blood serum. With abnormal spinal fluid such as those obtained from syphilitic patients, higher inhibition titers were obtained, as to be expected. Saliva gave definite inhibition though only in low titer, in conformity with the known presence in this secretion of small amounts of serum globulin. Only doubtful reactions were obtained with normal human semen and urine.

Experiments were also conducted to determine the ability of animal sera to inhibit the anti-human-globulin serum. As shown in the table, serum from horse, ox, and rabbit had no apparent inhibiting action. Rhesus serum weakened the clumping, even when highly diluted, but did not inhibit the reaction completely. This indicates the presence in rhesus serum of a serum globulin chemically related to, though qualitatively different from the globulins in human serum.

Summary and conclusions. 1. A new serological method of demonstrating human serum globulin has been described, based on its ability to inhibit the agglutinating action of anti-human-globulin serum for Rh-positive human red cells coated with Rh_0 univalent antibody.

2. Using this technic, further evidence was obtained that the Rh antibody coating sensitized Rh-positive cells is a serum globulin.

3. With the new technic it is possible to demonstrate the presence in normal spinal fluid, and in saliva of small amounts of serum globulin, in proportion to the known concentration in these fluids. Normal human semen and urine failed to inhibit the antiglobulin serum.

4. Umbilical cord serum inhibited anti-globulin serum to the same titer as adult serum.

5. Serum from horse, ox and rabbit did not inhibit the anti-human-globulin serum, while rhesus monkey serum merely weakened its reactions.

6. The new technic may find application not only in clinical medicine, but also in forensic work for the examination of blood stains, and in studies on biochemical evolution.

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17096. Relationship of Catalase Activity to Virulence in *Pasteurella pestis*.

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Huddleson[1] and Merz[2] observed that virulent *Brucella* organisms showed greater catalase activity than avirulent strains. This investigation was undertaken to determine whether a similar relationship exists for *Pasteurella pestis*. It was felt that such findings might be applied to the development of a rapid, inexpensive, *in vitro* method for virulence titration. Furthermore, such a relationship would indicate a basis for approaching the problem of virulence along the lines suggested by Jawetz and Meyer[3] who state, "It appears probable that the final criterion of virulence will have to rest on a biological and chemical identification of the bacterial enzyme systems which are responsible for the multiplication of the plague bacilli in the animal host."

Procedure. Huddleson's[1] method was modified as follows to measure the catalase activity of virulent and avirulent strains of *P. pestis*. Standard bacterial suspensions were prepared from twice-washed, 24 hour, heart infusion broth cultures suspended in phosphate buffer and adjusted to a turbidity of 100 ± 5 on the scale of the Klett-Summerson photoelectric

[1] Huddleson, I. F., Univ. Mich. Agri. Exp. Station, Technical Bull. 182, January, 1942.

[2] Merz, P., Uber die Katalasen der Brucellen, Inaugural Dissertation for the Degree of Doctor of Veterinary Medicine, University of Zurich, 1938.

[3] Jawetz, E., and Meyer, K. F., *Am. J. Path.*, 1944, **20**, 457.

TABLE I.
Relationship of Catalase Activity to Virulence in *Pasteurella pestis*.

Pasteurella pestis strains		Catalase determinations	Viable bacteria ($\times 10^8$/ml)	Virulence		Catalase activity (ml of H_2O_2)			
						Min. after adding bacterial suspension			
No.	Name			No. of titrations	LD_{50}	5	15	30	60
H-16	139-L	8	2.9	5	90	9.7 ± 1.0	15.4 ± 1.2	18.1 ± 1.8	19.3 ± 0.1
3	Yreka	8	3.3	5	110	10.3 ± 3.1	14.5 ± 2.8	16.6 ± 3.3	19.3 ± 0.1
14	F 9650	2	3.3	1	90	8.1	11.3	15.4	17.7
18	0 9817-C	2	2.7	1	50	10.8	13.9	16.7	17.7
5	327	8	2.2	5	20	8.5 ± 1.0	13.2 ± 2.1	15.6 ± 2.3	17.3 ± 0.7
13	I-72	2	2.3	1	30	9.7	12.6	14.8	17.2
1	Shasta (H-1)	2	3.0	2	230	14.6	14.9	15.4	17.1
17	" 412	2	2.6	1	240	6.5	11.2	14.6	16.7
2	" (H-2)	2	2.2	2	140	13.5	13.6	14.3	16.5
10	Webster	2	6.5	2	1100	12.8	13.4	14.7	16.4
12	de Rosa	2	2.4	2	3300	12.8	13.4	14.2	16.2
19	—	2	3.5	1	70	7.0	9.7	12.1	15.5
15	F 9581	2	1.7	—	—	7.4	10.1	13.7	14.7
11	—	2	2.1	1	2200	6.7	9.5	11.3	13.6
								Mean	16.7 ± 0.4
C-11	Soemedang	2	8.4	2	†	3.3	6.4	7.9	8.2
5	TRU	2	20.0	2	†	3.3	7.0	7.7	8.0
7	53 H-1	2	9.3	2	†	2.9	6.0	6.6	7.0
4	K-120	2	23.0	2	†	2.0	4.4	5.1	5.2
K-1	A-1122	2	6.9	2	†	3.4	4.7	4.9	5.0
C-12	14	2	8.0	2	†	2.1	4.0	4.6	4.9
6	Tijwidej	2	8.8	2	†	1.6	3.8	4.4	4.7
3	Java	2	5.6	2	†	1.2	3.1	3.8	3.8
10	EV 76	2	7.6	2	†	1.3	2.8	3.4	3.6
8	A 1122	2	11.0	2	†	1.1	2.7	3.1	3.4
9	Bombay	2	8.5	2	†	0.6	1.8	2.2	2.4
								Mean	5.1 ± 0.5

* LD_{50}—No. of organisms killing 50% of 8-14 weeks-old mice in 14 days. Reed and Muench.[8]

† 2×10^4 organisms injected subcutaneously into 8-12-weeks-old mice caused no deaths.

Catalase activity: ml of 0.1 N H_2O_2 decomposed in time interval shown.

[8] Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

colorimeter using a green filter (540 mu) and a 15 mm cuvet. Five ml of this suspension were pipetted into the substrate solution (25 ml of 0.48 N H_2O_2 in 0.03 M Sorenson phosphate buffer at pH 6.80) in a 250 ml Erlenmeyer flask shaken at 100 oscillations per minute. At 5, 15, 30 and 60 minute intervals, 5 ml samples were removed to beakers containing 3 ml of 7 N H_2SO_4 and titrated with 0.1 N $KMnO_4$. Blank titration showed that no spontaneous decomposition of H_2O_2 occurred during the experiment. Enzyme activity is proportional to the quantity of H_2O_2 decomposed.

The viable bacterial count of the standard suspensions was determined by surface plating, and virulence was titrated by injecting 0.2 ml of appropriate dilutions subcutaneously into 8-14 week old mice, 10 mice being used for each of 3 serial dilutions.

Results. The results are presented in Table I. Inspection of Table I reveals that the catalase activity of virulent strains was significantly greater than that of avirulent strains. In the 14 virulent strains studied, the enzyme activity, as expressed in ml of 0.10 N H_2O_2 decomposed in 60 minutes, ranged from 12.9 to 19.9 ml with a mean value of 16.7 ml. On the other hand, for 11 avirulent strains, values of 2.1 to 8.6 ml with a mean value of 5.1 were found. The difference between the mean values of virulent and avirulent organisms is statistically significant.

Some of the factors involved in the catalase activity of *P. pestis* were investigated and the influence of a number of conditions was studied. It was observed in early experiments that growth on such different media as heart infusion broth (Difco), nutrient agar (Difco), or horse blood agar was without effect on catalase activity. The number of viable organisms was observed to undergo a 5-fold decrease when the incubation period was extended from 24 to 72 hours. Despite this decrease, no differences in catalase activity were exhibited by standard suspensions prepared from cultures after 24, 48, or 72 hours of incubation. The stability of the enzyme was also demonstrated by experiments in which standard suspensions were stored at 5°C for as long as seven days without change in activity. A final substrate concentration of 0.40 N H_2O_2 was found to be most suitable for our purposes. No significant differences in catalase activity were observed over a temperature range of 24-32°C or with changes in shaking rate from stationary to 103 oscillations per minute.

The kinetics of catalase activity in *P. pestis* under the conditions outlined appear to be similar to other findings[4-7] reported for cell-free preparations of this enzyme and apparently follow the curve for a monomolecular reaction (Table I).

The use of catalase activity as an *in vitro* screening test for virulence is suggested by the differences between virulent and avirulent strains. Experiments are being continued to determine whether this test will distinguish varying degrees of virulence during the *in vitro* attenuation of some of the virulent strains studied.

Summary. 1. Optimal conditions for catalase activity in *P. pestis* were determined and the catalase activity of 14 virulent and 11 avirulent strains was measured.

2. The catalase activity of virulent strains was significantly greater than that of the avirulent strains.

3. The possibility of using catalase activity as a screening test for determining virulence *in vitro* has been indicated.

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