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TECHNICAL MANUSCRIPT 59

ELECTROPHORETIC PATTERNS OF
PLASMA FROM VACCINATED MONKEYS
FOLLOWING RADIATION
AND TULAREMIC INFECTION

JUNE 1963

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U.S. ARMY CHEMICAL-BIOLOGICAL-RADIOLOGICAL AGENCY
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The work reported here was performed under
Project 4B11-02-006, "Bacterial and Fungal
Agent Research," Task -01, "Selection of
Candidate Bacterial and Fungal Agents."
The expenditure order was 2070. This material
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Medical Bacteriology Division
DIRECTOR OF BIOLOGICAL RESEARCH

Project 1C022301A068

June 1963

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The method of Jencks *et al** was employed for electrophoresis of plasma proteins with the Spinco Model R electrophoresis apparatus. The animals were bled 16 times, three times prior to challenge and irradiation and at 13 intervals afterwards during the 30-day holding period. The plasma was separated within two hours following bleeding and kept frozen until the end of the 30-day test period. The plasmas of each monkey were then tested at one time in order to avoid day-to-day environmental variations and personal bias.

Table II shows the normal values for total protein and the percentages of the protein components of monkey plasma. All values falling outside of ± 2 standard deviations from the mean were considered significantly different.

TABLE II. NORMAL VALUES FOR MONKEY PLASMA COMPONENTS.

PROTEIN ^a	MEAN VALUE	LIMITS (± 2 STD. DEVIATION)	
Total, gm/100 ml	6.14	4.94	7.34
Albumin %	36.8	30.2	43.4
α_1 Globulin %	6.1	3.9	8.3
α_2 Globulin %	7.9	4.4	11.5
β Globulin %	20.5	13.8	27.1
γ Globulin %	28.2	21.1	35.3

a. Total protein based on 58 samples from 25 normal monkeys; other values based on 110 samples from 30 normal monkeys.

Figure 1 shows the mean changes that occurred in the albumin fraction. The VX group remained at a normal level, whereas the X group showed a gradual drop throughout the test period. The VX and X groups were from two different shipments of animals, and although they were about the same age and in apparent similar condition of health, the response to stressing with sublethal X-irradiation certainly appears to differentiate between the groups as to initial general physical condition.

* Jencks, W. P.; Jetton, M. R.; and Durrum, E. L. "Paper electrophoresis as a quantitative method," *Biochem. J.* 60:205-215. 1955.

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There was also a comparably rapid decrease in levels in the C and VCX groups and a slower, less pronounced drop in the VC group. Recovery toward normal values was most rapid in the VC group and least in the C group.

The α_1 fraction showed no significant change in any of the groups, although an upward trend was discernible in the VCX group.

Figure 2 shows the increased α_2 levels in the three challenged groups about seven days after respiratory infection, with the VCX group α_2 fraction remaining higher longer than that of the other two groups. After the second week there was a tendency toward increased levels among the VX and X groups.

In Figure 3, we see that the elevation of β globulin levels six days after respiratory challenge appeared only in the two vaccinated challenged groups, but not in the challenged only group.

Figure 4 shows the response observed in the γ globulin fraction. The VC and VCX group levels remained normal and, as might be expected, the γ globulin fraction of the C group increased significantly. The relationship of this increase to rise in agglutinin titer is shown in Figure 5. A slight rise in titer may also be noted in the VC and VCX groups, but it was not considered significant because it was not a fourfold increase above the titer at the time of challenge.

These analyses of the group means with regard to progressive changes in concentration of each fraction, with the exception of α_1 , clearly differentiated each challenged group. The changes in the albumin fraction appeared to correlate with severity of disease. The changes in the plasma protein fractions supplemented and supported the conclusions derived from all the other data collected in this experiment: namely, that sublethal X-irradiation 24 hours after respiratory challenge nullified the protection afforded by vaccination with *P. tularensis* LVS and that the response of the VCX group more nearly resembled that of the C group than of the VC group.

The variation encountered within each group, as mentioned earlier, necessitated the use of ± 2 standard deviations for evaluation and a few examples of the sort of data that went into the rather nice group means will make clear how data from a few animals may be atypical or misleading. Among the five monkeys in the normal group, one monkey ran low albumin and high γ globulin levels at -2 and +2 standard deviations, respectively, throughout the 35-day test period. Total protein was normal, the animal's appetite, gross appearance, and behavior seemed normal. There was, however, a slight weight loss in contrast to a slight gain by the others, and at sacrifice the spleen was enlarged and contained necrotic foci of undetermined origin.

In the X group, four of five monkeys behaved uniformly. The fifth exhibited a rise in albumin concentration rather than a dip as was observed in the other four at 22 to 27 days after X-irradiation. However, the initial (or pre-X-irradiation) albumin levels were lower than those of the others. The α_2 globulin was up from the eighth to fifteenth day, those of

the others were up from the 20th to the 29th day. Similarly, in the VX group, one monkey out of five had reduced plasma albumin levels from the eighteenth to twenty-ninth day.

In the C group, the possible effect of a low challenge dose (320 cells) was very distinct in one monkey; the albumin level dropped two standard deviations from the mean. The α_1 and α_2 levels remained normal, the β level rose to +2 standard deviations and, in sharp contrast to the other monkeys in this group, no change at all took place in the γ globulin fraction. This was consistent with a minimal agglutinin titer rise to 1:20. The other data collected on this animal further substantiated the mild course of the disease. In contrast, one monkey in the VCX group also inhaled 320 cells, but the disease was very severe and was reflected in the extreme changes in the plasma protein levels such as were observed in the other monkeys in this group.

The group data collected in this experiment reflect rather curious and perhaps significant differences in the physiological responses of vaccinated and nonvaccinated monkeys to combined sublethal X-irradiation and tularemic infection. In addition, from the data on the normal monkeys and comparison of the VX and X groups, we believe that electrophoretic analyses of plasma of laboratory animals for a week or two prior to their use in controlled experiments may represent a simple method for screening out those animals that vary significantly from the norm.

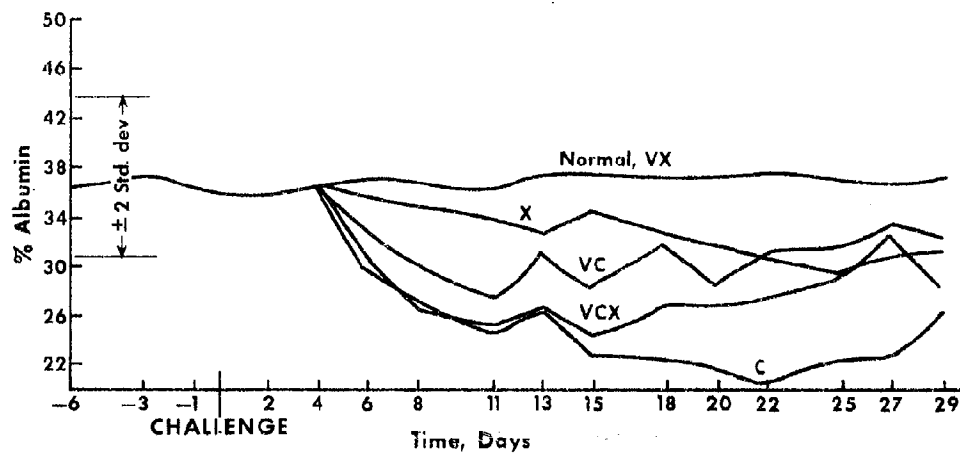
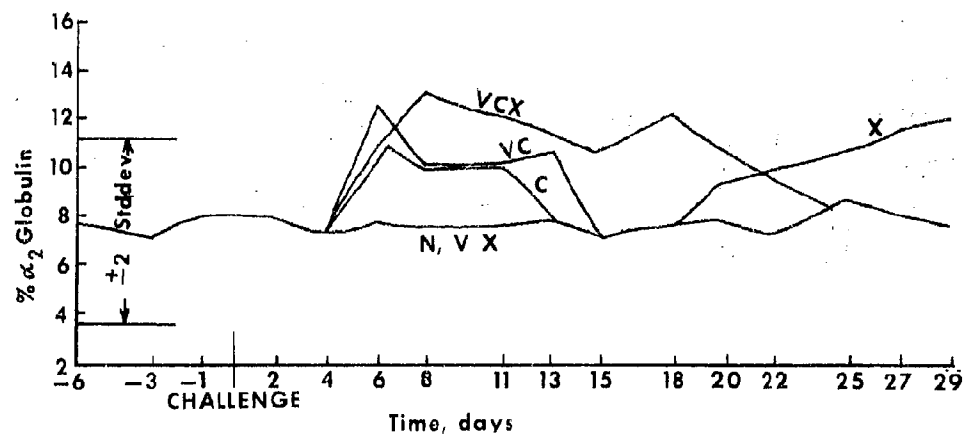
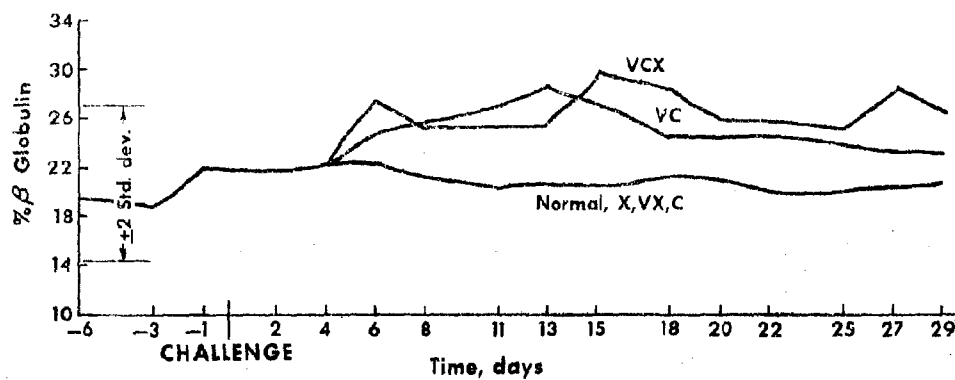
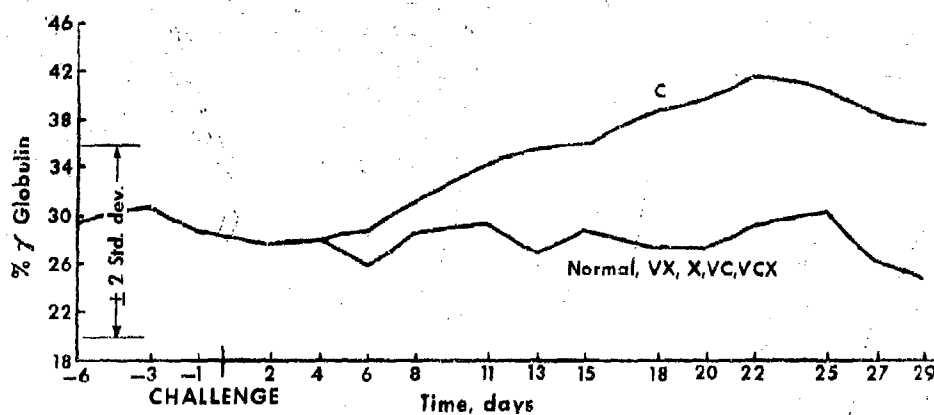


Figure 1. Plasma Albumin Levels.

Figure 2. Plasma α_2 Globulin Levels.

Figure 3. Plasma β Globulin Levels.Figure 4. Plasma γ Globulin Levels.

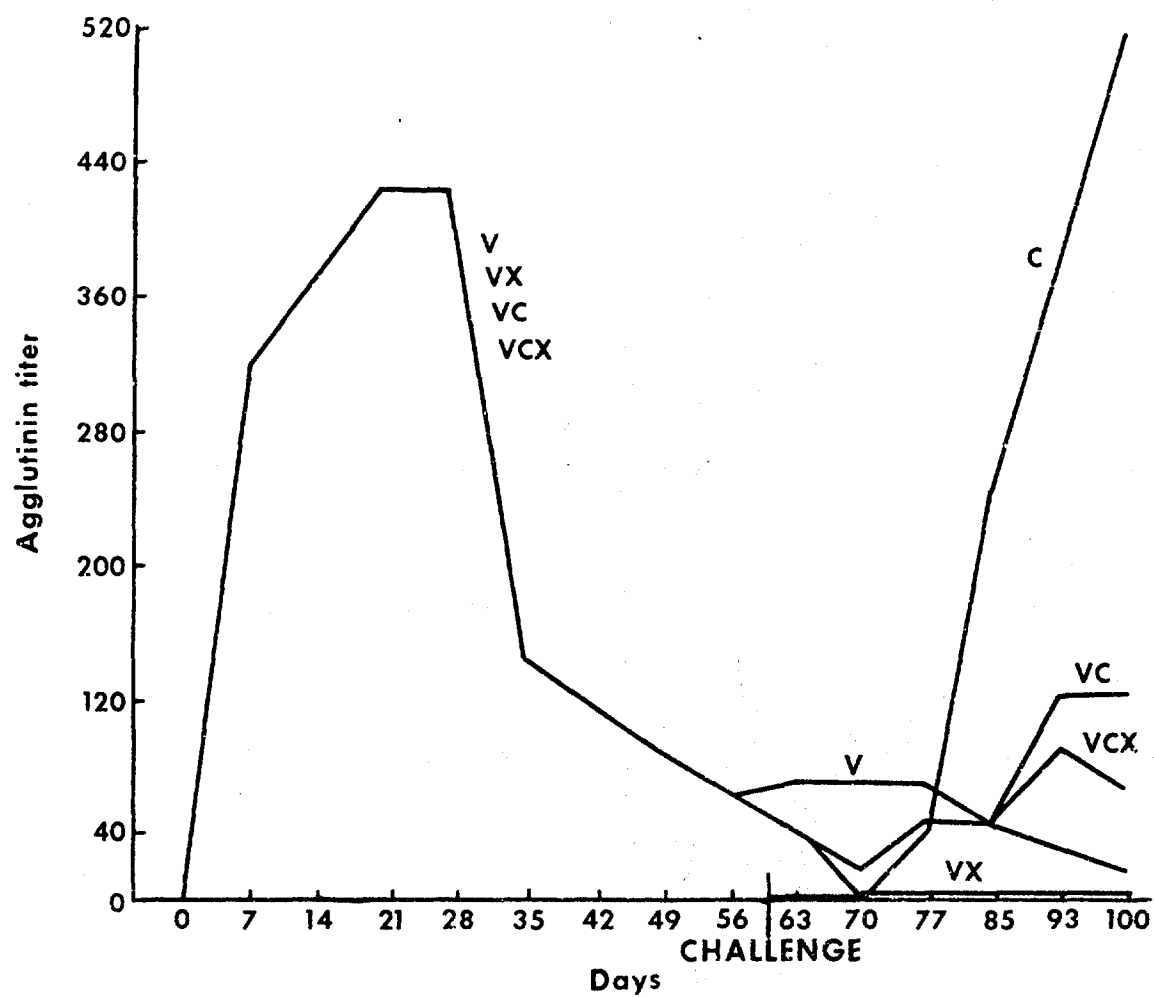


Figure 5. Agglutinin Titers.