mixture containing 100 $TCD_{50}$ of viruses and an equal amount of antiserum containing 20 units of antibody against its homologous virus. Twenty units represented a 20-fold concentration of that dilution giving 50-percent neutralization of 100 $TCD_{50}$ of virus.

This cooperative study has resulted in the differentiation of the 13 antigenically distinct viruses that are listed in Table 1. These viruses—some of which have been referred to in previous literature as "orphan viruses" (*1*) and others as "human enteric viruses" (*2*)—are now classified as the "enteric cytopathogenic human orphan (ECHO) group" (*5*). They share the following properties. (i) They are cytopathogenic for monkey and human cells in culture (*1–4*). All 13 prototype strains were isolated in cultures of monkey kidney cells, which for the strains tested proved to be more susceptible than HeLa cells. (ii) They are not neutralized by pools of the three types of poliomyelitis antiserum. (iii) They are not neutralized by antiserums for Coxsackie viruses that are known to be cytopathogenic in tissue culture, and they fail to induce disease in infant mice. (Animals less than 24 hours old should be used, for they have greater susceptibility.) (iv) They are not related to other groups of viruses recoverable from the alimentary tract (throat or intestine) by inoculation of primate tissue culture, such as herpes simplex, influenza, mumps, measles, varicella, and the ARD (acute respiratory disease) or APC (adenoidal-pharyngeal-conjunctival) group. (v) They are neutralized by human gamma globulin and by individual human serums; this indicates that they infect human beings.

Other studies of the ECHO viruses (more extensive for some than for others) have provided additional information. Complement-fixing antigens have been detected in the culture fluids of a number of viruses that have been tested (*1, 3*). All the viruses tested were ether-resistant. Ultrafiltration (gradocol membrane) measurements indicated sizes for types 1, 2, and 3 between 11 and 17 mμ (*1*). The size of type 10 is reported to be between 60 and 90 mμ (*2*). Plaque morphology of the ECHO viruses studied (types 1, 3, 4, 5, 6, 7, and 9) is sufficiently distinctive, except for type 7 (Garnett strain), to permit differentiation from polio virus plaques (*6*). The plaques of the ECHO viruses mentioned had irregular diffuse boundaries, and healthy cells could be found within the degenerated areas.

Kidney cells of different monkey species vary in their susceptibility to the ECHO viruses. Rhesus (*Macaca mulatta*) and cynomolgus (*M. irus*) cells are susceptible to all 13 types studied. Cells from the South American capuchin (*Cebus capucina*) were found to be resistant to types 1, 2, 3, 7, 8, 9, and 11 (*2, 6*). However, they were susceptible to type 10 (*2*). Cells from the African red grass military monkey (*Erythrocebus patas*), which were resistant to types 1, 2, 3, 4, 5, 6, and 9, were as susceptible as those from the rhesus monkey to the type 7 Garnett strain (*6*).

It is emphasized that this committee is not an authoritative body but rather a group of investigators who, together with others present at the Conference on Orphan Viruses, felt the need for a working approach to a classification of this heterogeneous assembly of viruses that were encountered while poliomyelitis studies were being made. Agreement within the committee was obtained by verification of the specificity of each prototype strain in at least two laboratories.

If requested by other investigators, the committee is prepared to assign numbers to new prototype strains that satisfy the criteria employed for differentiation of the strains listed in Table 1. To avoid unnecessary confusion in the literature, the committee is willing to function as a clearinghouse for characterization of new strains by comparison with established prototypes. In this way the distinction of new prototypes may be hastened.

The antigenic classification presented here is only a preliminary step toward understanding the role that these viruses of the human enteric tract play in disease. If and when any one of the established types is identified as the etiologic agent of a clinically distinct disease, it will be removed from the ECHO group of viruses.

COMMITTEE ON THE ECHO VIRUSES*
*National Foundation for Infantile Paralysis*

Table 1. List of antigenically distinct ECHO viruses.

| Type | Prototype strain | Geographic origin* | Illness in person yielding virus | Reference |
|---|---|---|---|---|
| 1 | Farouk | Egypt | None | (*1*) |
| 2 | Cornelis | Connecticut | Aseptic meningitis | (*1*) |
| 3 | Morrisey | Connecticut | Aseptic meningitis | (*1*) |
| 4 | Pesascek | Connecticut | Aseptic meningitis | (*1*) |
| 5 | Noyce | Maine | Aseptic meningitis | (*1*) |
| 6 | D'Amori | Rhode Island | Aseptic meningitis | (*1*) |
| 7 | Wallace | Ohio | None† | (*2*) |
| 8 | Bryson | Ohio | None | (*2*) |
| 9 | Hill | Ohio | None† | (*2*) |
| 10 | Lang | Ohio | None | (*2*) |
| 11 | Gregory | Ohio | None | (*2*) |
| 12 | Travis 2-85 | Philippine Islands | None | (*3*) |
| 13 | Hamphill 2-188 | Philippine Islands | None | (*3*) |

* Strains belonging to type 1 have also been recovered from the Philippine Islands (*3*) and from India (*1*). Strains belonging to types 8, 9, 10, 11, and 13 have also been isolated from healthy children in Mexico (*2*).

† Strains belonging to types 7 and 9 have been recovered from patients having the aseptic meningitis syndrome in West Virginia (*1*).

### References and Notes

1. J. L. Melnick, *Am. J. Public Health* 44, 571 (1954); *Ann. N.Y. Acad. Sci.* 61, 754 (1955); unpublished data obtained with A. D. Macrae, E. C. Curnen, J. T. Riordan, J. Emmons, S. Melnick, D. Banker, and P. N. Bhatt.
2. M. Ramos-Alvarez and A. B. Sabin, *Proc. Soc. Exptl. Biol. Med.* 87, 655 (1954); *Am. J. Public Health*, in press.
3. W. McD. Hammon and E. H. Ludwig, unpublished data.
4. J. F. Enders, unpublished data.
5. At a moment of conviviality, F. Duran-Reynals, Yale University School of Medicine, first suggested that viruses in search of disease be called orphan viruses.
6. G. D. Hsiung and J. L. Melnick, *Virology*, in press; unpublished data.

* Members of the committee are G. Dalldorf, J. F. Enders, W. McD. Hammon, A. B. Sabin, J. T. Syverton, and J. L. Melnick (chairman). The committee was called together as a result of a recommendation made at the Conference on Orphan Viruses that was held by the National Foundation for Infantile Paralysis in New York, 19–20 May 1955.

22 November 1955

## Calcium Uptake by a Coral

In the course of a series of experiments designed to test the usefulness of radioisotope tracers for the study of calcium deposition by corals, we have obtained some interesting data on the common Atlantic coral *Astrangea danae* (*1*). Pieces of living coral were placed in beakers one-third full of glass beads and containing 40 to 60 ml of sea water that had previously been filtered through No. 4 Whatman filter paper. Stirring and aeration were accomplished by means of jets of water-saturated air impinging on the surface. After the coral polyps became reextended, radioactive calcium-45 in neutralized sea water was added by pipette in amounts sufficient to give about 1000 counts per minute from 0.1-ml aliquots. All samples were spread to uniform area on copper planchets and counted at the same geometry with a 1.8-mg/cm² mica end-window Geiger-Müller tube. The high specific activity of the calcium-45 permitted very small additions of total calcium, never exceeding

Table 1. Calcium equilibrium between coral polyps and sea water. Counts given are means.

| Time (hr) | Counts/min per µl water | Counts/min per mg fresh polyp | Polyps (No.) |
|---|---|---|---|
| 6 | 10.0 | 9.3 | 5 |
| 11 | 10.9 | 9.0 | 6 |
| 23 | 10.0 | 7.0 | 2 |
| 31 | 9.6 | 9.3 | 3 |
| 50 | 9.4 | 8.7 | 4 |

Table 2. The calcium-45 content of sea-water samples after various times of contact with living and dead coral as compared with a sea-water control. All counts given are mean counts/min per µl of water.

| Time (hr) | Living coral* | Dead coral† | Control† |
|---|---|---|---|
| 1 | 10.4 | 3.5 | 3.2 |
| 11 | 10.6 | 3.3 | 3.5 |
| 23 | 11.0 | 3.2 | 3.8 |

* Mean of three experiments.
† Initial calcium-45 activity was one-third that added to the living coral.

0.1 µg/ml of the final solution, compared with about 400-µg/ml concentration in normal sea water.

At intervals, polyps were sampled by snipping off the distal half with fine scissors. These were blotted on filter paper, quickly weighed, dried on planchets, and counted. The counts were compared with those made on the sea water, aliquots being taken immediately before and after sampling the polyps. These data are summarized in Table 1, which covers polyps from six different colonies in two separate experiments.

Evidently isotope exchange equilibrium between the polyp-tissue calcium and outside sea-water calcium is reached in less than 6 hours. The exchangeable calcium of the tissues is apparently maintained at about 88 percent of the calcium concentration of the medium. Actually, this figure should be high, because the uncompensated errors from occluded sea water and from the slight differences in self-absorption in the counted samples both tend in that direction. The findings thus agree fairly well with Hosoi's (*2*) analytic data for tissue calcium in sea anemones (74.5, 72.8 and 66.3 percent of the concentration in medium, respectively, for three species).

In Table 2 the calcium-45 contents of sea-water samples after various times of contact with living coral colonies, or with dead corallum treated in various ways, are compared with blank series that consisted simply of sea-water samples stirred by air, in beakers. The temperature was maintained at 8° to 10°C by circulating sea water. At this temperature *Astrangea* does not appear to grow at all. The withdrawal of calcium-45 by isotopic exchange with the corallum was well below the 5 percent that could have been detected. No effect appeared to be produced by heavy feeding before an experiment, or even during an experiment. Tiny bits of ground fresh fish bone, when these occurred in the food mixture, attained radioactivities per unit volume of 10 to 20 times that of the medium.

The solutions in these experiments contained from 50 to 75 mg of $CaCO_3$, whereas the coral specimens represented 4 to 6 g of $CaCO_3$. It is evident, then, that isotopic exchange between corallum and medium involved only the free surface of the carbonate crystals and may consequently be assumed to have reached equilibrium rather quickly. This was unexpected, in view of the degree to which all our specimens of *Astrangea* have been riddled with boring sponges. It appears to promise that in experiments on rapidly calcifying reef corals, calcium-45 incorporated by exchange will be readily differentiable from that incorporated by active deposition; it also appears to promise that it will thus be possible to establish actual rates of deposition and to ascertain by what physiological factors the rates are controlled. One of us (T.F.G.) is embarking on such a study.

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### References and Notes

1. Contribution No. 786, Woods Hole Oceanographic Institution. This work was supported by Office of Naval Research contract Nonr 1238-(00). Calcium-45 was obtained from Oak Ridge National Laboratory on allocation from the U.S. Atomic Energy Commission. James McInnis of the Marine Biological Laboratory, Woods Hole, Mass., contributed the living coral.
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16 July 1955

## Rapid Method for Cultivation of Acid-Fast Bacilli

An accurate and rapid cultural method for the isolation of acid-fast bacilli is needed. A review of the available literature indicates that there are a number of cultural methods for the isolation of acid-fast bacilli that are less time-consuming than media employed for their routine isolation; but it would appear that each of them has its own particular technical disadvantages.

In the present method, 24 hour specimens of the sputum from patients suspected of having pulmonary tuberculosis were collected. The entire specimen of sputum was homogenized and decontaminated by the addition of an equal amount of 4-percent sodium hydroxide, which contained an indicator. This mixture was shaken vigorously for 10 to 20 minutes and then was incubated at 37.5°C for 30 minutes. Next it was neutralized by adding 25-percent hydrochloric acid dropwise. An equal amount of medium No. 1 (Table 1) was added to the specimen; this mixture was shaken vigorously in an erlenmeyer flask and then was incubated at 37.5°C for 24 hours. The cultural material was then centrifuged at 3000 rev/min for 15 minutes, the supernatant fluid was discarded, and four or five slides were made from the sediment. These were prepared with Ziehl-Neelsen stain and were studied for the presence of acid-fast bacilli.

If acid-fast bacilli were not found, 14 sterile, standard, glass slide preparations were made of the sediment and were air-dried. These were placed in sterile, standard, horizontal glass staining dishes and were covered with medium No. 2 (Table 2). The slides were then incubated at 37.5°C; each day a slide was removed, air-dried, stained by the Ziehl-Neelsen technique, and examined for acid-fast bacilli. If, at the end of 14 days, no acid-fast bacilli had been noted, the test was considered to have been negative. In each instance in developing this method, a sputum sample known to contain acid-fast bacilli was used as a control.

In Table 2 are summarized the results of employing the method and media described here in attempting to cultivate acid-fast bacilli from the sputa of 56

Table 1. Composition of media. Before human plasma, blood-water, and penicillin were added, the *p*H was adjusted to 7.0 and the preparation was sterilized in an autoclave for 15 min at 15-lb pressure.

| Compound | No. 1 | No. 2 |
|---|---|---|
| Lecithin (in alcohol)* | 0.4 g | 0.2 g |
| $Na_2HPO_4 \cdot 12H_2O$ | 18.0 g | 9.0 g |
| $KH_2PO_4$ | 2.0 g | 1.0 g |
| $MgSO_4 \cdot 7H_2O$ | 1.2 g | 0.6 g |
| Sodium citrate | 6.0 g | 3.0 g |
| $NH_4Cl$ | 10.0 g | 5.0 g |
| Iron ammonium citrate | 0.1 g | 0.05 g |
| Asparagine | 6.0 g | 3.0 g |
| Glucose | 10.0 g | 5.0 g |
| Tween 80 (10% soln.) | 4.0 ml | |
| Distilled water | 600.0 ml | 500.0 ml |
| Outdated liquid human plasma | 400.0 ml | |
| Blood-water (50%)† | | 500.0 ml |
| Penicillin | 100,000 units | 50,000 units |

* A 2-percent solution of lecithin in 95-percent alcohol is prepared; 20 ml is placed in medium container and gently evaporated to dryness.
† Blood-water is prepared by mixing equal parts of outdated blood-bank blood and sterile distilled water.