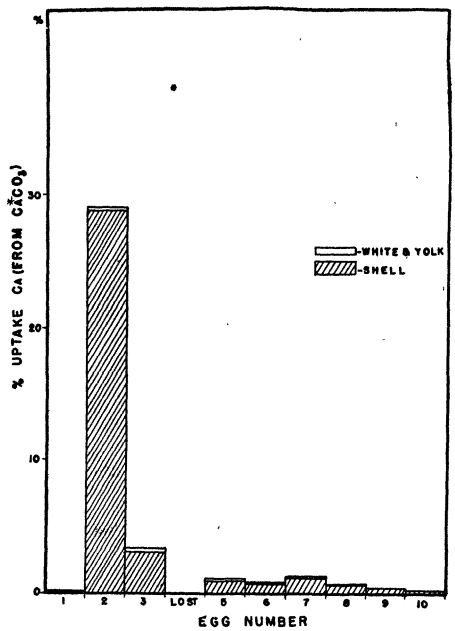

FIG. 1. Percentage of Ca^{45}, fed as a single dose of radioactive $CaCO_3$, appearing in successive eggs.

of interest, since it is in marked contrast to those for the white and the shell but is in good agreement with that previously reported for phosphorus uptake (*3*). A large portion (50%) of the calcium was excreted within 48 hr.

Another bird ("B") was then given a daily feed containing 1.7 g of active $CaCO_3$ for 10 consecutive days (multiple feeding trial). The results of this trial (Fig. 3) indicate that the percentage uptake of calcium from a given source (in this case active $CaCO_3$) became relatively constant in about 8 days after the first feeding.

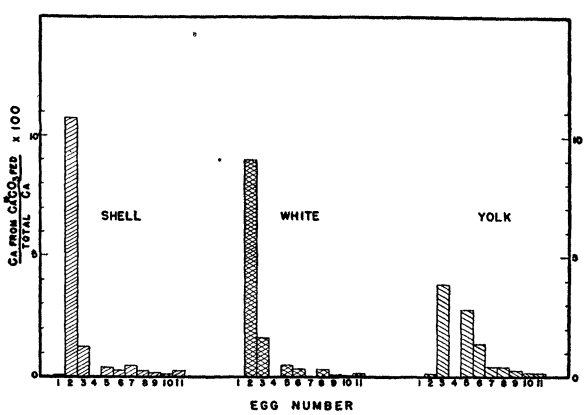

FIG. 2. Percentage of calcium that came from the active $CaCO_3$, appearing in shell, white, and yolk of successive eggs.

This is in striking contrast to the 15 days for P^{32} (*3*) but is still in excellent agreement with theory. Since the calcium fed in a given day appears in a number of eggs, it follows that the calcium in any given egg comes from the calcium fed on many different days. Thus, the recovery of calcium (expressed as a percentage of the daily calcium fed) in successive eggs in a multiple feeding experiment is a summation of the recoveries in successive single feeding experiments (see Fig. 3, hen "A").

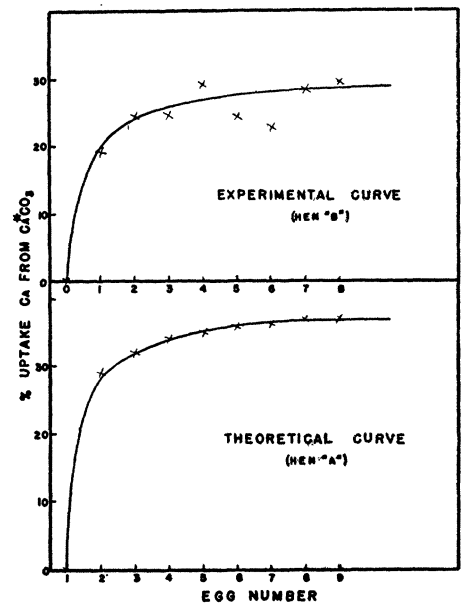

FIG. 3. Percentage of daily feed of calcium appearing in successive eggs in multiple feeding trial; theoretical curve (hen "A"), experimental curve (hen "B").

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Serologic Relationships of Mumps and Newcastle Disease

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The presence of neutralizing and antihemagglutinating factors against Newcastle disease virus (NDV) in half of patients convalescing from mumps suggests a possible relationship between the two viral agents. These cross-reacting factors were first observed in the course of an outbreak of mild meningo-encephalitis, suggestive of Newcastle disease because the epidemiologic and clinical findings fitted descriptions by Howitt *et al.* (*4*) of a disease in man which led to the formation of NDV neutralizing antibodies. Paired sera from cases of mild meningo-encephalitis showed little or no rise of neutralizing capacity against NDV, but a few pairs of mumps sera included as controls exhibited a sharp rise against this virus. Accordingly mumps sera were further investigated. Neutralization tests and calculation of neutralization indices were made by methods similar to those described by Howitt *et al.* (*4*), with few modifications. The California strain

No. 11,914 of NDV was employed throughout. Twenty-two mumps patients were studied, along with 23 control patients, 17 from the outbreak of mild meningo-encephalitis, and 6 having a clinical diagnosis of nonparalytic poliomyelitis. Thirteen of the mumps patients developed neutralization indices over 250, ten having indices of 1,000 and above, whereas only three of the sera from control patients showed neutralization indices over 250 and none went over 800. Antihemagglutination tests with mumps sera against NDV likewise demonstrated serologic relationships. Seven of 20 pairs of heat-inactivated mumps sera showed 4-to-64-fold rise of titer between the acute and convalescent phases. Four others showed titers of 1: 64–1: 256 in convalescent phase sera, titers well above those encountered in 20 pairs of sera from the control group of patients, none of which showed a rise of antihemagglutinating capacity against NDV.

The diagnosis of mumps in the patients studied has been considered elsewhere (*6, 9*). In addition to confirmatory epidemiologic and clinical findings, all of the patients showed evidence of recent mumps infection by complement fixation or by antihemagglutination tests, using the Enders strain of mumps virus. Mumps virus was isolated from the saliva or spinal fluid of 11 patients.

The positive results obtained in two types of serological tests against NDV in the sera of patients experiencing infection with mumps virus suggests that a diagnosis of Newcastle disease in humans should be made with caution, especially in the absence of virus isolation. Only five cases of human infection with NDV (*1, 2, 5*), all having mild conjunctivitis, have been known to be so confirmed. The presence of neutralizing and antihemagglutinating factors against NDV in convalescent phase mumps sera is difficult to interpret. The reactions may be due to nonspecific serum factors arising as result of infection rather than to specific antibodies. If the factors are actually antibodies, their presence would support the hypothesis that NDV and mumps virus are closely related. Burnet (*3*) first presented evidence for this in his work on receptor gradients. Kilham (*7*) has recently shown that NDV has a hemolytic activity closely resembling that demonstrated by Morgan, Enders, and Wagley (*8*) for the mumps virus. A more complete presentation of the data in this paper is in course of preparation. Further studies are needed to elucidate the full meaning of serologic and other relationships which appear to exist between NDV and mumps virus.

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Comments and Communications

Method for Isolation of *Actinomyces israeli* from Dento-Bacterial Plaque[1]

The dento-bacterial plaque is a minute mass of salivary debris and bacterial cells firmly attached to inaccessible tooth surfaces. The association of the plaque with the initial lesion of dental caries has been firmly established (Dietz, F. H. *J. D. Res.,* 1943, 22, 423; Stephan, R. M. *J.A.D.A.,* 1940, 27, 218). A microsectional study (Ennever, J., Robinson, H. B. G., and Kitchin, P. C. *J. D. Res.,* 1948, 27, 599) of the bacterial plaque has shown that bizarre, elongated, apparently branched, Gram-positive rods make up a large portion of the plaque stroma. As a result of this morphologic suggestion, a study was undertaken to determine whether *Actinomyces israeli* is a consistent component of the plaque flora.

After repeated trials it became apparent that uniform recovery of *A. israeli* from plaque material was not possible by the method of Rosebury, Epps, and Clark (*J. Int. Dis.,* 1944, 34, 131), even though their method affords an excellent means of cultivating the organisms from gingival scrapings, actinomycotic pus and other contaminated sources. It was felt that the sporadic isolations of *A. israeli* from the plaque centered about the physical difficulty of inoculating the culture medium. The following modifications of the Rosebury, Epps, and Clark method were instituted and have given consistently successful isolation of *A. israeli* from the dento-bacterial plaque.

The plaque mass, grown in the mouth on a tooth-bearing removable appliance, was removed with a sterile, hooked blade and placed in a micromortar containing 0.05 ml of autoclaved saline with Triton A-20 at 1: 200. A coarse suspension was prepared by trituration with a small, sterile glass pestle for several minutes. One visible granule was transferred by means of a capillary pipette to brain-heart infusion agar, streaked, and the preparation incubated anaerobically in an atmosphere of 5% CO_2 for six days.

Fifteen attempts to isolate *A. israeli* from this source have been uniformly successful. Care, however, must be exercised in seeding the visible granule. It is imperative that only a small quantity of the saline diluent accompany the granule. If too much liquid is introduced on the

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