essentially marine fishes "in rivers and lakes." This is a misunderstanding of the general trend of my statement, which, moreover, concerns Lake Sentani only.

In the paragraph under discussion, I state that there seems to be no reason whatever to use this theory for an explanation of the occurrence of sawfishes in Lake Sentani; neither are the facts of gradual upheaval, sustained by considerable geologic evidence, and the gradual replacement of salt water necessary for an explanation of the occurrence in this lake of other "marine" species, an opinion supported by the existence of such species in areas without previous gradual upheaval [for example, the Upper Digoel River near Tanah Merah, 450 km (not miles as I erroneously stated) from the sea]. On the other hand, this gradual upheaval and replacement of salt water may in some cases have helped marine species to get accustomed to fresh water.

I am well aware of the fact that several species of marine fishes, especially in the young stages, occasionally invade fresh water, probably more often than is generally assumed. This is why I cautiously used the expressions "essentially marine" and "not usually." The list of species showing this habit, as given by Herre, is interesting, but as a criticism it is not to the point.

In the large family of the Carangidae, several species are known to occur in outlets of rivers (brackish water), while some even may venture much farther upstream. However, in the Indo-Australian area the number of species showing this habit seems to be small; of the 58 species enumerated by Weber and De Beaufort from this region [*Indo-Australian Fishes* **6**, 192 (1931)] only one species is mentioned as entering rivers and occurring in brackish water, a second as occurring in the mouth of rivers, while the cited authors omitted to mention this habit for *Caranx melampygus* from New Guinea [Weber, *Nova Guinea* **5** (2), 249 (1908)]. Although they are evidently incomplete, these data stress the general tendency for a marine habitat in the group.

In the literature on the fishes of Netherlands New Guinea I did not meet with any data on carangid species invading really fresh water. The only locality where, during our voyage to New Guinea, we found fresh-water carangids was Lake Sentani, incidentally a place where upheaval could have been of some influence. If these fishes freely enter fresh water indeed, I wonder why we did not encounter them elsewhere in New Guinea.

I conclude that there is some reason for the opinion that upheaval and gradual replacement of salt water by fresh water helps to explain the occurrence of carangids in Lake Sentani. I am "more inclined to adopt this theory" in connection with the occurrence of carangids than in connection with the occurrence of sawfishes in the said lake, although our knowledge on the subject seems not yet sufficient to regard this as a definite explanation.

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26 March 1956

Sequences of Metabolic Events during Growth of Synchronized Bacteria

Achievement of synchronized division in growing cultures of *Escherichia coli* B and *Bacillus megaterium* has presented the possibility of a more precise determination of the sequence of metabolic events during growth and division of cells than has been possible previously. Such studies have been carried out in two different laboratories of the University of Pennsylvania, using methods that were developed or modified to be applicable to the two different organisms (*1*).

Growth of *B. megaterium* was synchronized by the chilling and warming of cultures that were growing in aerated, salt-glucose liquid medium. Samples were taken at frequent intervals. Determinations were made of (i) total cell substance by standard turbidity measurements; (ii) direct cell count in a Petroff-Hausser bacterial counting chamber; (iii) morphologic events on stained preparations by counting for the percentage of cells in the various mitotic stages; and (iv) synthesis of cell nucleic acid components by chemical analyses and ultraviolet absorption.

Similar studies were undertaken with cultures of *E. coli* B. Synchronized growth was initiated by addition of glucose as the only carbon source to synthetic liquid medium containing resting cells with a low RNA/DNA ratio. Cytologic studies of *E. coli* are omitted here.

The components chosen for analysis were (i) the ribose-containing compounds, which were determined by the Bial reaction; (ii) the deoxyribose compounds, which were determined by a modification of the Dische diphenylamine reaction; and (iii) the nucleic acid bases, purines, and pyrimidines, which were calculated from the absorption at 260 mμ read in the Beckman ultraviolet spectrophotometer. Absorption was measured on whole cells and extracted cells, either suspended in glycerin of the same refractive index as the cells or dissolved in 1*N* sodium hydroxide. All results are here expressed in terms of micrograms of nucleic acid per milliliter of culture.

Typical experiments are shown in Figs. 1 and 2. In the study with *E. coli* B (Fig. 1), it was found that, immediately after the addition of glucose, the ribose content increased rapidly. The amount of the deoxyribose component rose about the time that the turbidity began to increase. Also, after the increase in ribose content, there was an increase in the nucleic acid bases, which was often followed by division of the cells. The cycle was then repeated. These events are clearly separate in time.

The nonsimultaneous synthesis of the sugar components and the purines and pyrimidines indicates that these components must be considered individually and that readings on one should not be interpreted as representing the total picture for the particular nucleic acid under analysis at a particular time.

Cytologic studies of *B. megaterium* show that the cells, when they are chilled, come into the prometaphase and metaphase stages and are arrested there. When the cultures are warmed, progression through the mitotic stages is resumed, 50 percent or more of the cells being in the same stage at the same time. There is a sequence of chemical events

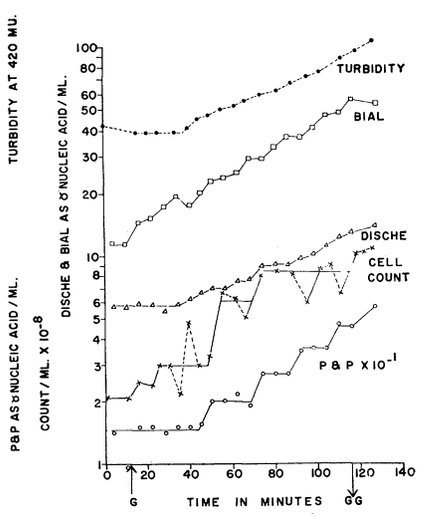

Fig. 1. *Escherichia coli* B. The culture consisted of 180 ml of "52" salt-ammonium sulfate medium at 37°C in a Klett sidearm cylinder, to which was added 28 ml of an overnight-aerated culture that had been chilled for 45 minutes to 5.5°C. The culture was aerated, and at 12 minutes 0.5 ml of 20 percent glucose solution was added (G). The glucose was exhausted from the medium at 110 minutes (GG). Samples were taken for analysis at the times indicated. The cells were counted under the microscope. The purines and pyrimidines (P&P) were determined by absorption at 260 mμ of the whole cells and of the extracts and the extracted cells. The values for the former were averaged with the sum of the latter values.

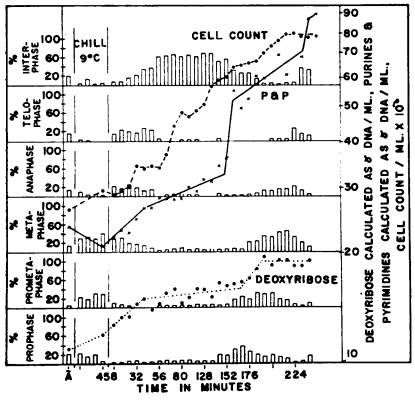

Fig. 2. *Bacillus megaterium* was grown in "M-9" salt-glucose medium at 34° C. The period of chilling was 45 minutes at 9°C. Two hundred nuclei were counted, and the phases were identified for each test interval. Deoxyribose was identified for each test interval with Dische reagent. Purines and pyrimidines (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

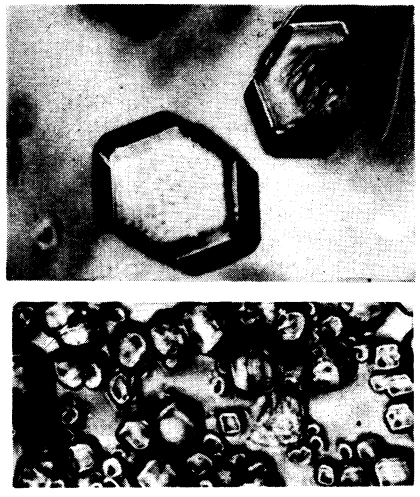

Fig. 1. Second crystallization of dodecahedral crystals in 1-percent sodium chloride. (Top) Late and (bottom) early crystallization seen under phase-contrast illumination.

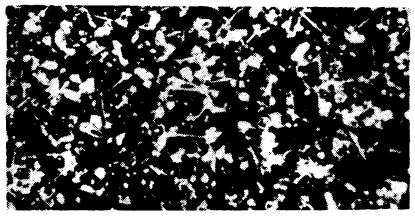

Fig. 2. Platelike crystals seen under dark-field illumination. Second crystallization in the presence of 5-percent ammonium acetate. The small whitish bodies are small plates seen on end and undergoing Brownian movement.

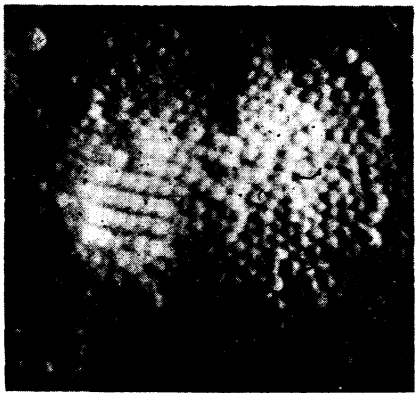

Fig. 3. Electron micrograph of pseudoreplica of dodecahedron residue dried on agar. Similar pictures have been obtained with the residue of platelike crystals. Several rows demonstrate the potentiality of these particles to form rectangular arrays. [The pseudoreplicas were made and the electron micrographs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