

Fig. 1. Monkey with occluders.

operation is polishing, which is done on a soft cotton buffing wheel with tripoli or some other polishing compound. If no sharp edges or roughness are found, the occluders are ready for cleaning and use.

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- We thank Ludwig von Sallmann, of the Na-tional Institute of Neurological Diseases and 5. Blindness, for performing the examinations.
- We have since learned from Austin Riesen that Leo Ganz, in the department of psychology at the University of Chicago, has been independently developing this technique for use with cats to produce, among other things, a colored Ganzfeld (!).
- 17 December 1958

## **Spatial Distribution of**

#### **Phoronopsis viridis Hilton**

Abstract. Individuals of the species Phoronopsis viridis Hilton exhibit an even distribution within their colonies. The distance between nearest neighbors is probably related to the space required for the operation of the lophophore. Distributions of other marine invertebrates are discussed briefly, together with the paleoecological implications of such knowledge.

The horizontal spatial distribution of Phoronopsis viridis Hilton was studied in three intertidal areas on the shores of Tomales Bay, California (1). Observations on the nearest-neighbor relationships of *Phoronopsis* were taken and analyzed after the method proposed by

Clark (2). Individuals of this species occur in clusters of thousands throughout the areas studied. While the clusters or colonies possibly develop as a response to very local environmental features, field examination revealed no obvious differences in the sediment at colonized and noncolonized sites. The first-nearest-neighbor relationships were observed for 384 individuals chosen at random within colonies.

The proportions of first-nearest-neighbor relations that are reflexive in each of the samples are shown in Table 1. The proportions for each locality are not significantly different from one another when tested by chi-square  $[Pr(X^2 \ge$ 1.004) > 0.05]. The number of reflexives (218) in the pooled data from all three sites was found to be significantly less than that expected for a random distribution (239)  $[Pr(X^2 \ge 4.66) < 0.05].$ This result is taken to indicate that individuals of P. viridis tend to be distributed evenly within the colonies.

The pattern of dispersion suggested by this analysis probably reflects direct interaction between individuals. After a heavy settlement, the growing animals must compete for space for expansion of the lophophore during feeding. Such competition would be expected to result in an even spacing of individuals in densely populated areas. At the borders of the colonies studied, individuals appear to be less crowded together and likely to be distributed at random. These circumstances are paralleled by the case of the clam Tellina tenuis reported by Holme (3). In feeding, this small clam sweeps the surface of the substrate with its inhalant siphon. The even spacing of Tellina observed by Holme was found to be statistically significant by Connell (4). The minimum distance between individuals is thus apparently related to mode of feeding in Tellina and Phoronopsis.

Connell, using a procedure similar to that employed here, studied the spatial distribution of two species of clams, Mya arenaria L. and Petricola pholadiformis Lamarck (4). He found that the clams were distributed in aggregations in the area of study but at random within the aggregations. He has suggested that this is the most common pattern for sedentary, bottom, filter feeders.

The pattern of spatial distribution of sedentary marine invertebrates can have important implications in marine ecology and paleoecology. Patterns of dispersion are important clues to larval behavior, interactions of individuals, and the nonuniformity of the physical environment. Knowledge of the kinds of patterns characteristic of particular taxonomic groups or modes of life may also be useful in the interpretation of the mode of formation of fossil assemblages. In this regard, such information can aid

Table 1. Proportion of nearest-neighbor relations that are reflexive in each of the samples.

Sample	Size	Proportion
1	99	0.53
2	114	0.59
3	171	0.58

in recognition of an assemblage that has been buried in place with a minimum of exposure after death. Patterns of spatial distributions of fossils have been determined by techniques similar to those employed here. Miller has used nearestneighbor relations in an attempt to determine current direction in an ancient, black-shale environment (5). For all of these purposes, more modern analogs are needed (6).

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#### **References and Notes**

- 1. Locality 1 was in the small cove immediately north of White Gulch; locality 2 was on the flats east of Lawson's pier; and locality 3 was on the flats near the creek on the south shore on the flats near the creek on the south shore of White Gulch (see U.S. Coast and Geodetic Survey Chart 5603, 1957). P. J. Clark, Science 123, 373 (1955). N. A. Holme, J. Marine Biol. Assoc. United Kingdom 29, 267 (1950). J. H. Connell, Invest. Shellfisheries Mass. Rept. No. 8 (1956) p. 15.
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  This investigation is part of studies supported by a grant from the Louis B. Block Foundation of the second statistics of the second statistics. and made possible by the cooperation of the Pacific Marine Station of the College of the Pacific

8 December 1958

# **Production of Spherules from** Synthetic Proteinoid and Hot Water

Abstract. When hot saturated solutions of thermal copolymers containing the 18 common amino acids are allowed to cool, huge numbers of uniform, microscopic, relatively firm, and elastic spherules separate. The place of this phenomenon in a comprehensive theory of original thermal generation of primordial living units is considered.

A comprehensive theory of the spontaneous origin of life at moderately elevated temperatures from a hypohydrous magma has been developed (1). The theory results from experiments which have yielded linked reactions in sequences akin to many in anabolism (1), materials which closely resemble protein in qualitative chemical composition and physical properties studied (2), and a biointermediate for nucleic acid, ureidosuccinic acid (3).

The material with attributes of synthetic protein, proteinoid, is easily produced by employing sufficient excess of



Fig. 1. Cell-like spherules prepared from 15 mg of proteinoid by 1 min of boiling with 3 ml of sea water  $(\times 1160)$ .

dicarboxylic amino acid in the thermal copolymerization of all of the common amino acids (2). Such products contain all of these same amino acids, are biuret-positive, can be salted in and subsequently salted out, reveal by endgroup assay mean chain weights of 3000 to 9000, and are split by proteinases and have other properties of natural proteins.

New conceptual difficulties arise, however, when attempts are made to fit some of the conditions employed into a comprehensive theory of the origin of life. One such problem is that posed by the presumed coagulation of proteins in the first living organisms produced at elevated temperatures. The other is the general problem of understanding modulation from a primitive hypohydrous organic magma (1) to the predominantly aqueous entity which the first organism is assumed to have been.

Table 1. Effect of concentration of aqueous sodium chloride on diameter of spherules.

Concen- tration of sodium chloride solution (%)	Diameter of spherules (µ)
0.15	1.4
0.31	1.6-1.8
0.63	1.6-1.8
1.25	2.3 - 2.7
2.5	2.0-2.1
5.0	0.7-0.9
10	0.4 - 0.6
20	Almost no spherules

The problem of thermal production of a hot "native protein" is seen not to be a real one when it is recognized that thermal denaturation of protein is obviated if the water content of the system is at a value low enough to represent only tightly bound water (4). It is also pertinent that proteins are protected from heat coagulation by other materials, notably nucleic acids (5), and that regardless of conceptual difficulties some bacteria and algae thrive in hot pools (6).

Against this background, it may be understood that coagulation might not occur in a hypohydrous prebiological system. Denaturation, however, is indicated for the hot proteinaceous material when it is, or was, first exposed to an aqueous environment. This presumed effect was recognized as possibly being of benefit in the primordial process if prebiochemical globules containing archeoprotein were to be denatured at their surfaces. One could visualize by this working hypothesis the origin of cell membranes (1).

The scores of experiments which have been performed in testing this concept have given with proteinoid (2) and hot water in the proper proportions huge numbers (typically 106 to 108) of spherules of uniform microscopic size (Fig. 1). These are usually 1.5 to 3  $\mu$  in diameter, the size varying with the conditions in the particular experiment. The spherules shown in Fig. 1 were prepared from 15 mg of proteinoid (2) and 3.0 ml of hot sea water with 1 min of boiling, after which the preparation was allowed to cool to room temperature. The celllike entities obtained retain their individual integrity for several weeks; if they are centrifuged at 2000 rev/min for 5 min and the supernatant liquid is removed, the spherules retain their shape and integrity.

Tendencies to aggregate, to coalesce, to lose shape, and to form spherules of different or of nonuniform size are influenced by the addition of substances to the hot water employed in the preparation. The spherules formed by adding hot water to the proteinoid (2) freshly formed prior to its washing by water, and ethanol, for instance, do not appear to be as tough as those prepared from washed proteinoid. The effect of one of the many added substances being studied on the size of the spherules is given in Table 1.

The entities obtained bear a relationship to cell models as previously reported (7) and to Oparin's coacervates (8). The mode of generation of the spherules from hot proteinoid and aqueous solutions in a thermal continuum, the properties of the units obtained, and the possible interpretations bearing on the origin of living cells are, however, significantly different.

The exact role, if any, of the somewhat vague concept of denaturation (9)in these studies is being investigated. The already abundant experiments demonstrate that the process described permits an unlimited variety of controlled variations in study of the effects on the spherules. These variations include the syntheses of proteinoid, the conditions of hydration, and the selection of the aqueous solution or suspension. The thermal homopolymer of glycine (10), for instance, does not form spherules. Also, shrinking or swelling can be induced by immersion of spherules in appropriate hypertonic or hypotonic solutions.

The experimental results as a whole are consistent with the total picture of thermal origins in a continuum (1-3). One inference derivable from these results is that spontaneous prebiological processes could have produced such enormous numbers of extensible cell-like membranes as to favor relatively the likelihood that some of these entities would also enclose enough spontaneously generated biochemical apparatus (1, 3) to permit replication in a sterile world (11).

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24 December 1958

# Learning in Rats with

# **Extensive Neocortical Damage**

Abstract. Albino rats with as much as 99 percent damage to the cerebral cortex were trained on a position habit in a simple T-maze. The operated rats were found to learn the problem as efficiently as normal animals even when a 30-second delay was imposed between the response and the food.

Numerous experiments have demonstrated learning in mammals that have been surgically deprived of their neocortex (1). When the conditioning technique is used, the results of these studies indicate that there are only slight differences in the final performance of the conditioned response in comparison to that evoked in normal animals. Few studies exist which have examined the learning ability of decorticate animals in an unrestrained, "free-moving" situation. such as traversing a single-unit T-maze. According to the work of Lashley (2), removal of as much as 80 percent of the neocortex in rats only slightly impairs the mastery of a position habit. The present study showed that rats subjected to extreme neocortical damage not only learn a position habit as readily as normal ones but are equally efficient when a delay of 30 seconds is interposed between the response and the reward.

Twenty-seven experimentally naive adult rats of the Wistar strain were used. Of these, 12 were subjected to varying amounts of cortical damage by the suction method. All operations were performed in one stage with deep ether anesthesia. From 3 to 4 weeks after surgery, all rats were trained to choose their nonpreferred position in a T-maze in order to obtain food. After the conclusion of the experiment the brains were removed from the operated rats, and the lesions were reconstructed on standard diagrams. Brains showing extensive subcortical damage were sectioned and stained.

The alleys of the T-maze were 4 in. wide and 4 in. high. The stem consisted of a start box and a choice chamber,

while each arm was composed of a delay chamber and an end box. These compartments, which were separated by guillotine doors, were 12 in. long. The floor and the top of the T were constructed of Plexiglas, the sides of plywood. Preliminary training involved giving each rat three trials per day in the maze for four consecutive days. The first two trials were "free," while on the third trial the rat was forced to the side it had avoided on the immediately preceding trial. Each end box contained a dish of wet mash from which the rat was allowed to eat for 10 seconds. No experimental delays were interposed during this period.

Determination of the nonpreferred side for each rat was made from the data on the last 2 days of preliminary training. On day 5, each rat was trained positively to the nonpreferred side, where food was present in the end box. No food was present in the end box on the preferred side. Eight control and five operated rats were trained without any delay. That is, as the animal made a choice of the left or right arm, the guillotine door separating the delay chamber from the end box was raised, allowing the animal to enter the end box. All of the remaining rats were delayed 30 seconds in the delay chamber following a correct or incorrect choice, after which the appropriate door was raised, allowing the animal access to the end box. The noncorrection method was used. Six trials were given daily with an intertrial interval of about 3 minutes. After early daily series of trials the rat was returned to its home cage and fed for 45 minutes. Training was continued until the animal made at least 10 correct responses on two consecutive days.

To reduce the operation of differential sensory cues coming from the delay chambers, the following precautions were taken. Visual cues were eliminated by enucleating the eyes of all animals. Olfactory cues were minimized by washing the floor of the maze after each trial and by placing a dish of food mash close to the incorrect end box. Auditory cues were reduced by the background noise of an air conditioner, and tactual cues were decreased by making the left arm identical with the right arm. Thus the development of secondary reinforcement from senses other than proprioceptivekinesthetic was minimum.

For the no-delay groups, the differences between the controls and the operated rats were far from statistically significant. The controls required a mean of 11.4 trials and a mean of 3.7 errors to reach the criterion. The means for the operated animals, on the other hand, were 12.8 trials and 3.9 errors. The average percentage of cortex damaged was 71.5. There was no relationship between lesion size and ease of learning the position habit. Table 1 presents the indi-



Fig. 1. Diagrams of the brains of those rats involved in the 30-second delay problem. Blackened areas indicate complete destruction of cortical surface.