experiments (*2*) similar to those reported by Crozier and have observed effects that can cause the discrepancies noted.

The isopiestic method (*3*) involves the equilibration, in an enclosed space, of solutions of nonvolatile solutes in the same solvent. At equilibrium, the activity of the solvent is the same in all the solutions. The use, at a fixed temperature, of a large quantity of a suitable saturated solution in contact with excess solute provides a fixed, known vapor pressure within the enclosed space. If droplets of a second solution are in equilibrium with this vapor, their composition is determined by their temperature and by the vapor pressure. Crozier's microscopic droplets of sodium chloride were deposited on Dri-Film or Teflon slides and exposed in a closed box to atmospheres of several different water vapor pressures. Observed drop diameters were converted to drop volumes, assuming hemispherical particles. The relationships between these volumes were considerably different from those predicted from ICT vapor pressure and density data (Fig. 1).

Crozier assumed that his droplets were at the same temperature as the air and the saturated solutions within his small observation box. In similar studies, however, we found that a significant temperature differential existed between the droplets and the air to which they were exposed. We passed air that was conditioned to various water contents and maintained at 25.0°C through an observation cell containing upper and lower windows. A microscope lamp, equipped with a water cell, illuminated the substage mirror of a microscope, upon whose stage the observation cell was placed. Droplets of NaCl solution (in the same size range as those of Crozier) were deposited upon a glass cover-slip about 10^{-2} cm thick, and the cover slip was inverted to form the upper window of the observation cell. An oil immersion lens was used to observe the particles through the glass, and a fine thermocouple was placed in the oil in contact with the glass surface.

The thermal resistance of the glass being relatively slight, the temperature of the particles could be taken as nearly equal to that of the thermocouple. Other sensitive thermocouples were employed to measure the air temperature and humidity. The microscope and all the apparatus were installed in a constant-temperature room at 25°C. Under these circumstances, it was observed that the cover-slip temperature started to rise as soon as the (water-cell equipped) microscope illuminator was turned on, and that it continued to rise until there was more than 0.5°C difference in temperature between glass and air.

In previous work, even larger variations in the cover-slip temperature had been noticed when the apparatus was located in a room where the temperature was not closely controlled. On warm days, the junction in the immersion oil indicated temperatures considerably higher than 25°C, despite the large volume (about 8000 cm^3) of 25.0°C air passing through the small cell (about 25 cm^3) each minute.

As a result, NaCl crystals on the cover-slip sometimes did not pick up moisture until the relative humidity of the 25.0°C air passing through the cell was raised to more than 80 percent, whereas 75.5 percent should initiate particle growth. It seemed reasonable to believe that heat was being conducted to the oil and the cover-slip by the microscope.

It is apparent that, when relatively large plane surfaces are used for the support of droplets, heat transfer from surface to droplet can introduce serious error. As a matter of fact, the resistance to heat transfer that is offered by the air near the particles is so great that the thermal resistances of the droplets themselves are of negligible effect. The particles are essentially at the same temperature as the surface that supports them, even though the nearby air may be warmer or cooler.

We have recalculated the equilibrium relative humidities that would have existed at the surfaces of Crozier's NaCl droplets, if his Teflon and Dri-Film surfaces (and his droplets) were, for example, 0.8°C warmer than the saturated solutions contained in his box. With water at 25.0°C, assuming equilibrium in water concentrations, the slide surface at 25.8°C would be exposed to a relative humidity of 95.4 percent instead of 100 percent. Similarly, saturated $(NH_4)H_2PO_4$ would yield a local relative humidity of 88.7 percent instead of 93.0 percent, and $(NH_4)_2SO_4$ would yield 77.4 percent instead of 81.1 percent. Employing these new relative humidity values, we have replotted a sample set of the droplet volumes recorded by Crozier, on Fig. 1. It will be observed that the revised plotting is in good agreement with the theoretical relationship between particle volume and relative humidity that is derived from ICT data. Similar treatment will yield similar results for the other droplets examined. The results obtained by Crozier could, therefore, be satisfactorily explained by the existence of a rather small temperature differential.

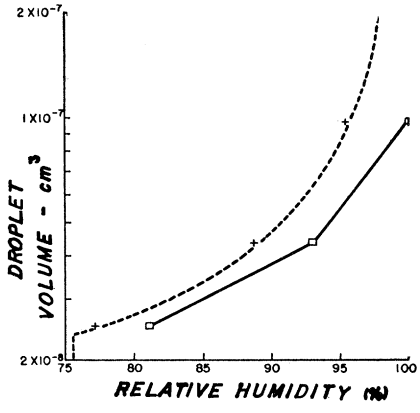

Fig. 1. Relationship of droplet volume to relative humidity. Solid line connects typical experimental points (□) obtained by Crozier (*1*). Broken lines are the calculated relationships based on vapor pressure and density of NaCl solutions; points marked (+) represent Crozier's data corrected as indicated in the text.

From Fig. 1, it is also apparent that the error that may be incurred by assuming, rather than determining, that isothermal conditions exist increases greatly as the relative humidity increases.

FRED W. LEAVITT*
SAUL KAYE†
Fort Detrick, Frederick, Maryland

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* Present address: department of chemical engineering, Rensselaer Polytechnic Institute, Troy, N.Y.

† Present address: Ben Venue Laboratories, Bedford, Ohio.

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Occurrence of Three Red Blood Cell Antigens in Rabbit as the Result of Interaction of Two Genes

In the course of the study of blood groups in the rabbit (*1*), isoimmunization techniques have been used to prepare specific antiserums that detect the presence of seven rabbit red blood cell antigens (labelled *A, B, C, D, E, F,* and *H*) (*2*). The mode of inheritance has been studied, and it has been shown that these antigens are controlled by five loci (*3*). A new isoantibody has been prepared that appears to identify an antigen resulting from the interaction of a pair of previously identified allelic genes.

The locus with which we are concerned has three alleles (Hg^A, Hg^D, and Hg^F), each of which gives rise to a detectable antigen. Thus, all rabbits must have antigen *A, D,* or *F,* or a combination of any two of these antigens. A new isoimmune serum appeared to contain three specific antibodies. One was identified as anti-*D*, and another as anti-*H*. The third antibody, called anti-*I*, was isolated by the absorption of the whole serum by cells of type *D* and by cells of type *AFH*. The resulting typing serum,

Table 1. Mean scores of cells of known phenotype when tested against specific antiserums.

Phenotype	Mean score
Pooled anti-A serum	
A	29.5
AF	31.9
AD	14.8*
Anti-D serum	
D	48.0
DF	53.8
AD	27.3*

* $P < 0.01$.

when tested against a panel of rabbit cells containing all our previously identified antigens, reacted strongly with cells that had antigens *A* and *D* and did not react with cells that were homozygous for *A*, *D*, or *F* or with cells that were heterozygous for *AF* or *DF*.

Matings were made to include all combinations of blood groups controlled by this locus; antigen *I* consistently appeared only when, as a result of segregation, the genes for *A* and *D* occurred in the animal. In all, 223 animals in our colony were typed, and of these 63 were found to be type *ADI*. In no case did *I* appear when neither *A* nor *D* was present. In no case was antigen *I* missing when both *A* and *D* were present. As the result of our attempts to maintain lines of inbred rabbits, we had available at least three generations of animals showing segregation for the genes Hg^A and Hg^D. The complete pedigree, shown in Fig. 1, indicates the relationships that must exist for the appearance of *I*.

In studying the effect of genotypes on the reactivity of cells, we compared the relative titers of various typing serums as they reacted with cells with antigen combinations resulting from the *Hg* allelic series. Anti-*A* was titrated against cells of animals of phenotypes *A*, *AD*, and *AF*; anti-*D* was titrated against cells of animals of phenotypes, *D*, *AD*, and *DF*. The scoring method suggested by Race and Sanger (*4*) was used to measure the titer of the typing serum. In every case the score for the *AD* cells was significantly ($P < 0.01$) lower than that for the other antigenic types (Table 1).

This finding, also reported in part by Joysey (5), is consistent with the concept that the interaction of genes Hg^A and Hg^D results in the formation of three products that perhaps involve a common substrate somewhere in the process of action so that the amount of antigens *A* and *D* is diminished. No attempt will be made at this time to give a physiological explanation of the reaction beyond the statement that two genes have clearly given rise to three antigens. The following genetic hypotheses may be rejected as inconsistent with the data: recessivity, and linkage between the *Hg* series and a gene giving rise to antigen *I*. The interaction postulated for the existence of human *f* may be similar to our findings, but *f* is dependent on *c* and *e* being on the same chromosome. In our example, Hg^A and Hg^D cannot be on the same chromosome.

The rare occurrence in offspring of cellular antigens that do not occur in either parent has been reported by Irwin (*6*), Thompsen (7), Boyd and Alley (*8*), and more recently by Rendel, Sorensen, and Irwin (*9*) and Levine *et al.* (*10*).

Our findings described here are of interest in three areas. First, the appearance in the rabbit offspring of antigens that are not present in either parent is one of a few cases in mammals. The use of blood groups in paternity cases is based on the premise that a child cannot have an antigen that is not present in either parent. Although the findings in the rabbit cannot, of course, be extrapolated to human blood groups, it is possible that such a phenomenon as that reported here may occur in human beings.

The second area of interest is that of the gene-antigen relationship. Since we have shown that two genes can give rise to three detectable antigens, the one gene-one antigen relationship obviously does not hold true for all situations.

Third, there have been several theories proposed on the mechanism for selective value of heterozygotes. The findings reported here support the theory of Rendel (*11*) and Haldane (*12*), among others, that the heterosis effect may be due to the fact that the products of the interaction of two alleles may be more effective than the product of either homozygote.

CARL COHEN
Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine

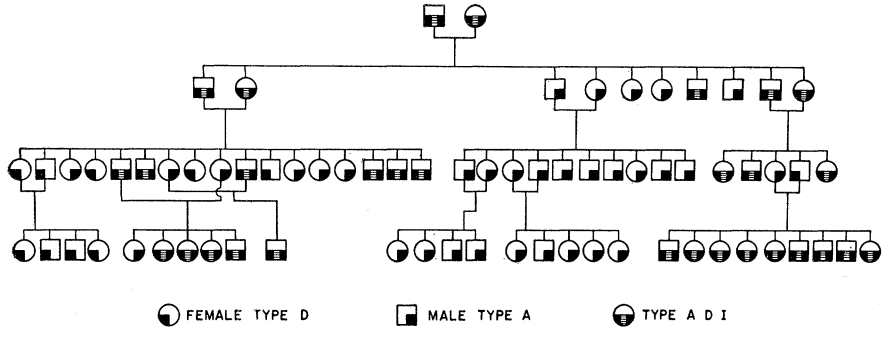

Fig. 1. Pedigree of a family of rabbits showing the inheritance of antigens *A*, *D*, and *I*.

References and Notes

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Efficacy of Striatal Shocks in Avian Conditioned Behaviors

A cursory investigation has shown that electric stimulation of the striatum of the pigeon can serve as a conditioned stimulus in both classical and instrumental situations. In three pigeons, silver-wire electrodes spaced 1.5 mm apart and insulated except at the tips were implanted in one hemisphere of the forebrain and affixed firmly to the skull. Leads were brought through the skin to allow direct connection with a stimulator. Such preparations could be maintained indefinitely without danger of infection or other complications. Post-mortem gross examination, correlated with histologic study of other specimens, suggested that the electrode tips were placed in the neostriatum intermediale just below tractus fronto-occipitalis (*1*). When delivered to this region, a very brief 60-cy/sec pulse of about 2 v produced discrete and highly consistent turning of the head in the direction opposite to the side stimulated. If the voltage was very slightly reduced, there was no observable response. If the voltage was very slightly increased, the result was "startle" in two birds, and indiscriminate jerking and struggling in the third.

One pigeon was trained to flex its leg in response to striatal stimulation. The unconditioned stimulus, an electric shock to the skin on the thigh, evoked a clearly unilateral leg flexion. A 0.75-sec striatal