immunoelectrophoresis with antiserums prepared against MUP from BALB/cAnN mice of both sexes. Extracts of both male liver and urinary bladder showed strong precipitin bands with the mobility of MUP, whereas extracts of male heart, lymph nodes, brain, spleen, thymus, lung, pancreas, and kidney, as well as extracts of female mouse organs, gave weak or negative reactions. Extracts of salivary glands of both sexes formed several arcs of varying intensity, mostly in the α-globulin region, and each extract showed some evidence of sex specificity.

The site of synthesis of MUP was investigated by the method of Hochwald *et al.* (*10*). Minced tissue from various organs of individual BALB/cAnN mice was incubated in roller tubes in a medium containing carbon 14–labeled lysine and isoleucine. A portion of dialyzed, concentrated culture fluid was mixed with sufficient protein (concentrated nondialyzable fraction from BALB/cAnN female urine) to produce clear precipitin arcs on microimmunoelectrophoresis at *p*H 8.6. After immunoelectrophoresis on microscope slides, autoradiographs were made by washing and drying the preparations and then applying film strips (*11*) to the dried agar surface for 2 weeks. Of the tissues studied (liver, kidney, urinary bladder, spleen, femoral bone marrow, thymus, mesenteric lymph node, pancreas, and salivary gland), only liver showed labeling of MUP; labeling was more intense with male liver than with female liver (Fig. 1). In the livers from female mice that had received testosterone subcutaneously (three doses of 10 mg of testosterone propionate injected at 48-hour intervals) there was an intermediate degree of labeling (Fig. 1).

These experiments demonstrate the synthesis of MUP by the liver, from which it is evidently released to the plasma and readily excreted in the urine; furthermore they furnish direct evidence of the suitability of the MUP system for study of hormonal control of the synthesis of a specific protein.

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Double Mating: Its Use To Study Heritable Factors in Dental Caries

Abstract. *When Osborne-Mendel female rats (white) were mated with both an Osborne-Mendel and an NIH Black rat male during the same breeding period, litters were born which contained both Osborne-Mendel (white) and crossbred (grey to black) offspring. The Osborne-Mendel and crossbred animals developed widely different levels of caries activity even though they were exposed to identical environmental conditions during the intrauterine, preweaning, and experimental periods. These findings are indicative of a strong heritable influence on the development of dental caries.*

The results of a recent study have shown that dental caries was appreciably more active in Osborne-Mendel (O-M) than in NIH Black rats (BR), and that the crossbreeds (O-M × BR) developed only slightly more activity than the BR's (*1*). These were noninbred "strains" of rats, and the results were based on comparisons between separate litters. Since variations in the oral and alimentary canal flora of the mother influence caries activity of her offspring (*2, 3*), it was conceivable that the results were due to some fluctuations in the maternal oral-intestinal flora.

Double mating, which had been used to study genetic factors in hamsters (*4*), provided an opportunity to compare both O-M and crossbred rats from the same litter. This was accomplished by caging an O-M female (white) with both an O-M and a BR male during the mating period. By using a white mother, positive identification of paternity could be determined, since the crossbred offspring are always dark colored. Twelve litters were born which contained both O-M (white) and crossbred (grey or black) offspring, and which varied in size from 4 to 15 animals and from 1 to 11 animals of a given color per litter.

When the animals were weaned at 21 days of age, two or three littermates were caged together in groups as follows: O-M's only, O-M's and crossbreeds together, and crossbreeds only. A total of 69 O-M and 60 crossbred animals were subjected to an 84-day caries test regimen as in an earlier study (*1*).

The results give very striking evidence that the difference between the dental caries experience of the two genetically different groups was the result of hereditary factors. The Osborne-Mendel animals developed significantly higher levels of caries activity on every basis of comparison than their crossbred littermates with which they were caged during the test period. The O-M's (35) averaged 10.8 carious teeth, 32.7 carious lesions, and 53.5 carious areas, while the crossbreeds (30) averaged only 9.2, 19.0, and 30.1, respectively. Double mating provided a unique situation by which genetically different animals were exposed to identical environmental conditions from conception to death. These results give more convincing evidence that the development of caries in these two "strains" of rats is affected by some hereditary influence than is given by the results of an earlier study in which comparable results were obtained with animals born in separate litters (*1*). It was particularly useful in this study that both the color of the BR and its characteristic response to the caries test challenge were dominant in the crossbred offspring. Identification of double-mated litters with BR mothers could not be made on the basis of color and would be of doubtful value, since the level of caries activity of crossbred rats with BR mothers was not significantly different from that of BR's, even when born in separate litters (*1*).

An environmental effect on caries activity was evident from the different levels of activity seen in animals of a given genetic background when they were caged with their own kind only or in mixed groups during the caries test period. The O-M animals caged sep-

arately (34) developed higher levels of caries activity than those caged with crossbreeds (35): carious teeth, 11.1 and 10.8; carious lesions, 36.9 and 32.7; carious areas, 62.3 and 53.5, respectively. Conversely, the crossbred animals caged separately (30) developed lower levels of activity than those in mixed groups (30): carious teeth, 8.5 and 9.2; carious lesions, 17.8 and 19.0; and carious areas, 28.4 and 30.1, respectively. These results suggest that the O-M's are more capable than the crossbreeds of supporting the "caries conducive flora" provided by the mother. Even though every animal was exposed to the same microbiota during the nursing period, that which was retained during the test period was only that which the animals within a given cage could support on the diet provided. Additional studies are under way to further identify heritable factors which may contribute to these results.

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25 June 1965

Hemadsorption-Negative Plaque Test: New Assay for Rubella Virus Revealing a Unique Interference

Abstract. *A simple and rapid plaque procedure has been developed for detecting and accurately assaying rubella virus in a noncytopathic virus–cell relationship. Plaque-formation is based on the development, in individual cells infected with rubella virus, of a unique type of intrinsic interference to infection with Newcastle disease virus. Rubella virus–infected cells challenged with Newcastle disease virus and tested for hemadsorption 15 hours later stand out as hemadsorption-negative areas. Individual living cells infected with rubella virus can be resolved under conditions allowing standard cloning procedures. In principle, the hemadsorption-negative plaque test can be used to search for a new class of noncytopathic, nonhemadsorbing viruses—those that induce an intrinsic interference to infection by any hemadsorbing virus.*

Research on the biochemistry, genetics, and teratogenic effects of rubella virus has been seriously limited by the lack of a test to score individual infectious particles and single infected living cells. We have devised a simple plaque assay for rubella virus as a noncytopathic virus under conditions that do not adversely affect viability of individual infected cells and, hence, allow isolation of virus or cell by standard cloning techniques. Detection of rubella virus depends on the development in infected cells of a unique interference to superinfection by Newcastle disease virus (NDV), a hemadsorbing, cytopathic myxovirus (*1*). In the test, rubella virus–infected cells (NDV-resistant) stand out as hemadsorption-negative plaques against a background of hemadsorption-positive (NDV-susceptible) cells.

The hemadsorption-negative plaque test is carried out as follows. Susceptible host cells (*2*) are grown to confluency in 60-mm petri dishes (Falcon Plastics No. 3002) containing 5 ml of attachment solution (*3*) supplemented with 3 percent fetal bovine serum (standard growth medium). This medium is removed at the time of infection, and rubella virus (*4*) contained in 0.3 ml of attachment solution is adsorbed for 60 to 120 minutes at 35°C. The plates are then rinsed once, flooded with 5 ml of standard growth medium, and incubated undisturbed at 35°C in an atmosphere of continuously flowing CO_2 and air. To reveal the rubella plaque areas at the end of the incubation period, usually 3 to 4 days, medium is removed from the infected monolayers and NDV is added at an attachment multiplicity, that is, a virus: cell ratio of 10 to 20 plaque-forming or hemadsorption-producing particles (*5*). Routinely, 0.1 ml of NDV stock, harvested as allantoic fluid 48 hours after infection of 9-day-old chick eggs, and 0.2 ml of attachment solution are applied, per plate, for 60 minutes at 37°C. Unattached virus is then removed, and the monolayer is rinsed once with 2 ml of attachment solution. To preclude spurious binding of red blood cells due to the presence of residual input virus on the surface of otherwise hemadsorption-negative (HAD^-) cells, the rinsed monolayer is exposed to 0.5 ml of antiserum to NDV at a concentration which neutralizes over 99 percent of the plaque-forming particle activity of NDV stock preparations within 15 minutes at 37°C, usually a 1:50 dilution of serum from immunized rabbits (*6*). Antiserum is removed after 20 minutes; the monolayer is washed once with 2 ml of attachment solution, flooded with 5 ml of this solution, and incubated for 15 ± 2 hours at 37°C to develop the hemadsorption-positive (HAD^+) state in all cells susceptible to NDV at the time of challenge. The single-cell hemadsorption test is completed as described (*5*), and care is taken to minimize elution of red blood cells and loss due to mechanical trauma in the washing procedure. Briefly, the medium is removed and replaced with 3 ml of a suspension of bovine erythrocytes (6×10^7/ml) in phosphate-buffered saline (*p*H 7). Red blood cell adsorption is carried out for 20 minutes at 4°C (cold room), and the plates are rinsed gently eight to ten times with copious amounts of cold, phosphate-buffered saline (*p*H 6) to remove unattached erythrocytes. Several milliliters of cold saline are left on the plate after the final rinsing. The hemadsorption-negative plaques stand out as dark areas when examined by a dark field–like illumination obtained by viewing the underside of plates held up to a light source from the ceiling. These plaques are stable in cold saline (*p*H 6) for 1 day, or they may be fixed with OsO_4 (*7*) and sealed in Gurr's water-mounting solution for a permanent record (Fig. 1).

As early as 20 hours after infection with rubella virus, challenge with NDV and application of the single-cell hemadsorption test reveal discrete foci which contain one or a few hemadsorption-negative cells. Figure 2 illustrates an area from a plate challenged with NDV 20 hours after infection with rubella virus. These small, nonhemadsorbing areas spread to encompass scores of cells as seen in Fig. 3, and in early infection often contain a few individual hemadsorbing cells that are also illustrated. Further incubation eventually