

10<sup>6</sup> r/hr. The peptide was hydrolysed with 6*N* HCl in evacuated tubes for 22 hours at 110°C. The resulting hydrolyzate was taken to dryness in a vacuum over NaOH pellets, dissolved in sodium citrate buffer of pH 2.2, and analyzed in a Spinco amino acid analyzer according to the method of Moore, Spackman, and Stein (2). Amino acids formed from other amino acids were identified by cochromatography on the column and also on paper with Hausmann's solvent system (see 3).

Table 1 shows that glutamic acid is transformed into aspartic acid, proline into glutamic and aspartic acid, methionine into  $\alpha$ -amino-*n*-butyric acid, histidine into aspartic acid, tyrosine into dihydroxyphenylalanine (DOPA), phenylalanine into tyrosine and dihydroxyphenylalanine, cysteine into alanine, and alanine into glycine. These data are in agreement with those of Row-

Table 1. Amino acid transformation as a result of gamma irradiation. The amounts of amino acids are given in micromoles (irradiated in 1 ml of buffer).

<i>Poly-L-glutamic acid</i>				
Dose (10 <sup>6</sup> r)	0	5.83	8.03	
Glutamic acid	5.6	2.8	0.95	
Aspartic acid	0	0.56	0.76	
<i>Poly-L-proline</i>				
Dose (10 <sup>6</sup> r)	0	3.11	7.65	9.83
Proline	15.7	8.6	3.6	2.3
Glutamic acid	0	1.4	1.0	1.1
Aspartic acid	0	0.11	0.18	0.22
<i>Glycyl-L-methionine</i>				
Dose (10 <sup>6</sup> r)	0	4.72	7.19	
Glycine	2.9	1.6	1.2	
Methionine*	1.9	0.91	0.38	
$\alpha$ -Aminobutyric acid	0	0.70	0.93	
<i>Glycyl-L-histidine</i>				
Dose (10 <sup>6</sup> r)	0	3.62	4.97	7.18
Glycine	5.6	2.4	1.9	0.58
Histidine	5.1	0.78	0	0
Aspartic acid	0	0.44	0.44	0.96
<i>L-leucyl-L-tyrosine</i>				
Dose (10 <sup>6</sup> r)	0	3.62	5.35	6.43
Leucine	3.4	2.3	1.6	0.72
Tyrosine	3.4	2.4	1.0	0.42
DOPA	0	0	0.17	0.18
<i>DL-phenylalanyl-glycine</i>				
Dose (10 <sup>6</sup> r)	0	2.41	5.28	7.01
Glycine	6.7	6.6	5.6	5.5
Phenylalanine	6.9	1.8	0.45	0
Tyrosine	0	0.04	0.29	0.20
DOPA†	0	0.08	0.15	0.16
<i>Glutathione</i>				
Dose (10 <sup>6</sup> r)	0	1.07	6.22	
Glutamic acid	3.9	3.7	2.8	
Glycine	3.9	4.2	3.2	
Cystine*	3.4	2.6	0.85	
Alanine	0	0.51	1.2	
<i>L-alanyl-L-leucine</i>				
Dose (10 <sup>6</sup> r)	0	3.33	5.82	10.26
Alanine	5.9	3.0	2.11	0.60
Leucine	5.9	1.3	0.85	0.54
Glycine	0	0	0.15	0.41

\* Methionine and cystine are partially destroyed during acid hydrolysis. † Small peaks for meta and ortho hydroxyphenylalanine were also observed.

bottom (4) who found that irradiation of tyrosine in aqueous solution produces dihydroxyphenylalanine and of Vermeil and Lefort (5) who irradiated phenylalanine and obtained *o*-, *m*-, and *p*-hydroxyphenylalanine. They also complement the findings of Grant *et al.* (6), of Markakis and Tappel (7) who reported that cystine can be changed into alanine, and those of Kopoldova *et al.* (8) who detected  $\alpha$ -amino-*n*-butyric acid, as well as threonine, alanine, aspartic acid, and serine, among the decomposition products of methionine.

For the formation of aspartic acid from glutamic acid one could either postulate decarboxylation of the gamma-carboxyl group of glutamic acid by oxidation of the gamma carbon atom or splitting between the beta and gamma carbons with subsequent fixation of CO<sub>2</sub> on the beta carbon atom (9). To test these hypotheses, poly-L-glutamic acid was irradiated in the presence of NaHCO<sub>3</sub> labeled with C<sup>14</sup>. After hydrolysis and chromatography, a radioautogram indicated activity in the position for aspartic acid thus suggesting that the second possibility is plausible. The occurrence of the C<sup>14</sup>-labeled CO<sub>2</sub> in the glutamic acid itself might be explained by a recarboxylation of a decarboxylated glutamic acid free radical. A third unidentified, ninhydrin negative, radioactive product was also observed. Similarly, gamma irradiation of poly-L-proline (10) in the presence of C<sup>14</sup>-labeled NaHCO<sub>3</sub> yielded labeled glutamic and aspartic acid, Fig. 1 (11).

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10. The poly-L-proline was a gift of Dr. Ephraim Katchalski.
11. Supported by Atomic Energy Commission Contract No. AT (30-1)2735. An equipment grant from the Charles F. Kettering Foundation is acknowledged.

17 January 1963

## Siphonophores and the Deep Scattering Layer

Abstract. *Bathyscaphe dives in the San Diego Trough have revealed a close spatial relation between siphonophores and the deep scattering layer as recorded by precision depth recording echo-sounders. Measurements of gas bubbles within the flotation structures of Nanomia bijuga captured in a closing net in an ascended scattering layer indicate that these are very close to the resonant size for 12-kcy/sec sound. Because such organisms are capable of making prolonged vertical migrations, and are widespread geographically, they are very probably the major cause of stratified zones of scattering throughout the oceans of the world.*

Despite intensive study, the question of the causes of ubiquitous, mid-depth zones of oceanic, sonic reverberation generically known as the deep scattering layer (DSL), has evaded a satisfactory answer. The general interpretation of the acoustical evidence is that diminutive fishes with gas-filled swim bladders are the principal scatterers. More frequently, though, net hauls taken through scattering layers at their daytime depths return virtually empty or with the catch dominated by euphausiids. These hauls have not demonstrated that the populations of these small shrimps are large enough to account for measured reverberation volumes (1, 2).

Recent observations from the U.S. Navy bathyscaphe *Trieste* have revealed that colonial hydrozoan jellyfish known as siphonophores are probably the primary cause of the deep scattering layer. Six dives were made from January to October 1962 off San Diego, site of the discovery of the deep scattering layer (3, 4). Scattering conditions were recorded either on an EDO depth-finding system or Precision Depth Recorders (PDR), or both, from surface ships while the *Trieste* was ascending. Typically (5, 6) the layer was located between 260 and 440 m during daylight hours and usually displayed an upper and lower component. Observations from the bathyscaphe permitted general identification of organisms thought capable of scattering 12-kcy/sec sound. Whenever possible, counts were made, and by computing the angle of view and the maximum range at which the various types of organisms could be recognized, the volume of water containing a given number of

organisms could be estimated. This permitted comparison of population densities with the results obtained with the net. These observations have been related to the depths and nature of the recorded scattering layers. The general results are summarized here.

Both components of the deep scattering layer have consistently been associated with siphonophores. Euphausiids were also observed in close relation with the upper component, but seldom in large numbers. The mid-water prawn, *Sergestes similis* consistently showed a correlation in depth with the lower component. Bathypelagic fish, primarily myctophids and *Cyclothone* species were frequently observed in relatively large concentrations, but always at depths well below recorded scattering. Schools of squid of the *Loligo* type were observed above, below, and at the layer on two dives. On two other occasions, immature Pacific hake, *Merluccius productus*, were associated with a shallow, intensely reflective layer centered above the deep scattering layer at about 200 m (7).

In view of the generally accepted concept of the faunal composition of deep scattering layers, these observations are most surprising. Negatively, there is a consistent lack of peak populations of midwater fish from these regions, even though it has been possible to observe and count them at other depths. Positively, there is a striking relation between the depths of recorded scattering layers and concentrations of siphonophores (8).

These observations led us to consider the evidence for siphonophores as a major cause of deep scattering layers. This can be conveniently discussed under the requisites for such organisms suggested by Marshall (9) and Tucker (6).

Essentially, siphonophores consist of concentrations of individuals specialized for various functions distributed along a highly contractile central axis. When fully extended, the siphonophore colonies may be 75 cm long and have relatively large structures in relation to the wavelength of 12-kcy/sec sound, but the watery nature of these parts should be transparent to sound energy. One of the major groups of siphonophores, the Physonectae, however, is characterized by the presence of a small, gas-filled individual bubble attached to the apex of the colony which functions as a flotation device (10). Parts of siphonophores similar to those observed at scattering layer depth were

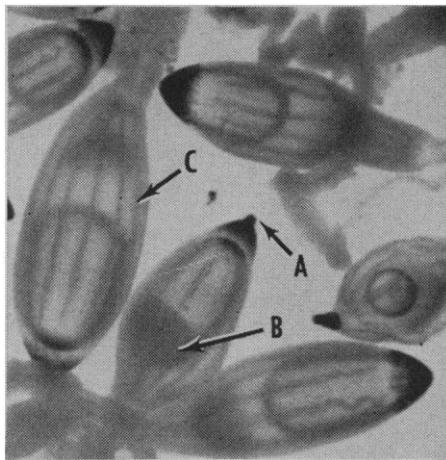


Fig. 1. Pneumatophores of *Nanomia bijuga* (about  $\times 12$ ) with contained gas bubbles. A, Pore; B, gas gland; C, longitudinal muscle band.

collected in a large closing net the night after a dive when the layers had already migrated to surface waters. Thus these specimens very probably represent the species observed *in situ*. These have been identified as *Nanomia bijuga* (11). Fifty of their pneumatophores, and the gas-bubbles which still remained in them 3 weeks after fixation in 10 percent Formalin, were measured under a  $24\times$  dissecting microscope (Fig. 1). The following average sizes are given in millimeters: pneumatophore, length, 3.27, width, 1.16; bubble of gas present at time of measurement, length, 1.49, width, 0.97. The size of the gas-bubble at great depths is not known. There is little doubt that one is present at these depths since an entity which reflects light in a manner similar to an air bubble can clearly be seen in these siphonophores from the bathyscaphe. These bubbles are covered only with a thin, watery skin, and they should respond to acoustic energy as perfect resonators.

According to theory (12), the size of resonant bubbles for 12-kcy/sec sound at 260 to 440 m depth is 1.27 to 1.64 mm; thus it is highly probable that these pneumatophores greatly enhance sound-scattering characteristics. Further, there is a marked size-depth distribution; the smaller colonies are generally located at shallower depths. This matches the requirement of resonant-size which is directly proportional to depth. The possibility that resonant bubbles might be associated with sound-scattering organisms was postulated from the outset of investigations of the deep scattering layer (4, 13). Heretofore, however, it was assumed that these

were the swim bladders of fish (see 2).

Little is known of the bathymetric distribution of physonectids, but peak populations of *Nanomia bijuga* were observed in close correspondence with the components of deep scattering layers on three dives made in July, September, and October 1962. Populations as high as 300 per 1000 m<sup>3</sup> were estimated on this latter dive (14). Another unidentified species of siphonophore was associated with the deep scattering layer in dives in February 1962.

Undisturbed colonies of *Nanomia bijuga* are usually seen perfectly motionless in a "feeding position" with tentacles stretched out in all directions. When stimulated by the pressure wave of the moving bathyscaphe they contract, pull in the outstretched processes, shorten the longitudinal axis of the colony, and swim with remarkable speed by frequent pulsations of their numerous swimming bells. This action is so fast and coordinated that if the organisms are horizontal they can easily be mistaken for fish. Further, on the basis of their anatomy, it can be theorized that it would not be necessary for them to maintain such swimming action to ascend to the surface. Rapid upward movement would cause an expansion of the gas within the pneumatophore which would make them still more buoyant and result in an accelerated rate of rise as the decrease in pressure became proportionately greater with shallower depth. Obviously, venting of expanding gas would be necessary to avoid explosion and to control the speed of ascent. Such a pore, guarded by a sphincter muscle, is present in *Nanomia bijuga* and other physonectids (10). This pore is located in the middle of the pigmented, nipple-like tip of the pneumatophores (Fig. 1).

Siphonophores can voluntarily sink from the surface in rough weather, and it would seem that descent to scattering layer depths could be accomplished by actively expelling gas by contraction of the well-developed longitudinal muscles which are present in the wall of pneumatophore (Fig. 1) and reduction of surface area by contraction of the stem-like stolon and tentacles. The latter movement can alter the center of balance so that the colony will tip over; thus active downward swimming is also possible. *Nanomia* colonies disturbed by the *Trieste* have been observed to act in this way. Gas would have to be produced under in-

creasing pressure in order to regain hydrostatic balance. The experiments of Jacobs (10) indicate that this species is capable of relatively rapid compensation to sudden changes of pressure of half an atmosphere. The gas is produced by a well-developed gland and giant cells located at the base of the pneumatophore. Protrusion of extensible processes which would increase the surface area, and therefore their resistance to sinking, might aid in this process. It is generally agreed that vertical movements of scattering layers are related to changes in deep-sea illumination (1). It is therefore of interest to note that Mackie recently reported that the sister species, *Nanomia cara*, is phototactic, and responds to light stimuli by swimming (15). The pigment effector cells which he describes may very well play a role in controlling vertical movements. Apparently, then, though full documentation is lacking, this type of siphonophore is ideally suited to perform long diurnal migrations with little expenditure of energy.

Siphonophores make up a large portion of the plankton in the warmer oceans of the world. The species of our bathyscaphe observations, *Nanomia bijuga* is cosmopolitan in distribution (11) and is considered the most common physonectid in the waters adjacent to the California coast (16). Certain anomalies of siphonophore distribution are also informative. For example, few species are reported from arctic and antarctic waters; the latter region develops deep scattering layers only spasmodically.

Thus it appears that physonectid siphonophores fulfill all the prerequisites of a major scattering organism. In view of this evidence we suggest that the primary cause of diffuse zones of scattering recordable on echo sounders in mid-depths off the California coast—and very probably throughout the warm water oceans of the world—are such organisms.

That this has not been considered before is attributable primarily to the fragile nature of these colonies so that it is impossible to sample them adequately with conventional nets (17). On contact with mesh or bridle, the tentacles stick by their stinging cells, the individuals making up the colony break away, and most are lost through the large meshes of high-speed nets which have been most commonly used in scattering-layer research. Further, because of their pellucid nature they

are extremely hard to photograph and could easily have been missed in studies of the deep scattering layer with cameras and underwater television (18). On the other hand, several biologists who have descended in bathyscaphes have reported seeing siphonophores at depths of the deep scattering layer, although they did not relate them to zones of scattering (19).

The Trieste siphonophore observations indicate that we are dealing with a zone of mid-water predators—a living net—stretched across the world's oceans. Obviously populations of such magnitude must play a key role in the overall economy of the oceans (20).

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28 March 1963

## Electroconvulsive Threshold Elevation: From Daily Stimulation of Adrenalectomized Animals

**Abstract.** *The elevation of electroconvulsive threshold, which develops in cats during repeated daily measurements thereof might result from an increased production of deoxycorticosterone. Bilateral adrenalectomy followed by maintenance on deoxycorticosterone and cortisone in fixed dosages did not prevent subsequent elevation of the threshold in either cats or miniature dogs. The elevation rate in the adrenalectomized dogs exceeded that in the intact control dogs. This elevation, which resembles tolerance, in the intact cat or miniature dogs, is not dependent on an increased production of adrenocortical hormones; it may more likely be the result of cerebral rather than extracerebral adaptation.*

A significant increase in electroconvulsive thresholds (ECT) develops in cats during periods when regular daily determinations are made. After the elevation has occurred, threshold returns almost to original levels if occasional rather than daily measurements are made (1).

This "tolerance" to repeated electrically induced convulsions might result from an increased production of deoxycorticosterone (1), which has been re-

ported to cause elevation of the threshold in rats (2). Electroconvulsions might cause an increased production of this hormone, since adrenal hyperplasia has been described in rats so treated (3). This hypothesis could be tested by removing the adrenals and maintaining the animals on fixed doses of corticosteroids before and during the periods of daily electrostimulation. The purpose of this report is to show that the elevation in the electroconvulsive