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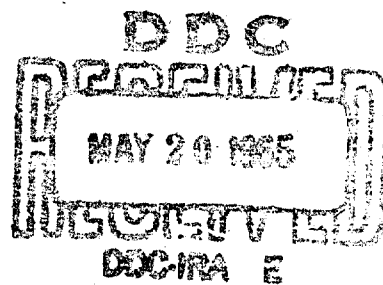
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TECHNICAL MANUSCRIPT 222

RETEST REACTION  
IN TUBERCULOUS MONKEYS

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U.S. ARMY BIOLOGICAL LABORATORIES  
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 222

RETEST REACTION IN TUBERCULOUS MONKEYS

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Project 1C522301A059

April 1965

In conducting the research described in this publication, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

ABSTRACT

Retest tuberculin reactions in rhesus (Macaca mulatta) monkeys to 0.2 ml of 1:10 dilution of veterinary-type old tuberculin given intradermally into the eyelid produced responses as seen by eye closure at 6 hours. With readings made at  $\frac{1}{2}$ , 6, 24, and 48 hours, the earliest virginal site reaction was seen at 24 hours. There was a general tendency for the degree of response as seen by eye closure to be less at retesting. Several animals at retest produced negative or questionable responses at 6, 24, and 48 hours, but these were inconsistent in any one animal from one retest to the other.

## I. INTRODUCTION

A recent histopathologic investigation on the tuberculin retest phenomenon\* has renewed interest in this type of reaction. Although the basic gross characteristics of the retest reaction were observed many years ago, the method extensively reported was the scratch-type test and reactions were observed in humans for the most part. In the recent report referenced above, tuberculin was administered intradermally in guinea pigs. The purpose of the present investigation was to compare the gross nature of the retest tuberculin reaction in rhesus monkeys after intradermal skin testing with the reactions elicited during the virginal response. The conventional intrapalpebral test technique was used i.e., injection of tuberculin in the eyelid, which provided a small, confined, and definitely delineated area for this retesting.

## II. MATERIALS AND METHODS

Six rhesus monkeys, found to be tuberculin-positive upon routine skin testing, were secured for this investigation. Which eyelid used for the original palpebral tests was recorded for each animal but the magnitude of the positive response was not provided.

For the present investigation the standard method for tuberculin testing monkeys at Fort Detrick was used. The antigen used was veterinary-type old tuberculin (OT) diluted 1:10. Intradermal injections were made in the eyelid, using 0.2 ml of the diluted OT. The original description of this skin test method by Schroeder\*\* states that 0.1 ml of 1% purified protein derivative was injected. After skin testing, the results can be read as the degree of eyelid closure, and can be observed without recapturing or restraining the animal.

Virginal skin tests were given as described in the eyelid that was not used for the initial determination of tuberculin hypersensitivity. Observations were recorded for the virginal-site skin test and subsequent retests at 15 minutes, 6, 24, and 48 hours. Retests were given in the same eyelid from which data had been recorded. The first and second retests were given one and three weeks after the virginal test.

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\* Arnason, Barry G., and Byron H. Wahsman. 1963. The retest reaction in delayed sensitivity. Lab. Investigation 12:737-747.

\*\* Schroeder, C.R. 1938. A diagnostic test for the recognition of tuberculosis in primates: A preliminary report. Zoologia, N.Y. 23:397-400.

At the conclusion of the investigation the animals were sacrificed and necropsied. Pathologic tissues were cultured for presence of mycobacteria.

### III. RESULTS

During the course of this investigation all the monkeys remained in apparent good health. They were lively, had normal appetites, and exhibited no gross signs of serious illness.

At the virginal skin test period all animals had negative responses at the 15-minute and 6-hour readings (Table 1). All had reactions at 48 hours that were positive; all but one animal had reactions at 24 hours that were definitely positive. The one animal, Q45, had a questionable response at the 24-hour reading for this virginal testing.

During the first retest, one week later, none of the animals exhibited a 15-minute response, but all animals did show a positive response at 6 hours. The majority of reactions at this time period were not greater than the maximum virginal area reactions. At this first retesting all animals had positive reactions at 24 hours, but two animals (Q31, R61) had negative reactions at 48 hours.

The second retest, three weeks after the virginal tests, produced results that were, in general, less than those observed in the first retest. One animal (R61) had a questionable response at 24 hours. At 48 hours this questionable response continued in that animal. Also, at 48 hours a second animal (Q45) produced a questionable response and still another (6A15) had a definitely negative response. The animal that had the negative response at 48 hours also had a negative response at 6 hours. A positive reaction in this animal was seen at 24 hours, but was less than that observed for the other retest and the virginal area reaction in this animal.

In both series of retest reactions there was a general tendency for the reaction to reach its peak development at 24 hours; however, this was not observed in all animals. The magnitude of these retest reactions in the majority of animals was not as great as that of the virginal reactions.

Upon necropsy all animals were observed to have lesions compatible with tuberculosis and positive cultures were secured from each animal.

TABLE 1. VIRGINAL AND RETEST TUBERCULIN REACTIONS IN MONKEY EYELID  
(Reactions read by degree of eyelid closure)<sup>a/</sup>

Animal No.	Skin Test Number <sup>b/</sup>	Time after Testing when Observations Recorded			
		15 min	6 hr	24 hr	48hr
Q45	1	0	0	±	1/3
	2	0	1/2	1/2	1/4
	3	0	1/3	1/3	±
Q31	1	0	0	1/3	3/4
	2	0	1/2	3/4	0
	3	0	1/3	1/3	1/3
R61	1	0	0	1/4	1/2
	2	0	1/2	1/2	0
	3	0	1/2	±	±
6A15	1	0	0	1	1
	2	0	3/4	1	1/2
	3	0	0	1/3	0
I34	1	0	0	1	1
	2	0	2/3	3/4	1/3
	3	0	1/2	2/3	2/3
I26	1	0	0	1/2	1
	2	0	1	1	3/4
	3	0	1/3	1/3	1/4

- a. 0 = negative response  
 1 = complete eye closure  
 ± = non-measurable induration with erythema
- b. 1 = virginal test  
 2 = 1st retest (7 days)  
 3 = 2nd retest (21 days)

#### IV. DISCUSSION

Only one of the two characteristics ordinarily associated with the retest reaction was observed in the tuberculin retest reactions in monkeys using intradermal injection in the eyelid. Retest reactions develop sooner than virginal area reaction, i.e., 6 hours vs. 24 hours; and the size of the retest reaction at 6 hours is greater than it is at 24 hours. The acme of development of the retest reaction may vary with (i) the animal species used, (ii) the difference in test site, or (iii) the antigen. Arnason et al. showed that the acme of development of the retest reaction in guinea pigs' flank skin from tuberculin occurred at 6 to 8 hours.\* In a human subject retested with coccidioidin in the forearm, the acme of development was seen at 12 hours.\*\* In the present investigations, with readings made at 6 and 24 hours without intervening readings, the acme of development of the retest reaction in monkey eyelid using tuberculin was seen at 24 hours.

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\* Arnason, Barry G., and Byron H. Wahsman. 1963. The retest reaction in delayed sensitivity. Lab. Investigation 12:737-747.

\*\* Sinski, James T., and Paul J. Kadull. Observations on the retest reaction in a coccidioidin-sensitive individual. Trans. 9th VA-Armed Forces Coccidioidomycosis Study Group, Los Angeles. (1964) (In Press). Also: February 1965. Retest reaction in a coccidioidin-sensitive individual, (Technical Manuscript 183). Special Operations and Medical Investigation Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

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13. ABSTRACT

Retest tuberculin reactions in rhesus (Macaca mulatta) monkeys to 0.2 ml of 1:10 dilution of veterinary-type old tuberculin given intradermally into the eyelid produced responses as seen by eye closure at 6 hours. With readings made at ½, 6, 24, and 48 hours, the earliest virginal site reaction was seen at 24 hours. There was a general tendency for the degree of response as seen by eye closure to be less at retesting. Several animals at retest produced negative or questionable responses at 6, 24, and 48 hours, but these were inconsistent in any one animal from one retest to the other.