onstrate the secretion of SCA into the fluid content of the organ. These findings are in keeping with the data reported previously (1,2).

SCA was also found to be present within the ejaculatory ducts. This explains the reactivity of prostate extracts with anti-SCA sera(1,2), because the ejaculatory ducts necessarily are included in these extracts.

Summary. Fluorescein-conjugated anti-seminal plasma immune globulin stains specifically human and rabbit seminal spermatozoa. Testicular spermatozoa lack reactivity with this reagent for SCA. The study of the components of the male genital tract of the rabbit indicates that SCA is produced in the seminal vesicle.

We gratefully acknowledge the opportunity of learning much about the technical aspects of these procedures during visits to the laboratories of Drs. E. Witebsky, and E. H. Beutner, Univ. of Buffalo School of Medicine, and Drs. B. Seegal and K. C. Hsu, College of Physicians and Surgeons, New York.

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The Kinetics of Heat Inactivation of Bovine Serum Agglutinins for Brucella.* (27271)

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Hess and Roepke(1), by a chromatographic method, demonstrated the presence of agglutinins for Brucella in bovine sera with low titers from herds apparently free of brucellosis. The method distinguished these apparently nonspecific agglutinins from the classical type of agglutinin in sera from Brucella-infected cows.

Differential tests to distinguish between nonspecific and specific agglutinins based on heat inactivation of the nonspecific agglutinin were devised by Hoerlein(2), Morse *et al.*(3) and Amerault *et al.*(4). The acidified plate antigen (APA) test of Rose and Roepke (5) also differentiated the nonspecific from the specific agglutinins.

Field studies have shown that the 18-hour tube agglutination test at 56°C(3) could not consistently distinguish the serum of Brucella-infected cows from the serum of cows which contained the nonspecific agglutinin; however, the 15-minute tube agglutination test at 65°C(4) gave results in better agreement with epidemiological, bacteriological, and clinical information. These data suggested that nonspecific agglutination reactions might be caused by several nonspecific agglutinins of differing heat labilities.

This report provides quantitative data on studies of heat lability of 3 types of agglu-

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tinins for Brucella in various bovine sera.

Materials and methods. Sera containing nonspecific agglutinins for Brucella were obtained from cows that met the following criteria(6): 1) The serum agglutination titers were greater than or equal to 1:100 and were negative on both the APA test at a pH of 3.7 and the 15-minute tube test at 65°C. 2) The cows were from herds with a history of no previous exposure to Brucella and in which all other animals in the herd had negative serum agglutination tests at a dilution of 1:50. 3) Bacteriological examinations at parturition did not reveal Brucella in the colostral milk and the placental cotyledons. Furthermore, the calves were born at term and were apparently healthy. 4) At necropsy, Brucella were not isolated from the spleen, liver, kidneys, or the visceral and supramammary lymph nodes.

Sera containing specific agglutinins were obtained from cows from which *Br. abortus* had been isolated. Preliminary tests on sera from 9 infected cows determined the range of heat inactivation rates. The 2 sera representing the extremes of high and low inactivation rates were studied in detail.

Serum used as a nonagglutinating control was obtained by pooling sera from 20 to 30 cows selected on the basis of negative Brucella agglutination tests at a dilution of 1:6. Prior to heating, titers of both specific and nonspecific sera were adjusted to levels suitable for agglutinin assay (1:100 to 1:300) by dilution with the control serum pool.

Antigens. The *Br. abortus* tube test and plate test antigens were furnished by the Agricultural Research Service, U. S. Dept. of Agriculture, Beltsville, Md.

Inactivation and agglutinin assay. The kinetic constants were calculated from measurements of the agglutinins remaining after a given period of exposure to heat. The sera containing specific and the nonspecific agglutinins were handled identically as follows: the lower half of a 15 × 150 mm test tube was immersed in a mechanically agitated water bath. Temperature of the bath was controlled to within ± 0.3°C by a bimetallic thermostat. Temperature of the test solution was obtained from a thermometer, graduated in 0.1°C, and placed with the bulb in close proximity to the base of the tube.

Two ml of serum, adjusted to a suitable titer in control serum, was placed in the immersed tube. After 4 minutes were allowed for temperature equilibration (zero time) and at all subsequent predetermined times, a 0.4 ml aliquot was removed from the tube with a pipette. To halt the inactivation reaction in a uniform manner and thus obtain a more precise estimate of the period of exposure to heat, the aliquot was removed with a cold pipette and quickly distributed in proportional amounts into a series of 5 or 6 test tubes to which 4 ml of cold antigen was added immediately. The titration tubes were then incubated at 37°C for 48 hours.

After incubation, the tubes were centrifuged in a Servall SS-1 centrifuge at a 2000 rpm (480 × *g*) setting for 5 minutes, including time for acceleration. Both the speed and time of centrifugation were critical in adjusting the sensitivity of the test. After centrifugation, the upper 2 ml of the supernatant fluid was withdrawn with a pipette, placed in a photoelectric turbidimeter (Libby Photronreflectometer)§ and the turbidity reading recorded. The instrument was previously standardized by adjusting the maximum deflection of the galvanometer while 2 ml of standard antigen was in the cuvette; this corresponded to no agglutination. A distilled water blank was used to determine minimum deflection, which corresponded to complete agglutination. Intermediate turbidity values were obtained by diluting the standard antigen in saline. The graph of these values was linear.

A plot of the turbidimetric data *vs* the reciprocal of the serum dilution was made, and the agglutinin titer was arbitrarily selected at the intercept of 50% turbidity. One to 5 replicate titer determinations were made on each serum sample at the specified tempratures. The results of the titer determinations were reproducible within 5%.

The formulas applicable to first order reactions were used on the basis of the results obtained. The velocity constant, *k*, was calcu-

§ R. L. Libby, Pearl River, N. Y.

lated for each determination by use of the formula(8): $k = \frac{2.303}{t_2 - t_1} \log \frac{C_1}{C_2}$, where C_1 = titer at time t_1 in minutes and C_2 = titer at time t_2. As an alternative method, which was equivalent, k was evaluated from the slope of the line. The half-life in minutes was calculated by using the formula(7): $t_{1/2} = \frac{0.693}{k}$.

Results. Fig. 1 shows that a direct linear relationship exists between the logarithm of the reciprocal of the titer and the time of the heat treatment which indicates that the inactivation was a first order reaction.

Two types of nonspecific agglutinin for Brucella could be distinguished from the rates of inactivation at different temperatures. In the upper part of Table I the data are arranged into 2 groups depending on the lability of the agglutinins at 56°C. There was a 2- to 3-fold difference in the half-life values between the 2 types. The heat inactivation rates were not only different at a given temperature, but also the change of inactivation rates with the same change in temperature was distinctly different.

Temperatures above 63°C inactivated the nonspecific agglutinins at a rate too fast to be measured by these methods. Conversely, specific agglutinins were only partially inactivated after 30 minutes at 63°C (Table I). Hence, the quantitative rate differences of the heat inactivation of both specific and nonspecific agglutinins can best be directly compared at 63°C. At this temperature they differed by at least a factor of four.

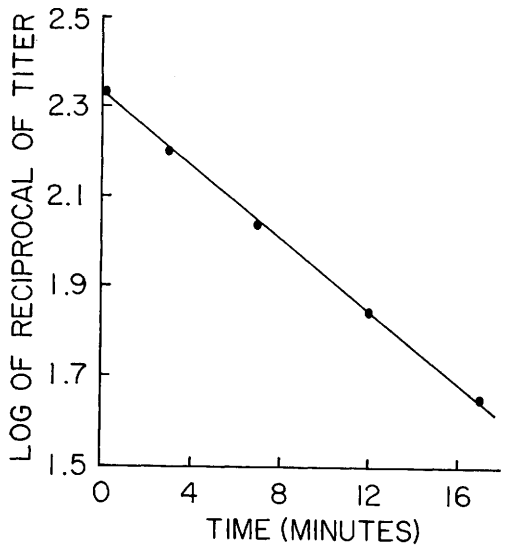

FIG. 1. A representative graph that shows the straight line relationship between time and the log of reciprocal of titer. This is nonspecific serum 1199 at 62°C.

TABLE I. Bovine Serum Agglutinins for Brucella; Half-Life at Various Temperatures.

Temp., °C	Nonspecific agglutinins: 56°C stable (from 3 cows) Mean*	Range	56°C labile (from 2 cows) Mean*	Range
	Min.		Min.	
61	30.6	30.2–31.6	8.8	6.0–10.5
62	11.0	8.2–13.3	7.0	5.3– 8.2
63	7.6	5.2– 9.1	4.6	3.8– 5.4
65	‡	—	‡	—

Temp., °C	Specific agglutinins: Least stable Mean†	Range	Most stable Mean†	Range
	Min.		Min.	
61	>40.0	—	>40.0	—
62	>40.0	—	>40.0	—
63	29.5	29.0–30.0	>40.0	—
65	18.7	15.1–22.4	30.8	30.0–31.5

* Each value represents mean of 15 to 18 determinations.

† Means of 2 to 6 determinations representing extremes in heat lability; serum agglutinins from 7 other infected cows fell between these values on 1 or 2 determinations.

‡ Inactivated too fast to be measured accurately.

Discussion. These data indicate 3 general types of agglutinin for Brucella in bovine serum. The 2 types of nonspecific agglutinins are commonly found in serum from animals with low agglutinin titers in herds with a negative history of Brucella infection. In addition to the 5 cows in this report, tissues from 21 other cows and milk from an additional 14 that met the criteria for nonspecific serum agglutinins stated under *Materials and methods* were cultured for Brucella. No isolations were obtained.

These data also support the work of Rose (8), which shows that at least 2 types of protein are presently designated as nonspecific agglutinins and both types are distinguishable from specific agglutinin by the APA test since both types differ from the agglutinin commonly associated with Brucella in-

fection in the cow. Within the limits of this study, the considerable differences in rates of inactivation also permit one to distinguish both types of nonspecific agglutinins as well as the specific agglutinin by use of a combination of both 56°C and 65°C heat inactivation tests.

The extremes in the range of rates of heat inactivation of specific agglutinins from the 9 cows studied, happened to be represented by one cow with a 12-month history of infection (least stable) and another cow with a known infection of the order of 6 weeks (most stable). The number of sera involved precludes any interpretation of this observation.

Summary. Two types of nonspecific agglutinins for Brucella were distinguished through kinetic studies of their inactivation by heat. Rates of inactivation of specific and nonspecific agglutinins differ to an extent that can be accounted for only by a difference in molecular species. Heat inactivation of nonspecific and specific agglutinins for Brucella is a first order reaction.

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Metabolism of Specifically Labelled Glucose in Adipose Tissue from Alloxan-Diabetic Rats.* (27272)

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Metabolic studies with adipose tissue *in vitro*, using specifically labelled glucose, have suggested the phosphogluconate oxidation pathway as a major route of glucose metabolism. Milstein(1) comparing the oxidation of glucose-1-C^{14} and glucose-6-C^{14} to CO_2 in rat adipose tissue concluded that this pathway was the principal route of glucose metabolism and was preferentially stimulated by insulin. Likewise, in adipose tissue from alloxan-diabetic animals, the conversion of glucose carbon-1 to CO_2 was found to be proportionally more depressed than was glucose carbon-6 to CO_2, suggesting a selective decrease in this pathway. Winegrad and Renold(2), reporting on the effects of insulin added *in vitro* on the oxidation of specifically labelled glucose to CO_2 in a similar system, corroborated the findings of Milstein. However, they also measured the recovery of labelled carbon in tissue fatty acids and found that although insulin greatly increased the incorporation of both carbon-1 and carbon-6 into fatty acids, the ratio of C1/C6 in fatty acids in both control and insulin-stimulated tissues remained approximately 0.5. Subsequently, in experiments(3,4) in which both concentration of glucose and/or insulin were independently varied, this ratio persisted with all experimental combinations, suggesting that the action of insulin was to accelerate glucose entry into the metabolic pathways and not to stimulate selectively any specific pathway of glycolysis.

The purpose of this report is to extend the above studies to include not only the oxidation of specifically labelled glucose to CO_2

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