Reversal of sulphonamide action in *Escherichia coli* (B_{12} auxotroph) by vitamin B_{12}

Sulphonamides are known to block sequentially the synthesis in *Escherichia coli* of methionine, xanthine, serine, thymine, valine[1,2] and glycine[3]. At each of these steps, the inhibition index is increased in presence of vitamin B_{12}[2,3]. Since the requirement of vitamin B_{12} for growth of *E. coli* B_{12} auxotroph can be met completely by methionine[4], it was of interest to study the growth inhibition by sulphanilamide (SA) in this mutant as influenced by the two metabolites.

The mutant strain[4] of the organism was grown in the medium of GREEN AND SEVAG[5] with additions as shown (Table I). Growth was measured after 24 hours incubation at 30° and expressed in terms of galvanometer deflections in a Klett-Summerson photoelectric colorimeter at 660 mμ.

TABLE I

COMPARATIVE ACTIVITY OF METHIONINE AND VITAMIN B_{12} IN OVERCOMING SA GROWTH INHIBITION OF *E. coli* B_{12} AUXOTROPH

Additions to 10 ml basal medium	*mg SA added* 0	0.5	0.7	1.0
	Growth at 24 hours			
1. None	0	0	0	0
2. Methionine, 100 μg	48	0	0	0
3. Methionine, 1 mg	48	0	0	0
4. Vitamin B_{12}, 2 mμg	47	46	17	10
5. Vitamin B_{12}, 20 mμg	49	48	39	23
6. As in (3) + xanthine 0.25 mg	48	30	17	14
7. As in (6) + serine 0.2 mg	50	49	36	19

It is observed (Table I) that, in presence of vitamin B_{12}, a higher concentration of SA is required to inhibit growth than when methionine is used. The protective effect of vitamin B_{12} is more pronounced at the higher concentration of the vitamin and can be simulated by a combination of methionine, xanthine and serine.

These observations suggest that, although methionine could, interchangeably with vitamin B_{12}, serve the requirement for growth of the mutant, this vitamin assumes additional functions in a condition of stress caused by SA bacteriostasis analogous to its *p*-aminobenzoic acid potentiating action already observed[2] with the wild strain under similar conditions. That the requirements for growth and for metabolic activity with respect to specific systems need not necessarily be the same is known[6].

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[1] K. C. WINKLER AND P. G. DE HAAN, *Arch. Biochem.*, 18 (1948) 97.
[2] W. SHIVE, *Ann. N.Y. Acad. Sci.*, 52 (1950) 1212.
[3] H. R. ALIMCHANDANI AND A. SREENIVASAN, *Nature* (communicated).
[4] B. D. DAVIS AND E. S. MINGIOLI, *J. Bacteriol.*, 60 (1950) 17.
[5] M. N. GREEN AND M. G. SEVAG, *Arch. Biochem.*, 9 (1946) 129.
[6] E. E. SNELL, in *Bacterial Physiology*, edited by C. H. WERKMAN and P. W. WILSON, Academic Press, Inc., New York, 1951, pp. 215–255; W. D. BELLAMY AND I. C. GUNSALUS, *J. Bacteriol.*, 48 (1944) 191.

Received September 17th, 1955

Relationship between skew diffusion gradient curves and axial ratios of rod-shaped particles

It is a well-known phenomenon that from experiments with filamentous molecules skew diffusion gradient curves are obtained, in contrast to the symmetrical curves obtained from those with spherical particles. Thus POLSON[1], GRALÉN[2] and JULLANDER[3] found that in plotting diffusion curves of cellulose derivatives, which are known to be filamentous, the curves obtained are asymmetrical. KAHLER[4] made similar observations in his diffusion experiments on solutions of sodium thymonucleate, and NEURATH AND SAUM[5] obtained skew diffusion curves in their work on tobacco mosaic virus. GRALÉN[2] showed that the diffusion constant of rod-shaped particles could be calculated from the following semi-empirical equation:

$$D_c = D_0 (1 + K_1 c)$$

where D_0 is the diffusion constant at infinite dilution, K_1 is a constant and c is the concentration. JULLANDER[3] showed with nitrocellulose in acetone or amylacetate that K_1 is proportional to the molecular weight. Solutions of such rod-shaped particles also behave anomalously in osmotic pressure measurements in that the ratio between their osmotic pressure and concentration does not follow the van 't Hoff's law except at infinite dilution. In general the equation

$$p = \frac{RTc}{M} (1 + BcM) \tag{1}$$

holds for such substances. In this equation p is the osmotic pressure, R is the gas constant, T is the absolute temperature, M is the molecular weight and B is a constant related to the particle dimensions. For an uncharged rigid rod-shaped particle it equals

$$\frac{N \pi d l^2}{4 M^2}$$

See OSTER[6], ZIMM[7]. In this equation N is Avogrado's number, d is the diameter and l is the length of the particle. This equation is related to the covolume *i.e.* the volume swept through the liquid when a rod-shaped particle is rotated through an angle of 360° round its short axis. The osmotic pressure can also be expressed by the equation:

$$p = \frac{RTc}{M} \frac{D_c}{D_0} \tag{2}$$

POLSON[8]. This equation was derived from the assumption that some of the rotational Brownian motion round the short axis is transferred to translatory motion through impacts with molecules of similar size and shape in higher concentration. This transfer of rotational into translatory motion would take place less frequently in dilute solutions, hence the increase in diffusion constants at higher concentrations and the skew diffusion curves. From equations 1 and 2 it follows that

$$\frac{D_c}{D_0} = 1 + BcM$$

Substituting the value for B in this equation and expressing the molecular weight in terms of molecular dimensions, *i.e.*

$$M = \frac{\pi d^2 l N \delta}{4}$$

where δ is the density of the material, we have

$$\frac{D_c}{D_0} = 1 + \frac{l c}{d \delta} = 1 + \frac{l c V}{d} \tag{3}$$

V is the partial specific volume.

Using equation 3 the axial ratios (l/d) of molecules in different preparations of nitrocellulose have been calculated. These results are recorded in Table I together with those calculated from sedimentation and diffusion measurements by JULLANDER[3]. For the calculation of the axial ratios the values of K_1 as determined by JULLANDER were used.

The axial ratios l/d calculated from K_1 values as determined by GRALÉN as well as the axial ratios calculated from the diffusion and sedimentation constants and the partial specific volume as determined by the same author are recorded in Table II. In Fig. 1 the relationship between the values as calculated by JULLANDER AND GRALÉN from sedimentation and diffusion measurements using HERZOG, ILLIG AND KUDAR's[9] equation and those calculated from equation 3 are recorded graphically. Three of GRALÉN's values which showed abnormally large deviations were omitted as were two of JULLANDER's which showed negative K_1 values. JULLANDER regarded these two values as due to artifacts resulting from poor initial boundary formation in his diffusion experiments. Considering the difficulties involved in the calculation of K_1 values, the overall agreement between the axial ratios calculated by the two different methods must be considered good. Very good agreement is shown between the axial ratios for sodium thymonucleate calculated from equation 3 and from diffusion, sedimentation and partial specific volume measurements of KAHLER[4]. KAHLER calculated an axial ratio l/d of 284 while equation 3 yields a value of 257. Unfortunately the tobacco mosaic virus preparation investigated by NEURATH AND SAUM[5] was too inhomogeneous in particle size to allow a calculation of the axial ratio for this virus.

It must be noted that the good agreement between the two methods of estimation of axial ratios is in part due to the fact that in both methods the partical specific volume is used. Comparison between these axial ratios and those obtained from X-ray measurements might give a measure of the extent to which a filamentous molecule is hydrated.

TABLE I

AXIAL RATIOS l/d OF DIFFERENT PREPARATIONS OF NITROCELLULOSE AS CALCULATED FROM EQUATION 3 AND K_1 VALUES COMPARED TO THOSE CALCULATED FROM SEDIMENTATION AND DIFFUSION MEASUREMENTS

$c = 0.01$ g/ml $\bar{V} = 0.576$ ml/g

Material	K_1	*l/d from equation 3*	*l/d from S and D* JULLANDER
e h	1.08	187	230
$V\ F_{120}$	0.68	118	110
$V\ F_3$	0.10	17	42
$V\ F_{1/2}$	0.22	37	24
L n t	1.05	182	130
H n t	0.54	94	110
M p t 128	1.75	304	230
F K 2	0.35	60	190
F K 3	1.03	179	210
F K 6	1.01	175	190
F K 9	3.87	668	650

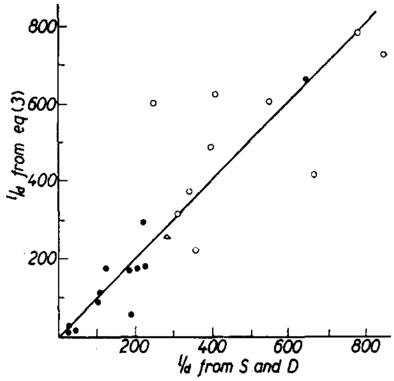

Fig. 1. Axial ratios l/d calculated from the asymmetry of diffusion curves compared to those calculated from sedimentation and diffusion measurements. The points are from JULLANDER's and the circles from GRALÉN's work on nitrocellulose. The triangle is KAHLER's result on sodium thymonucleic acid.

TABLE II

AXIAL RATIOS l/d OF DIFFERENT PREPARATIONS OF NITROCELLULOSE AS CALCULATED FROM EQUATION 3 AND FROM S AND D VALUES OF GRALÉN

$c = 0.01$ g/ml $\bar{V} = 0.576$ ml/g

Material		*l/d from equation 3*	*l/d from S and D* GRALÉN
Unbleached American linters	4.2	729	870
Bleached American linters	2.4	417	670
Chlorite bleached linters	4.5	780	780
Sulphate cellulose	5.6	970	550
Sulphite cellulose	5.3	920	550
Holocellulose spruce	3.5	607	250
Cellulose from holocellulose	1.3	225	360
Rayon pulp No. 1	3.6	624	410
No. 2	3.5	607	550
No. 3	2.0	347	380
No. 4	4.7	815	220
No. 5	1.8	312	320
No. 6	2.8	490	400

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[1] A. POLSON, *Kolloid Z.*, 83 (1938) 173.
[2] N. GRALÉN, *Thesis*, Uppsala University, 1944.
[3] I. JULLANDER, *Arkiv Kemi, Mineral. Geol.*, 21A (1945) 1.
[4] H. KAHLER, *J. Phys. and Colloid Chem.*, 52 (1948) 676.
[5] H. NEURATH AND A. M. SAUM, *J. Biol. Chem.*, 126 (1938) 435.
[6] G. OSTER, *J. Gen. Physiol.*, 33 (1950) 445.
[7] B. H. ZIMM, *J. Chem. Phys.*, 14 (1946) 164.
[8] A. POLSON, *Nature*, 157 (1946) 406.
[9] R. O. HERZOG, H. ILLIG AND H. KUDAR, *Z. Phys. Chem.*, A167 (1934) 329.

Received August 23rd, 1955