CONDUCTIVITY AS APPLIED TO STUDIES OF BACTERIAL METABOLISM

II. PARALLELISM BETWEEN AMMONIA AND CONDUCTIVITY IN NUTRIENT GELATIN CULTURES OF PUTREFACTIVE ANAEROBES[1]

L. B. PARSONS AND W. S. STURGES

Laboratory of the Cudahy Packing Company, Omaha

In a previous paper the possible value of conductivity measurements in studies of bacterial proteolysis was discussed and data were presented demonstrating a direct proportionality between conductivity change and ammonia production in the case of two anaerobes (*C. flabelliferum* and *C. sporogenes*). The investigation has been extended to a representative group of strongly proteolytic anaerobes to ascertain whether a correspondingly definite relationship exists for these organisms also.

Nutrient gelatin was selected as the medium for this study because of its universal use and because it lends itself readily to conductivity measurements. Bacto nutrient gelatin (dehydrated), adjusted to pH 8.0, was employed.

Following are the cultures and their sources:

C. sporogenes	Army Medical School
C. flabelliferum	Cudahy Laboratory
C. bifermentans	Lister Institute
C. histolyticum	Lister Institute
C. Reading	Lister Institute
C. parasporogenes	Lister Institute
C. putrificum	Yale Bacteriological Laboratory

Invigorated cultures were inoculated into 25 cc. portions of medium in large culture tubes and incubated in hydrogen at 20° and 37°C.

[1] Presented at twenty-seventh annual meeting of the Society of American Bacteriologists, Madison, Wisconsin, December 29 to 31, 1925.

Measurements of electrical conductivity were made at 30°C. Ammonia was determined by the Folin method using 1 cc. samples. Control tubes of sterile media were analyzed and appropriate conductivity and ammonia blanks subtracted from all subsequent determinations. Changes in specific conductivity in reciprocal ohms (Δk), and changes in ammonia nitrogen were tabulated. To facilitate interpretation of the data Δk was plotted against milligrams of ammonia nitrogen per 100 cc. (ΔN) for each organism at each temperature. These $\Delta k - \Delta N$ plots in all cases appeared to be straight lines.

The curves for all organisms except *C. histolyticum* had the same slope. This line gave the equation

$$\Delta N = \Delta k \times 1.95 \times 10^4$$

This is the same proportionality constant which the writers previously found for *C. flabelliferum* and *C. sporogenes*. *C. histolyticum* also gave a linear relationship which was better expressed by the slightly different constant 1.87×10^4.

To gain an idea of the applicability of these constants, data have been tabulated showing Δk and ΔN (both calculated and observed) and the per cent deviation of the calculated from the observed value for each culture. The data for *C. sporogenes*, *C. flabelliferum* and *C. bifermentans* are given for 20° only since the first two have been reported previously for 37°C. and the last was not run at 37° owing to an oversight.

The calculated ΔN values in tables 1 to 6 were computed using the constant 1.95×10^4 while those in table 7 were obtained with the constant 1.87×10^4. Had the former constant been used for *C. histolyticum* the deviations would have been within 10 per cent but in all instances the calculated values would have been greater than the observed. It will be noted that in most cases where the ammonia value was very low large percentage deviations were obtained. This is not indicative of any inherent weakness in the conductivity method but is explicable by the fact that analyses for ammonia were invariably carried out on 1 cc. samples in order to maintain identical conditions for all determinations. Obviously slight unavoidable titration errors and possible variations in the initial composition of the media will yield relatively large errors when the ammonia content of the sample is low. If one were interested in obtaining measurements of such low con-

TABLE 1

Ammonia conductivity relationships.—C. sporogenes, 20°C.

AGE	Δk	ΔN		PER CENT DEVIATION
		Calculated	Observed	
days				
2	0.9×10^{-3}	17.5	20	−12.5
4	4.6×10^{-3}	90	86	+4.6
6	8.3×10^{-3}	162	158	+2.5
10	18.3×10^{-3}	357	363	−1.6
16	21.1×10^{-3}	412	433	−2.1

TABLE 2

Ammonia conductivity relationships—C. flabelliferum, 20°C.

AGE	Δk	ΔN		PER CENT DEVIATION
		Calculated	Observed	
days				
2	1.1×10^{-3}	21	14	+50.0
4	8.1×10^{-3}	158	162	−2.5
6	12.3×10^{-3}	240	248	−3.2
10	17.6×10^{-3}	343	363	−5.5
16	22.5×10^{-3}	438	458	−4.4

TABLE 3

Ammonia conductivity relationships—C. bifementans, 20°C.

AGE	Δk	ΔN		PER CENT DEVIATION
		Calculated	Observed	
days				
2	2.7×10^{-3}	52	46	+13.0
4	8.5×10^{-3}	166	154	+7.8
6	16.3×10^{-3}	318	319	−0.3
10	22.6×10^{-3}	441	438	+0.7
16	26.8×10^{-3}	523	525	−0.4

centrations of ammonia it would doubtless be possible by the choice of larger samples and by careful observation of the initial conductance to reduce the deviations to a minimum.

TABLE 4

Ammonia conductivity relationships—C. Reading

AGE	20°C.				37°C.			
	Δk	ΔN		Per cent deviation	Δk	ΔN		Per cent deviation
		Calculated	Observed			Calculated	Observed	
days								
2	0.8×10^{-3}	15	13	+15.4	13.0×10^{-3}	254	255	−0.4
4	4.0×10^{-3}	78	79	−1.3	17.0×10^{-3}	332	331	+0.3
6	8.4×10^{-3}	164	159	+3.1	25.0×10^{-3}	488	483	+1.0
10	15.2×10^{-3}	296	309	−4.2	29.3×10^{-3}	572	565	+1.3
16	22.6×10^{-3}	441	461	−4.3	30.2×10^{-3}	588	599	−1.8

TABLE 5

Ammonia conductivity relationships—C. parasporogenes

AGE	20°C.				37°C.			
	Δk	ΔN		Per cent deviation	Δk	ΔN		Per cent deviation
		Calculated	Observed			Calculated	Obsevred	
days								
2	0.8×10^{-3}	15	12	+25.0	13.6×10^{-3}	266	263	+1.1
4	5.2×10^{-3}	102	122	−16.4	21.2×10^{-3}	414	402	+3.0
6	11.5×10^{-3}	224	218	+2.8	23.2×10^{-3}	452	471	−4.0
10	21.1×10^{-3}	412	409	+0.7	26.7×10^{-3}	521	517	+0.8
16	25.2×10^{-3}	492	489	+0.6				

TABLE 6

Ammonia conductivity relationships—C. putrificum, 37°C.

AGE	Δk	ΔN		PER CENT DEVIATION
		Calculated	Observed	
days				
6	5.3×10^{-3}	103	94	+9.6
10	17.5×10^{-3}	341	335	+1.8
16	19.6×10^{-3}	382	390	−2.0

Note: Cultures of *C. putrificum* did not show digestion at 37° until 6 days and at 20°C. no appreciable digestion was noted up to 16 days.

While it has been shown that change in conductivity exactly parallels change in ammonia content it must be borne in mind that the measured conductivity is the sum of the conductances of the ammonium ions and all other ions which are formed, both basic and acidic. It is not probable that any considerable quantity of basic ion other than ammonium would result from the proteolytic decomposition of gelatin. The fact that the same constant serves equally well for computing ammonia from conductivity for the first six anaerobes indicates that the average conductivity of the various acidic ions produced is practically identical although this does not necessarily mean that the acids

TABLE 7

Ammonia conductivity relationships—C. histolyticum

AGE	20°C.				37°C.			
	Δk	ΔN		Per cent deviation	Δk	ΔN		Per cent deviation
		Calculated	Observed			Calculated	Observed	
days								
2	6.4×10^{-3}	119	118	+0.8	27.6×10^{-3}	516	494	+4.5
4	19.5×10^{-3}	365	354	+3.1	24.3×10^{-3}	455	423	+7.0
6	24.1×10^{-3}	450	442	+1.8	32.3×10^{-3}	605	605	0.0
10	28.3×10^{-3}	530	536	−1.1	39.4×10^{-3}	737	723	+1.9
16	39.9×10^{-3}	747	758	−1.4	38.1×10^{-3}	713	712	+0.1
2	6.0×10^{-3}	112	118	−5.1	28.5×10^{-3}	532	525	+1.3
4	18.4×10^{-3}	344	333	+3.3	33.7×10^{-3}	630	617	+2.1
6	23.7×10^{-3}	442	407	+8.6	36.4×10^{-3}	680	675	+0.7
10	28.3×10^{-3}	530	539	−1.6	47.7×10^{-3}	894	928	−3.6
16	36.5×10^{-3}	682	701	−2.7	42.1×10^{-3}	786	793	−0.9

are the same either in character or quantity. It would appear, therefore, that the acidic ions in cultures of *C. histolyticum* have a greater average conductance than the acidic ions of the other anaerobes. This probably implies that *C. histolyticum* produces acids of a lower mean molecular weight than do these other anaerobes.

The relationship already shown to exist between conductance and ammonia for two anaerobes seems to be generally true for organisms of this type. Many possible applications to metabolic studies will suggest themselves.

SUMMARY

1. The conductivity method as a measure of ammonia, formerly proposed and employed in studies of *C. flabelliferum* and *C. sporogenes* by the writers, has been applied to cultures of six other representative proteolytic anaerobes.

2. The relationship expressed by the equation,—change in NH_3N = change in conductivity $\times$ 1.95 $\times 10^4$,—held true to within 10 per cent for cultures grown at 20° and 37°C.

3. *C. histolyticum* gave values better expressed by the constant 1.87 $\times 10^4$.

4. The method promises to be of considerable use in metabolic studies.