Fractionation of Sera from Guinea Pigs at Different Stages of *Brucella abortus* Infection.* (24446)

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Previous work showed that guinea pigs infected with *Brucella abortus* could not be reinfected while living organisms of the original infection were demonstrable in the tissues. then resistance waned until animals regained susceptibility(1). Subsequent investigations demonstrated that mononuclear phagocytic cells and humoral factors played an important role in resistance(2). Qualitative and quantitative alterations in serum protein components as a result of other infections have been demonstrated(3,4,5). Results obtained by electrophoretic fractionation of serum protein components during the course of experimental brucella infection are here reported.

Materials and methods. Male guinea pigs weighing 400 to 500 g and kept on diet of Purina rabbit feed, green grass and water were inoculated subcutaneously with 3 x 10^7 *Brucella abortus* organisms (strain SAS). Infection was allowed to progress ½, 1, 2, 3, 4, 5, 8, 10 and 12 months. Animals were bled at end of each period and serum separated after overnight refrigeration. Total protein determinations were made by modification of Kingsley's method(6). Sera were fractionated at room temperature for 16 hrs at 5 milliamperes in Spinco Model R—Series D paper electrophoresis cell with Whatman 3 mm filter paper strips and barbital buffer pH 8.6, ionic strength 0.075. Strips were dried and stained by Durrum's method(7). Stained strips were scanned, ionograms plotted and areas under curves integrated with Spinco Analytrol.

Results. Five fractions appeared as shown in Fig. 1: Albumin, and alpha-1, alpha-2, beta and gamma globulins. All fractions, except gamma globulin, migrated towards the anode. Fifteen days after inoculation infection was well established as shown by abundant growth of brucella from the spleen, and positive agglutination titers of 1:50 to 1:100. At this stage of infection the electrophoretic pattern was the same for infected animals and uninfected controls (Fig. A). Guinea pigs infected for 1 and 2 months gave agglutinin titers of 1:200 and 1:400 respectively but again the electrophoretic patterns were not significantly different from the normal. Three to 4 months after infection, however, marked differences between uninfected controls and infected animals were evident (Fig. 1). Average agglutinin titer at this stage of infection was 1:1,600 and overall changes in the electrophoretic pattern may be attributable to an increase in gamma globulin. Five to 8 months after infection the average agglutinin titer was 1:800 and a decrease in the gamma globulin concentration was evident. Twelve months after infection the electrophoretic pattern closely approximated that of the uninfected controls and the agglutinin titer ranged from 1:160 to 1:400.

Quantitative data in terms of percentage composition and their conversion to concentration of protein components in terms of mg/ml offered a better evaluation of the changes observed during infection (Table I). Total protein in controls remained fairly constant throughout the experiment, averaging 62 mg/ml and relative concentration of all components exhibited little variation. In infected animals total protein increased from 61 mg/ml at 15 days to a high of 66 mg/ml at 3 months, then decreased to 56 mg/ml at 12 months. Gamma globulin concentration increased approximately 50% 3 to 4 months after infection, as compared to uninfected controls, and then returned to low levels. Albumin concentration fell from 23 mg/ml 15 days after infection to 16 mg/ml at 4 months. This decrease coincided with an increase of gamma globulin. At the end of 12 months albumin concentration was 17 mg/ml while no gamma globulin increase was apparent. This latter

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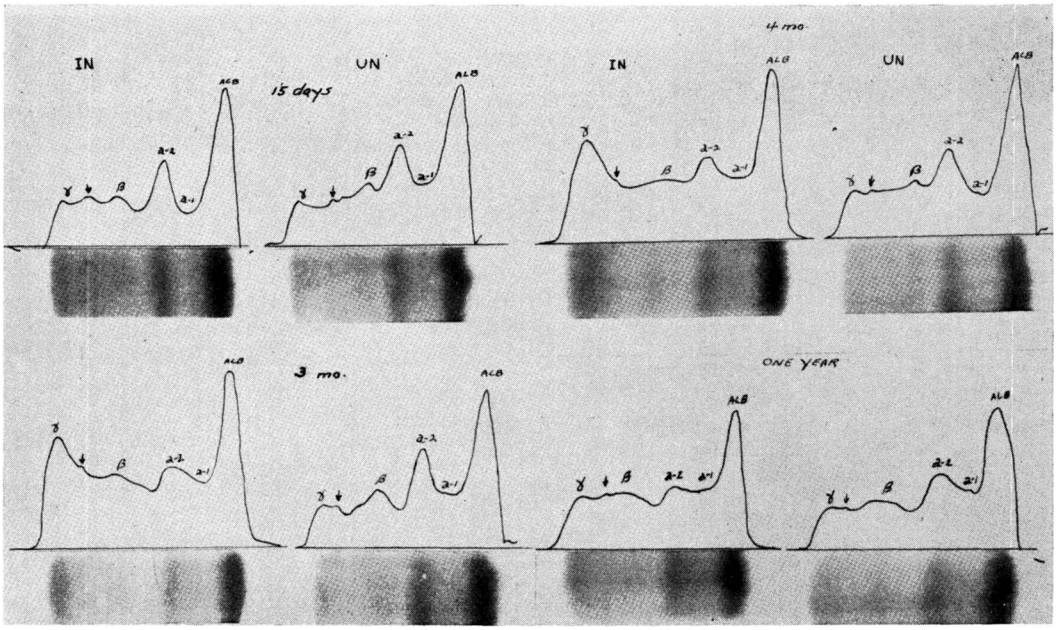

FIG. 1. Elecrophoretic patterns of sera of uninfected and infected guinea pigs during different stages of experimental *Br. abortus* infection. Arrows indicate origin.

effect may be due to decrease in the total protein in infected animals at this stage of infection.

Increase in globulin with a decrease in albumin seems a characteristic serum alteration in many diseases. The fall in serum albumin has been thought to follow serum globulin increase and to depend upon a regulatory

TABLE I. Average Concentration and Percentage of Serum Protein Components in Infected and Uninfected Guinea Pig Sera.

Duration of infection, mo		No. of animals	Components: Albumin Conc.	Albumin %	α-1 Conc.	α-1 %	α-2 Conc.	α-2 %	β Conc.	β %	γ Conc.	γ %	Total protein, mg/ml
½	IN	4	23	38	3	4	13	21	8	14	14	23	61
	UN	4	23	37	3	6	13	21	10	16	12	20	61
1	IN	12	23	36	3	5	13	21	9	15	14	23	62
	UN	5	24	39	3	6	14	23	7	12	12	20	60
2	IN	4	21	34	4	6	13	21	10	16	14	23	62
	UN	4	23	37	5	8	16	25	7	11	12	19	63
3	IN	8	18	27	5	9	13	19	10	15	20	30	66
	UN	4	23	37	4	6	15	24	10	16	11	17	63
4	IN	5	16	26	4	6	11	18	6	9	26	41	63
	UN	4	22	37	2	3	15	26	8	13	13	21	60
5	IN	5	19	31	7	11	12	19	9	14	15	25	62
	UN	4	23	37	3	4	16	25	8	13	13	21	63
8	IN	4	19	30	4	7	15	24	10	16	14	23	62
	UN	4	23	36	4	7	14	22	10	16	12	19	63
10	IN	4	22	35	3	5	14	22	8	13	15	25	62
	UN	3	22	36	4	6	11	18	10	17	14	23	61
12	IN	4	17	30	6	11	12	21	8	15	13	23	56
	UN	3	21	35	4	7	14	23	9	15	12	20	61

Stand. dev. for protein components from uninfected sera is 0.9 to 1.

mechanism to keep osmotic pressure within certain limits(8). The rise in globulin has been attributed to (a) antibody formation, (b) alteration of relative rates of production and utilization of albumin and globulin, and (c) traumatic shock(8,9,10). Alpha-1 and alpha-2 globulin concentration did not vary more than 3-4 mg ml throughout the experiment. Changes in concentration for both alpha-globulins occurred within the 4-5 month infection period (Table I). The beta-globulin fraction exhibited very little change. Lack of variation in this fraction at 3-4 months, when significant alterations were taking place, could be due to the fact that as the gamma-globulin rose in concentration it tended to mask the increase, if any, of the beta-fraction in electrophoretic patterns.

Summary and conclusions. 1. New serum components were not detected in infected animals. 2. Significant increases in gamma-globulin fraction were observed during 3-4 month infection period. 3. The rise observed in gamma-globulin coincided with highest agglutinin titers in sera of infected animals during 12 months experimental period. 4. Absorption studies are being carried out in an effort to determine if the gamma-globulin rise is due mainly to antibody formation.

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Influence of Urine Volume on Output of Corticoids by Normal Human Subjects.* (24447)

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It has been noticed that the output of adrenal cortical steroids appeared to be dependent upon urine volume. This does not imply that the cortex was activated by changes in water balance, but rather that alteration in filtration and/or reabsorption in the kidney with urine flow influenced steroid output. Lloyd (1) has presented data to demonstrate this relationship in Addisonian and normal patients. Marks and Leaf(2), Kalant(3) and Lazo-Wasem *et al.*(4) have confirmed the results in dog, rat, and guinea pig. This is a report of the influence of urine volume on urinary steroid output in normal subjects under various conditions of salt and water load. As a corollary, renal clearances of steroids were also determined.

Methods. Our tests were performed on 24 normal medical students. Three different procedures were adopted to provide short and long term evaluation of the problem. 1. *Long term study (day by day).* Students on a relatively constant diet and in good health, collected 24 hour urine specimens kept in the cold and preserved with small amount of glacial acetic acid. The subjects voluntarily restricted water to a minimum, or drank water to excess, or maintained a normal water intake throughout the 24 hours. In another series of tests the subjects took 40 mg of hydrocortisone/day in a divided dose while controlling water intake. Each sample was

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