more closely related to human type A strains circulating in the middle 1930's than to other known influenza virus strains, including the virus of swine influenza.

PAUL BROWN
D. CARLETON GAJDUSEK
J. ANTHONY MORRIS
National Institute of Neurological Diseases and *Division of Biologics Standards, National Institutes of Health, Bethesda, Maryland 20014*

References and Notes

1. P. W. Brown, D. C. Gajdusek, J. A. Morris, *Amer. J. Epidemiol.*, in press; P. W. Brown, K. M. Chen, D. C. Gajdusek, J. A. Morris, *ibid.*, in press.
2. F. M. Burnet and E. Clark, *Influenza* (Macmillan, Melbourne, 1942).
3. E. O. Jordan, *Epidemic Influenza* (American Medical Association, Chicago, 1927).
4. T. Fujii, *Endemic Diseases in the Caroline Islands. Collection of Medical Essays 3.* (South Seas Government Office, Tokyo, 1934), cited by E. E. Hunt, Jr., N. R. Kidder, D. H. Schneider, *Hum. Biol.* **26**, 21 (1954).
5. *Catalog of Research Reagents* (Research Reference Reagents Branch, Collaborative Research Program, NIAID, NIH, Bethesda, 1968).
6. Box titrations were performed in which serums were diluted in twofold steps from 1:10 to 1:1280, and in which virus was diluted in twofold steps to include a range of approximately 1 to 32 TCID. The following virus strains were used in tests: A/WS/-/33, A/PR/8/34, A/BH/-/35, A/Hickcox/-/40, A/Cam/-/46, A1/Ann Arbor/1/57, A2/Taiwan/1/64, all isolated from humans; and A/Swine/1976/31, A/Equine-1/Prague/56, and A/Equine-2/Miami/63. Neutralizing antibody was detected in hemadsorption-inhibition tests on primary, rhesus monkey kidney cell cultures supplied by the Tissue Culture Section of the Division of Biologics Standards, NIH. All serums were simultaneously tested against a given virus, and a triplicate virus titration was included in each test run. Antibody titers are expressed as the reciprocal of the highest serum dilution, before addition of other test reagents, at which hemadsorption was completely inhibited.
7. C. H. Andrewes, P. P. Laidlaw, W. Smith, *Brit. J. Exp. Pathol.* **16**, 566 (1935); T. Francis, Jr., and T. P. Magill, *J. Exp. Med.* **63**, 655 (1936); M. R. Hilleman, F. J. Flatley, S. A. Anderson, M. L. Luecking, D. J. Levinson, *N. Engl. J. Med.* **258**, 969 (1958).
8. F. M. Davenport, A. V. Hennessy, T. Francis, Jr., *J. Exp. Med.* **98**, 641 (1953); R. E. Shope, *ibid.* **63**, 669 (1936).
9. T. Francis, Jr., and R. E. Shope, *ibid.*, p. 645 (1936).
10. F. M. Burnet and D. Lush, *Brit. J. Exp. Pathol.* **19**, 17 (1938).
11. T. Francis, Jr., *Ann. Intern. Med.* **39**, 203 (1953).
12. Insufficient virus was present at a level of one or two TCID for antibody titers to be accurately determined; and the four TCID virus level, although most sensitive in distinguishing differences in the amount of antibody to the several reacting strains, was in some cases still of marginal reproducibility. Above four TCID of virus, antibody titers were reproducible; but at progressively higher virus dosage levels, titers were increasingly depressed to a point that differences in the amount of antibody to reacting strains of virus tended to be abolished.
13. Statistical analysis was performed by Dr. P. Shaughnessy, who used several parametric and nonparametric tests which showed uniform agreement on the following results: $P > .05$ for SW versus WS and PR/8 versus BH; $P < .05$ for SW or WS versus PR/8 or BH, and for SW, WS, PR/8, and BH versus the other six virus strains.
14. We thank C. Shaw and Miss D. Stein for technical assistance.

2 June 1969

Reef Coral from Aldabra: New Mode of Reproduction

Abstract. *A unique mode of asexual reproduction in recently collected specimens of* Goniopora *(Scleractinia) is reported. Skeleton is absent from new polyps; the skelton develops independently of the parent colony as the new polyps themselves increase. The young colonies eventually become detached. The cycle seems to be a response to a sandy habitat, a conclusion reached by analogy with* Fungia *and* Manicina.

The shallow waters of the atoll of Aldabra are generally poor in coral growth (*1*). Areas of densest growth are most often found just inside the lagoon, in the region of the passages through the atoll rim. Current action is generally stronger here, and the better circulation is presumably an important factor favoring coral growth. In one area just inside the lagoon at Passe Gionnet, consisting of scattered coral patches and open sand, there is a patch of coral measuring approximately 20 by 5 m; the patch is composed almost entirely of the coral *Goniopora stokesi* Edwards and Haime (a new species record for Aldabra and the region). This coral occurs as small hemispherical colonies, between 2 and 8 cm in size, which lie unattached on a soft substrate consisting of a clean, white, detrital calcareous sand.

Where it grows *Goniopora* covers about 80 percent of the substrate surface. Small growths of the marine phanerogam *Halophila* and the algae *Caulerpa* and *Halimeda* also occur. The coral patch extends up to the prop roots of a mangrove fringe surrounding a lagoon islet, and for much of the day the corals are shaded by mangrove trees. At low spring tides the area is covered by about 1 m of water (tidal range approximately 2.5 m).

The *Goniopora* polyps are usually expanded during the day (at low water), a habit which is unusual in corals (*2*), but which has been recorded (*2*, *3*) in other species of this genus in other regions. The columns of the polyps may extend by as much as 5 cm from the corallum and are brown-mauve in color. Another feature is that in the retracted state, the polyps are still not withdrawn entirely into the skeletal calice; the columns project about 2 to 3 mm. Even the action of a preservative fails to increase retraction (Fig. 1), and this condition must, therefore, be taken to be normal for these specimens.

By far the most unusual feature, however, is that of small, closely attached, spherical masses of polyps (referred to here as polyp-balls) borne by the principal colonies. In one of our preserved specimens (Fig. 1) measuring 6 cm in diameter, about 25 percent of the surface area is obscured by some 40 polyp-balls. These polyp-balls are of all sizes between 3 mm and 2 cm, and bear anything from 1 to about 30 polyps. Each possesses its own small spherical calcareous corallum (skeleton) (Fig. 2), but surprisingly, there is no rigid connecting tissue between the principal corallum and the polyp-ball coralla; they are held in place by soft tissue alone. Each polyp-ball is derived from an individual polyp. No feature of this kind in corals has ever been described before, or otherwise made known to us (*4*).

Examination of two sets of dried specimens (originally two colonies bearing polyp-balls) and seven preserved specimens (several with polyp-balls), together with the facts of their occurrence, leads to the conclusion that the polyp-balls are part of the life cycle of the species. The attached nature of what are evidently young colonies suggests that they represent a form or phase of asexual reproduction. By an unusual mode of budding, young colonies could develop and grow in the attached polyp-ball state until they are about 2 cm in diameter, after which they detach. They then gradually assume a hemispherical shape with an epithecal base. Development of epitheca results from the partial withdrawal of fleshy tissues which previously surrounded the entire polyp-ball skeleton. Epitheca also serves as a useful means of dis-

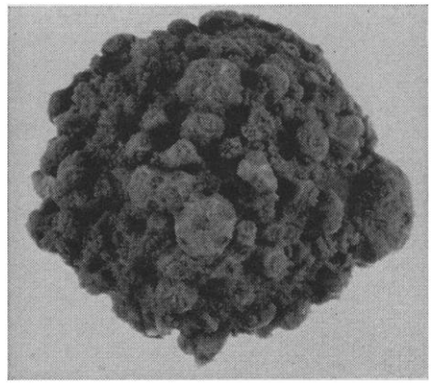

Fig. 1. Preserved colony of *Goniopora stokesi* showing the protuberances of the polyp-balls (× 0.75).

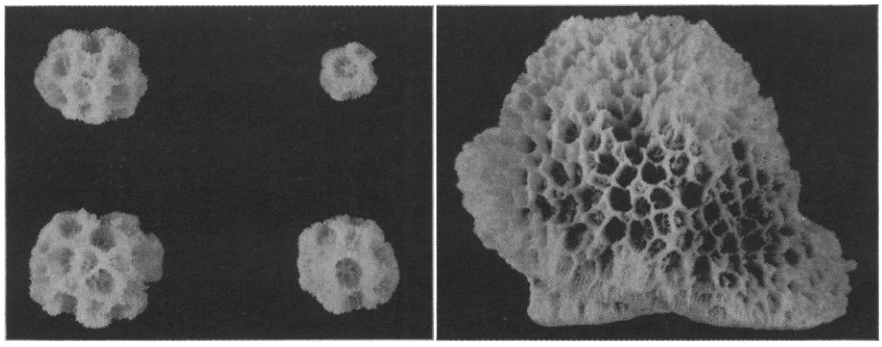

Fig. 2 (left). Dried and cleaned skeletons of polyp-balls at various stages of development, all from the same colony (× 0.9). Fig. 3 (right). Dried, cleaned specimen in side view showing the flat base and slightly irregular hemispherical shape (× 0.75).

tinguishing polyp-balls from small adult colonies in dried specimens. The absence of any attached colonies larger than 2 cm in diameter or of detached colonies smaller than 2 cm in diameter points to this being the critical size. The essential controlling factor of size may be gravity, which would place an increasing strain on the connecting tissues and either cause them to rupture, or induce in them a detachment mechanism.

The principal modes of asexual reproduction in corals are intratentacular, extratentacular, and transverse division (*5*). The latter mode is relatively rare in corals as a group, being almost entirely restricted to members of the Fungiidae. The two former modes may be further subdivided into special cases. Extratentacular budding is produced either by invagination of tissues outside the ring of tentacles, or by the union of marginal filaments of the broader subsidiary mesenteries with a new opening formed outside the tentacular ring. In either case, the skeletal manifestation is virtually the same, that is young calices appear between, rather than within, mature calices. This latter feature is clearly seen in both adult colonies (Fig. 3) and polyp-balls (Fig. 2), the usual mode of increase in *Goniopora* and other Poritid genera being extratentacular (*5*). This suggests that the polyp-ball formation is essentially a development of this method rather than a completely distinct phenomenon. The most problematic feature of these polyp-balls, however, is that of the absence of connecting skeletal tissue; the very long polyps that this species possesses or the inability of the species to retract the polyps completely, or both, may be the reason. In most other corals exhibiting extratentacular budding, the new polyps commence growth below the outermost skeletal surface, that is within the corallum. A corresponding skeletal part must develop to accommodate the new polyp, and the resulting new calice will be an integral part of the parent skeleton. If, however, a new polyp were to form very close to the oral end of the column, within the region never fully withdrawn, adjustment within the corallum would not be necessary. When the young polyp in this latter case began to lay down its own skeleton, this would be held to the parent colony by soft tissues alone. Our preliminary examination suggests that the point of attachment of polyp-balls is often close to the oral end of the column, but because of the preservation of the specimens a precise relationship could not in many cases be established.

Another possible explanation of this condition, although unlikely, is that the polyp-balls result from the settlement and growth of coral planulae upon the adult colonies. This could represent an extreme case of the affinity of many invertebrate larvae, including those of corals, for areas of growth of adult colonies.

A temporary attached phase in coral growth is rare, but, as Yonge (*6*) and Goreau and Goreau (*7*) have shown for the other main examples (*Manicina* and various Fungiid genera), this is associated with the colonization of unstable soft substrates, an environment which is unfavorable to most other corals. Survival is principally due to marked sediment clearing propensities and righting reactions. Although in *Manicina* the method of performing these processes is entirely different from that in the Fungiidae, there is a broad connection between this ability and the large size of the polyps. The fixed stage allows the corals to grow to a sufficient size to cope with these substrate conditions. These combined characteristics give these corals a competitive advantage.

A parallel may be drawn for *G. stokesi*. Colonies are commonly expanded during daytime, lie unattached on a sandy substrate, and pass through an attached phase during youthful stages. The colonies possess large fleshy polyps capable of great extension. Such polyps shed sediment efficiently and might even enable colonies to right themselves. Patches of colonies of this species would correspond to the clones and multiple clones of *Fungia*.

Finally, mention must be made of the systematic significance of these polyp-balls. Although mode of reproduction is usually taken to be an important factor in distinguishing genera and higher scleractinian groups, the corals described here are not thought to represent a new genus because (i) apart from the polyp-balls themselves, they exhibit all the skeletal features typical of the genus *Goniopora*; (ii) as already mentioned, polyp-ball formation accompanies the more usual extratentacular budding typical of *Goniopora* in the same colonies; and (iii) our specimens closely resemble others belonging to the established species *G. stokesi* (*8, 9*). We conclude that this species exhibits a remarkable ability, without parallel in other corals, to modify its mode of asexual reproduction in response to environmental differences, the prime factor for polyp-ball formation being a sandy substrate (or perhaps a particular type of sandy substrate). Polyp-ball reproduction may represent incipient speciation, and its presence or absence in *G. stokesi* could be indicated by subspecies names.

B. R. ROSEN

School of Physics,
University of Newcastle upon Tyne,
Newcastle upon Tyne, England

J. D. TAYLOR

Department of Zoology, British Museum (Natural History), London, England

References and Notes

1. J. C. F. Fryer, *Trans. Linn. Soc. London Ser. 2* **14**, 397 (1911).
2. T. A. Stephenson, *Sci. Rep. Gr. Barrier Reef Exped.* **1**, 389 (1940).
3. C. M. Yonge, *ibid.* **1**, 13 (1930).
4. J. W. Wells, personal communication.
5. ———, in *Treatise on Invertebrate Paleontology: Coelenterata*, R. C. Moore, Ed. (Univ. Kansas Press, Lawrence, 1956).
6. C. M. Yonge, *Pap. Tortugas Lab.* **29**, 185 (1935).
7. T. F. Goreau and N. I. Goreau, *Biol. Bull.* **118**, 419 (1960).
8. H. M. Edwards and J. Haime, *Ann. Sci. Nat. Bot. Biol. Vegetale Ser. 3* **16**, 41 (1851). Original description of *Goniopora stokesi*, but an original specimen was not seen.
9. J. S. Gardiner and P. Waugh, *Sci. Rep. Murray Exped.* **6**, 242 (1939). Specimens in the collection of the British Museum (Natural

History) were examined. None is preserved, but we were able to infer that some had once been polyp-balls because of their small size and lack of epitheca. Some of the more mature specimens, larger in size and with epitheca, showed none of the irregularities seen in our own adult colonies. These probably did not bear polyp-balls. In other respects these corals were all very similar.

10. The corals were observed and collected while one of us (J.D.T.) was engaged in ecological studies on the atoll of Aldabra in the western Indian Ocean (46°24′E, 9°24′S). These investigations are part of the Royal Society Expedition to Aldabra. The specimens which were collected are now in the British Museum (Natural History). We thank J. W. Wells for commenting on the manuscript and G. R. Adams for the photographs.

5 June 1969 ■

Adenosine Triphosphatase Sensitive to DDT in Synapses of Rat Brain

Abstract. *The insecticide DDT selectively inhibits the action of a Na^+,K^+, Mg^{2+}-adenosine triphosphatase found in the nerve ending fraction of the rat brain. As judged by the concentrations of inhibitors that give 50 percent of enzyme inhibition, DDT was approximately 1000 times more toxic than its non-insecticidal analog, 1,1-dichloro-2,2-*bis(p-*chlorophenyl)ethylene. The degrees of inhibition of this enzyme system by various toxic and nontoxic DDT analogs were closely related to a general toxicity in vivo of these compounds. Moreover, the extents of inhibition of this enzyme system by DDT were much higher at low temperatures, an indication of a causal relation between poisoning in vivo by DDT and the inhibition in vitro of the Na^+,K^+,Mg^{2+}-adenosine triphosphatase system.*

The toxicity of DDT may result from its ability to disrupt the transport mechanisms of sodium and potassium ions in the nervous system (*1*). However, the biochemical mechanisms by which DDT causes such disruptions are unknown.

Chlordane and other chlorinated hydrocarbon insecticides partially inhibit the adenosine triphosphatases in rabbit brain (*2*), and also there is an adenosine triphosphatase that is particularly sensitive to DDT in the rat brain (*3*).

There are at least three different groups of adenosine triphosphatases existing in the nervous system: Na^+,K^+, Mg^{2+}-adenosine triphosphatases (*4*), which are particularly sensitive to ouabain; Mg^{2+},Ca^{2+}- or contractile adenosine triphosphatase, which is sensitive to Mersalyl (*5*); and other Mg^{2+}-adenosine triphosphatases (*4*, *6*). The former two enzymes may take part in the mechanisms of ion transport in the nervous system.

In view of the possibility that a causal relation might exist between the enzymatic system involving active ion transport mechanisms and the sites of DDT attack, we have expanded our earlier study (*3*) to partially characterize the DDT-sensitive adenosine triphosphatase and to study the effects of other DDT analogs on the enzyme system.

The enzyme source was the acetone powder preparation of the nerve ending fraction (*3*). The preparations were either suspended in 0.05*M* tris(hydroxymethyl)aminomethane - hydrochloride (tris-HCl) buffer (*p*H 7), or in buffer similar to the one developed by Skou (*7*) (*p*H 7.6), containing 0.25 mole/liter of sucrose, 0.03 mole/liter of imidazole, 1 mmole/liter of ethylenediaminetetraacetate, and 0.1 percent deoxycholic acid. The preparations were homogenized at 0°C for 3 minutes with a small Potter-Elvehjem homogenizer. The homogenate was centrifuged for 30 minutes at 20,000*g*. The precipitate was resuspended in the same medium to contain 0.1 to 0.2 mg/ml of protein. A portion (0.2 ml) of the enzyme preparation was added to 1.6 ml of a standard incubation mixture containing 0.1*M* NaCl, 20 m*M* KCl, and 6 m*M* $MgCl_2$ in 0.03*M* tris-HCl buffer (*p*H 7.6) (*8*) or in 1.6 ml of a standard assay mixture at *p*H 7 as described (*3*). In all cases the system was first incubated at 24°C with Mersalyl (10^{-6} mole/liter, Sigma) for 10 minutes (added with 18 μl of distilled-deionized water). The DDT and its analogs (*9*) were added to the system with 18 μl of ethanol (95 percent); the system was maintained for an additional 10 minutes. To assay the adenosine triphosphatase activity, 1 μmole of adenosine triphosphate (ATP) (disodium salt) was added with 0.2 ml of tris-HCl buffer, and the system was maintained for 30 minutes, normally at 24°C. The total scale of assay procedure for inorganic phosphorus was 15 ml (*10*). The enzyme activity (24°C) of average preparations was in the order of 15 nmole per milligram of protein per minute of ATP hydrolyzed at *p*H 7.6. In all cases, an identical set of tests was made with the same enzyme sources, except that they were first incubated with $10^{-4}M$ ouabain (Nutritional Biochemicals). This portion of the enzyme activity was subtracted from the total activity to estimate the Na^+,K^+,Mg^{2+}-adenosine triphosphatase activity. On average, 37 percent of the total activity of these enzyme preparations was due to the adenosine triphosphatases sensitive to ouabain at *p*H 7.6 and 24°C.

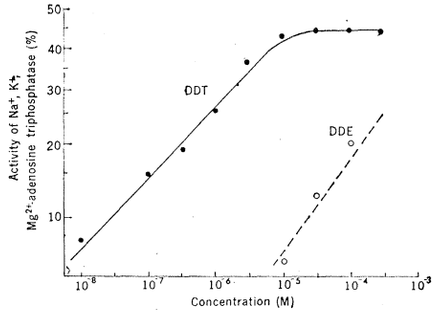

Fig. 1. Relation between the concentration of DDT and DDE and the degrees of inhibition of Na^+,K^+,Mg^{2+}-adenosine triphosphatases. Degrees of inhibition of the enzyme was plotted in probability units to give a linear relation against log-concentration of inhibitors (*16*).

To establish the general identity of the adenosine triphosphatase sensitive to DDT, (i) the Mg^{2+},Ca^{2+}-adenosine triphosphatase was isolated from the rat brain homogenates at *p*H 7 by the method of Puszkin *et al.* (*5*), and (ii) the Na^+,K^+,Mg^{2+}-adenosine triphosphatases of the acetone powder preparation (*3*) from the nerve ending fraction of the rat brain were partially activated by the enzyme solution and the assay buffer mixture of Skou at *p*H 7.6 in the presence of $10^{-6}M$ Mersalyl (*5*). The Mg^{2+},Ca^{2+}-adenosine triphosphatase was more sensitive toward DDE than to DDT (inhibition of $10^{-5}M$ DDT and DDE was 33.7 percent and 47.1 percent, respectively). It cannot, therefore, be the DDT-specific, target enzyme. This was in agreement with the previous observation that $2.5 \times 10^{-4}M$ Mersalyl mainly inhibited Mg^{2+},Ca^{2+}-adenosine triphosphatase so that the remaining enzyme became more sensitive to DDT than to DDE (*3*).

When the Na^+,K^+,Mg^{2+}-adenosine triphosphatase was tested against DDT and DDE (Fig. 1) it became evident that a portion (approximately 50 percent) of this enzyme system was particularly sensitive to DDT but not to DDE. Since the DDT-inhibition curve showed a sharp break at 50 percent, it appeared likely that there were more than one Na^+,K^+,Mg^{2+}-adenosine tri-