longer adsorbs on the skin. On the other hand, the intrinsic characteristics of the skin could remain constant but with increased antibody production accompanying growth, the skin may become preempted with antibodies (13), thus rendering the skin nonreactive with passive antibody.

Summary. Passive cutaneous anaphylactic-like (PCA-like) reaction could be elicted in the skin of newly hatched and young chickens with chicken antiserum. The reaction occurred within 20 min following antigen injection, reacted specifically with the antiserum, and the reaction could be prevented with antihistamine. The present findings indicate that differential susceptibilities to anaphylactic reaction exists depending on the developmental stage of the chicken.

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Neutralization of Mycobacteriophages by Sera of Patients with and without Sarcoidosis* (33401)

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Mankiewicz (1, 2) reported that sera of patients without sarcoidosis and who harbored intestinal mycobacteriophages possessed phage neutralization activity against the carried phage or against mycobacteriophage D.W. In contrast, sera of patients with active sarcoid did not possess an appreciable amount of serum antibodies to their own intestinal mycobacteriophage or to phage D.W. Mankiewicz' inability to demonstrate antiphage antibodies in sera of patients with sarcoidosis was supported by the work of Bönicke (3), however, his report failed to name the test phage.

Since the antigenicity of mycobacteriophages R1, D29, and Leo has recently been characterized (4), it was reasoned that applying the same standard bacteriophage neutralization techniques to sera of patients would greatly increase the possibility of demonstrating the presence of neutralizing antibody. Therefore, the present study was undertaken to determine if the sera of patients, both with and without sarcoidosis, contain antibodies to mycobacteriophages D29, Leo, or R1.

Methods. Sera. Sera were obtained from six patients with active sarcoidosis hospitalized in the Ohio State University Hospital. Sixty-four additional sera came from patients with biopsy-proven sarcoidosis hospitalized at the Eastern North Carolina Sanatorium, Wilson,

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[1] NIH Career Development Awardee.

North Carolina.[2] Sera from 81 patients none of whom had sarcoidosis were obtained at the Ohio State University Hospital outpatient clinic. No attempt was made to determine or compare the tuberculin hypersensitivity status of any of the patients. All sera were stored in stoppered tubes at —20° until the day of testing. Each was thawed slowly and tested at the appropriate dilution.

Mycobacteriophages and assay bacteria. Mycobacteriophages D29, R1, and Leo were the same as described previously (4). *Mycobacterium smegmatis* ATCC no. 607 was used as the assay bacterium.

Neutralization. Stock phage was diluted in broth to approximately 5×10^8 plaque-formers/ml and unheated serum was diluted 1:50 in broth. Then 0.9 ml of the diluted serum was preincubated at 37° and 0.1 ml of the diluted phage was mixed with the prewarmed serum. After 30-min incubation, the concentration of surviving phage in the neutralization mixture was determined: a sample was removed, diluted 1:50 or more to stop neutralization, diluted further if necessary; and a sample from the last dilution of the mixture was plated in duplicate or quadruplicate. Each plate usually had 200–250 plaques. The phage control consisted of 0.1 ml of the diluted phage (described above) mixed with 0.9 ml of prewarmed broth at 37°. Two samples were removed immediately. Each sample was diluted separately and plated in quadruplicate. This process was repeated at the end of the experiment and the mean plaque count from the 16 plates (approximately 4000 total plaques) was used to calculate the initial phage concentration. The activity of each serum is expressed in terms of its K value (4). The K value is a quantitative characteristic of a serum. The mathematical expression for the reaction is

$$K = 2.3\, D/t \times \log P_0/P$$

in which D represents the final dilution of serum used, P_0/P the reciprocal of the surviving phage fraction at the time t. Sera with K values greater than 0.002 were judged to have evidence of some neutralizing activity (5). Sera with K values less than 0.002 were judged to have neutralizing activity or antibody.

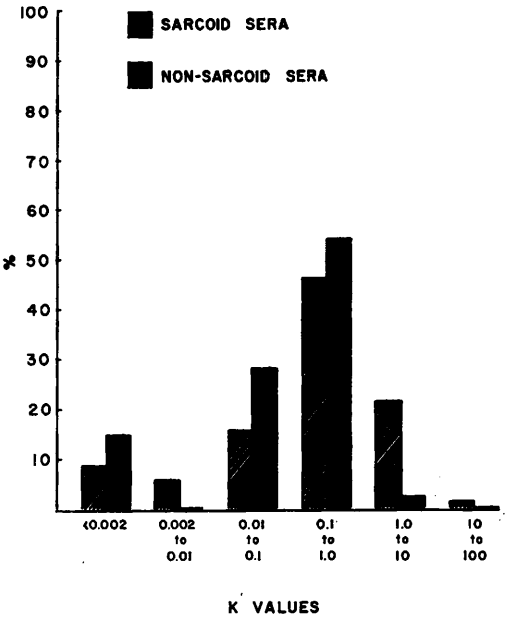

FIG. 1. Distribution of the frequencies of K values against mycobacteriophage D29 of 70 sera from patients with sarcoidosis and of 81 sera from patients without sarcoidosis.

Results. Figure 1 presents the percentage distribution of K values, over a five decade span, of the patients' sera against mycobacteriophage D29. Of all the sarcoid sera, 8.6% had K values less than 0.002, indicating absence of neutralizing antibodies; however, the remaining 91.4% possessed neutralizing activity with K values greater than 0.002. Of the nonsarcoid sera, 14.8% lacked detectable neutralizing antibody, whereas 85.2% had neutralizing activity. Approximately 50% of both the sarcoid and the nonsarcoid sera were found to have K values within the 0.1–1.0 decade. A difference was found between sarcoid and nonsarcoid sera with respect to the percentage of each having K values greater than 1.0. As shown in Fig. 1 the former was 22% and the latter 2%.

Figure 2 presents the percentage distribution of K values, over a five decade span, of

[2] The sera were a gift from Dr. Ralph A. Vogel, Veterans Administration Hospital, Atlanta, Georgia. The patients were originally studied by Dr. Frances E. Noblin; subsequently, their sera have been studied by others interested in the immunologic aspects of sarcoidosis.

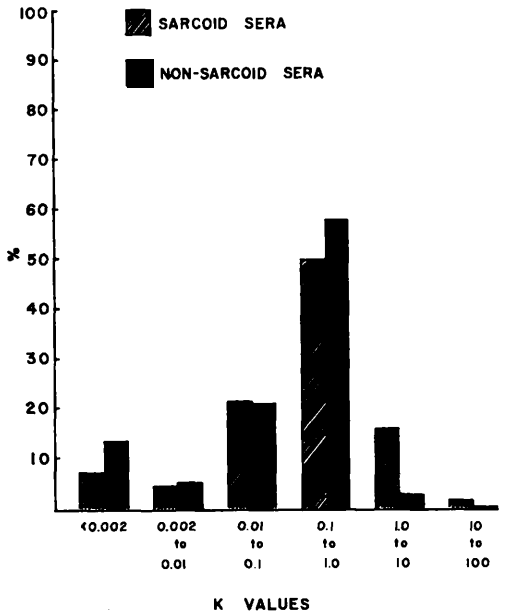

FIG. 2. Distribution of the frequencies of *K* values against mycobacteriophage Leo of 70 sera from patients without sarcoidosis.

the patients' sera against mycobacteriophage Leo. Most of the sarcoid sera (92.9%) had neutralizing activity against phage Leo, while 86.5% of the nonsarcoid sera had antibodies to phage Leo. As shown the percentages of the sarcoid and of the nonsarcoid sera were about the same within each decade of *K* values except for the 1.0–10 decade. In this decade there was a larger percentage of the sarcoid than of the nonsarcoid sera (20 vs 2%). None of the nonsarcoid sera were found to have *K* values greater than 10, whereas 1.4% of the sarcoid did.

Figure 3 depicts the percentage distribution of *K* values, over a five decade span, of the patients' sera against mycobacteriophage R1. All of the nonsarcoid sera and essentially all of the sarcoid sera (98.5%) had neutralizing activity against phage R1. However, the percentages of the sarcoid and of the nonsarcoid sera within the 0.01–0.1, 0.1–1.0, and 1.0–10 decades of *K* values were different. In particular, the results showed that 97.5% of nonsarcoid sera had neutralizing activity against phage R1 with *K* values ranging between 1.0 to 10, whereas in the sarcoid group only 25.8% possessed similar *K* values. On the other hand, a large number (53.0%) of the sarcoid sera were found to have *K* values between 0.1 and 1.0 while none of the nonsarcoid sera had *K* values in this decade.

Discussion. Since these resuls are quantitative and those of Mankiewicz (1, 2) or Bönicke (3) are qualitative, direct comparisons cannot be made. Mankiewicz' reported results (1, 2) were minimal in that no data were given on the number of sera of patients with sarcoidosis tested for phage neutralization. Also, her report (1) did not clearly indicate which mycobacteriophages were used nor did it give the extents of neutralization of the phages tested. Bönicke's results were even less clear as to what had been tested; however, his conclusion was in agreement with Mankiewicz with respect to the absence of neutralizing activity in sera of patients with sarcoidosis. The difference between these results and those of Mankiewicz (1, 2) and Bönicke (3) may be accounted for by one or more of the following possibilities. (a) Different mycobacteriophages were probably used to measure neutralization. (b) Mankiewicz'

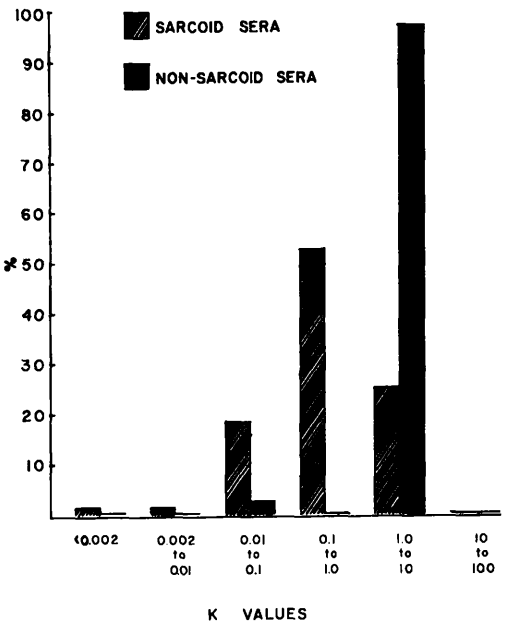

FIG. 3. Distribution of the fequencies of *K* values against mycobacteriophage R1 of 70 sera from patients with sarcoidosis and of 81 sera from patients without sarcoidosis.

and Bönicke's sampling of sera from a population of patients with and without sarcoidosis may have been too small to represent adequately the two groups. (c) Different techniques were used to measure neutralization. It would appear that the standard neutralization technique used in the present investigation may be more sensitive than that of Mankiewicz or Bönicke because of its quantitative aspects and therefore identifies more sera with neutralizing activity. The method used by the latter was not quantitative. Eloquent descripitions of quantitative methods for neutralization have been described and that of Adams (6) for the *Escherichia coli*-T phage is classic. Bowman (7) showed that the same technique could be applied to mycobacterial phage–antiphage system.

The present experiments indicate that mycobacteriophage R1 would be the best mycobacteriophage for comparing antibodies titers to mycobacteriophages, particularly in the nonsarcoid sera group (see Fig. 3), since almost 100% of these sera had antibodies to this phage at K values in the 1.0–10 decade. These findings could be related to the fact that *M. butyricum*, which is naturally lysogenic with this phage (8), may be ubiquitously distributed in nature. However, this reason does not explain the failure of patients with sarcoidosis to produce R1 antibodies as extensively with K values in the 1.0–10 decade.

Summary. Of 70 sera from different patients with sarcoidosis serum neutralization activity could be demonstrated in 91–98% against mycobacteriophages D29, Leo, or R1. Of 81 sera from nonsarcoid patients, neutralizing activity was demonstrated in 83–85% against phages D29 and Leo, and in 100% against phages R1. In test using phage R1, the magnitude of the K values of sera of patients not having sarcoidosis was much larger than those of patients with sarcoidosis.

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Influence of Induction of Hepatic Microsomal Enzymes by Phenobarbital on Toxicity of Organic Phosphate Insecticides* (33402)

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The possibility that the toxicity of pesticides and other environmental chemicals may be increased by prior exposure to drugs capable of inducing synthesis of hepatic microsomal drug-metabolizing enzymes is a problem of considerable concern. Since the presence in harvested crops of small quantities of the pesticides used in agriculture is frequently unavoidable, information is needed on the extent to which enzyme induction alters the toxicity of important pesticides. Most of the cholinergic organic phosphates that are commonly used as insecticides are phosphorothioates which must undergo oxidative desulfuration to become anticholinesterase agents. This activation reaction is catalyzed by a microsomal oxidase and drug-induced increases in its activity accelerate

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