the rabbit- γ -globulin chain B and B piece were not significantly different.

2. Tryptophan was determined spectrophotometrically on the intact protein and cystine was determined as cysteic acid after oxidation of the protein. Oxidation of *S*-carboxymethylcysteine gave three products; one of these was eluted from the 150 cm. column of the Analyzer very close to the position of cysteic acid and was unstable to hydrolysis with acid.

3. In contrast with a previous report, hydroxyllysine could not be detected in either human γ -globulin or rabbit γ -globulin.

We are indebted to Dr S. Moore and Dr W. H. Stein, The Rockefeller Institute, New York, for much helpful advice on the Spinco model 120 Amino Acid Analyzer, and to Dr S. Moore for information and results before publication. We are also indebted to Dr N. M. Green, St Mary's Hospital, London, for advice on the determination of tryptophan. We thank Dr R. A. Kekwick, The Lister Institute, London, S.W. 1, for a sample of purified human γ -globulin, Mr P. S. Diamond for valuable technical assistance and Mrs Sara Ash for many calculations. Financial support from the National Science Foundation, U.S.A., the U.S. Public Health Service and the Wellcome Trust is gratefully acknowledged.

REFERENCES

- Anderson, W. F. & Gill, T. J., III (1962). *Biochim. biophys. Acta*, **58**, 558.
Askonas, B. A., Farthing, C. P. & Humphrey, J. H. (1960). *Immunology*, **3**, 336.

- Beaven, G. H. & Holiday, E. R. (1952). *Advanc. Protein Chem.* **7**, 319.
Brand, E. (1946). *Ann. N.Y. Acad. Sci.* **47**, 187.
Cohen, S. (1963). *Nature, Lond.*, **197**, 253.
Eastoe, J. E. (1955). *Biochem. J.* **61**, 589.
Fleischer, S., Hardin, R. L., Horowitz, J., Zimmerman, M., Gresham, E., Turner, J. E., Burnett, J. P., Stary, Z. & Haurowitz, F. (1961). *Arch. Biochem. Biophys.* **92**, 329.
Fleischman, J. B., Porter, R. R. & Press, E. M. (1963). *Biochem. J.* **88**, 220.
Hannig, K. (1959). *Clin. chim. Acta*, **4**, 51.
Heimer, R., Woods, K. R. & Engle, R. L. (1962). *Proc. Soc. exp. Biol., N.Y.*, **110**, 496.
Melamed, M. & Green, N. M. (1963). *Biochem. J.* (in the Press).
Michaelis, L. & Schubert, M. P. (1934). *J. biol. Chem.* **106**, 331.
Moore, S. (1963). *J. biol. Chem.* **238**, 235.
Porter, R. R. (1959). *Biochem. J.* **73**, 119.
Schram, E., Moore, S. & Bigwood, E. J. (1954). *Biochem. J.* **57**, 33.
Smith, E. L., McFadden, M. L., Stockell, A. & Buettner-Janusch, V. (1955). *J. biol. Chem.* **214**, 197.
Spackman, D. H. (1960). In *Instruction Manual and Handbook, Beckman/Spinco Model 120 Amino Acid Analyzer*, part 10. Palo Alto: Beckman Instruments Inc.
Spackman, D. H., Stein, W. H. & Moore, S. (1958). *Analyt. Chem.* **30**, 1190.
Waldschmidt-Leitz, E., Bretzel, G. & Keller, L. (1960). *Naturwissenschaften*, **11**, 254.
Walsh, K. A. & Brown, J. R. (1962). *Biochim. biophys. Acta*, **58**, 596.

Biochem. J. (1963) **88**, 234

The Molecular Weights of the Peptide Chains of γ -Globulin

By R. H. PAIN

Department of Immunology, St Mary's Hospital Medical School, London, W. 2

(Received 29 January 1963)

Mild reduction of γ -globulin with mercaptoethanol in aqueous solution breaks five interchain disulphide bonds, allowing the separation of two fractions, termed chains A and B (Fleischman, Pain & Porter, 1962). The molecular weights of the peptide chains A and B of horse γ -globulin were reported as 105 000 and 40 000 respectively. It became apparent that the persistence of aggregate in the solutions examined had given rise to high values with the Archibald technique. More accurate results have now been determined for chains A and B by using short-column equilibrium sedimentation (Van Holde & Baldwin, 1958). Determinations of the molecular weights of the

products of reduction of papain-digestion piece I (Fleischman, Porter & Press, 1963) and of whole horse γ -globulin have also been carried out.

MATERIALS

γ -Globulin. Horse and rabbit γ -globulins were separated from sera on a diethylaminoethylcellulose column equilibrated with 17.5 mM-sodium phosphate buffer, pH 6.5. Solutions were concentrated to 4 g.dl.⁻¹, sterilized by filtration and stored at 3°.

Peptide fragments of γ -globulin. These were prepared as described by Fleischman *et al.* (1963).

Other materials. Crystalline ribonuclease and crystalline bovine plasma albumin were purchased from Armour

Pharmaceutical Co. Ltd. Hen ovalbumin was prepared by the method of Kekwick & Cannan (1936). Avidin was prepared by Dr N. M. Green (Green, 1963).

Guanidine hydrochloride was prepared from guanidine carbonate (analytical reagent) by the method of Anson & Stanley (1941).

Buffers. The following buffers were used for equilibrium studies: (i) 8M-urea-0.2M-KCl-0.05M-potassium phosphate, pH 7.5; (ii) 8M-urea-0.2M-KCl-0.1M-formic acid, pH 3.5; (iii) 0.15M-KCl-7.8 mM-potassium phosphate, pH 7.7; (iv) 0.18M-KCl-0.02M-glycine, adjusted to pH 3.5 with HCl. For each run in 8M-urea, fresh buffer was made up and its density measured.

The sedimentation and diffusion experiments were performed with 0.1M-KCl-0.02M-tris, adjusted to pH 7.49 with HCl; $\eta/\eta_0 = 1.004$ and $\rho = 1.0028$ (25°).

EXPERIMENTAL

Sedimentation equilibrium. Sedimentation-equilibrium runs were made and results calculated by the method of Van Holde & Baldwin (1958). A 12 mm. double-sector cell was used for columns of solution between 1 and 3 mm. deep, and a Kel-F multichannel cell (Yphantis, 1960) for columns of 0.8 mm. depth. The refractive increment for the solute, n_D^0 , was determined for each dialysed solution by using synthetic-boundary cells. Dilutions were made by weight into glass bulbs of 0.2 ml. capacity fitted with 3 cm.-long capillary necks to minimize evaporation. Determinations of molecular weights were made with protein concentrations between 0.15 and 1.5 g.dl.⁻¹.

To locate the menisci accurately, particularly where higher-molecular-weight components or high speed caused broadening of the outer meniscus, photographs were taken at a bar angle of 90° as soon as the rotor attained running speed. Plates were measured with a projection microscope (constructed by Precision Grinding Ltd., Mitcham, Surrey, with modifications to the specification of Dr J. M. Creeth) reading directly to 2μ at $\times 50$ magnification.

Values of apparent molecular weight, M_{app} , were extrapolated to zero concentration by plotting $1/M_{app}$ against concentration, c .

Diffusion. In the diffusion experiments buffer was used as the upper solution in all cases, and protein solution in the concentration range 0.25–1.4 g.dl.⁻¹ as the lower solution. The apparatus was a Gouy diffusimeter (Caspary & Kekwick, 1957), modified by Dr J. M. Creeth to incorporate Rayleigh optics. The methods used were as previously described (Gosting & Morris, 1949; Akeley & Gosting, 1953; Creeth, 1958). Total fringe numbers were measured from Rayleigh photographs and reduced height-area diffusion coefficients calculated from at least eight Gouy

photographs (Gosting & Morris, 1949; Akeley & Gosting, 1953). A calibration run with sucrose at a concentration, $c/2$, of 0.7299 g.dl.⁻¹ gave a diffusion coefficient of 5.171×10^{-6} cm.² sec.⁻¹ at 25.00°, in good agreement with $D(\bar{c}) = 5.172 \times 10^{-6}$ cm.² sec.⁻¹ (Gosting & Morris, 1949; Akeley & Gosting, 1953).

Partial specific volume. Partial specific volumes were calculated by the graphical method described by Kraemer (1940). Densities were measured in a 15 ml.-volumetric-flask type of pycnometer with a calibrated capillary neck. For measurements in 8M-urea buffers, each concentration of protein was dialysed separately against buffer.

Concentrations were determined by measurement of the extinction at 280 m μ . The specific extinction coefficient at 280 m μ was determined for horse γ -globulin; a value of 13.8 was obtained after taking into account moisture and ash content. In neutral 8M-urea buffer, at 280 m μ , $E_{1\%}^{1cm}$ was 13.6. For horse γ -globulin in KCl-phosphate buffer, pH 7.7, $\bar{v}$ was 0.739 ± 0.007 , and for horse- γ -globulin chain A in 8M-urea, pH 3.5, $\bar{v}$ was 0.726 ± 0.010 .

Molecular weight of γ -globulin. This was calculated from values of S^0 and D^0 by using the Svedberg (1925) equation. Correction factors were eliminated and charge effects minimized by making all measurements at 25° near the mean isoelectric point in KCl-tris buffer, pH 7.49, except for v , for which the KCl-potassium phosphate buffer, pH 7.7, was used.

Concentrations of protein solutions for sedimentation and diffusion experiments were based on refractive increments obtained from Rayleigh photographs in the synthetic-boundary and diffusion cells respectively, a value of $dn/dc = 0.00174$ being used.

RESULTS

The molecular weights of some well-characterized proteins were determined under the conditions shown in Table 1.

Peptide chains of γ -globulin. In Table 2 are listed the molecular weights of the peptide chains obtained by reduction (chains A and B) of whole γ -globulin, by papain digestion (piece I) of whole γ -globulin, and by reduction of piece I (A piece). All the reduced chains were measured in 8M-urea buffers because of aggregation in the absence of urea. The values of $S_{25,w}$ obtained from runs in 8M-urea buffer, pH 3.5, for horse- γ -globulin and rabbit- γ -globulin chains A at 0.52 g.dl.⁻¹ are 2.17s and 2.39s respectively; this result, combined with the results for the two species in Table 2,

Table 1. *Molecular weights of some characterized proteins*

Experimental details are given in the text.

Protein	Buffer	M_w ($M_z = 17\ 300$)	Concn. (g.dl. ⁻¹)
Ribonuclease	KCl-phosphate, pH 7.7	14 900	1.0
Ovalbumin	KCl-phosphate, pH 7.7	44 800	0.8
Bovine plasma albumin	8M-Urea, pH 7.5, with 5 mM- <i>p</i> -chloromercuribenzoate	65 800	0.36
		57 700	0.65
Avidin	3M-Guanidine-HCl	18 700	0.33

Table 2. *Molecular weights of constituent chains of γ -globulin*

Experimental details are given in the text. All values of M_w are extrapolated to zero concentration.

Protein	Buffer	M_w	Value of $\bar{v}$ used
Horse- γ -globulin chain A	8M-Urea, pH 3.5	50 300	0.726
Horse- γ -globulin chain B	8M-Urea, pH 7.5	19 400	0.721
Rabbit- γ -globulin chain B	8M-Urea, pH 7.5	20 000	0.721
Rabbit- γ -globulin piece I	8M-Urea, pH 3.5	40 700	0.726
Rabbit- γ -globulin A piece	8M-Urea, pH 3.5	21 600	0.726

Fig. 1. Concentration-dependence of the apparent M_w of horse- γ -globulin chain A (O) and of horse- γ -globulin chain B (●) in 8M-urea. Δ , Apparent M_z for horse- γ -globulin chain A (for details, see the text). Measurements of chain A were made at pH 3.5 and of chain B at pH 3.5 and pH 7.5. Experimental details are given in the text.

indicates a degree of similarity in structure between horse and rabbit γ -globulins. The concentration-dependence of molecular weight for these peptides in the presence and absence of urea is shown in Figs. 1 and 2.

A sedimentation-equilibrium experiment was performed to simulate the presence of relatively small soluble aggregate in a solution of protein. Solutions of ovalbumin at a concentration of 0.8 g.dl.⁻¹ containing 0, 2.5, 6.0 and 12.5% (expressed as a percentage of the total protein concentration) of γ -globulin were run at speeds of 14 290, 17 980 and 21 740 rev./min. in a multi-channel cell. Weight-average (M_w) and z-average (M_z) molecular weights were calculated and are given in Table 3 together with the theoretical values of M_w and M_z . M_w could not be measured at higher speeds for proportions of γ -globulin of more than 2.5% owing to excessive broadening of the outer meniscus.

Fig. 2. Concentration-dependence of the apparent M_w of rabbit- γ -globulin piece I in 8M-urea, pH 3.5 (Δ), and in KCl-glycine buffer, pH 3.5 and I 0.20 (O). Experimental details are given in the text.

Horse γ -globulin. The concentration-dependence of the uncorrected sedimentation and diffusion coefficients, shown in Figs. 3 and 4 respectively, is described by the equations:

$$\left(\frac{1}{S^0} \frac{dS}{dc} \right)_{25^\circ} = -5.68 \times 10^{-2} \text{ dl.g.}^{-1};$$

$$S_{25,w}^0 = 7.75 \times 10^{-13} \text{ sec.}$$

$$\left(\frac{1}{D^0} \frac{dD}{dc} \right)_{25^\circ} = -6.89 \times 10^{-2} \text{ dl.g.}^{-1};$$

$$D_{25,w}^0 = 4.80 \times 10^{-7} \text{ cm.}^2 \text{ sec.}^{-1}$$

where c refers to the concentration characteristic of the experiment, i.e. the mean plateau-region concentration in the sedimentation-velocity experiments and half the protein concentration in the diffusion experiments. From these results and the measured value of $\bar{v}$, namely 0.739, the molecular weight is given by

$$10^6 \times 1/M_{app.} = 6.62 - 0.08c$$

giving an extrapolated value of $M = 151\,000$.

DISCUSSION

The values of M_w obtained for previously characterized proteins show good agreement with the accepted values of 17 700 for avidin (Green, 1963), 13 683 for ribonuclease (Hirs, Moore & Stein, 1956*a, b*), 45 000 for ovalbumin (Halwer, Nutting

Table 3. *Dependence of weight-average and z-average molecular weights of mixtures of ovalbumin and γ -globulin on speed*

Experimental details are given in the text. The concentration of γ -globulin is expressed as a percentage (w/w) of the total protein concentration.

Concn. of γ -globulin (%)	0		2.5		6		12.5	
$10^{-5}\omega^2$	M_w (app.)	M_z (app.)	M_w (app.)	M_z (app.)	M_w (app.)	M_z (app.)	M_w (app.)	M_z (app.)
22.4	47 100	56 000	49 200	54 000	50 500	65 000	56 900	78 200
35.4	46 500	53 400	48 300	60 600	48 700	64 000	53 600	68 500
51.8	44 800	48 300	44 900	51 600	—	53 900	—	59 400
Calculated values	45 000	45 000	46 700	53 200	50 400	62 800	57 200	78 700

Fig. 3. Concentration-dependence of the sedimentation coefficient of horse γ -globulin at pH 7.49 and I 0.12. Experimental details are given in the text.

Fig. 4. Concentration-dependence of the diffusion coefficient of horse γ -globulin at pH 7.49 and I 0.12. Experimental details are given in the text.

& Brice, 1951; Fevold, 1951) and 67 500 for bovine plasma albumin (Harrington, Johnson & Ottewill, 1956). The values for bovine plasma albumin in 8M-urea show the expected sign for concentration-dependence of M_{app} . The slightly high value for ribonuclease is similar to those found for old samples by Yphantis (1960), and the relative values of M_w and M_z shown in Table 1 confirm that this is due to size heterogeneity (cf. Richards & Schachman, 1959; Yphantis, 1959). These results, together with those of Cecil & Snow (1962), confirm that this method is reliable for proteins in concentrated urea buffers. Reproducibility and linearity of plots of $1/M_{app}$ against c for separate runs with the multichannel cell show that the difficulties of evaporation with such solvents have been largely overcome by the techniques used.

The error in setting $c_1 = c_1^0$ at r' for determining M_w by 'method III' (Van Holde & Baldwin, 1958) has been calculated for all runs and $(c_1^0/c_1)_{r'}$ is found to vary from 1.005 to 1.04, depending on the length of column and the speed of rotation. This error will only have an appreciable effect on M_w when (d^2n/dr^2) is large, and in fact no correlated deviation of $1/M_{app}$ from the least-squares plot of $1/M_{app}$ against c has been found. For a few proteins, when dn/dr at the outer meniscus is measurable, 'method I' has been used to verify the value of M_w calculated by 'method III' in cases where $(c_1^0/c_1)_{r'} = 1.04$, and good agreement has been obtained.

Measurement of molecular weights in the presence of aggregate. The molecular weights of horse- γ -globulin peptide chains A and B determined by equilibrium sedimentation are substantially lower than the values quoted by Fleischman *et al.* (1962), where the Trautman plot of Archibald approach-to-equilibrium data was used (Trautman & Crampton, 1959). This discrepancy is attributed to the presence of small amounts of aggregated material, probably not more than 10 times the size of the monomer. That aggregate is present in solutions of both chains A and B in 8M-urea buffers is shown by the broadening of the outer meniscus and by an upward curvature in the plot of $(1/r)(dn/dr)$ against n , the slope and shape of this plot depending on the speed of rotation. When equilibrium is established at a series of increasing speeds, the apparent M_w decreases until it reaches a constant minimum value, and this provides a criterion for measuring the true molecular weight of the monomer peptide chain in the solvent concerned. Since 'method III' involves the use of the refractive increment of the initial solution n_0^0 , the calculated value of M_w will be low by a factor corresponding to the contribution of the aggregate to n_0^0 . Thus it would be preferable to use a treatment such as 'method II' or Lamm's (1929) plot which does not involve a knowledge of the initial concentration, c_0 . Use of the latter has confirmed its poorer resolution of aggregates (see, for example, Schachman, 1959) whereas for short

columns 'method II' is less precise and higher speeds are required to reach a minimum value of the slope of $(1/r)(dn/dr)$ against n . Values of M_z calculated in this way by 'method II' for horse- γ -globulin chain B lie within $\pm 5\%$ of the mean value for M_w , however, so that the value of M_w is not likely to be more than 5% low.

To justify further the use of the above approach for obtaining the molecular weight of a slow component, solutions of ovalbumin containing small amounts of γ -globulin to simulate a small aggregate were allowed to come to equilibrium in a multichannel cell at three different speeds. The results are compared in Table 3 with the theoretical M_w and M_z , showing reasonable agreement at low speed. Increasing the speed leads to decreases in the apparent M_w and M_z values, and to a value of M_w for ovalbumin close to 45 000 in the presence of 2.5% of γ -globulin. In this model system, the 'aggregate' is only four times the size of the monomer.

For a run on chain A at 17 250 rev./min. with 0.8 mm. columns (see Fig. 1) the value of apparent M_z extrapolated to zero concentration was such that $M_z = 2M_w$, that is, similar to the value for horse- γ -globulin chain A reported earlier. Thus it is reasonable to suppose that aggregation is the basis of the apparent discrepancy between the Trautman-plot M_z and the equilibrium M_w values reported here. Taking a simplified case where the monomer is accompanied by 5% of n -mer, for the apparent M_z to equal twice M_w , n must equal 7. Assuming S to be proportional to $M^{\frac{1}{2}}$, the sedimentation coefficients of chain B and its aggregate in 8M-urea buffer would be 0.6s and 2.2s. It would be difficult to detect heterogeneity in such a system by boundary-spreading experiments.

Peptide chains of γ -globulin. The value of 40 700 obtained for rabbit- γ -globulin piece I in neutral 8M-urea is significantly lower than the value quoted by Charlwood (1959). The glycopeptide associated with this piece (Fleischman *et al.* 1963) is removed by propionic acid and, presumably, by 8M-urea. If a value of 2000 is assumed for the glycopeptide, M_w for piece I would then become 42 800. Further, in potassium chloride-glycine buffer, pH 3.5, the negative slope of the plot of $1/M_{app}$ against c for piece I (Fig. 2) indicates some concentration-dependent association that could lead to an apparent discrepancy with measurements made at finite concentrations. The value for piece I is supported by the sum of the weights of A piece and chain B.

There is a strong tendency for these peptide chains to aggregate. In the absence of urea and at 25°, horse- γ -globulin and rabbit- γ -globulin chains B form dimers, the former with little or no evidence of reversibility, whereas chains A and A pieces

form mixtures of aggregates. The behaviour of rabbit- γ -globulin piece I in aqueous solution suggests a certain degree of reversible interaction that is inhibited by urea, whereas the tendency of A piece to aggregate is still present in 8M-urea buffer.

Horse γ -globulin. The extrapolated value of 151 000 for the molecular weight of horse γ -globulin agrees reasonably well with the sum of the constituent chains, according to the structure proposed by Fleischman *et al.* (1963).

The M_{app} obtained from sedimentation and diffusion measurements is virtually independent of concentration. The only other examination of γ -globulin that involved a study of the concentration-dependence of the diffusion coefficient was that of Cammack (1962), where a large negative slope was obtained for the plot of $1/M_{app}$ against c for rabbit γ -globulin. The latter finding is supported by the Archibald data for rabbit γ -globulin (Charlwood, 1959; Cammack, 1962). This difference between the concentration behaviour for these two investigations may well be due to a species difference. One possible explanation for a negative slope for the plot of $1/M_{app}$ against c is a concentration-dependent association. The present work has shown that there is a marked difference in the tendency to associate of fragments from rabbit γ -globulin and from horse γ -globulin. Human γ -globulin exhibits a behaviour most readily explained by reversible association when subjected to low ionic strength near the isoelectric point (Doty & Edsall, 1951; Cann, 1953), and it is possible that this tendency exists to a very slight extent for rabbit γ -globulin even at higher ionic strengths.

SUMMARY

1. Weight-average molecular weights have been determined in urea buffers for the products of reduction of γ -globulin and of the papain-digestion piece I by the sedimentation-equilibrium method.
2. The discrepancy with previous higher values has been demonstrated to be due to the unavoidable presence of small amounts of aggregate.
3. The molecular weight of horse γ -globulin has been established at 151 000 by combining values of S^0 , D^0 and $\bar{v}$.

The author is indebted to Dr R. A. Kekwick for providing facilities at The Lister Institute, London, to Dr J. M. Creeth in particular for guidance in the measurement and interpretation of diffusion data, and to Dr P. A. Charlwood for helpful discussion. The technical assistance of Mr A. McKay is gratefully acknowledged. This work was made possible by generous grants from the National Science Foundation, U.S.A., the U.S. Public Health Service and the Wellcome Trust.

REFERENCES

- Akeley, D. F. & Gosting, L. J. (1953). *J. Amer. chem. Soc.* **75**, 5685.
- Anson, M. L. & Stanley, W. M. (1941). *J. gen. Physiol.* **24**, 679.
- Cammack, K. A. (1962). *Nature, Lond.*, **194**, 745.
- Cann, J. R. (1953). *J. Amer. chem. Soc.* **75**, 4218.
- Caspary, E. A. & Kekwick, R. A. (1957). *Biochem. J.* **67**, 41.
- Cecil, R. & Snow, N. S. (1962). *Biochem. J.* **82**, 255.
- Charlwood, P. A. (1959). *Biochem. J.* **73**, 126.
- Creeth, J. M. (1958). *J. phys. Chem.* **62**, 58.
- Doty, P. & Edsall, J. T. (1951). *Advanc. Protein Chem.* **6**, 70.
- Fevold, H. L. (1951). *Advanc. Protein Chem.* **6**, 187.
- Fleischman, J. B., Pain, R. H. & Porter, R. R. (1962). *Arch. Biochem. Biophys.* Suppl. 1, 174.
- Fleischman, J. B., Porter, R. R. & Press, E. M. (1963). *Biochem. J.* **88**, 220.
- Gosting, L. J. & Morris, M. S. (1949). *J. Amer. chem. Soc.* **71**, 1998.
- Green, N. M. (1963). *Biochem. J.* (in the Press).
- Halwer, M., Nutting, G. C. & Brice, B. A. (1951). *J. Amer. chem. Soc.* **73**, 2786.
- Harrington, W. F., Johnson, P. & Ottewill, R. H. (1956). *Biochem. J.* **62**, 569.
- Hirs, C. H. W., Moore, S. & Stein, W. H. (1956a). *J. biol. Chem.* **219**, 623.
- Hirs, C. H. W., Moore, S. & Stein, W. H. (1956b). *J. biol. Chem.* **221**, 385.
- Kekwick, R. A. & Cannan, R. K. (1936). *Biochem. J.* **30**, 226.
- Kraemer, E. O. (1940). In *The Ultracentrifuge*, p. 58, Eqn. (110), by Svedberg, T. & Pedersen, K. O. Oxford University Press.
- Lamm, O. (1929). *Z. phys. Chem.* **A143**, 177.
- Richards, E. G. & Schachman, H. K. (1959). *J. phys. Chem.* **63**, 1578.
- Schachman, H. K. (1959). *Ultracentrifugation in Biochemistry*, p. 204. New York and London: Academic Press.
- Svedberg, T. (1925). *Zsigmondy-Festschr.: Kolloidzshr.* **36**, (suppl.) 53.
- Trautman, R. & Crampton, C. F. (1959). *J. Amer. chem. Soc.* **81**, 4036.
- Van Holde, K. E. & Baldwin, R. L. (1958). *J. phys. Chem.* **62**, 734.
- Yphantis, D. A. (1959). *J. phys. Chem.* **63**, 1742.
- Yphantis, D. A. (1960). *Ann. N.Y. Acad. Sci.* **88**, 586.

Biochem. J. (1963) **88**, 239

The Role of Molybdenum in Nitrate Assimilation by *Agrobacterium tumefaciens*

By C. K. RAMAKRISHNA KURUP AND C. S. VAIDYANATHAN
Department of Biochemistry, Indian Institute of Science, Bangalore 12, India

(Received 14 December 1962)

Agrobacterium tumefaciens initiates specifically the crown-gall disease of plants. Relatively little is known about the metabolic characteristics of the bacterium since most of the biochemical researches have been concerned with the tumour tissue itself (Klein & Link, 1955). Vardanis & Hochster (1961a, b) have studied the nucleic acid and glucose metabolism of the bacterium. A study of its nitrogen metabolism has been undertaken in this Laboratory to try to elucidate the mechanism and biochemical aspects of tumour induction.

The requirement of molybdenum in nitrate assimilation is specifically due to its involvement in enzymic nitrate reduction. This element has been shown to be the metal component of nitrate reductase from *Neurospora* (Nicholas & Nason, 1954a, b), soya-bean leaves (Nicholas & Nason, 1955a; Evans & Hall, 1955). *Escherichia coli* (Nicholas & Nason, 1955b; Taniguchi & Itagaki, 1960) and *Pseudomonas aeruginosa* (Fewson & Nicholas, 1961), and to undergo a reversible oxidation-reduction from the 5+ to the 6+ oxidation

state during enzyme action (Nicholas & Stevens, 1956; Fewson & Nicholas, 1961). The present results on growth and nitrate-reductase activity under conditions of tungstate inhibition and molybdenum deficiency indicate that molybdenum is required for the nitrate metabolism of *Agrobacterium tumefaciens* also.

MATERIALS AND METHODS

Cultural conditions. *Agrobacterium tumefaciens* (strain B6) was grown in modified Czapek-Dox medium (Salle, 1954) containing 30 g. of sucrose and 3 g. of NaNO₃ or 4.68 g. of ammonium sulphate/l. of medium. This was distributed in 200 ml. amounts in 1 l. conical flasks, sterilized by autoclaving for 15 min. at 15 lb./in.² pressure, sown with a suspension of cells from potato-agar slopes and incubated in the dark at 30° with occasional shaking.

Determination of growth. In the nitrate medium the bacterium completed the lag phase of growth after about 18 hr. and the exponential phase about 48 hr. after inoculation. Thereafter the growth was stationary till about 96 hr. This pattern was unaffected by the presence of molybdate