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

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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.

Table 2. Days necessary to detect bacilli. Fifty-six sputa were examined by each cultural method.

Cultural method	Positive sputa (No.)	Avg. No. of days for growth
Medium No. 1 and No. 2	30	2.5
ATS medium	23	31.1

patients suspected of having pulmonary tuberculosis but in whom the sputa were reported as being negative with the Ziehl-Neelsen technique. As parallel and as control cultures, American Trudeau Society medium (ATS) was used as described by Willis and Cummings (*1*).

It is to be noted that 22 of the sputa cultivated were positive in all media employed. Twenty-five sputa were negative in all media used. Eight sputa were positive in medium 1 or 2, and one sputum was positive in ATS medium but not in medium 1 or 2. In the cultures that were positive, growth of acid-fast bacilli was detected in an average of 2.5 days when medium 1 or 2 was employed, while an average of 31.1 days was required for the detection of growth in the ATS medium.

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19 July 1955

Desynaptic Pseudoassociations in Secale montanum

Unusual meiotic chromosome associations were observed at diakinesis in an experimental plant of the grass, *Secale montanum* Guss. The plant was obtained by pollinating an Iranian strain of *S. montanum* with x-rayed pollen of the same strain. Observations were limited, for the plant was accidentally destroyed after a single fixation of pollen mother cell materials had been collected. Nevertheless, it is felt that the observations should be recorded.

The plant proved to be heterozygous for an induced reciprocal translocation, but more striking were the peculiar chromosome associations at diakinesis (Fig. 1*A*). These unusual bivalents and translocation configurations involved homologous chromosomes that lay side by side, paired along their entire lengths through late diakinesis, yet connected only at their very ends. The connections, which were simply strands of matrix rather than true chiasmata, were always terminal and connected only corresponding regions of the homologous chromosomes. As is shown in this report, these matrical strands were not effective in maintaining the associations in metaphase I.

Similar meiotic configurations have been designated as "quasibivalents" (*1*), "pseudobivalents" (*2*), and as "*s-s* associations" (*3*). In terminology, I follow Walters, who uses the original term *pseudobivalent* to describe ". . . configurations which have a bivalent-like appearance, but which are not formed by chiasmata" (*2*). However, a more general term, *pseudoassociation,* must be proposed to include the translocation configurations recorded here. Under this terminology, matrical strands that connect the chromosomes are called "pseudochiasmata."

Individual pollen mother cells exhibited both normal and pseudoassociations. An estimated 80 percent of the diakinesis cells contained one or more pseudoassociations. Only six diakinesis cells could be completely analyzed, however. Of the six cells, two showed $5_{II_n} + 1_{IV_n}$, two showed $1_{II_n} + 4_{II_p} + 1_{IV_p}$ (Fig. 1*A*), and two cells showed $5_{II_p} + 1_{IV_n}$ (n is normal association and p is pseudoassociation). No univalents were observed at diakinesis, but one or more was found in 94 percent of the first metaphase cells. Apparently desynapsis of the diakinesis pseudoassociations was the source of metaphase I univalents. At metaphase I, the univalents moved to the poles ahead of chromosomes of normal configurations (Fig. 1*B*). Early movement of univalents toward the poles was indicated by the fact that only four lagging chromosomes were seen in 83 anaphase I cells, although first metaphase cells contained an average of 3.7 univalents per cell. About 20 percent of the anaphase I cells showed unequal chromosome distribution. Further stages of meiosis appeared rather normal. Only two out of 54 anaphase II cells contained laggards, and only 7.8 percent of the microspores contained micronuclei.

That the pseudochiasmata were, indeed, matrix strands rather than true chiasmata was suggested, not only by their appearance, but also by the following considerations. Desynapsis showed the terminal connections to be ineffective in maintaining pseudoassociations through the first metaphase of meiosis. If the connections were true chiasmata, it would be difficult to explain their consistent terminal location. It is unlikely that chiasmata would originate only at the chromosome ends. Neither could the terminal position of the strands be explained as products of terminalization, for the chromosomes did not open out as do normal bivalents.

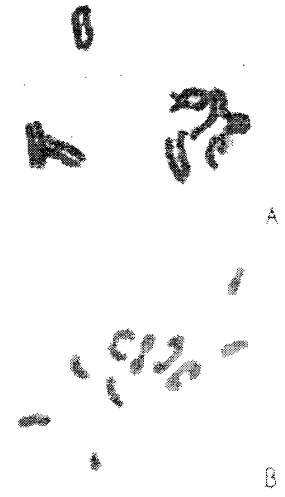

Fig. 1. (*A*) Diakinesis, one normal bivalent, four pseudobivalents, and one pseudotranslocation association of four chromosomes; (*B*) Metaphase I, four bivalents and six univalents.

The pseudoassociations showed interesting deviations from the usual meiotic chromosome behavior. Homologous chromosomes ordinarily synapse along their entire lengths in zygotene and pachytene. In diplotene and diakinesis they separate along most of their lengths but remain connected at chiasmata. Resulting configurations have led to the conclusion that homologs repel each other after pachytene and that they are held together only by chiasmata thereafter (*4*). In diakinesis, the chromosomes of pseudoassociations remained paired along their entire lengths and gave no suggestion of repulsion. Furthermore, the unusual side-by-side association was maintained through late diakinesis without benefit of chiasmata. The terminal strands of matrix could not explain the side-by-side association, for strands were present only at the distal ends of the chromosome arms. Apparently the chromosomes remained paired only through some sort of mutual attraction.

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15 July 1955

New Type of APC Virus from Epidemic Keratoconjunctivitis

Epidemic keratoconjunctivitis (EKC) is an eye infection with sharply defined clinical characteristics, especially the development of round subepithelial corneal