FACTORS CONTROLLING BACTERIAL DISSOCIATION[1]

LIKE very many other species of bacteria, *Brucella abortus* exhibits the phenomenon of dissociation, that is, changes in colony form, culture characteristics, cell morphology, biochemical reactions, immunological reactions and virulence. The usual change is from the antigenically active Smooth (S) type to Intermediate (I) and antigenically inactive Rough (R), Brown (Br) and other types.[2]

In the course of some experiments which had been started in an attempt to study the nature of dissociation in *Brucella abortus* significant differences in the amount of dissociation after urea treatment were encountered when different strains were used.[3] It, therefore, seemed desirable to test the relative influence of possibly inherent versus environmental factors upon the degree of dissociation, with the object of systematic selection of strains with different dissociation "potentials."

Such investigations were made possible by utilizing a new technique of single cell isolation[4] which permitted studies with pure lines (clones) thus established. For the purpose of comparative studies it was necessary to establish first a standard set of conditions in which the degree of dissociation of different cultures could be compared. Thus, it was decided to express as "dissociation rate" the percentage of dissociated colonies observed on plates made from 10 days old broth cultures (beef infusion, buffered to pH 6.8) each of which had been inoculated with one Smooth colony. A comparison was then made between dissociation rates of individual colonies arising from various single cells of one stock-culture (BAI's strain Number 19-9) and dissociation rates of individual colonies all of which originated from one colony of one isolated single cell of the same culture. The data in the accompanying table show that the progeny of a single cell isolated from a heterogenous population exhibited similar dissociation rates with a relatively small range of variation (B in Table 1), while the progeny of different single cells from the same population showed varying dissociation rates (A in Table 1). Subsequently, progenies from many single cells, isolated from various strains, were tested and the similarity of dissociation rates within clones was confirmed (see Table 1). Differences between clones were found to be statistically highly significant.[5] Substrains thus established and kept on agar slants at low temperatures have so far retained their original dissociation rates for more than six months. A systematic search for clones with low dissociation rates was successful.

TABLE 1

	A	B	C	D	E	F
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
	1	44	74	28	7	0.0
	7	45	76	32	7	0.0
	24	47	78	36	7	0.0
	25	47	80	36	7	0.3
	35	50	81	41	9	3
	37	52	82	42	9	3
	37	52	87	43	9	4
	43	54	88	44	13	5
	44	54	91		14	13
	50	56	95		15	
	55	56	95			
	55	60				
	59	60				
	63	61				
	69	68				
Dissociation constant*	40% ± 5.09	54% ± 1.74	84% ± 2.23	38% ± 1.99	10% ± 0.95	3% ± 1.27
Number of colonies counted	1640	1586	1777	1220	1687	1929

A: Dissociation rates of individual colonies originating from many single cells isolated from strain 19–9.
B: Dissociation rates of individual colonies originating from one single cell isolated from strain 19–9.
C–F: Examples of dissociation rates of colonies from strains started from single cells.
* Dissociation Constant = most representative dissociation rate of a population = $\bar{p}$ of Hendricks, *Poul. Science,* 14: 365.

On the basis of results which were obtained when the effect of a number of environmental factors on these inherent dissociation rates were studied, it appears that dissociation rates should be considered as the ability of newly arising variants to establish themselves within a population. This infers that the dissociation rate, as defined here, is only an indicator for primary inherent differences, such as growth rate or

[1] The U. S. Bureau of Animal Industry is contributing to the cost of this work.

[2] B. S. Henry, *Jour. Infect. Dis.,* 52: 374, 1933.

[3] W. Braun, *Jour. Bact.,* 46: 222, 1943.

[4] K. I. Johnstone, *Jour. Path. and Bact.,* 55: 159, 1943.

[5] For example, in an analysis of variance between clones B, C, D, E and F, according to methods described by Hendricks, *Poul. Science,* 14: 365, an F value of 413.65 was obtained, which, for the appropriate degrees of freedom, corresponds to a P level of considerably less than 1 per cent.

viability of variants, and is, therefore, affected by environmental or inherent changes which affect these factors. Some experimental proof for this concept has been obtained. For example, an increase in the density of the population appears to increase the dissociation rate. In such altered environmental conditions the absolute degree of dissociation is changed, but the relative differences between two clones, such as a high dissociating one and a low dissociating one, are retained. Another example is provided by the results obtained when the effect of the pH of the environment was studied. Thus, a high dissociating strain showed 50 per cent. dissociation at pH 6.6 and 2 per cent. at pH 7.4, while a low dissociating strain showed 1 per cent. dissociation at pH 6.6 and none at pH 7.4. Tests with other strains, as well as observations with buffered and unbuffered broth, provided further proof that the pH of the environment affects the relative degree of dissociation (by affecting growth rates?), however, always within the limits of the inherent factors which determine dissociation rate; *i.e.*, environmental influences which lower the dissociation rate will decrease the dissociation rate of a high and a low dissociating strain proportionally. It should now be possible to test the relationship between dissociation rate and rate of multiplication, ability of variants to establish themselves within a population, etc., by starting broth cultures with a known number of low dissociating Smooth cells and low dissociating Rough cells and subsequent daily counts of the number of each type present.

Work with strains started from single cells has also indicated (1) that all formerly described types of variants as well as several so far undescribed types can arise among the progeny of a single cell; (2) that the type of dissociation, *i.e.*, whether primarily *S* to *R* or *S* to *Br*, etc., differs between clones, and (3) that the ability to withstand toxic effects (urea solutions of high concentration) differs between clones and can be subjected to systematic selection. These apparently inherent characteristics are now being studied and special attempts are being made to establish the feasibility of selection for immunizing power. Also, an analysis of actual chemical differences between variants has been started.

A detailed account of the work on dissociation rates will be published in the near future.

WERNER BRAUN
DIVISION OF VETERINARY SCIENCE,
UNIVERSITY OF CALIFORNIA, BERKELEY

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A NEW REAGENT FOR VITAMIN A

VITAMIN A can be estimated biologically, optically or chemically. Sometimes it is desirable to make determinations in the field and for such purposes only chemical methods are suitable. Antimony trichloride is the only reagent commonly used in this way. It has, however, disadvantages not found in the reagent to be described.

This material is "Super-Filtrol,"[1] a commercial adsorbent made from the aluminum silicate mineral, Montmorillonite. It is cheap, easily handled and requires no precautions in its use except that both it and the oils to be tested be anhydrous.

When even small amounts of vitamin A in solution in petroleum ether, chloroform, benzene or carbon tetrachloride are brought into contact with the reagent it immediately becomes bright blue. All polar solvents prevent or destroy the color which can not be eluted as such from the particles of the solid. This color may be utilized as a measure of vitamin A potency in fish liver oils, and presumably for the analysis of food extracts if they are anhydrous. Materials other than fish oils have not been tested.

The color may be estimated visually by comparison with a series of standards prepared by mixing varying proportions of powdered cobalt glass with the reagent. These are standardized against oils of known potency and may be conveniently prepared in steps of about 15,000 I.U. The color may be measured more accurately by a reflection type photoelectric colorimeter which may be made very simply by utilizing a diverging and variable slit between the photocell and the colored surface.

The color may best be developed by placing about 10 gm of "Super-Filtrol" in a small flask and covering it with petroleum ether. To this add a known weight or volume of vitamin A-containing material either as solution or just as oil, and shake. The blue color forms at once. For field use it has been found convenient to deliver the oil from the blunt end of a 20-gauge, hypodermic needle on a 1 cc syringe. Drops of the order of 7 mg are delivered with an accuracy of about 3 per cent. Two such drops, containing as little as 28 I.U. (2,000 I.U. per gm) will make a color on 10 gm of the reagent.

Shantz, Cawley and Embree[2] have shown that in the case of the antimony trichloride reaction a dehydration takes place, splitting off the terminal alcohol group and forming "anhydro vitamin A." This reaction is probably common to most or all of the materials

[1] Manufactured by the Filtrol Corporation, Vernon, Calif.

[2] E. M. Shantz, J. R. Cawley and N. D. Embree, *Jour. Am. Chem. Soc.*, 65: 901, 1943.