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10. We thank D. H. L. Evans for advice, B. G. Cragg and E. G. Gray for reading the manuscript, and E. Mansell for technical assistance. One of us (L.E.W.) is a postdoctoral fellow of NIH.

27 December 1965

Deep Scattering Layer Migration and Composition: Observations from a Diving Saucer

Abstract. *The distribution of a myctophid fish and physonect siphonophores observed during dives in the Soucoupe off Baja California closely correlates with scattering layers recorded simultaneously with a 12-kcy/sec echo sounder. These organisms were observed while they were migrating vertically, and at their night and daytime levels. They are capable of rapid, extensive changes in depth.*

Many meso-pelagic animals undergo diurnal vertical migrations of several hundred meters. Keyed to ambient light (*1, 2*), they move upward from mid-depths at dusk, remain near the surface throughout the night, then descend at dawn to their daytime levels. Parallel movements of ubiquitous, stratified zones of sonic reverberation in the oceans discovered during World War II (*3*) led Johnson (*4*) to the conclusion that similar organisms were the cause of these deep scattering layers. In the intervening 20 years diverse methods have been used in an effort to specifically identify the responsible animals (*5*). The preponderance of evidence implicated meso-pelagic fishes, particularly the Myctophidae (*5, 6*). Of acoustic importance, many of these diminutive lantern fishes have gas-filled swim bladders of such a size as to be resonant for the sound pulses used (12 to 24 kcy/sec) (*7*), and most layers studied by appropriate methods are sharply peaked in narrow frequency bands. This strongly implies resonant scattering from bubble-containing organisms (*8*). Recently, direct observations from the bathyscaphe *Trieste* showed that another type of organism at the opposite end of the phylogenetic scale, siphonophores of the suborder Physonectae (*9*), must be considered as the cause of some layers (*10*). These polymorphic coelenterates consist of various types of individuals arranged along a contractile stem. Because of its gelatinous nature, the colony should have a sound impedance similar to that of water. A terminally born, buoyant individual (pneumatophore), however, generates and retains carbon monoxide bubbles which approximate the resonant size for echo sounder frequencies (*11*). Thus, much of the acoustical evidence implicating fishes with swim bladders (*5, 8, 12*) is also applicable to physonects.

The causative animals, in addition to being efficient sound scatterers, must form widely distributed populations spatially related to the layers. Obviously, they must also undergo vertical migrations of the extent and rate of the recorded layers. Both fishes and physonects are inadequately sampled by standard net tows, and only scant data on their migratory movements are available from this source. Earlier observations from submersible vehicles also leave this question in doubt. Because of operational limitations, the *Trieste* observations had been made on vertical penetrations of the layers at their daytime depths, and apparently the French bathyscaphe dives have also been confined to daylight hours (*13*). With the Cousteau Soucoupe Sous Marine "diving saucer" (*14*), four dives were made on 3 and 4 February 1965, in about 1300 m of water, approximately 10 miles (16 km) southeast of Cape San Lucas, Baja California. Observations were made while the layers were at the surface; at intermediate depths while the layers were migrating upward and downward; and in the upper regions of the main layer while it was at its daytime level. The saucer is a small, two-man vehicle which is limited to a depth of 300 m. This is too shallow to penetrate through most scattering layers at their daytime levels, but maneuverability, ease of launching and recovery, and hovering ability make it ideally suited for such an operation. Thus, more than 14 hours of a 36-hour period were spent in underwater observations, and two complete cycles of scattering layer migrations were studied in detail. This probably constitutes the first *in situ* observation of migrating myctophids and physonects, and permits correlation of these organisms with components of a complex scattering layer.

High-resolution echo sounder records show that multiple and double component layers are common (*2, 5*). The deep scattering layer off Cape San Lucas was no exception. On 31 January, and during the dives, scattering layers were surveyed from the Scripps Research Vessel T-441 with a hull-

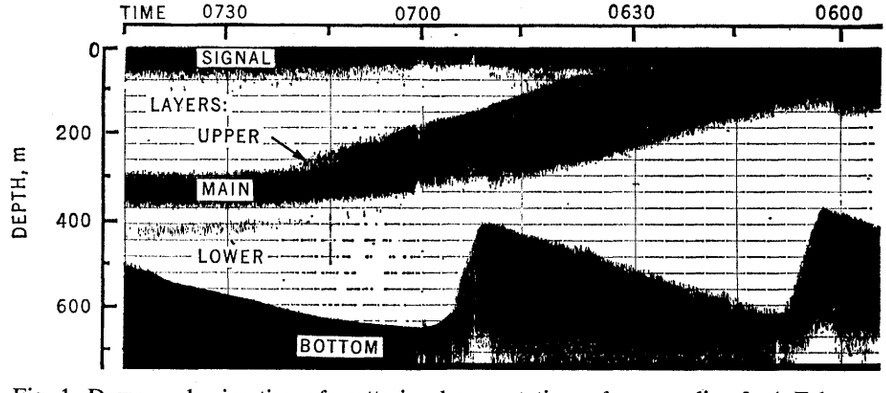

Fig. 1. Downward migration of scattering layers at time of saucer dive 3, 4 February 1965. Resolution between the two components of the "main" layer is vague in the photograph, but clearer in the actual recording. During most of the time, vessel T-441 was drifting. For the brief periods, from about 0601 to 0610 and again from 0650 to 0710, the ship was under way, returning to station. At these times screw and water noise affected the resolution and intensity of the recording, and details of the separation of the "lower" layer from the "main" layer were lost. Time is recorded from right to left, and the 20-fathom (about 40-m) depth lines are broken at 3-minute intervals. Bottom is being recorded on the second cycle, and 732 m should be added for its true depth.

mounted, UQN 12-kcy/sec transducer, a Gifft Precision Sonar Transceiver run at high gain with a 0.017-sec signal length, and recorded on a Precision Depth Recorder model V. Heavy surface scattering was present at night. At dawn a layer separated from the surface zone and performed a dramatic downward migration of approximately 300 m in 90 minutes. (Figure 1 presents the echogram obtained on 4 February during the time of saucer dive 3, and is typical of the morning migrations.) When the migration was about two-thirds completed, a diffuse upper layer split from the main layer at the 220-m level and disappeared from the record. Simultaneously, a lower layer separated, dropped down to a depth of 420 m, and faded out. Indications of two components were evident in the main layer during its downward migration. These components then merged as the layer leveled off at about 300 m. During late morning the main layer thinned, again showed a suggestion of two components, and became unrecordable during midday. Evening ascent of the main layer began gradually at midafternoon with an increase in rate of rise just at sunset. From this point, upward migration was completed in about 2 hours. On two of the observation days, weak, discrete sound reflectors (*15*) were present above the main layer and rose immediately above it, masking any possible evidence of re-formation of an upper layer. Diffuse scattering recorded at high gain also contributed to this masking. At dusk the lower layer rose rapidly, at a maximum rate of 12 m/min, eventually fusing with the main layer. The nature and behavior of the layers during the 3 days of observation were essentially the same. Slight variations could be attributed to changes in the gain settings of the transceiver and recorder, and extraneous noise.

During the dives, personnel in a skiff, using a directional listening device, tracked the position of the saucer by its 37-kcy/sec pinger. Communications between the skiff and saucer were made at 20-minute intervals by underwater telephone. Movements and depths of the layers were recorded by the RV T-441 within a mile (1.6 km) radius of the dive site. This information was transmitted to the saucer via the skiff, and adjustments in saucer depth were made accordingly. During ascent and descent of the saucer, almost continuous observations were made with the use of two 1000-watt flood lamps and a 150-watt spotlight. To avoid attracting or repelling animals while the saucer was hovering before, during, and after passage of the layers, series of 2-minute observations were made with the lights on, alternating with 3-minute waits in darkness. For the first 1½ minutes of the observational periods, the regular flood lamps were used. An additional 2.5-kw "movie light" mounted at the end of an extendable arm was turned on for the last 30 seconds of the observation periods. In Figs. 2 and 3, respectively, numbers of myctophids and physonectid siphonophores are shown at the time and depth they were sighted during dives 3 and 4.

Dive 3 started before dawn, while the deep scattering layer was still at the surface (Fig. 2). A concentration of myctophids, one of three possible species, referred to here as the "silver myctophid" (*16*), was observed from 30 to 110 m. These were adult fish ranging from about 8 to 10 cm in length. Between 50 and 60 m, they swarmed in the saucer lights, swimming in a series of quick, random movements of about 1 m, alternating with motionless, short pauses. They avoided the brightest region of the light field and were repelled by the movie light. A few lantern fish followed the saucer downward, and the lights were briefly turned off to discourage their departure from their natural depth. After the saucer leveled off at 190 m to begin the series of 2-minute observations, three myctophids were seen before the layers migrated past the saucer. These were a different species from the near-surface population and were motionless, suspended vertically, head down (*16*). Eight silver myctophids were seen during passage of the layer, swimming downward at a 45° angle. Ignoring the saucer lights, they made rapid flights of several meters, paused momentarily, changed direction, and continued downward. The top of the main layer had leveled off at 278 m. As the saucer descended to 275 m, five silver myctophids swam upward into view. At 300 m, large numbers of this species swarmed in the saucer lights. Their actions were similar to those observed at the surface. As the saucer ascended, several

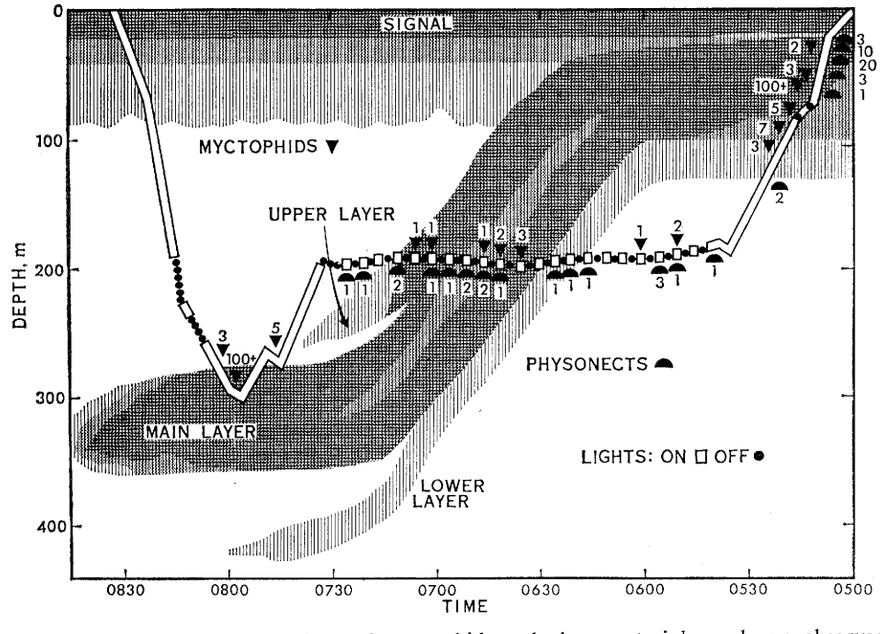

Fig. 2. Distribution and numbers of myctophids and physonect siphonophores observed during downward migration of scattering layers during dive 3. The time scale of the original precision depth recorder echogram represented by Fig. 1 has been compressed 6 to 1. Depth course of the saucer was established by its upward-directed Atlas echo sounder pinging on the sea-air interface. Its trace has been converted to the same depth and time scales as the diagram. The open bars represent periods when the lights were on; the black dots, times when lights were off. Depth checks of the saucer were made by recording the saucer on the RV T-441 echo sounder, and the presented depth trace is considered accurate within the limits of the open bars (about 10 m). Numbers of myctophids sighted are shown above the depth course; the physonects, below.

of the fish followed upward, apparently drawn by the lights. The floods were turned off. At 258 m, when lights were turned on momentarily, no myctophids were seen. At 210 m, ambient light was discernible, and the lights were once again switched on. From 100 m to the surface, increased ambient light reduced the effectiveness of the lamps and limited the field of vision.

Three types of physonectid siphonophores were observed: (i) a large species, about 60 to 70 cm in stem length when fully extended, and similar in appearance to *Nanomia (= Stephanomia) bijuga* (*16*), but with fewer feeding individuals (gastrozooids); (ii) smaller forms (20 to 40 cm in length), either younger colonies of the same, or a closely related species; and (iii) a small (20 to 30 cm) form, lacking concentrated pigmentation in its zooids. These will be referred to, respectively, as "large *Nanomia* type," "small *Nanomia* type," and "small pelucid type."

On descent of the saucer, a mixed population of physonects was centered above the silver myctophids at 35 m (Fig. 2). Some were swimming straight down. Those observed at 180 to 200 m, before the layers had migrated downward, were small, inactive *Nanomia* and pelucid types. The three siphonophores seen at the time of passage of the bottom of the layer were the large *Nanomia* type. When they were actively swimming down at about a 45° angle, with tentacles contracted and stems half drawn up, their motion was straight and continuous, typical of synchronous contractions of the swimming bells (nectophores) (*17*). Their rate of descent was more rapid than that of the myctophids. A mixed population of large and small *Nanomia* types was observed during passage of the main layer. Their swimming actions were similar, and they displayed a mild avoidance response to the lights. The last three siphonophores, observed at the time of passage of the upper layer, were of the small pelucid form. These could be seen only when close to the observation port when the movie lights were on. They swam downward, anterior end leading, in spirals about 1 m in diameter. As they entered the brightest region of the light field, they reversed their position, twisted into a double spiral, spread their tentacles, and hung motionless (*18*). Apical bulbous structures, which I took to be their pneumatophores, seemed to collapse and fall to one side. No other physonects were seen for the duration of the dive.

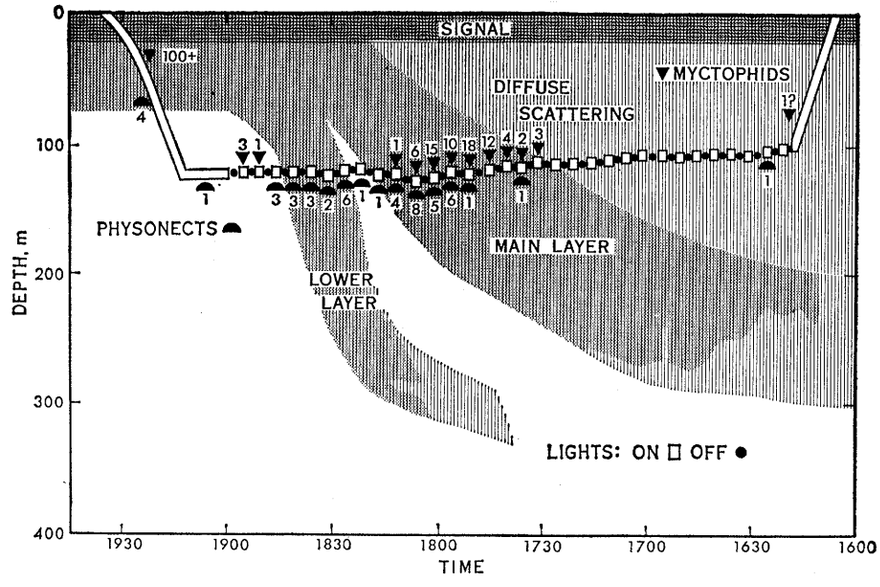

Fig. 3. Distribution and numbers of myctophids and physonect siphonophores observed during upward migration of the layers during dive 4. Treatment is the same as in Fig. 2.

Dive 4 began in late afternoon, when the main layer had risen about 100 m from its midday position (Fig. 3). Only one questionable myctophid was seen at the edge of the light field during descent, and only one small physonect was seen during the second observation period at 105 m. Over the next hour, 12 observation periods were devoid of either type of organism. Just as the upper part of the main layer passed the saucer, silver myctophids were sighted. At first only a few were seen, but their numbers increased as the middle of the main layer passed. Their counts then dwindled abruptly. The behavior of the lantern fish was strikingly different from that displayed during their downward migration. They were swimming in slow, zigzag motions, of about 2 m. Some were swimming upward at about a 45° angle; others were almost horizontal. The fish appeared to be mildly attracted to the lights. None were sighted during the passage of the lower layer. The four myctophids seen after passage of the layer were smaller and sluggish in action, and their scales did not reflect light as strongly as those observed in the main layer. They appeared to be a different species (*16*).

Physonect sightings were concentrated at the times of passage of the bottom part of the main layer and the lower layer (Fig. 3). About half of those observed were the large *Nanomia* type. These were rising rapidly in approximately the reverse posture of those seen descending. I got the impression they were revolving slowly around their stem axis. Their half-contracted stems streamed behind like a tail on a kite at a lesser angle than their actively pumping, more erect anterior end. They were always observed slanting away from the light field. Some of the smaller *Nanomia* types were undulating from side to side, indicating asynchronous contractions of nectophores (*17*). They were distracted by the lights, and some, by further contractions of their stems, assumed a horizontal swimhing posture or turned in large irregular circles.

As the saucer ascended slowly on its jets, four physonects were observed at 70 to 60 m. Between 30 to 20 m, the saucer was surrounded by the silver myctophids. They swarmed in our lights and were attacked by squid.

The relationship of myctophids and physonects to scattering layers can be summed up as follows. During dive 3, made through the layers at the surface, while the layers were descending, and in the top of the main layer at its daytime depth, at least 233 myctophids were sighted coincidentally with scattering layers, and six at other depths (three of these were attracted upwards from the depth of the layer by the saucer lights); 49 physonects

were sighted in relation to the layers, and nine elsewhere. During dive 4, made while layers were ascending and after they had surfaced, at least 161 myctophids were observed in the layers, and five at 100 to 200 m at other times; 45 physonects were in phase with the layers, and five were at 100 to 200 m at other times. Results of dives 1 and 2 made at the same times on the previous day were essentially similar. Other organisms capable of scattering 12-kcy/sec sound were observed (for example, squid, heteropods, euphausiids, sergestid shrimps, pelagic crabs, and unidentified fishes). None, however, were spatially related to the layers.

One cannot enter an environment without modifying it. The saucer lights appear to have been the main stimulus affecting the behavior of the organisms (*19*). The differences in phototactic response of the fish and physonects obviously influenced their observed abundance (*20*). Regardless, efficient sound scatterers need not be present in large numbers to account for the layers (*5*), and many of the organisms were seen when the lights were first turned on. The striking correlation of their distribution with the layers is convincing evidence that we are dealing with the causative scatterers. Very probably, the silver myctophids were the primary cause of the main layer. These were concentrated in its upper component. The lower component of the main layer and the lower layer appear to be associated with the *Nanomia*-type physonects, the large *Nanomia* type predominating in the latter feature. (Because of depth limitation of the saucer we were not able to penetrate these components at their daytime depths.) The small, pelucid physonects may be the cause of the ill-defined upper layer, but the observations are far from conclusive on this point. While individual myctophids or physonects were not followed throughout the course of vertical migration, again the correlation of populations with movements of the layers leaves little doubt that they do undertake these journeys.

Dissections of three specimens of each of the three possible silver myctophid species demonstrate that all have well-developed, prolate-shaped swim bladders with large gas glands (*21*). To conveniently evaluate these swim bladder sizes as acoustic resonators, their computed volumes were converted to radii of free spheres (1.9 to 3.4 mm). It is of interest to relate these data to the detailed acoustic analysis of deep scattering layers in the Atlantic by Hersey, Backus, and Hellwig (*8*). Theoretically (*22*), the swim bladders of the silver myctophids would fit these authors' "mid-frequency" layer whose peak resonance varied between 6 and 11 kcy/sec, and changed as the 1/2 power of the frequency during upward migration (their equipment was frequency limited during downward migration). They suggest this would indicate a causative organism capable of maintaining a constant gas volume (*23*). Extrapolating from previous measurements of physonects (*10, 11*), bubbles within *Nanomia*-type siphonophores closely correspond to their "high-frequency" layer. In these cases, peak resonance varied between 15 and 25 kcy/sec and changed as the 5/6th power of the hydrostatic pressure; this was indicative of expansion and contraction of gas bubbles, and would be more probable in physonects rather than fishes, since pneumatophores are capable of stretching, and excess gas can be vented.

A significant difference between results reported here and those made from the *Trieste* in the California Current (*10*) is that the spatial relationship between myctophids and physonects is reversed. In the earlier study, physonects were associated with a main layer at a daytime depth of 300 m, and myctophids (*Stenobrachius* [*-Lampanyctus*] *leucopsarus* and *L. mexicanus*), the adults of which lack large, functional swim bladders, were concentrated between 450 and 700 m. More recent, unpublished data show that in the summer months these fishes are frequently associated with a deep layer recordable only by 12-kcy/sec echo sounders as it migrates to the surface. These are probably the populations sampled long ago by Tucker's (*6*) net hauls, whereas his shallower layer, attributed to euphausiids, was probably caused by physonects.

Investigations by deep submersibles in other water-mass regimes should provide additional information on the relative importance of these two types of scatterers and identify other causative organisms.

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15. High-resolution echo sounders frequently record a strong individual target in the form of a hyperbola These are generally located at daytime depths above the deep scattering layer and migrate upward just above the layers. See R. S. Dietz, *Sci. Am.* **207**, 44 (Aug. 1962), and Hersey and Backus (*5*).
16. Since I lacked a method of collecting midwater organisms from the saucer, specific identification of fauna unfamiliar to me was impossible. At the time of the dives, RV T-441 personnel dip-netted under a night light and made plankton tows with a 0.5-m net. Squid, euphausiids, and shrimp were taken, but no myctophids or physonects. However, excellent motion picture footage of the latter organisms was obtained from the saucer. These films were viewed by E. H. Ahlstrom of the Bureau of Commercial Fisheries, and C. L. Hubbs and myctophid specialist R. L. Wisner of the Scripps Institution. In Wisner's opinion, based on size, shape, behavior, and vertical and geographical distribution, the myctophid in question is one of three species, *Lampanyctus omostigma, L. parvicauda,* or most likely *Myctophum aurolaternatum.* For the purpose of this report, because of its light-reflecting properties, I refer to it as "silver myctophid." The smaller, black, quiescent species seen in low numbers out of phase with the layers resembled *L. mexicanus.* On the basis of viewed motion picture films and geographic distribution, A. Alvarino of the Scripps Institution suggests that the large *Nanomia*-type physonect is probably *Halistemma* (= *Stephanomia*) rubra.
17. G. O. Mackie, *Proc. Roy. Soc. London Ser. B.* **159**, 366 (1964). Off San Diego, I have also observed *Nanomia bijuga* from the saucer, migrating downward in a scattering layer in an upright position similar to that described by Mackie as "reverse swimming." However, the siphonosome was contracted to one side in an "S" shape. A series of rapid nectophore pulsations jetted the colony downward about a meter at a time. Brief rests would follow these movements, and the colony would rise slightly, which indicated positive buoyancy. These alternating swim and rest actions continued until the colony disappeared from view below me.
18. A similar behavior has been observed in captive calycophoran siphonophores and termed the "veronica" display by G. O. Mackie and D. A. Boag, *Pubbl. Staz. Zool. Napoli* **33**, 178 (1963).
19. While adjusting ballast, or maneuvering, the saucer creates a loud, low-frequency noise. None of the organisms appeared to be affected by the din.

20. Differences in light-reflecting pigmentation of the organisms is also a factor in estimating relative population densities. For example, when flood lamps were used, I estimate that the silver myctophid could be seen at distances up to 7 m; the large *Nanomia*-type physonect with orange-pigmented gastrozooids was recognizable at up to 5 m; and the small *Nanomia*-type could be seen only at distances of up to 3 m. The half-angle of the cone of vision from the saucer port is about 45° in water; thus these volumes compute to 359, 131, and 28 m^3, respectively. Use of the movie light at least doubles these distances and the volumes increase eight times. I did not keep a separate record of sightings while using the regular floods and the movie light. Because of these variables, I have not attempted to reduce numbers of organisms observed to a common volume denominator.
21. Examination of specimens provided by R. L. Wisner was done by R. Capen. Descriptions and measurements of these and other bathypelagic fishes are in manuscript.
22. U.S. Natl. Defense Res. Committee Div. 6, *Summary Tech. Rept.* (1948), vol. 7. Andreeva and Chindonova (*12*) have recently considered the sheer modulus effect on bubbles, which theoretically enhances resonance at 300- to 400-m depths.
23. J. Kanwisher and A. Ebeling, *Deep-Sea Res.* **4**, 211 (1957), have questioned, on a physiological basis, whether physoclistous, swim-bladdered fishes can secrete and absorb gas rapidly enough to maintain neutral buoyancy throughout their vertical range. In this respect, note that the silver myctophids observed on their downward migrations were swimming rapidly. In contrast, their upward swimming was more leisurely, and upward migration of the main layer associated with these fish was slow, never exceeding 2 m/min and taking several hours to complete.
24. I thank R. Kientzy for skilled saucer piloting, and N. Shenton and the Westinghouse team for logistic support. R. Nubigin, W. Bunton, R. Bradley, and I. Davies assisted in operations at sea. J. Flynn aided analysis of data. G. Curl, E. Buffington, W. Batzler, G. Pribble, E. Hamilton, and G. Pickwell have given advice. Conversations with R. Backus have been helpful.

17 January 1966

Mammary Glands of Pregnant Rats: Development Stimulated by Licking

Abstract. *At the end of gestation, the mammary glands of pregnant rats that have been prevented from licking their ventral surfaces by neck collars are about 50-percent less developed than those of control animals. Neither the burden nor the stress effect of the collar is an alternative explanation. A considerable proportion of mammary development during pregnancy is thus caused by the female's own licking.*

Although suckling and other stimulation of the ventral surface of the rat are known to maintain lactation and cause mammary growth after parturition, there is no evidence that sensory stimulation contributes to mammary development during pregnancy (*1*). Yet by the time the female gives birth her mammary glands have increased considerably in size and have begun to produce milk (*2*).

A striking behavioral feature of the pregnant rat is intensified licking of her own ventral surface. Recently we reported that licking of the nipple lines and of the genital and pelvic regions increases markedly with the advance of pregnancy, whereas licking of the more anterior body parts, of the head, forepaws, shoulders, and upper back, tends to decline (*3*).

Since sensory stimulation is necessary for postpartum mammary function, and since self-licking is prominent in the behavior of the pregnant rat, it seems reasonable to ask whether self-licking stimulates mammary development during pregnancy. Mammary development was assessed at the end of gestation in rats that had been prevented from licking themselves throughout pregnancy and in several groups of controls that had been allowed to lick.

The main group of nulliparous pregnant rats (*4*) were prevented from licking by attachment around the neck of an 8.7-cm-wide full rubber collar so designed that the female could not extend her head beyond the collar's edge and lick her ventral surface (Fig. 1, *5*).

To control for the burden of wearing a full collar, a second group wore notched collars, equal in weight to the full collars but having 5-cm notches cut out to allow the females to reach under the collar and lick their bodies. A third group, uncollared throughout pregnancy, provided normative data on mammary development.

Because the full collar not only prevented licking but also interfered with various normal behavior patterns, interference that could cause stress (*6*), it was necessary to add another control group in which licking was allowed but in which stress was present. This fourth group therefore wore no collars but were injected twice daily with 0.25 ml of 2-percent formalin, a procedure reported to produce a stress reaction shown by enlargement of the adrenal glands, without interfering with pregnancy (*7*). A fifth group, injected with distilled water, served as controls on the formalin injection.

Samples of mammary gland were obtained after the females had been killed on the 22nd day of pregnancy, just prior to parturition. The left abdominal mammary gland was removed and fixed in 10-percent formaldehyde solution for 24 hours; a 0.5-mm piece of tissue close to the anteriormost nipple was then cut away,

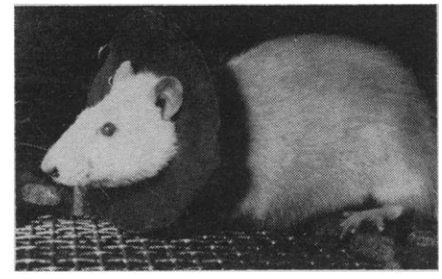

Fig. 1. A pregnant rat wearing a full collar.

dehydrated and cleared, and embedded in paraffin before being serially sectioned at 5 μ and stained with hematoxylin and eosin. One section from each gland was chosen randomly whenever the developmental level was assessed.

As a measure of mammary development, the proportion of secretory tissue to total glandular tissue was obtained by projecting the image of a section on a paper of uniform weight and by tracing the image in detail; the area representing the entire gland section was then cut out and weighed, as were the smaller areas representing secretory tissue. Secretory tissue was thus determined as a weight percentage of the total gland section. This weighing method is comparable to a standard planimeter technique (*8*); it yields results that correlate positively with ratings of the density of alveoli within the lobules, as well as with the amounts of secretion within them.

Figure 2 shows that the mammary glands of full-collared rats contained only about half as much secretory tissue as those of each control group. An analysis of variance for all groups was significant (F, 36.23; $p<.001$), as was the difference between the full-collared group and each control group, revealed by Duncan's new multiple-range test at the 1-percent level.

Since the notched-collared group did not differ significantly from the uncollared group, the burden of the collar is

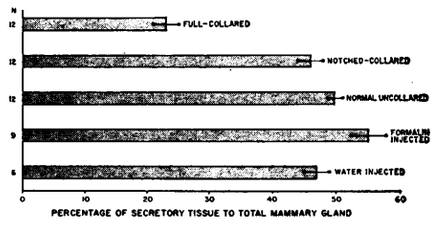

Fig. 2. Mammary development during pregnancy in the five groups of rats. Mean ± SE, •—|—•.