STUDIES ON THE ANTIGENIC STRUCTURE OF VIRULENT AND NONVIRULENT BRUCELLAE WITH THE AID OF THE AGAR GEL PRECIPITATION TECHNIQUE*

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OLITZKI AND SULITZEANU (1957, 1958) examined the antigen-antibody reaction between *Brucella suis* antisera and their corresponding antibodies by the Ouchterlony (1948) technique and noted the appearance of at least 6 precipitation lines.

Further experiments of Olitzki and Sulitzeanu (1958) distinguished the different antigens of *Br. suis* by chemical and physical procedures and determined the conditions under which a maximal number of precipitation lines in agar could be produced. Sulitzeanu (1958) showed that no one of the precipitation lines could be correlated with the presence or absence of agglutinins in the immune serum. He found, however, a good correlation between agglutinating and passive protection power of sera. For all these experiments *Br. suis* was chosen because of the ease with which strongly precipitating antisera could be obtained against it.

The general plan of the present work was as follows : rabbits were immunized with antigens derived from different strains of the genus *Brucella*. These antisera were tested for their precipitating power against homologous and heterologous antigens. Finally, quantitative titrations similar to those employed by Feinberg (1956) and absorption tests were carried out, in order to determine whether there exist differences in the antigenic structures of *Br. abortus*, *Br. melitensis* and *Br. suis*.

MATERIAL AND METHODS

The experimental procedures have been described in the earlier papers of Olitzki and Sulitzeanu (1957, 1958) and Sulitzeanu (1958). Certain modifications of the techniques and a few additional details are given below :

Strains

Br. melitensis, strain 5, *Br. suis*, strain 39, and *Br. abortus*, strains 2308 and 19 were employed by Olitzki (1953) in experimental infections of mice with the aid of the mucin technique and subcultured for 6 years on trypticase soy agar. The vaccine strain Rev. I of *Br. melitensis* was isolated by Herzberg and Elberg (1955) and employed as vaccine strain by Elberg and Faunce (1957), while the virulent strain 6015 of *Br. melitensis* was used by them as challenge. The strain designated as Ru is identical with that employed by Vershilova (1954) and Vershilova and Kokorin (1954) in immunization of individuals exposed to brucellosis infections. Another *Br. abortus*, strain 19, was kindly provided by Dr. H. S. Cameron, School of Veterinary Medicine, University of California, Davis, California, and was designated as strain 19 Da.

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Preparation of antigens for immunization and preparation of immune sera

The antigens were prepared by the same method as those of *Br. suis* described by Olitzki and Sulitzeanu (1958). For the preparation of immune sera a mixture was prepared which contained 7·65 ml. Kleerol, 1·35 ml. Arlacel A, 9·0 ml. physiological saline and 180 mg. acetone-killed and dried bacteria. The adjuvant mixture contained 10 mg. of bacteria per ml. Groups of rabbits received subcutaneously 4 doses of 1·0 ml. and then 4 doses of 2·0 ml. at weekly intervals. Between the fourth and the fifth injection a 2 week interval was left and a preliminary examination of the serum carried out. Blood was obtained by cardiac puncture 2 weeks after the eighth injection.

Antigens for gel precipitation

The bacteria were repeatedly disintegrated in a disintegrator (Mickle, Mill Works, Gomshall, Surrey). Since the experiments of Sulitzeanu (1958) showed that the insoluble sediments do not produce additional lines, no further attempts to remove them by centrifugation were made.

Agar for gel precipitation

Difco Bacto-Agar was used through all experiments. Since preliminary experiments showed that at lower than physiological salt concentrations sharper precipitation lines appear, the following quantities of salts were added to 1 litre of 1 per cent agar: 0·353 g. NaH_2PO_4, 0·639 g. Na_2HPO_4 and 0·172 g. NaCl. The resulting pH was 7·2. Since each plate contained only 7·0 ml. of agar and 0·7 ml. of antigens and antibodies in physiological saline distributed in seven holes, this low salt concentration of the agar proved to be favourable for the development of the precipitation lines.

Diffusion test

In order to assure a constant reproduction of the results, all experiments were carried out on small Petri dishes with a diameter of 5·0 cm. The holes for the antigens and sera were made with the aid of cutters kindly provided by Dr. C. Lamanna, Scientific Director, Naval Biological Laboratory, Oakland. All the figures attached to this paper show in 2-fold magnification the position of the holes, which was never changed during the entire work. This method, introduced by Feinberg (1956) also had the advantage that volumes no greater than 0·1 ml. per hole could be employed. Since the diffusion of the reagents from the peripheric holes was practically possible only in one direction from the periphery to the centre, the reaction lines appeared quickly and the first results were observed after an incubation of 20 hr. The plates were incubated in plastic bags closed with rubber bands. Cotton-wool moistened with water served to prevent drying.

RESULTS

In the first 3 experiments an undiluted immune serum was placed in the central hole and undiluted bacterial antigens around it. The results of these experiments are demonstrated in Figs. 1, 2 and 3. In all these figures a diffuse precipitation zone appears around the serum source, and was designated as D. This zone was then followed by 6 sharp lines, which are easily visible in Figs. 1 and 2 opposite the *Br. suis* antigen. Generally, the thickness of these lines decreased from the centrum to the periphery. Line 6, nearest to the central serum source, was the thickest, and lines 1 and 2, nearest to the peripheric antigen sources, were the weakest and were not regularly produced with each antigen. The only exception was the appearance of an extremely strong line near the antigen source of *Br. abortus*, strain 19. This crescent-shaped line around the antigen sources A is well visible in Figs. 1 and 3. Those lines situated nearest to the serum source, such as lines 4, 5 and 6, were produced by all strains. It seems, therefore, that they were produced by non-specific antigens. On the

other hand, the several crossings of the lines (*e.g.* in Fig. 1 between antigen sources M and A and in Fig. 2 between M and A, M and S and between S and A) indicated that there existed quantitative differences of the antigenic pattern.

Since these experiments with undiluted sera and antigens did not permit final conclusions, further experiments were carried out, where antigens were placed around the central hole containing diluted antiserum. An experiment of this kind is reproduced in Fig. 4, where the central hole contains *Br. suis* antiserum diluted 1/3. The lines 1, 2 and 3 have completely disappeared. There remained only the diffuse precipitation zone around the serum source, the thick line 6, the sharp and thin line 5 and the more thick and diffuse line 4. The 2 latter appeared opposite *Br. melitensis* and *Br. suis* and disappeared opposite *Br. abortus* strain 19, while line 6 was common for all the 3 antigens. On the other hand, there appeared again the sharp crescent-shaped precipitation line near the source of *Br. abortus* 19, which was already shown in Figs. 1 and 3. In order

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EXPLANATION OF PLATES

FIG. 1.—Diffusion pattern of three antigens reacting with undiluted *Br. suis* antiserum. I.S. Suis = *Br. suis* immune serum. A = *Br. abortus* 19; M = *Br. melitensis* 6015; S = *Br. suis* 39.

FIG. 2.—Diffusion pattern of three antigens reacting with undiluted *Br. melitensis* antiserum. I.S. MEL. = *Br. melitensis*, strain 5, immune serum. Antigens the same as in Fig. 1.

FIG. 3.—Diffusion pattern of three antigens reacting with undiluted *Br. melitensis* antiserum. I.S. REV. I = *Br. melitensis*, strain Rev. I, immune serum. Antigens as in Figs. 1 and 2.

FIG. 4.—Diffusion pattern of three antigens reacting with *Br. suis* antiserum, diluted 1 : 3. Abbreviations as in Fig. 1.

FIG. 5.—Varying amounts (mg.) of *Br. melitensis*, strain 6015, antigen reacting with *Br. melitensis*, strain 5, immune serum.

FIG. 6.—Varying amounts (mg.) of *Br. abortus*, strain 2308, antigen reacting with *Br. suis* antiserum.

FIG. 7.—Varying amounts (mg.) of *Br. melitensis*, strain Rev. I, antigen, reacting with *Br. melitensis*, strain 5, antiserum.

FIG. 8.—Varying amounts (mg.) of *Br. melitensis*, strain Rev. I, antigen reacting with its homologous antiserum.

FIG. 9.—Minimal amounts (μg.) of *Br. melitensis*, strain 6015, and strain Rev. I antigens reacting with *Br. suis* immune serum. Rev = non-virulent strain Rev. I; Vi = virulent strain 6015.

FIG. 10.—Minimal amounts (μg.) of *Br. melitensis*, strain 6015, and *Br. suis*, strain 39, antigen reacting with *Br. suis* immune serum. Abbreviations as in Fig. 1.

FIG. 11.—Varying amounts (mg.) of the vaccine strain Ru reacting with *Br. melitensis* strain Rev. I antiserum.

FIG. 12.—Varying amounts (μg.) starting from 625 μg. of *Br. melitensis*, strain 6015, antigen reacting with its homologous antiserum.

FIG. 13.—Varying amounts (μg.) starting from 625 μg. of *Br. melitensis*, strain 6015, antigen reacting with *Br. melitensis*, strain Rev. I, antiserum.

FIG. 14.—Varying amounts (μg.) starting from 1250 μg. of *Br. melitensis*, strain Rev. I, antigen reacting with *Br. melitensis*, strain 5, antiserum.

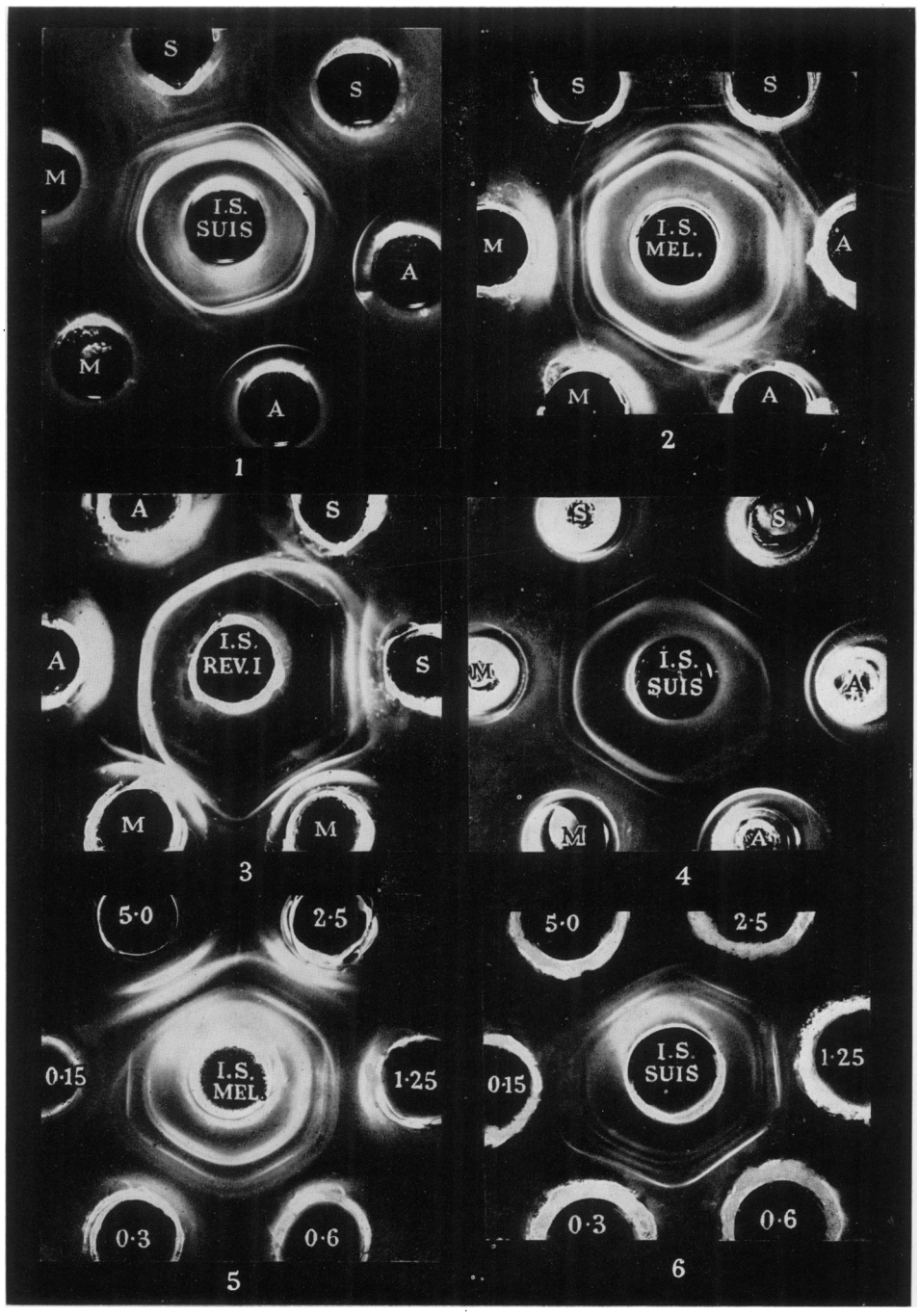
FIG. 15.—Varying amounts (μg.) starting from 1250 μg. of *Br. melitensis*, Strain Rev. I, antigen reacting wilh its homologous antiserum.

FIG. 16.—*Br. melitensis*, strain 6015, antigen reacting with varying dilutions of its homologous immune serum. AGMEL = antigen of *Br. melitensis*; I.S. = homologous immune serum.

FIG. 17.—*Br. abortus*, strain 19, antigen reacting with varying dilutions of its homologous immune serum. AG19 = antigen of *B. abortus*, strain 19. IS = homologous immune serum.

FIG. 18.—*Br. suis* antiserum reacting with antigen of *Br. melitensis*, strain 6015, *Br. abortus*, strains 19 Je. and 19 Da.

FIG. 19.—*Br. suis* antiserum antisera reacting with antigen *Br. abortus*, strains 2308, 19 Je. and 19 Da.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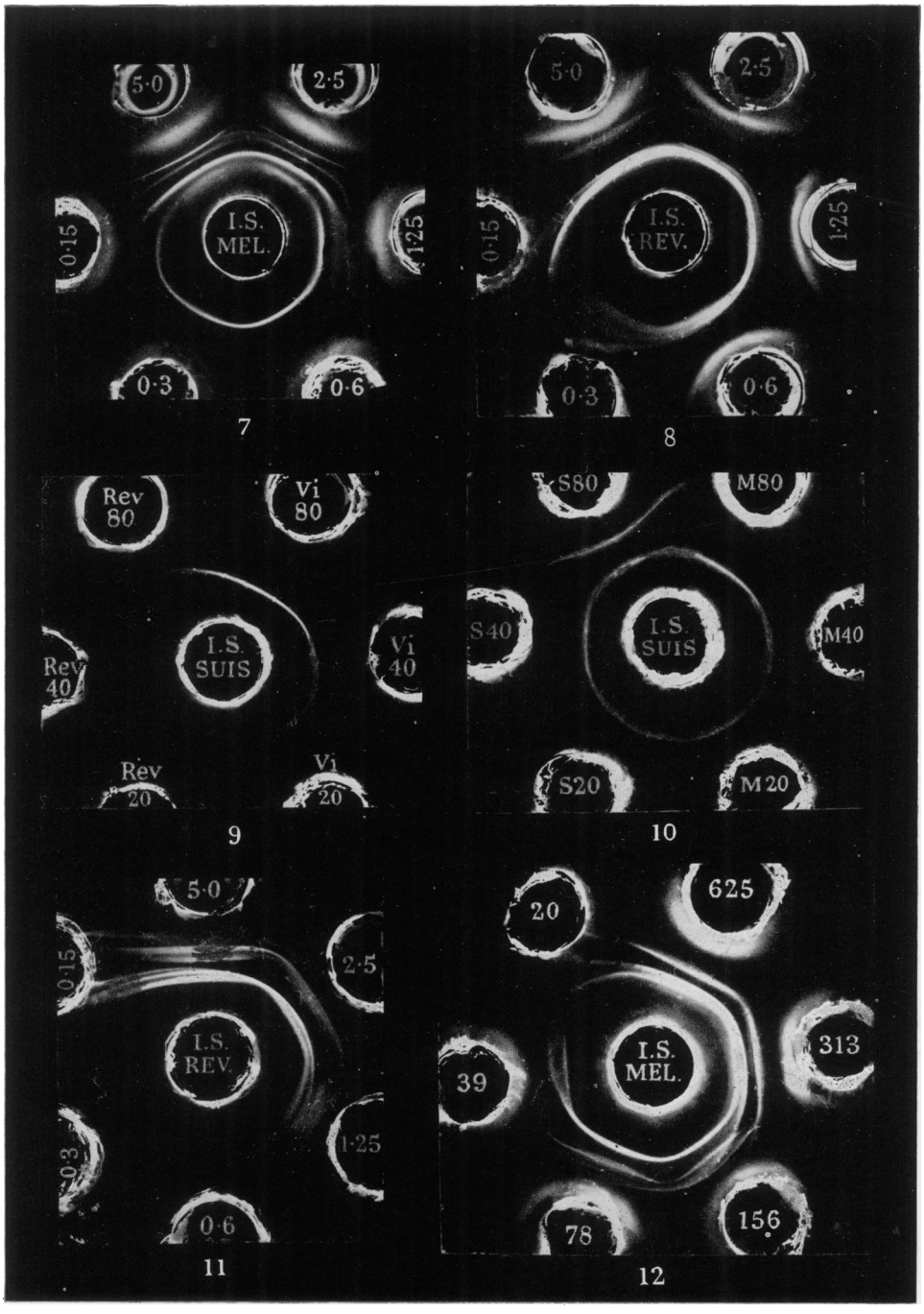
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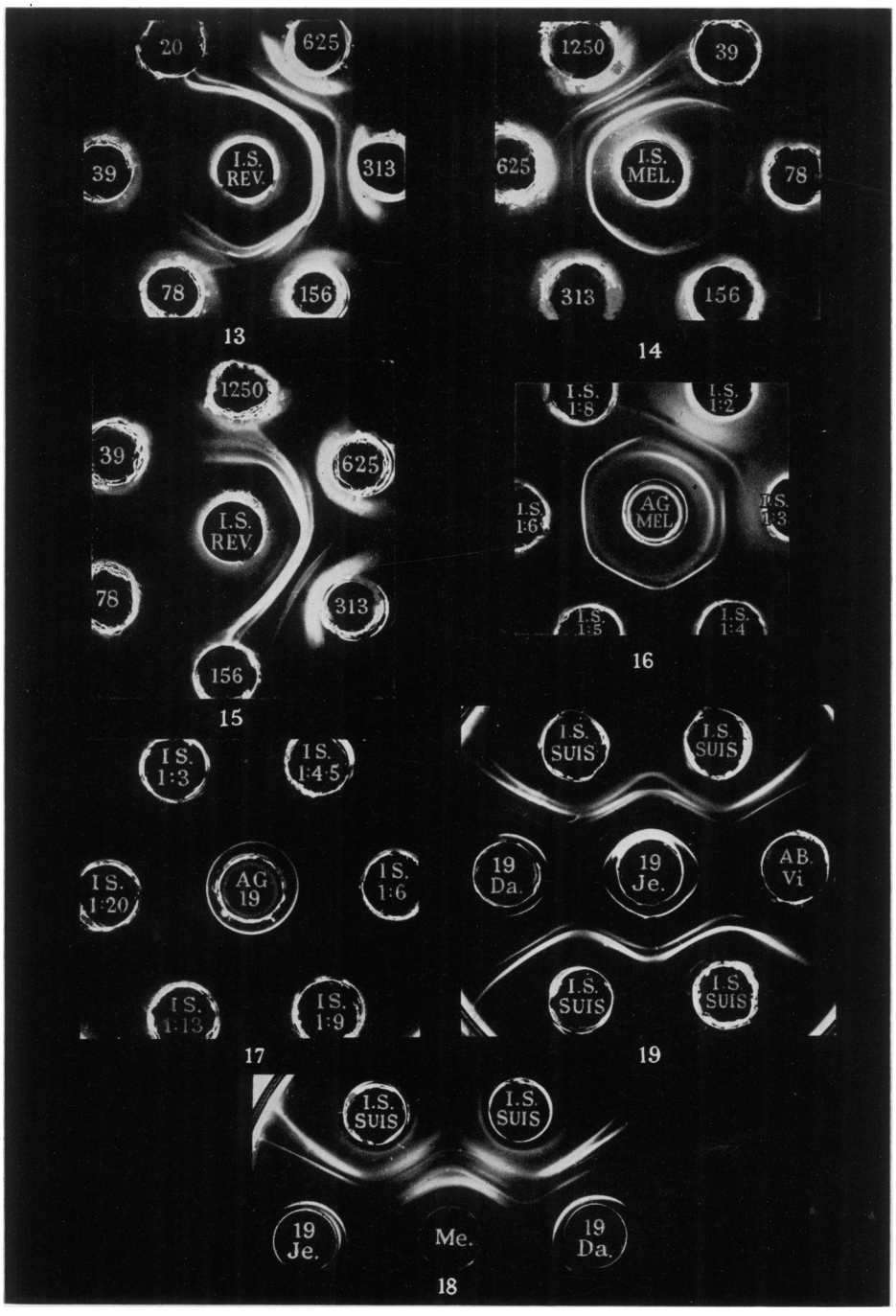

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to determine whether these and similar diffusion patterns were the results of quantitative or qualitative differences in the antigenic structures, the following experiments were carried out with varying amounts of bacterial antigen placed around a constant amount of undiluted antiserum.

A typical experiment is shown in Fig. 5. Around the central source of 0·1 ml. antiserum varying quantities of *Br. melitensis* strain 6015 antigen were placed. The figure shows that the diffuse zone D, and the lines 6, 5 and 4 still persisted at 0·15 mg. Line 6 became split up in 2 partial lines. Other lines, as the crescent-shaped line 1 appeared only in the vicinity of at least 0·3 mg. and line 2 at 2·5 mg. antigen. Another experiment of this kind is presented in Fig. 6, where varying quantities of *Br. abortus*, strain 2308, antigen had reacted with *Br. suis* antiserum. The lines 6, 5, 4 and 3 appeared in a range from 5·0–0·15 mg. antigen. However, opposite the hole containing 0·3 mg. of antigen the lines were more distinct than opposite all the other holes containing varying amounts of antigen.

While the virulent strains *Br. melitensis* 6015 and *Br. abortus* 2308 produced line 6 even in the presence of 0·15 mg. of antigen, the vaccine strain *Br. melitensis*, Rev. I, showed a somewhat weaker precipitating potency. Fig. 7 shows that with *Br. melitensis* immune serum line 6 appeared still opposite 0·15 mg. of antigen, while according to Fig. 8 the reaction almost disappears at 0·3 mg. of antigen. All the other reactions appeared in the presence of great amounts of antigen ranging from 5·0 to 1·25 mg. with the exception of the line 1, which in the reaction with the strain 5 antiserum was still visible in the presence of 0·15 mg. and with the Rev. I antiserum in the presence of 0·6 mg. of antigen.

In order to determine the minimal precipitating quantities of the virulent strains, smallest quantities of the antigens were brought in contact with the corresponding antisera. Fig. 9 and 10 show the results of 2 titration experiments. In Fig. 9 the precipitating potency of the virulent strain *Br. melitensis* is compared with that of the nonvirulent strain Rev. I towards a *Br. suis* antiserum. While the antigen of the virulent strain still reacted when only 20 μg. of bacterial substance are present, the reaction of Rev. I antigen did not appear, although 80 μg. were present. In Fig. 10 the precipitating activities of *Br. melitensis*, strain 6015, and *Br. suis* antigen were compared. The lines 6 of both strains appeared in the presence of 20 μg., while line 5 of *Br. suis* still appeared in the presence of 80 μg. of antigen.

Relatively small amounts of bacterial substance of the vaccine strain Ru. reacted with *Br. melitensis* (5) antiserum while with *Br. suis* and *Br. melitensis* (Rev. I) antiserum positive reactions were observed in the presence of at least 625 μg. of bacterial substance as demonstrated in Fig. 11.

In order to determine whether the line which still appears at minimal antigen concentrations is identical with line 6, experiments were started with maximal amounts of 625 and 1250 μg. of bacterial substance, decreasing to 39 and 20 μg. respectively. These experiments are summarized in Fig. 12–15. Fig. 12 and 13 show the reactions of *Br. melitensis*, strain 6015, in 2 different antisera. In both of these all lines have disappeared at 39 μg. of bacterial substance with the exception of line 6 which reached 20 μg. in Fig. 12 and 39 μg. in Fig. 13.

Fig. 14 and 15 show corresponding experiments with strain Rev. I. Line 6 in both figures reached the limit of 156 μg. and in Fig. 14 traces of a diffuse line are still visible at 78 μg.

The result of these and other experiments, which were not presented in photo-

graphs, are summarized in Table I. The table shows that lines 5, 6 and D were produced by all strains. There existed only quantitative differences between the different strains. The virulent *Br. melitensis*, strain 6015, produced these lines with minimal quantities of bacterial substance, while from other strains greater amounts had to be present. Lines 2, 3 and 4 were rarely produced by the vaccine strain *Br. abortus* 19. This strain, however, produced regularly a strong line 1 near to its antigen source.

TABLE I.—*The Minimal Amounts of Disintegrated and Dried Bacterial Substance Required for the Production of Precipitation Lines in the Presence of* 0·1 *ml. of Undiluted Immune Serum*

Antiserum.	Antigen. Species.	Strain.	Minimal amount of dried bacterial substance (μg.) which produced precipitation lines. 1	2	3	4	5	6	D
Br. suis (39)	*Br. melitensis*	6015	—	5000	1250	313	156	20	78
		Rev. I	—	—	2500	1250	625	156	156
		5	—	5000	1250	625	156	78	156
	Br. suis	39	—	—	—	1250	78	20	78
	Br. abortus	2308	—	—	313	156	156	156	156
		19	1250	—	—	5000	1250	156	156
		Ru.	2500	2500	2500	1250	1250	625	625
Br. melitensis (5)	*Br. melitensis*	6015	313	2500	156	156	156	20	78
		Rev. I	156	2500	1250	1250	625	156	156
		5	2500	2500	1250	625	313	156	156
	Br. suis	39	—	2500	313	625	313	20	156
	Br. abortus	2308	—	—	313	313	156	156	78
		19	2500	—	—	—	2500	313	313
		Ru.	—	2500	1250	313	313	78	78
Br. melitensis (Rev. I)	*Br. melitensis*	6015	—	313	313	156	78	20	20
		Rev. I	625	2500	2500	313	313	156	156
		5	—	—	5000	5000	2500	156	156
	Br. suis	39	—	—	—	2500	313	156	156
	Br. abortus	2308	—	5000	625	625	625	156	156
		19	2500	—	—	—	313	313	313
		Ru.	2500	1250	1250	1250	1250	625	625
Br. abortus (19)	*Br. melitensis*	6015	—	1250	1250	313	78	39	78
		Rev. I	—	—	2500	1250	625	78	78
		5	—	1250	625	625	625	10	78
	Br. suis	39	—	—	2500	625	625	78	78
	Br. abortus	2308	—	2500	2500	1250	313	10	78
		19	625	2500	2500	625	78	78	78
		Ru.	—	1250	156	156	78	78	78
Br. abortus (2308)	*Br. melitensis*	6015	—	—	2500	625	156	78	78
		Rev. I	—	—	2500	1250	313	156	156
		5	—	—	—	1250	1250	156	156
	Br. suis	39	—	—	5000	625	625	156	78
	Br. abortus	2308	—	—	2500	625	156	78	78
		19	1250	—	—	625	625	156	156
		Ru.	—	—	625	625	156	78	78

In the following experiments the antigens were placed in the centre of the plate and reacted with varying serum dilutions placed in the peripheric holes. Fig. 16 presents such an experiment, in which *Br. melitensis* antiserum reacts with the antigen of strain 6015. According to the reversion of the experimental

conditions the lines D and 6 were nearest the peripheric holes, followed by lines 5 and 4. The experiment shows that lines 5 and 4 were still visible opposite the serum dilution 1 : 8, while lines D and 6 already disappeared at the serum dilution 1 : 3. The majority of the strains reacting with different immune sera showed a similar diffusion pattern. Only a few lines were produced in the presence of diluted immune serum, mainly lines 4, 5 and 6. The highest serum titres were generally obtained with line 5. The only strain which showed another diffusion pattern was *Br. abortus*, strain 19. Lines 4, 5 and 6 never appeared in the presence of diluted immune serum. Instead, line 1 appeared around the antigen source and was still visible up to a serum dilution of 1/20, as shown in Fig. 17.

In order to prove that this antigen line of *Br. abortus* 19 was really different from the other lines which appeared at higher serum dilutions, we placed antigen of *Br. abortus* 2308 in one sector, the antigen of strain 19 in a second, and a mixture of both antigens in a third sector around the source of diluted Rev. I antiserum which yielded the highest titres of lines 4 and 5. The result showed that line 1 of strain 19 appeared everywhere, where its antigen was present, as an additional line, and never as a continuation of another antigen line. In order to ascertain that this peculiar antigen line of strain 19 is common to strains 19 of different origin, the Jerusalem strain 19 was compared with the Davis strain 19. Fig. 18 shows the results of such an experiment. As immune serum a *Br. suis* antiserum was used, which produced all 6 lines regularly. In the centre of the 3 antigen sources was placed *Br. melitensis*, strain 6015, on the right side was strain 19 Da. and on the left side was strain 19 Je. The figure shows that the antigens of strain 19 Je. and 19 Da. also produced the sharp line 1, while *Br. melitensis*, which produced all 6 lines showed a more diffuse, bride line which was situated in a wider distance from its antigen source and did not constitute a continuation of lines 1 of the 2 strains 19. Fig. 20 shows a similar arrangement of both strain 19 antigens. In the 3 central holes 3 different *Br. abortus* antigens were placed : the non-virulent strains 19 Je and 19 Da. and the virulent strain 2308. These antigens reacted with 2 weak *Br. suis* antisera, one producing lines 4, 5 and 6, and the other only lines 5 and 6. Nevertheless, there appeared around the serum sources of both vaccine strains the additional line 1, which did not appear around the serum source of the virulent strain. The results of all these experiments are summarized in Table II. The table shows that the only lines which appeared at higher serum dilutions were lines 4 and 5, with the exception of strain 19 which produced line 1, while the titres for line 6 generally were lower. The height of the titres for line 5 depended completely on the antiserum. Two *Br. melitensis* antisera precipitated all antigens at dilutions ranging from 1/13·5 up to 1/35·0, a *Br. suis* antiserum at dilutions from 1/4·5 up to 1/20, while a *Br. abortus* antiserum yielded titres from 1/3 up to 1/6.

Absorption experiments

In the following experiments graded dilutions of an antiserum were absorbed with graded amounts of homologous and heterologous antigens and the lines produced by the absorbed antisera compared with those produced by the original antiserum. Table III shows the results obtained with a *Br. melitensis* antiserum. It shows that after treatment with small amounts of antigens the serum lost the property of producing lines 1, 2 and 3 and mostly also line 4. The only lines which still persisted were lines 5 and 6. There were no significant differences in

TABLE II.—*The Titres of Different Precipitating Immune Sera*

Immune serum.	Antigen. Species.	Strain.	Reciprocals of final dilutions producing lines. 1	4	5	6
Br. melitensis (5)	*Br. melitensis*	6015	—	20·0	13·5	4·5
		Rev. I	—	20·0	13·5	4·5
		5	—	20·0	20·0	6·0
	Br. suis	39	—	20·0	13·5	4·5
	Br. abortus	2308	—	20·0	30·0	6·0
		19	20·0	—	—	—
		Ru.	—	20·0	25·0	6·0
Br. melitensis (Rev. I)	*Br. melitensis*	6015	—	20·0	20·0	6·0
		Rev. I	—	20·0	25·0	6·0
		5	—	20·0	20·0	4·5
	Br. suis	39	—	20·0	20·0	4·5
	Br. abortus	2308	—	20·0	35·0	6·0
		19	20·0	—	—	—
		Ru.	—	20·0	35·0	4·5
Br. suis (39)	*Br. melitensis*	6015	—	13·5	13·5	3·0
		Rev. I	—	—	20·0	4·5
		5	—	13·5	20·0	4·5
	Br. suis	39	—	—	9·0	9·0
	Br. abortus	2308	—	3·0	4·5	3·0
		19	20·0	—	—	—
		Ru.	—	20·0	6·0	3·0
Br. abortus (19)	*Br. melitensis*	6015	—	—	9·0	—
		Rev. I	—	—	6·0	—
		5	—	—	3·0	—
	Br. suis	39	—	—	3·0	—
	Br. abortus	2308	—	—	4·5	—
		19	20·0	—	—	—
		Ru.	—	—	4·5	—

the combining power of the different antigens. At a serum dilution of 1/4 and an antigen concentration of 2·4 mg. per ml., *Br. melitensis*, *Br. suis* and *Br. abortus* were able to remove all precipitins. These results proved that the antigens involved in the above described precipitation reactions were shared by all strains of the genus *Brucella*, and that only quantitative differences between the strains existed.

DISCUSSION

The experiments described above showed that the same soluble antigens of *Br. suis*, which were revealed by Olitzki and Sulitzeanu (1957) by the Ouchterlony (1948) technique, are present in all strains of the genus *Brucella*. However, in order to produce all the 6 precipitation lines, a long immunization of rabbits with the aid of adjuvants was necessary. There existed certain rules in the appearance of the precipitation lines, when quantitative methods were applied. If graded quantities of antigens were titrated in the presence of a constant amount of undiluted immune serum, then smallest amounts of antigen were sufficient for the production of the lines nearest to the source of serum, mainly lines D and 6. For the production of all the other lines higher quantities of bacterial extracts were required. The regularity of the appearance of the lines decreased with their

TABLE III.—*Absorption Experiments with a* Br. melitensis *Antiserum*

Serum diluted.	Absorption with antigen. Strain.	mg. per ml.	Remaining precipitation lines with *Br. abortus.*	*Br. suis.*	*Br. melitensis.*
1/2	*Br. melitensis* (6015)	1·2	6	6	6
		0·6	6	6	6
		0·3	6	6	5, 6
		0·15	6	6	5, 6
1/4		2·4	—	—	—
		1·2	—	—	6
		0·6	—	—	6
		0·3	—	6	5, 6
		0·15	—	6	5, 6
1/2	*Br. suis* (39)	2·4	—	6	5, 6
		1·2	5, 6	5, 6	4, 5, 6
		0·6	4, 5, 6	5, 6	4, 5, 6
1/4		2·4	—	—	—
		1·2	—	—	6
		0·6	—	—	6
		0·3	—	6	5, 6
		0·15	6	6	5, 6
1/2	*Br. abortus* (2308)	2·4	—	6	5, 6
		1·2	5, 6	5, 6	5, 6
		0·6	5, 6	5, 6	5, 6
1/4		2·4	—	—	—
		1·2	—	—	6
		0·6	—	—	6
		0·3	—	6	4, 5, 6
		0·15	6	6	4, 5, 6

distance from the serum source. The only exception was the line 1 of *Br. abortus*, strain 19, which appeared with a great regularity in a great distance from the serum source just on the edge of the antigen source. There existed also quantitative differences in the ability of the different strains to produce the most common lines. Line 6 was produced by the virulent strains *Br. melitensis* (6015) and *Br. suis* (39) with a great regularity in the presence of a few μg. of bacterial substance, while the different vaccine strains required generally higher quantities. However, differences from serum to serum occurred. *Br. abortus* antigens required in their homologous immune sera generally somewhat lower quantities for the production of line 6 than in *Br. melitensis* and *Br. suis* antisera.

If a constant amount of antigen was reacting with graded quanties of antiserum, then the highest titres were obtained with line 5, which in certain cases appeared even at serum dilutions of 1/35. These results obtained with 2 different methods, one using decreasing amounts of antigen, and the other one decreasing concentrations of antiserum, show that the relative amount of a single antigen in an antigen mixture is not always correlated with its precipitinogenic potency. Antigen 6 which seems to be the main antigen of the bacterial extracts did not produce serum titres as high as those produced by antigen 5. The same observation was made with the antigen 1 of strain 19. Although high concentration of bacterial substance was required in order to make its line visible, it appeared in serum dilutions as high as 1/20 proving its relatively high potency as precipitinogen. This antigen produced antibodies even in antisera against strains, which themselves were unable to give rise to the appearance of the line, when

used as antigens in the precipitation test. However, the appearance of its specific antibody in all antisera produced in the course of this investigation proves its high potency as precipitinogenic substance.

The absorption tests showed that there existed only quantitative differences in the presence of the different antigens in all strains of the group. The most persistent antibodies against the antigens 5 and 6 were finally removed under favourable conditions, *i.e.*, a serum dilution of 1/4 and an amount of absorbing antigen in a concentration of at least 2·5 mg. per ml. independent from its origin.

There remains only to clear up the relationship of the antigens described above with those described by Braun, Burrous and Phillips (1957) and Phillips, Braun and Plescia (1958), which were characterized as DNA-proteins and gave also several precipitation lines. Experiments are now in progress, to compare them with the antigens described above.

SUMMARY

Br. abortus, *Br. melitensis* and *Br. suis* possess at least 6 soluble antigens, which could be demonstrated by the aid of the agar gel precipitation technique. These antigens differed in their relative concentration in bacterial extracts of different origins and in their ability to produce antibody titres in immune sera. No antigen specific for a single species was demonstrated among these antigens.

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