was readily detectable with the experimental lidars used, even in daylight, provided the angle of view was high—that is, with minimum path attenuation. Echoes were also returned on occasion by what appeared to be clear blue sky, but at heights at which patches of cirrostratus had been visible a short time earlier, or were present elsewhere in the sky.

Lidar has great potential for making cloud observations. Although the systems used here were prototypes, their performance was reliable and straightforward, and I consider that the promise of lidar in this role may be fairly readily realized. Although single-shot observations have great value, higher pulse-repetition frequencies are most desirable. For all purposes, and particularly for operational applications in meteorology and aviation, immediately available recorded displays are needed, with the capability of developing range : height or height : time sections.

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3. The experimental lidar program was initiated and directed by M. G. H. Ligda. The equipment, which includes components lent by Lear Siegler Corp., was developed under the direction of R. C. Honey. F. G. Fernald, A. Smith, and G. Davis helped with the observations and data reduction. Work sponsored by Lear Siegler Corp. and ONR.

27 May 1965

Major Urinary Protein Complex of Normal Mice: Origin

Abstract. *Mouse serum contains protein having the same charge density and molecular size as the major urinary protein complex of mice. Mouse liver (but not eight other tissues examined) incorporated amino acids labeled with carbon-14 into the complex in vitro. The degree of incorporation was greater in livers from males than from females, and was intermediate in livers from females treated with testosterone.*

The family of proteins excreted in the urine of normal mice, designated the major urinary protein (MUP) complex, is of particular interest since both the phenotype (*1*) and the quantity excreted (*2*) are under the influence of hormones. The protein complex has a weight-averaged molecular weight of 17,800 (*3*) and migrates in moving-boundary (*3*), paper (*2, 3*), or agar-gel electrophoresis as a prealbumin—that is, it migrates faster than albumin when *p*H is above the isoelectric point of albumin. Furthermore, the complex exhibits electrophoretic heterogeneity: electrophoresis in agar gel at *p*H 5.5 yields three components. A phenotypic classification of inbred strains of mice has been made on the basis of mobilities and relative amounts of these components (*1*). Most strains showed phenotypic differences in MUP complex between males and females; moreover, pooled urine from female mice that had been treated with testosterone showed the electrophoretic pattern characteristic of males of the same strain, while urine from castrated males showed the same pattern as that from normal females (*1*).

Immunochemical studies of the origin of MUP have yielded conflicting results: namely, that it is present in serum and probably originates in the liver (*4*) and that it is not present in serum and originates in the kidney (*5*). We now report immunochemical experiments of which the results are direct evidence that MUP is synthesized by the liver.

Urine from BALB/cAnN male mice was dialyzed exhaustively against distilled water and freeze-dried. The nondialyzable fraction was dissolved in 0.9 percent NaCl at a concentration of 0.02 percent. This solution and samples of BALB/cAnN male serum (undiluted or diluted 1 : 2) were placed in alternate wells of an Ouchterlony plate and allowed to react with rabbit antiserum prepared against BALB/cAnN male urinary protein. A single precipitin line formed and showed a reaction of identity.

These same samples and the same antiserum were used in an immunoelectrophoresis experiment in barbital buffer at *p*H 8.2 (*6*). Precipitin arcs formed by urine and serum occupied the same position, anodal to the arc formed by serum albumin with rabbit antiserum prepared against BALB/cAnN male serum.

Our results, in complete agreement with those of Rümke and Thung (*4*), demonstrate that mouse-serum protein immunochemically similar to the MUP complex also exhibits the same mobility. Further evidence of identity came from the following experiment. The urine and serum samples described and the antiserum prepared against urinary protein were allowed to diffuse in agar from troughs placed normal to one another (*7*). Straight precipitin lines formed. The angle between the precipitin line and the mouse-serum trough averaged 58.2 ± 1.3 deg; that between the precipitin line and the urinary-protein trough, 60.3 ± 0.5 deg (*8*). These results indicate no difference in the diffusion coefficients (*7*).

Thus the MUP present in serum has not only the same charge density but also the same molecular size as excreted MUP. Demonstration of the presence of MUP in serum confirms an earlier suggestion (*3*); however, the work of Rümke and Thung (*4*) and other observations (*9*) show that MUP constitutes only a small portion of the mouse-serum prealbumin.

Organ extracts, prepared by grinding fresh tissue in a Potter-Elvehjem homogenizer with 9 volumes of Locke's solution, were examined by immunodiffusion in Ouchterlony plates and by

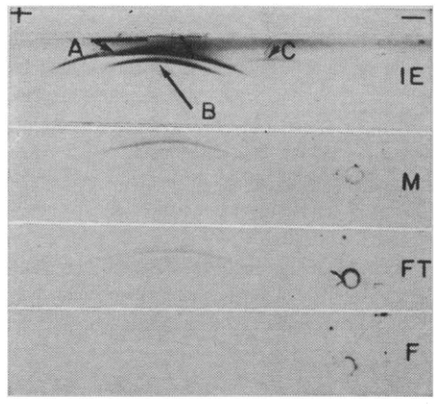

Fig. 1. Incorporation of labeled amino acids into MUP. Arc *A* shows different intensity of labeling, depending on the source of the liver; whether arcs *B* and *C* represent portions of MUP itself is not known, but there was little difference in their degrees of labeling. *IE*, immunoelectrophoretic pattern; *M*, *FT*, and *F*, autoradiographs of culture fluid of liver from individual male, testosterone-treated female, and normal female mice, respectively.

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-