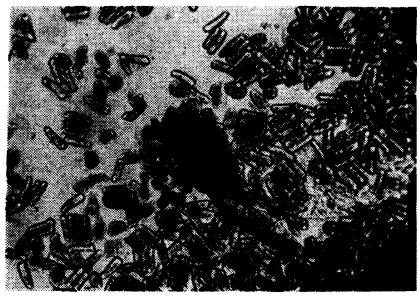

Fig. 1. Parenchyma cells in chlorotic translucent leaf spots on Triumph sweetpotato separated by virus action.

had an enzyme function, and its true nature was proved by the approach-grafting transmission technique to be a virus.

In the autumn of 1955, exactly 1 year later, under similar growing conditions in the same greenhouse, the translucent spots were again prevalent on Triumph leaves. It was then possible, by virtue of the rapid indexing-transmission technique [E. M. Hildebrand, *Science* **123**, 506 (1956)] to index this material on potential indicator plants, and on Scarlett O'Hara morning glory it induced typical symptoms of internal cork virosis within 8 days. Repeated in quadruplicate, this experiment gave identical results each time it was performed—that is, Scarlett O'Hara morning glory showed typical virus-induced symptoms within 8 days. Thus, the translucent spots on the Triumph variety were shown to be symptoms of internal cork disease.

Recent microscopic examination of the translucent leafspots again revealed cell separation that resulted from dissolution of the calcium pectate intercellular substance, presumably by enzyme action. The palisade and spongy parenchyma cells from young spots, when separated from each other, open to view their contents, in which chloroplastids are in great abundance and apparently unaffected. This study makes possible micrurgical studies on the physiology and pathology of these living cells of Triumph sweetpotato leaves.

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9 December 1955

Marine Fishes in Fresh Water

At the risk of pursuing a subject too far, I again wish to correct some errors on the subject of marine fishes in fresh water. I have just read the interesting letter from M. Boeseman, of the Leiden Natural History Museum, on "Fresh-water Sawfishes and sharks in Netherlands New Guinea" [*Science* **123**, 222 (10 Feb. 1956)].

After speaking of *Pristis microdon* and its occurrence in both fresh and salt water, he goes on to say "For some species, there are strong indications of breeding in fresh water, a well-known habit of the specimens in Lake Nicaragua." What does he mean by "specimens," sawfish or sharks? The reference is probably to sharks. But no one has ever presented any proof that sharks breed in Lake Nicaragua. Contrary to the oft-published statement, Lake Nicaragua is not landlocked, since it is connected with the sea by its outlet, the San Juan river.

Bigelow and Schroeder, in their monumental work on the sharks of the western Atlantic, mention rapids in the upper San Juan and seem to believe that these prevent sharks from passing up or down the river. From extended observation of fish migrations in tropical rivers with rapids, I have no doubt that in the rainy season sharks are able to make the trip in either direction. Bigelow and Schroeder merely infer that the Lake Nicaragua shark is landlocked and breeds in the lake, but they do not present any proof and do not directly claim that such is the case.

Further on Boeseman is inclined to adopt the theory of gradual upheaval and gradual replacement of salt water by fresh water to explain the presence of essentially marine fishes in rivers and lakes. As an example he cites "jacks (Carangidae)—which do not usually invade fresh water by free will."

This was an unfortunate selection, for certain species of jacks freely enter fresh-water rivers and lakes when they are available. *Caranx sexfasciatus,* which occurs from China and Japan to Australia, and from South Africa to the Hawaiian and Society Islands, enters fresh water in large numbers, usually remaining until they are a year or year and a half old. *Caranx ignobilis,* another jack of equally wide range, also enters fresh water but stays until it is between 2 and 3 years old. These two species are in such abundance in certain lakes that important fisheries at the outlets depend largely on them.

I have records of six species of jacks being taken in Philippine lakes and rivers. In some of these lakes a snapper (*Lutianus argentimaculatus*) is common and is the basis of an important local fishery. In Tahiti and in Luzon I have taken snake eels in mountain streams. When these species and such coral-reef dwellers as species of parrot-fish (Scaridae) are taken in rivers, and stinging scorpion fish (Scorpaenidae) are taken in hill stream rapids 40 km from the sea, one may well revise his ideas about the adaptability of marine fish to life in fresh water. One is hardly safe in excluding any group of littoral fishes. In the Solomon Islands schools of *Abudefduf metallicus,* family Pomacentridae, typical of coral reefs, lived in a deep pool between rapids in a river on Malaita Island.

In the Indo-Pacific tropics at least, a great variety of marine littoral fishes may occur in fresh water. Deep-sea fishes, as well as those of the open sea, may safely be excluded from life in fresh water, but one cannot say offhand that any shore fish is incapable of voluntarily entering and living in fresh water.

Albert W. C. T. Herre
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21 February 1956

I gratefully accept the opportunity to reply to a few criticisms that occur in A. W. C. T. Herre's discussion of my previous article on "Fresh-water sawfishes and sharks in Netherlands New Guinea" [*Science* **123**, 222 (10 Feb. 1956)]. In general, I want to state that the indicated errors are mere misinterpretations.

Referring to my paragraph on *Pristis microdon* and the Lake Nicaragua species, Herre assumes that the word *specimens* refers to sharks. Since the whole first part of this paragraph deals with sawfishes, sharks not even having been mentioned in this or the previous two paragraphs, the word *specimens* evidently concerns sawfishes only. Accordingly there is no reason to discuss Herre's statements on sharks in Lake Nicaragua.

Concerning the sawfishes in Lake Nicaragua, Bigelow and Schroeder [*Fishes of the Western North Atlantic* **2**, 39, 40 (1953)] write as follows: "While it may not be strictly landlocked there, in a topographic sense, any more than it is up the Amazon, the fact that Sawfishes breed in the Lake and are rather sluggish in habit makes it likely that most of the local inhabitants are permanent residents." As evidence they give a footnote: "Females taken there dropped their young at the time of capture (Marden, Nat. Geogr. Mag. **96**, 1944: 184)." Although it remains possible that copulation takes place in the sea, the quoted statements seem fairly conclusive.

I completely agree with Herre that sharks are able to pass rapids during the rainy season, but this does not interfere at all with my cautiously formulated statement on the migratory possibilities of sawfishes. Moreover, sawfishes are much more sluggish than sharks and probably experience more difficulties when they try to overcome obstacles.

Herre further infers that I am inclined to adopt the theory of gradual upheaval and gradual replacement of salt water by fresh water to explain the presence of

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

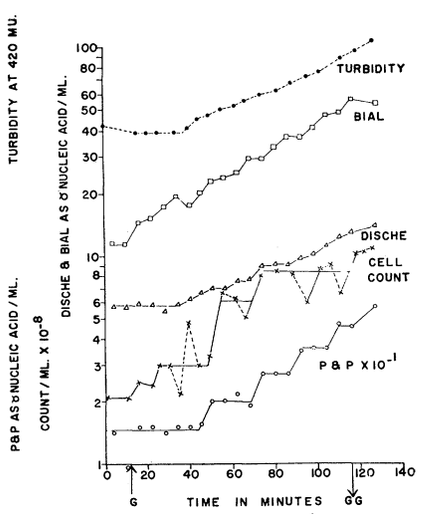

Fig. 1. *Escherichia coli* B. The culture consisted of 180 ml of "52" salt-ammonium sulfate medium at 37°C in a Klett sidearm cylinder, to which was added 28 ml of an overnight-aerated culture that had been chilled for 45 minutes to 5.5°C. The culture was aerated, and at 12 minutes 0.5 ml of 20 percent 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.