 Mrep per pass, and the total dosages to which the samples were exposed ranged from 2.10 to 52.5 Mrep.

The chemicals used were of standard analytic grade. Calcium bicarbonate was prepared by passing carbon dioxide through a water suspension of calcium carbonate. The samples, which were irradiated at room temperature, were withdrawn from the polyethylene bags, and the oxalic acid, which was precipitated as calcium oxalate, was regenerated and derivatized. Table 1 gives the characterization of oxalic acid for all series investigated.

These results were repeated when glass vials were used as containers. Owing to the porosity of the polyethylene bags, gas analyses were not attempted.

Oxalic acid was not detected on irradiation of sodium carbonate solutions. Blank tests, carried out with distilled water, were also negative. Formic acid was likewise not observed to form in these reactions. The formation of oxalic acid from ammonium carbonate is explained by the fact that ammonium carbonate is a mixture of ammonium bicarbonate, ammonium carbamate, and ammonia (*2*).

Percentage yields of oxalic acid were calculated as 100 times the number of grams of oxalic acid formed per gram of solute. G values (number of molecules

Table 1. Identification of oxalic acid.

Test	Theoretical		Found	
Neutralization equivalent CHO · 2HO	63		62.5	± 1.0
Oxalic acid dihydrate, mp °C	100*	(*1*)	100	± 0.2
Oxalic acid, mp °C	189.5*	(*3*)	190	± 0.2
Benzylthiuronium oxalate, mp °C	193*	(*4*)	193	± 0.2
Aniline blue formation (*5*)	+		+	
Infrared spectrum of calcium oxalate†				

* Mixed melting points with authentic materials showed no depression. (All melting points were carried out on Kohfler hot stage.)
† Spectrum agreed with standard calcium oxalate curve.

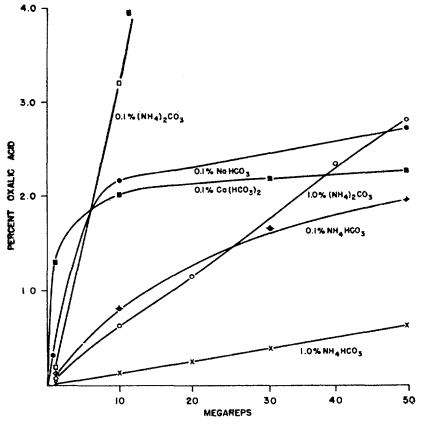

Fig. 1. Oxalic acid yield versus irradiation dose.

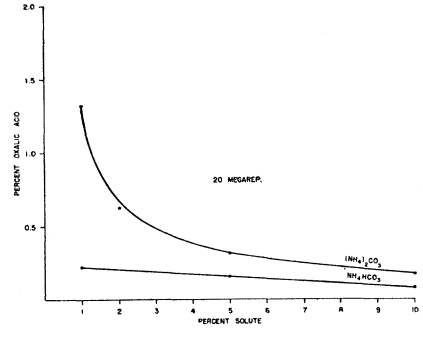

Fig. 2. Oxalic acid yield versus concentration of solution.