spleen apoferritin hydrolysate, equivalent to 0.8 mg of protein, is shown in Fig. 1. The hydrolysis was carried out with 6*N* HCl at 100° C for 18 hr. The following amino acids were present as ninhydrin-reactive substances: tyrosine, phenylalanine, the leucines, valine, glutamic acid, aspartic acid, glycine, serine, arginine, lysine, cystine (as cysteic acid), and methionine. Threonine was observed in trace amounts on chromatograms employing larger quantities of protein. Histidine was not observed in Fig. 1 because of its low sensitivity to the ninhydrin reagent, although its presence was reported by Mazur and Shorr (*5*). The relative quantitative estimations are in agreement with those given by Mazur and Shorr (*5*), except that, in addition to the amino acids listed by these authors, aspartic acid, glycine, serine, and threonine were detected chromatographically.

Paper chromatography of horse spleen ferritin showed the same amino acid composition as that of apoferritin but, in addition, it was observed that some of the iron, liberated by hydrolysis, had migrated in the phenol direction as a definite yellow spot. A chromatogram of ferric iron alone did not show this movement. It was found that the migration of iron on the chromatogram of the ferritin hydrolysate was not characteristic of ferritin, for identical movement was seen in a paper chromatogram run on Amigen (commercial pancreatic casein hydrolysate), to which ferric iron had been added in amounts approximating that contained in ferritin. In all these chromatograms, however, the amino acids moved to their relative positions with the collidine-lutidine solvent, whereas the iron remained on the phenol abscissa. Thus, the iron appears to be conjugated with one or more of the amino acids in the phenol run, but the association is disrupted by the collidine-lutidine solvent.

One-dimensional chromatograms (phenol solvent) of several single amino acids with added iron showed that all the amino acids tested were capable of moving iron from the starting point in varying degrees. Each amino acid could be classified according to the amount of iron it carried (Table 1).

TABLE 1

Amino acid	Amount of iron carried by the amino acid*
Histidine	++++
Alanine	++
Arginine	++
Isoleucine	++
Leucine	++
Lysine	++
Phenylalanine	++
Tyrosine	++
Valine	++
Threonine	+
Serine	+
Aspartic acid	+
Glutamic acid	+
Glycine	+
Control (iron)	No migration

* The system of grading, ++++ to +, is used to designate the amount of iron carried by the amino acid.

Thus, the iron spot, noted on the paper chromatograms of hydrolyzed ferritin and Amigen + iron, is probably due to the fact that such amino acids as histidine, the leucines, arginine, lysine, phenylalanine, and valine are associated with a considerable amount of iron and move to about the same position in phenol.

It is also apparent from Table 1 that histidine conjugates a greater quantity of ferric iron than do other amino acids. Furthermore, the strong affinity of histidine for iron has been reported (*7*). It is possible that a correlation might be found between the histidine content of proteins and their iron-binding capacity.

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Mass Mortality of Fish Associated with the Protozoan *Gonyaulax* in the Gulf of Mexico[1]

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This is the first reported observation of the concurrent appearance of a red tide, luminescent water, and immense numbers of the dinoflagellate protozoan, *Gonyaulax,* with mass mortality of fish on the eastern coast of North America. The episode occurred in the summer of 1949 in a salt water lagoon known as Offatt Bayou along Galveston Island (Figs. 1, 2). Three previously unreported associated biochemical phenomena were revealed by water analyses and offer new clues to the still-undiscovered mechanism of destruction of fish.

From time to time since 1902, comparable but more extensive destruction of fish has been concomitant along the west coast with the appearance of dinoflagellates (particularly *Gonyaulax*), luminescence, and the rusty red water commonly called a red tide (*7, 14*). Similar losses of marine life have occurred in Florida (*4*), but another dinoflagellate, *Gymnodinium,* has been incriminated. Historically, *Gonyaulax* has not only been associated with the mass mortality of marine life, but the species *G. catenella* is known to be the cause of the disastrous poisonings in man by mussels along the Pacific coast (*10–14*). Since 1941, when there were 346 cases with 24 deaths, there have been only a few cases, because state laws forbid the gathering of shellfish during the

[1] Acknowledgment is made of the assistance of R. Z. Finchum and O. K. Mullen, of the Texas Game, Fish and Oyster Commission, in the collection of samples and in making field observations.

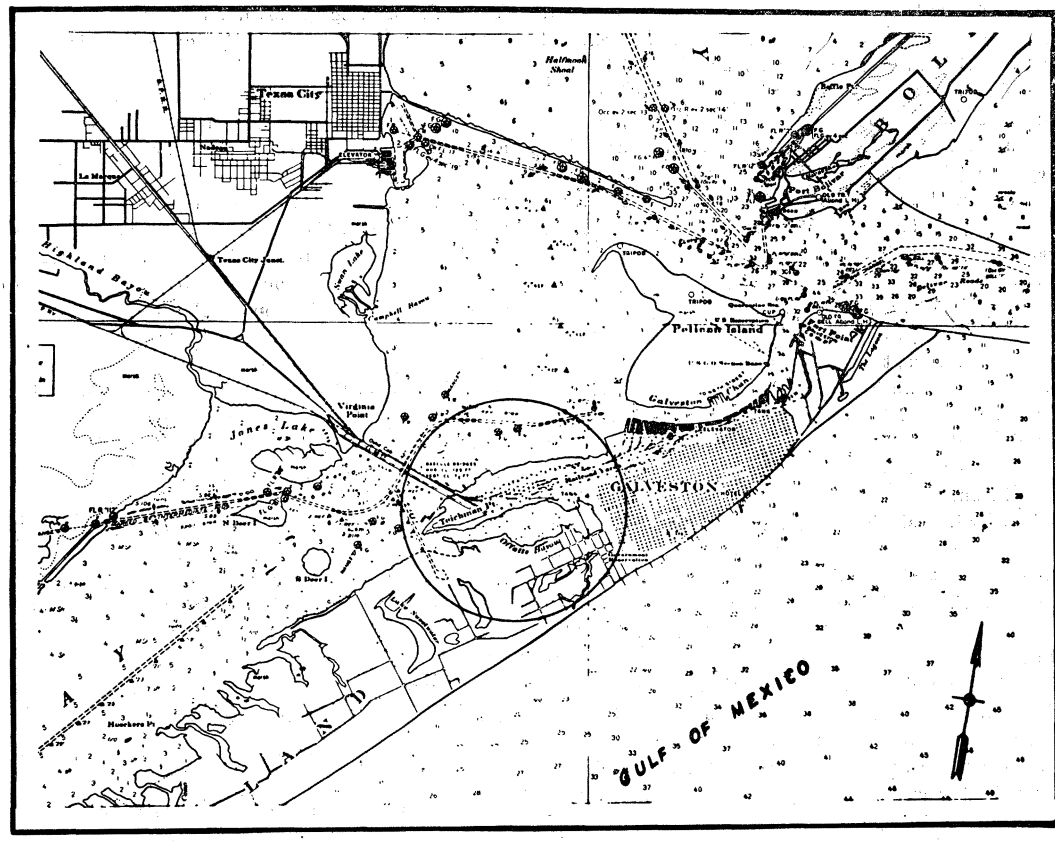

FIG. 1.

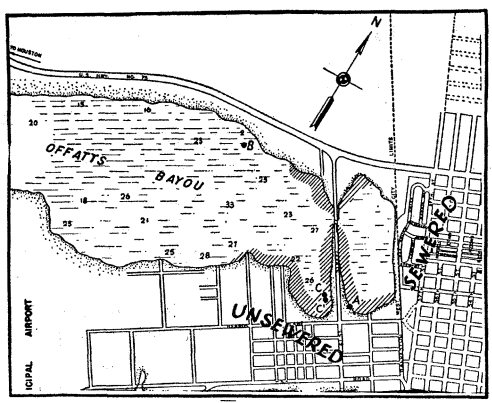

FIG. 2.

season when *G. catenella* may synthesize the poison. The toxin, which is approximately 10 times as potent as strychnine for mice (*11*), is transmitted to man only in shellfish because in the latter the viscera (in which the poisonous protozoa are concentrated), as well as the muscular portion of the fish, are eaten. Poisonings caused by *G. tamarensis* have also been reported from Nova Scotia along the North Atlantic (*2*). Oysters can concentrate the poison but have caused little trouble, because their harvesting during the summer months was popularly abandoned long ago.

Since oysters are the only edible shellfish in the vicinity of Galveston, it is not surprising that there are no records of local poisonings. It is probable, however, that red tides have occurred more often than they have been reported. In fact, when Cabeza de Vaca (*1*) visited this region in 1528, he listed a "time when the fish die" in addition to other customary aboriginal appellations for the seasons. Certain Indian tribes along the Pacific forbade their members to eat mussels during red tides or luminescent water, and the sudden extinction of one band may have been caused by this toxin (*10*). It has been suggested that the red tide with its associated phenomena can explain three famous biblical Egyptian plagues when the water turned to blood, followed by an unbearable stench and swarms of flies.

The initial recognition that the local protozoan was a dinoflagellate was made by Ludwik Anigstein. Further observations showed that it was a chain-making, armored form with the sharply broken girdle of the genus *Gonyaulax* (Fig. 3). Its other characteristics indicate it is either *G. catenella* Whedon and Kofoid or a similar new species (*8, 9, 15, 16*). Microscopic examinations have been made of both living and preserved specimens. The illustrations of the latter differ only from the appearance of the living animal in the absence of flagella (Figs. 3, 4). Unfortunately, the preservation of an insufficient number of specimens has prohibited the special technique required for defining the sutures of the cellulose plates of which

the armor is composed. The local species makes longer chains than have been reported for *G. catenella*, but only failure to delineate the sutures has prevented its identification as that species. Actually, this failure implies identity, because indistinct differentiation of the plates is characteristic of *G. catenella* (*16*).

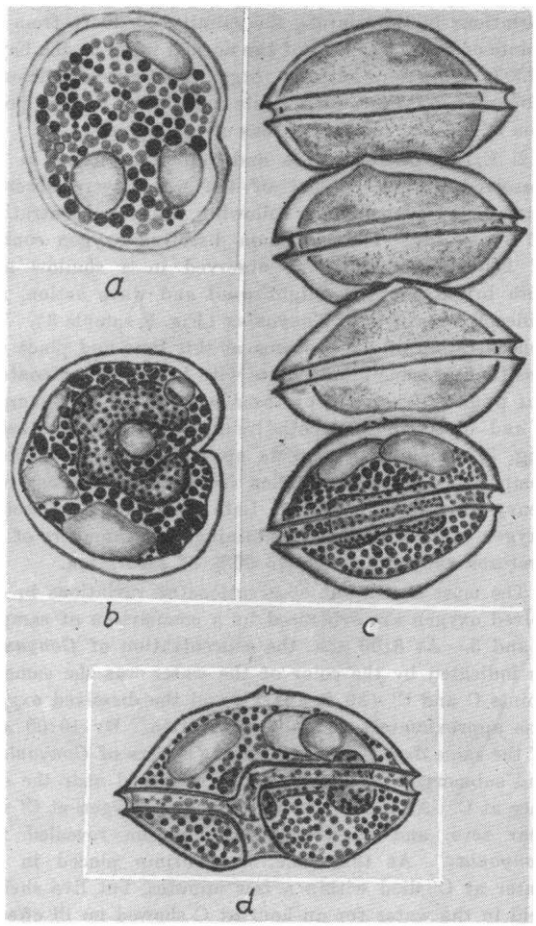

FIG. 3. Camera lucida drawings of local *Gonyaulax*, by J. B. Cross. ×1,300. No flagella, because all illustrations made of specimens preserved in glychrogel; *a*, apical view, apparent double wall is actually girdle; *b*, antapical view, showing nucleus, Giemsa; *c*, dorsal view of short chain; *d*, ventral view showing sulcus and sharp break in girdle.

The two other similar chain-forming *Gonyaulax* can be eliminated readily from consideration. In *G. series* Kofoid and Rigdon (*6*), the central members of a chain are much larger than the terminal members. This is obviously not true of the local species (Fig. 4*a*). In *G. catenata* (Levander), the apical and antapical views have a kidneylike shape, and the nucleus appears as a short, thick club with bulbous ends (*5*). These characteristics are entirely different from the horseshoe-shaped nucleus and almost circular shape of the local species in comparable views (Figs. 3*a*, 3*b*). In these respects, as well as in size, the local species agrees with *G. catenella*, and the shape of the nucleus has been considered by Martin to be more reliable for identification than plate formation (*9*). Measurements on 51 animals from 2 slides gave a median length of 13.5 μ (range, 10.5–17.0, with a giant of 22), a width of 25.2 μ (range, 21.5–30.5), and a transdiameter of 20.7 μ (range, 16.5–24.0). The microphotographs (Fig. 4) show the protozoan filled with vesicles containing a translucent nongranular substance. Such vesicles are found in the peripheral zone of the animal, but the larger central region is packed with the granular bodies of varying sizes that are pictured in the drawings.

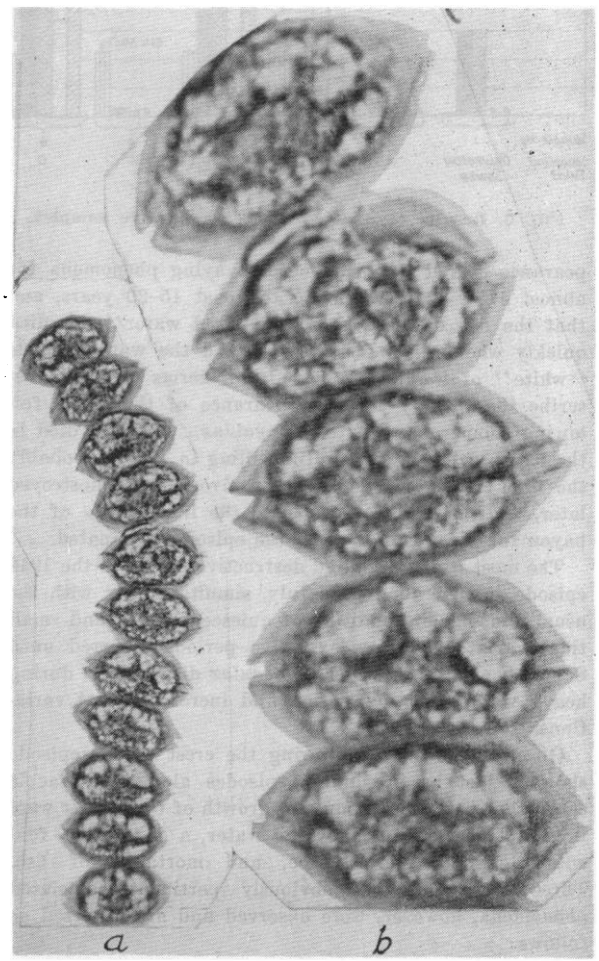

FIG. 4. Microphotographs by F. W. Schmidt. *a*, chain of 11 individuals ×600; *b*, part of *a*, ×1,800, to show detail of armor, girdle, and vesicles.

The virulent poison isolated from *G. catenella* and capable of killing man and other mammals has little effect on fish and does not explain the mass destruction of marine life that has been concurrent with an abundance of these protozoa (*13*).

Gunter attributed the mass mortality of fish in Offatt Bayou, occurring yearly, with one exception, from 1936 through 1941 (*3*), to the stagnation in the inner portions of the bayou plus the accumulation and anaerobic decomposition of organic matter during the summer months. Near-by residents and fishermen have reported the ap-

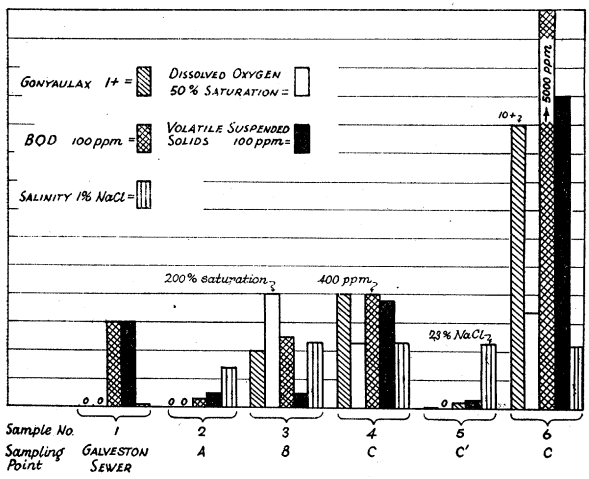

FIG. 5. Results of analyses of representative samples.

pearance of red tides and accompanying phenomena for almost every summer during the past 15–20 years, and that the fish did not die in the "red water" but died quickly when the redness settled and the water became "white" or "milky." The latter terms evidently describe the slightly turbid appearance of the water following submergence of the *Gonyaulax*. Few fish died in the bayou following the initial killing in 1949. Probably the first catastrophe left few survivors to be destroyed later, and fish did not return to the inner areas of the bayou for a week or so after the episode terminated.

The most widespread and destructive period of the 1949 episode started about mid-July, simultaneously with the usual midsummer condition of quiescent water and small tidal variations. Less extensive periods recurred until September 10, after which *Gonyaulax* disappeared during heavy wind and wave action and increased tidal variations.

Observations made following the crest of the episode showed, just as in similar episodes along the Pacific coast, that periods of abundant growth of *Gonyaulax* were invariably accompanied by red water, a distinctive, foul odor, brilliant luminescence, and mortality of fish. Three additional and obviously pertinent associated phenomena, however, were observed and are reported as follows:

1. There were indications that small amounts of sewage pollution favored the growth of the protozoan. The most widespread and destructive visitation followed immediately after heavy rainfalls had flushed sewage-polluted surface water into the bayou from unsewered areas west of the city limits (Fig. 2). Further, those loci at which heavy growths recurred frequently were in wind-sheltered areas of the bayou, where there is light pollution from private septic tanks. Since the effluents from these tanks enter through spoil banks and dredge fillings below mean low tide, it was never possible to detect gross sewage pollution at any of these points. None of Galveston's municipally operated sewers empties into the bayou, but for comparison, sample 1, Fig. 5, shows approximately the composition of the city's sewage. The composition of sewage of maximum strength found flowing into the bayou is represented by sample 2, Fig. 5, which was from a private combined sewer of very small flow. Salinity tests at successive distances from obvious points of entry of sewage demonstrated rapid multiple dilution with the salty bayou water. Even in moderately quiescent water and during periods of slight variations in tidal levels, the salinity 10–20 ft from the points of entry was that of the normal water in the bayou (2.3% NaCl). Coliform organisms were sufficiently numerous, however, to indicate significant sewage pollution of most of the inner areas of the bayou.

2. Extraordinarily wide and rapid variations in the dissolved oxygen content of the water were observed during and immediately following heavy concentrations of *Gonyaulax*. The maximum dissolved oxygen content of 200% saturation was observed in a shallow area with bright sunlight, slight wind and wave action, and widespread growth of *Gonyaulax* (Fig. 5, sample 3). The concentration of the protozoa at this time and place was less than at some other points with lower oxygen content, but practically all the protozoa were living. In samples 4 and 6, there were both living and dead *Gonyaulax*. Fig. 5, sample 4, represents approximately the average composition of samples taken from quiescent areas with heavy growth in sunlight. Late at night the dissolved oxygen content of water containing large numbers of the protozoa ranged from 40 to 90% of saturation.

The most significant observations of variations in dissolved oxygen are evidenced by a comparison of samples 4 and 5. At 8:00 A.M. the concentration of *Gonyaulax* as indicated by the color of the water was the same at points C and C′ (30 ft apart), and the dissolved oxygen was approximately at 100% saturation. By 10:00 A.M. of the same day, the red material (masses of *Gonyaulax*) had submerged deeply at C′ but remained near the surface at C. At 11:00 A.M. the dissolved oxygen at C′ was near zero, and microscopic examination revealed few *Gonyaulax*. At this time, live shrimp placed in the water at C′ died within a few minutes, but live shrimp held in the water for an hour at C showed no ill effects. Similar results were obtained several times. In quiescent areas with a heavy growth of *Gonyaulax*, the dissolved oxygen would remain high, but whenever the mass of organisms rapidly submerged and the water was quiescent, the dissolved oxygen would decrease rapidly. No causes for the submergence were suggested by the present findings.

3. The values of biochemical oxygen demand (BOD) for the samples associated with *Gonyaulax* were extraordinary and far in excess of what might be explained by sewage or any other organic pollutant entering the bayou. That the protozoan contributed heavily to the organic content and the BOD is implied by the fact that the variations in the observed values are roughly proportional to the number of *Gonyaulax*. This is illustrated by the range of values in samples 3 through 6. In sample 6, the BOD was approximately 5,000 ppm, and the water was as intensely discolored by the concentration of the protozoan as though a large amount of ferric hydroxide had been added. It seems logical to assume

that these extraordinarily high values of BOD and consequent anaerobic conditions in the water were attributable to the *Gonyaulax* and were important contributing factors in the mass mortality of fish in Offatt Bayou.

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The Synthesis of L-Ascorbic Acid Uniformly Labeled with C^{14} [1]

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The synthesis of L-ascorbic acids isotopically labeled in different positions is of great interest in biochemical research. Recently Burns and King (*1*) have described a synthesis of 1-C^{14}-L-ascorbic acid.[2] We wish to report the synthesis of uniformly enriched L-ascorbic acid.[2] Our synthesis was patterned after that of Reichstein and Grüssner (*4*) and adapted to working on a semimicro scale.

A mixture of uniformly enriched sucrose, glucose, and fructose was prepared by allowing bean leaves to photosynthesize for 24 hr in an atmosphere of $C^{14}O_2$ (*2*).

The sucrose was hydrolyzed, and a mixture consisting mainly of glucose was precipitated by the addition of absolute ethanol. The mixture, weighing 392 mg and having a specific activity of 1.4 μc/mg, was hydrogenated in an alkaline aqueous medium using Raney nickel. The sorbitol produced was oxidized to sorbose with *Acetobacter suboxydans* by the method of Wells, Stubbs, Lockwood, and Roe (*6*). After the fermentation the organism was removed by centrifuging, and the supernatant liquid was passed through Amberlite IR-100-H and Duolite A-4 ion exchange columns to remove ionic impurities. Crystallization and decolorization with Darco G-60 gave 344 mg of pure sorbose, mp 163°–165° C.

The sorbose was diluted with 135 mg of carrier, and acetonated, using freshly distilled dry acetone, and sulfuric acid as a catalyst. The diacetonesorbose was extracted from unreacted material and monoacetone derivative with ether. Oxidation of the diacetonesorbose with 6% potassium permanganate was carried out in alkaline medium (*3*), the unattacked material from the first oxidation being removed by ether extraction and reoxidized. The two batches were carried through separately to the last step. The reaction mixture was filtered and acidified to pH 2, after which the diacetone-2-ketogulonic acid was extracted with ethyl acetate and refluxed with water for 40 min to effect deacetonation. Lyophilization gave white, powdery 2-ketogulonic acid, mp 170°–171° C. The methyl ester was prepared by passing a large excess of diazomethane into a methanolic solution of the acid at −15° C. By crystallization from methanol-acetone, 68 mg of white crystals, mp 145°–150° C, was obtained. There was obtained in addition 220 mg of residual oil, consisting partly of methyl 2-ketogulonate, which could not be induced to crystallize and was therefore diluted with 205 mg of carrier and carried through the last step. Treatment under nitrogen of the methanolic solutions of the ester with stoichiometric amounts of 4.65*N* sodium methoxide in methanol and 2.60*N* hydrogen chloride in methanol, gave, after purification according to the method of Szent-Györgyi (*5*), 30 mg of L-ascorbic acid having specific activity of 0.80 μc/mg, and 70 mg having specific activity 0.16 μc/mg. The products melted at 189°–190° C, and gave a single spot of proper R_f value on a paper chromatogram.

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[1] Research carried out under the auspices of the Atomic Energy Commission.

[2] According to suggestions for the naming of isotopically labeled compounds proposed at the Chemistry Conference, Brookhaven National Laboratory, and shortly to be published, these acids would be known as L-ascorbic acid-1-C^{14}_1, and L-ascorbic acid-ue-C^{14}_6, respectively.