On the Problem of Naturally Occurring Aberrant Strains of Brucella. (21224)

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Recently, increased attention has been paid to the isolation of naturally occurring Brucella strains which do not meet the established criteria for differential diagnostic characteristics of *Brucella suis, B. abortus* and *B. melitensis*(1-6). It has been claimed that many strains may show biochemical characteristics of one "species", but serologic characteristics of another. For example, strains have been described that require no added CO_2, produce H_2S, grow in the presence of basic fuchsin or thionin, and agglutinate in the presence of monospecific *abortus* sera but not in monospecific *melitensis* sera(2). Such strains have been interpreted as "biochemically *melitensis*, except for H_2S, serologically *abortus*". Obviously, these strains could have originated from "typical" abortus strains by 2 mutational steps, *i.e.* a spontaneous change in CO_2-requirement and a spontaneous change in resistance to thionin. Marr and Wilson(7) have reported that the spontaneous rate of mutation from added CO_2-requirement to lack of such requirement in *B. abortus* is approximately 2×10^{-10}. In other words, any population containing 2×10^{-10} CO_2-requiring *B. abortus* cells is likely also to contain one mutant cell in which a spontaneous genetic change has caused the abolition of the added CO_2-requirement; all descendants of this cell would display similar properties. The rate af which determinants of other taxonomic characteristics of Brucella, such as dye-resistance and H_2S production, are likely to mutate never has been established. Also no data are available to indicate whether such mutants, once they have arisen, would be capable of competing successfully with their "typical" parent-type *in vivo*.

Methods. To investigate these points, the mutation rate to dye-resistance was determined with the help of the fluctuation test (8) for 4 strains of *B. suis* and 4 strains of *B. abortus*. The results indicate an average mutation rate of 6×10^{-10} (Table I). The

TABLE I. Rates of Mutation to Dye-Resistance in Smooth Strains of *Brucella abortus* and *Brucella suis*.

Parent strain	Mutation rate $\times 10^{-10}$	Remarks
B. abortus, Weybridge 544-14-342 (CO_2-requiring)	9.9	Screened on 2-1 agar containing 1:30000 thionin
B. abortus, Spink 1301 (CO_2-requiring)	5.3	
B. abortus, 6232 (CO_2-requiring)	7.2	
B. abortus, NIH 313 (air-growing mutant)	6.6	
B. suis, NIH No. 6	6.8	Screened on 2-1 agar containing 1:50000 basic fuchsin
Weybridge P-2	9.1	
Spink 1330	6.9	
PSIII	5.2	
"	4.1	
"	4.4	
"	4.7	

ability of such mutants to compete with their parent type *in vivo* was then tested by infecting 188 guinea pigs subcutaneously with various known mixtures of dye-resistant mutant cells and their respective "typical" parental cells. All animals were sacrificed 4 weeks after infection and the proportion of dye-resistant cells in the recovered Brucella populations was determined by streaking ground and diluted samples of individual spleens and regional lymph nodes on both plain 2-1 agar and 2-1 agar containing dye (basic fuchsin in the case of *B. suis*, thionin in the case of *B. abortus*.

The *results* showed that most dye-resistant mutants can compete successfully with their "typical" parent type *in vivo* (Table II). Despite some variation among individual animals of each group, in general the proportion of aberrant cells recovered from the animals was similar to the proportion of such cells in the original inoculum. One exception was the basic fuchsin-resistant mutant isolated from *B. suis*, PSIII, which failed to compete successfully with its fuchsin-susceptible parent type and was practically eliminated following

TABLE II. Recovery of Mutant Strains of Brucella from Guinea Pigs following Mixed Inoculation with Their "Typical" Parent Type. All data are based on the recovery from five animals per group.

Type inoculated	Total No. of cells inoculated/animal	% recovery from spleen on dye-medium in comparison to recovery in plain medium	
		Avg	Range
B. abortus, 6232 = parent	8400	0*	
" , *thionin-resistant*	7000	100	
46% thionin-resistant + 54% parent	15400	40	25– 66
B. suis, NIH No. 6 = parent	790	0	
Idem	79	0	
B. suis, NIH No. 6, *basic fuchsin-resistant*	740	100	
50% NIH No. 6 basic fuchsin-resistant + 50% parent	1530	66	50– 77
95% " + 5% "	1564	95	65–119
B. suis, PSIII = parent	1100	2	<1– 3
" , *basic fuchsin-resistant*	1130	100	
50% PSIII basic fuchsin-resistant + 50% parent	2230	5	<1– 13
		% recovery from spleen in air in comparison to recovery under CO_2	
B. abortus, Weybridge 544-14-342 (CO_2-requiring) = parent	8300	0	
" , *air-growing mutant*	6300	100	
43% air-growing mutant + 57% parent	14600	66	41–100

* Recovery from plain media was excellent in all cases listed in this column, which proved that each inoculum dose used constituted at least 1 ID_{100}. On dye-medium controls of certain resistant mutants did not yield exactly 100% recovery in comparison with plain medium (*e.g.*, 6232 thionin-resistant mutant consistently yielded 92% recovery on dye-medium compared with plain medium); in these cases experimental data have been adjusted on a comparative basis of 100% for control (*e.g.*, 92% = 100% or average of 37% observed in experiment of line 3 = 40% following appropriate adjustment).

mixed infection with parent and mutant cells. Data were also obtained for the *in vivo* competition between CO_2-requiring *B. abortus* cells and mutants capable of growing without added CO_2 (Table II). The latter appeared to have a particularly high selective value as indicated by their tendency to increase in proportion following mixed infection with their CO_2-requiring parent cells.

Efforts also have been made to determine whether it is possible to select from aberrant strains a number of mutant types that would more closely resemble standard strains. Table III shows 2 examples of such efforts. Two strains were obtained from Weybridge, England, which in their biochemical reactions resembled *B. abortus*, requiring CO_2, producing H_2S, and being capable of growing on basic fuchsin but not on thionin. However, according to the Weybridge investigators these 2 strains are serologically melitensis since they show a high degree of agglutination in monospecific melitensis sera. It was relatively simple to isolate from these strains mutants which did not require added CO_2 for growth. From these mutants, in turn, other mutants were isolated which were able to grow well on thionin. As shown in the second and fourth line of Table III, these mutant strains now resemble typical melitensis strains except for H_2S production. So far it has not been possible to devise a screening technique which would permit the isolation of non H_2S-producing mutants from a population of H_2S-producing cells.

Discussion. Provided that the selective values observed for most of these mutants in guinea pigs reflect similar selective values in natural hosts, it does not appear too surprising that with increased attention towards the characterization of naturally occurring strains an increasing number of aberrant strains has been uncovered. The figures for spontaneous occurrence of mutants with aberrant diagnostic characteristics show that such types are likely to occur among any population of

TABLE III. Isolation of Mutants with "Typical" Characteristics from "Atypical" Strains.

Strain	Colony type	CO_2 requirement	H_2S production on days 1	2	3	4	Growth in presence of: 1:50000 B. fuchsin	1:30000 thionin	Agglutination in monospecific sera; Abortus	Melitensis	Interpretation
1301	S	+	+	+	+	+	+	—	++10	++160*	Biochemically abortus, serologically melitensis
Mutant from 1301	S	—	+	+	+	+	+	+			Now typical melitensis, except for H_2S
783	S	+	+	+	+	+	+	—	—	+++160*	Biochemically abortus, serologically melitensis
Mutant from 783	S	—	+	+	+	+	+	+			Now typical melitensis, except for H_2S

* Serological data from Weybridge; not rechecked because of lack of monospecific sera.

Brucella cells larger than 1 x 10^9. Since many of these "aberrant" types apparently are not eliminated in competition with the more typical parent type, they should persist in any area where the incidence of brucellosis is relatively high. Furthermore, it can be assumed that under certain environmental conditions, perhaps in certain hosts, these aberrant types may attain an even higher selective value and thus replace their normal parent type. A survey of competition between these types in hosts other than the guinea pig should be of interest in this respect. Therefore, the *in vivo* experiments described above, recently were extended to studies with mice and the results obtained with 128 mice were identical to those previously obtained with guinea pigs. But of still greater interest would be a study of competition between aberrant and typical strains in their natural hosts, *i.e.*, cattle, swine, or goats.

Even though the reliability of some of the serological tests with monospecific sera has been questioned, any subsequent confirmation of lack of significance of the serological data reported by others would in no way affect the validity of the data and considerations presented here: accepted diagnostic characteristics are subject to spontaneous mutational changes and many of the resulting aberrant types possess considerable selective value *in vivo*. These observations, therefore, reemphasize the dangers of utilizing characteristics subject to simple mutational changes for taxonomic purposes(9), and add weight to the argument that the now recognized species of Brucella represent varieties rather than valid species(4,5,10). However, from a practical standpoint it may remain useful to retain the so-called species of *B. abortus, B. suis* and *B. melitensis* as a reference standard, even though they may merely represent the most common biotypes of one species.

Summary. "Typical" *Brucella abortus* and *B. suis* strains were found to mutate spontaneously to dye-resistance (thionin and fuchsin, resp.) at an average rate of 6 x 10^{-10}. Most of the resistant mutants, and also mutants displaying altered CO_2-requirements, competed successfully with their typical parent type *in vivo*. The significance of naturally occurring aberrant *Brucella* strains and taxonomic problems have been discussed in the light of these findings.

1. World Health Organization Brucellosis Information Series No. 93, 1952.

2. Stableforth, A. W., WHO/Bruc/56, 1952.

3. Pickett, M. J., Nelson, E. L., and Liberman, J. D., *J. Bact.*, 1953, v66, 210.

4. Renoux, G., and Carrère, L., *Ann. Inst. Pasteur* (Paris), 1952, v83, 277.

5. Renoux, G., *ibid.*, 1952, v82, 289.

6. Cruickshank, J. C., *J. Hyg.*, 1954, v52, 105.

7. Marr, J., and Wilson, J. B., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 438.
8. Luria, S. E., and Delbrück, M., *Genetics*, 1943, v28, 491.
9. Luria, S. E., *Bact. Rev.*, 1947, v11, 1.
10. Evans, A. C., *Publ. Health Rep.*, 1923, v38, 1945.

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Isotopic Uric Acid in Gouty and Rheumatoid Arthritis Patients Treated with Probenecid and Phenylbutazone.* (21225)

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(Introduced by Fred R. Griffith, Jr.)

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There has been a clinical impression, not very well documented, that phenylbutazone (Butazolidin;† 3, 5-dioxo-1,2,-diphenyl-4-n-butylpyrazolidine) can lower the serum urate concentration markedly without causing a comparable excretion of "extra" uric acid. It was reported that both phenylbutazone and probenecid (Benemid;‡ p(di-n-propyl sulfamyl)benzoic acid) caused a significant increase in urinary uric acid output in 2 gouty subjects but failed to do so in 3 patients with rheumatoid arthritis(1). Both drugs tended to cause a decrease in the serum urate levels although the effect was less regular in the gouty subjects than in the subjects with rheumatoid arthritis. In an attempt to elucidate the mechanism involved, isotope studies were carried out on 3 more gouty patients and 4 more patients with rheumatoid arthritis. The body uric acid pool size and turnover rate were determined during control and drug periods, and urinary uric acid excretion was determined by the isotope dilution method. The results indicate that within the experimental design used, both drugs cause statistically significant uricosuria only in subjects with increased body stores of uric acid.

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‡ Benemid was kindly supplied by Sharp and Dohme, Philadelphia, Pa.

Experimental. All patients were hospitalized and were kept under close observation. Their diet was uniformly low in purines and constant in calories, carbohydrate, fat and protein. The only medications allowed in addition to the test drugs were codeine (P.R.N. for pain) and non-barbiturate hypnotics. All urine was collected *ad libitum* and pooled into convenient samples. Uric acid was isolated from one aliquot of each pool for N^{15} determination and another aliquot was taken for uric acid determination by the isotope dilution method(2). The uric acid pool size and turnover rate were determined during the control period as previously described(2) and each patient was then placed on one of the test drugs for approximately one week. The uric acid pool size and turnover rate were determined in the second week of that period, during which the patient continued the same therapeutic regimen. The first drug was then discontinued and the second one given for approximately one week. During the second week of this therapeutic period the uric acid pool size and turnover rate were again determined. The daily dose schedule for probenecid was 3 tablets of 0.5 g each, and for phenylbutazone was 2 tablets of 200 mg each.

Results. The pool of miscible uric acid in normal individuals as determined in this and other laboratories(3) is usually not greater than 1200 mg (29 mMols U A nitrogen), and may be less in women. From Tables I and II it is apparent that only 2 patients, D. W., and A. B., were within normal limits. The other