

collisional broadening of CO by CO₂ (7), since the Venus atmosphere is almost entirely CO₂. The temperature exponent in Eq. 1 is that for quadrupole-quadrupole interactions, which are expected to dominate the collisional broadening of this transition. The 1972 NASA nightside model of the Venus atmosphere (8) was used for the temperature and pressure profiles. We calculated the brightness temperature T_B for a normal ray by numerically integrating the radiative transfer equation between altitudes of 60 and 130 km. Above 130 km the contribution to T_B is negligible; at 60 km a brightness temperature of 320°K was used for the upwelling radiation, which is based on the measurements of Ulich (9). Since the angular extent of the planet is much less than the antenna beam width, we obtained the disk temperature T_D by integrating T_B over the planet, assuming spherical symmetry. The fractional absorption of CO was then determined from the relative values of T_D at the center of the spectral line and 8 Mhz away, respectively. We estimate that the errors due to the numerical calculation process are less than 1 percent.

Three of the CO mixing ratio profiles used in the calculation are shown in Fig. 2. The corresponding calculated spectra are given in Fig. 1 with the measured spectrum. Because of pressure broadening of the CO line, our measurement is relatively insensitive to CO below ~ 80 km. In the range from 85 to 100 km the measurement is very sensitive to the CO mixing ratio profile. Above ~ 105 km the measurement is sensitive only to the total column density of CO, because of the dominance of the Doppler broadening which is a weak function of altitude.

Profile A of Fig. 1 is that calculated by Liu and Donahue (10) for an eddy diffusion coefficient of $10^6 \text{ cm}^2 \text{ sec}^{-1}$, and gives the best fit to our measurement of the profiles reported by Liu and Donahue and by Sze and McElroy (11). However, profile A implies an absorption feature that is significantly narrower and slightly stronger than given by the measurements, an indication that it gives too much CO at higher altitudes and too little at lower altitudes. A preliminary comparison of our measurements with the CO distribution recently calculated by Dickinson and Ridley (12) indicates that their calculated distribution has more nightside CO than implied by our measurement. Further measurements of the spectral line reported here will determine the extent of diurnal variation of CO in the upper atmosphere of Venus.

Profile B illustrates a CO mixing ratio profile that is consistent both with our measurement and with the infrared measurement of Connes *et al.* (4), which is sen-

sitive to CO at lower altitudes. Profile C differs from profile B only in having more CO in the altitude region from 87 to 102 km and illustrates the sensitivity of our measurement to this altitude region. Because our measurement provides quantitative information on CO at higher altitudes on Venus than has been previously available, it should lead to the development of more precise photochemical models of the upper atmosphere of Venus.

Note added in proof: On 9 November 1975 we detected the 115-Ghz microwave line of CO in the spectrum of Mars also. The 11-m-diameter antenna of the National Radio Astronomy Observatory located on Kitt Peak, Arizona, was used for this observation.

R. K. KAKAR
J. W. WATERS

*Space Sciences Division, Jet Propulsion
Laboratory, Pasadena, California 91103*

W. J. WILSON

*Electronics Research Laboratory,
Aerospace Corporation,
El Segundo, California 90245*

References and Notes

1. A. H. Barrett, *Mem. Soc. R. Sci. Liege* **8**, 197 (1963).
2. W. M. Sinton, *J. Quant. Spectrosc. Radiat. Transfer* **3**, 551 (1963).
3. V. I. Moroz, *Mem. Soc. R. Sci. Liege* **9**, 406 (1964).
4. P. Connes, J. Connes, L. D. Kaplan, W. S. Benedict, *Astrophys. J.* **152**, 731 (1968).
5. B. L. Ulich and R. W. Haas, *Astrophys. J. Suppl. Ser.*, in press.
6. J. W. Waters, W. J. Wilson, F. I. Shimabukuro, *Science*, in press.
7. D. A. Draeger and D. Williams, *J. Opt. Soc. Am.* **58**, 1399 (1968).
8. R. B. Noll and M. B. McElroy, *NASA SP-8011* (1972).
9. B. L. Ulich, *Icarus* **21**, 254 (1974); unpublished data. We obtained the brightness temperature at 2.6-mm wavelength by interpolating between the measured values of $300^\circ \pm 21^\circ \text{K}$ at 2.1 mm and $355^\circ \pm 10^\circ \text{K}$ at 3.5 mm, respectively.
10. S. C. Liu and T. M. Donahue, *Icarus* **24**, 148 (1975).
11. N. D. Sze and M. B. McElroy, *Planet. Space Sci.* **237**, 63 (1975).
12. R. E. Dickinson and E. C. Ridley, *J. Atmos. Sci.* **32**, 1219 (1975).
13. We thank Drs. S. Gulkis, F. W. Taylor, and S. Kumar for helpful discussions. This report presents the results of one phase of research carried out at the Jet Propulsion Laboratory, California Institute of Technology, under contract NAS 7-100, sponsored by the National Aeronautics and Space Administration. The work at the Aerospace Corporation was jointly supported by an internal research program and by the National Science Foundation (grant MP573-04554).

20 October 1975

Protein Purification: Adsorption Chromatography on Controlled Pore Glass with the Use of Chaotropic Buffers

Abstract. *Chromatography on controlled pore glass in combination with chaotropic buffers makes possible, in a single step, protein purifications of several hundredfold. The new emphasis is on highly selective controllable adsorption. The method is useful for the purification and concentration of proteins from large volumes of complex media and for the purification of proteins that are poorly soluble or tend to aggregate in aqueous solution. D(-)-β-Hydroxybutyrate dehydrogenase, a mitochondrial membrane-bound protein, several soluble proteins, and staphylococcal α toxin, which can be purified directly from large volumes of culture medium, are used to illustrate the method.*

Controlled pore glass (CPG), first introduced by Haller, has been used for the separation of a number of biologically interesting compounds (1). In most of these applications, the separation is based mainly on differences in molecular size. We now describe a new procedure for the purification of proteins which emphasizes the use of adsorption chromatography on CPG in combination with chaotropic buffers. The high adsorption affinity of CPG allows rapid concentration and buffer replacement for proteins from large volumes of salts, sugars, or culture media. Selectivity in the elution of proteins is achieved by the use of different chaotropic eluting agents, by varying the pH, and by changing the ionic strength. Purifications of several hundredfold have been achieved with this method. The technique is illustrated in the purification of several types of proteins, including a membrane protein, several soluble proteins, and an exotoxin.

The column packing chosen was CPG-

10-350 (75- to 125-μm particles; Electro-Nucleonics). The particular lots used had mean pore diameters of 313 to 368 Å with surface areas of between 62 and 75 m²/g. The pore size selected precludes the entry of membranous material into the inner volume of the CPG, effectively limiting the adsorption process to soluble proteins or small aggregates. Used CPG can be reclaimed either by washing in a mixture of persulfate and sulfuric acid for several days or by heating on a steam bath with 10 percent nitric acid for several hours, followed in either case by repeated washing with water to neutrality. However, the beads are sensitive to strong alkali, as well as to hydrofluoric acid, and both should be avoided.

The application of the CPG method to the purification of the apoenzyme form of D(-)-β-hydroxybutyrate dehydrogenase (BDH) (E.C. 1.1.1.30) is of interest since BDH is a mitochondrial membrane-bound protein that has a tendency to aggregate in

ordinary solutions (2). The addition of LiBr, a chaotropic salt, to the extraction medium minimizes this tendency to self-associate, and thus facilitates chromatographic purification to homogeneity.

The supernatant from phospholipase A digestion of beef heart mitochondria (2), which contains the soluble enzyme, is adjusted to 25 mM tris(hydroxymethyl)aminomethane (tris)-HCl, pH 7.5, 0.4M LiBr, 5 mM dithiothreitol, and 5 to 10 mg of protein per milliliter. Approximately 95 percent of the BDH activity and half of the 5-g protein sample are adsorbed. The purification of BDH from 73 g of mitochondrial protein is shown in Table 1. The product is homogeneous as studied by polyacrylamide gel electrophoresis (Fig. 1A, gel 6). The apodehydrogenase (apoBDH) is soluble, devoid of lipid, and inactive until reactivated specifically by incubation with phospholipids containing lecithin. The availability of the apoenzyme with these special properties makes possible direct studies of protein-lipid interactions and of the way in which such interaction leads to biological activity (3). During purification of apoBDH, the complex mixture of adsorbed proteins is fractionated into several distinct protein groups before apoBDH elution (Fig. 1A), indicating the power of the method in terms of general application for protein purification. The use of a second, smaller CPG column allows easy sample concentration and further purification, if necessary (Table 1).

Recoveries of BDH activity from the purification and concentration columns are approximately 20 to 30 percent and 100 percent of the applied activity, respectively. The yield from 73 g of mitochondrial protein was 14 mg of apoBDH with a specific activity approximately 100-fold greater than that from the supernatant of phospholipase A digestion. The purification

Table 1. Elution scheme for chromatography of D- β -hydroxybutyrate apodehydrogenase on CPG. The columns for the purification and concentration of apoBDH are summarized in terms of the elution steps. The bed volumes of CPG were 250 ml and 11 ml, respectively. The concentration column serves both to concentrate the product and to make possible additional purification.

Elution step	Composition of eluent*	Elution volume (ml)	
		Purification column	Concentration column
1	Sample applied†	780	2630
2	1M phosphate, pH 6.5‡	500	20
3	1.5M phosphate, pH 8.15§	500	
4	1M phosphate, pH 8.15§	1500	45
5	0.75M phosphate, pH 8.15§	1500	100
6	0.05M phosphate, pH 6.5	500	20
7	0.1M tris-HCl, pH 7.5	500	25
8	0.1M tris-HCl, pH 7.9, 1M LiBr¶	1400	
9	0.1M tris-HCl, pH 8.0, 1M LiBr¶	800	60
10	0.1M tris-HCl, pH 8.15, 1M LiBr¶	750	95
11	0.2M tris-HCl, pH 8.25, 2M LiBr#	500	

*All buffers contain, in addition to the components listed, 5 mM dithiothreitol, which was used to stabilize the apoBDH. †The sample preparation is described in (2) and in the text. ‡This buffer replaces the LiBr (from the column loading), which would otherwise elute apoBDH at pH 8.15, the pH used for the elution of contaminant proteins. §Buffers 3 to 5 removed most contaminant proteins prior to elution of BDH (with buffers 8 to 10). The first 50 ml of effluent of buffer 4 contained a white precipitate (Fig. 1, gel 3). Elution with buffer 5 was continued until the effluent was clear and colorless, yielding a contaminant protein with a molecular weight of about 36,000 (Fig. 1, gel 5). ||Buffers 6 and 7 decreased the pH and phosphate concentration of the columns to prevent the formation of a lithium phosphate precipitate and to allow a graded increase in eluent pH for apoBDH elution. ¶Elution of apoBDH began at about pH 7.9 in the presence of 1M bromide ions. Contaminant proteins still adsorbed to the CPG began to elute at pH 8.1. Thus, a narrow pH slot accomplished the apoBDH elution and further purification. #This buffer yielded a clear, yellowish-green effluent which contained much protein and about 5 to 15 percent of the applied apoBDH activity. This step was not included in the routine elution. Purified apoBDH-containing fractions from buffers 8 to 10 (2630 ml) were adjusted to pH 7.0 to 7.5 prior to loading onto the concentration column at levels of up to 2 mg of protein per milliliter of CPG.

procedure has been scaled up and 150 mg of purified apoBDH has been obtained from 400 g of protein from bovine heart mitochondria, in a volume of 40 liters.

Adsorption chromatography on CPG can also be used to effectively separate the more conventional soluble proteins. For example, bovine serum albumin, chymotrypsinogen A, lysozyme, and myoglobin can readily be separated from one another. The mixture (2 mg of each in 0.01M potassium phosphate buffer, pH 6.5) was applied to a column containing 3 ml of CPG-10-350. The serum albumin eluted with this buffer. The sequential use of three different buffers, four column volumes each [(i) 0.10M potassium phosphate, pH 6.5; (ii) 1.0M KI and 0.01M potassium phosphate, pH 6.5; and (iii) 0.5M potassium phosphate, pH 8.15], allowed the selective elution of chymotrypsinogen, lysozyme, and myoglobin, respectively.

Adsorption chromatography onto CPG is an effective and rapid purification technique for staphylococcal α toxin. The staphylococci were grown on yeast extract medium (4), and the cells were removed by centrifugation. The supernatant (2800 ml), adjusted to pH 6.8, was then passed directly onto a column containing 500 g of CPG-10-350, resulting in the quantitative adsorption of hemolytic toxin activity. The CPG was washed with five column volumes of 0.05M potassium phosphate buffer, pH 6.8, and the toxin was then eluted with 1M potassium phosphate, pH 7.5.

Adsorption chromatography onto CPG is an effective and rapid purification technique for staphylococcal α toxin. The staphylococci were grown on yeast extract medium (4), and the cells were removed by centrifugation. The supernatant (2800 ml), adjusted to pH 6.8, was then passed directly onto a column containing 500 g of CPG-10-350, resulting in the quantitative adsorption of hemolytic toxin activity. The CPG was washed with five column volumes of 0.05M potassium phosphate buffer, pH 6.8, and the toxin was then eluted with 1M potassium phosphate, pH 7.5.

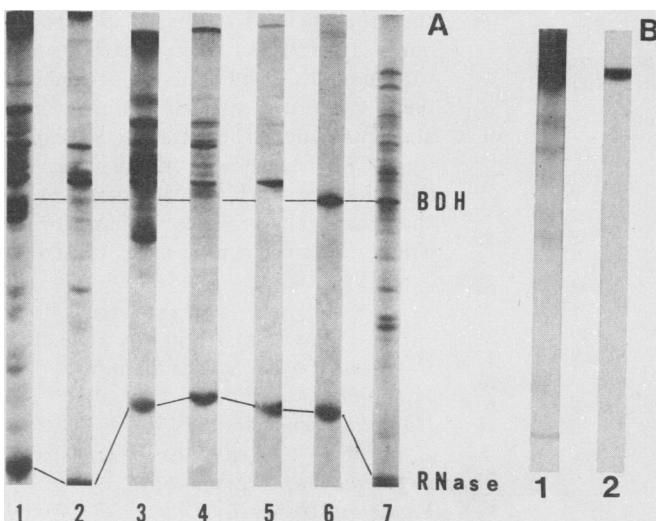


Fig. 1. Polyacrylamide gel electrophoresis of fractions obtained in the purification procedures for BDH and staphylococcal α toxin. (A) The fractionation procedure for the purification of BDH is outlined in Table 1. (Gel 1) Beef heart mitochondria (60 μ g of protein). (Gel 2) The column effluent from step 1 (Table 1). (Gel 3) Residue from centrifugation of the turbid effluent obtained at the elution front (first 50 ml) with buffer 4. (Gel 4) The remainder of the effluent from buffer 4. (Gel 5) The effluent from elution with buffer 5. (Gel 6) Purified BDH, 10 μ g of protein, obtained by elution with 1M LiBr, pH 7.9 to 8.1 (specific activity, 100 μ mole of NAD⁺ reduced per minute per milligram of protein). (Gel 7) The last effluent, obtained with buffer 11 (Table 1). The gels were stained with amido black. Ribonuclease A (10 μ g of protein) was added as an internal standard to each gel. The positions of BDH and ribonuclease are indicated. Electrophoresis was in acetic acid-urea (7). (B) Staphylococcal α toxin was purified by electrophoresis at pH 8.9 under nondissociating conditions (5). Gel 1 shows the electrophoretic pattern of a 150- μ g sample of the precipitate obtained from the growth medium after the pH was adjusted to 6.8 and ammonium sulfate was added to 90 percent saturation (5). Gel 2 shows the purified α toxin after chromatography on CPG and DEAE.

The α toxin eluted under these conditions is 95 percent pure. Any small amounts of residual protein contaminants were removed on diethylaminoethyl (DEAE)-Sephadex in 0.05M potassium phosphate buffer, pH 8.2. The α toxin preparation after DEAE-Sephadex chromatography appeared to be homogeneous on polyacrylamide gel electrophoresis at pH 8.9 (Fig. 1B); 98 percent was form B (5). It seems likely that form A is a breakdown product derived from form B, since a previous method of purification (5), which involved more extensive handling, yielded 20 percent as form A. Furthermore, the glass procedure removed a proteinase from the α toxin which may be responsible for the conversion of the form B to the form A. The total recovery of hemolytic units (4) was 81 percent; 190 mg of homogeneous α toxin was obtained and had a specific activity of 2.8×10^4 hemolytic units per milligram of protein. Contaminating δ toxin activity, as assayed by relative hemolytic activity on rabbit and human erythrocytes, was reduced from 20 percent in the growth medium to 0.1 percent in the protein eluting from the CPG column.

Our new procedure for purification of α toxin illustrates another advantage of selective adsorption chromatography. The use of a single CPG column allowed the purification of about 200 mg of α toxin from a growth medium containing about 100 g of peptides and amino acids, that is, the purification was approximately 500-fold. The enrichment is even severalfold greater in that salts and sugars are present as well. For this application, ion exchange is of little use because α toxin adsorbs poorly at the concentration of salts in the growth medium. The CPG and DEAE adsorption procedures together make possible the production of large amounts of homogeneous α toxin in 1 to 2 days with a threefold better yield, as compared to previous procedures.

The use of adsorption chromatography on CPG combined with chaotropic buffer elutions for protein purification is still largely empirical. The nature of the eluting solute significantly affects the degree of protein-CPG interaction (Table 2). In general, the elution efficiency of the various salts follows their order in the chaotropic series (6). At pH 8.15, thiocyanate, nitrate,

iodide, and bromide elute BDH. However, BDH is not eluted by buffers at pH 8.15 containing 0.75M to 1.5M phosphate, 1M acetate, ethanol at concentrations ranging up to 40 percent (by volume), or 0.5M guanidinium hydrochloride (inactivation occurs when BDH is exposed to ethanol concentrations in excess of 40 percent or concentrations above 0.7M guanidinium hydrochloride). The role of the cation in apoBDH elution is somewhat less variable than that of the anion. However, choline chloride elutes apoBDH more efficiently than do the other chloride salts.

The recovery of α toxin from CPG columns also depends upon the nature of the eluent (Table 2). The potency of elution varies with the anion tested in a manner similar to that observed during apoBDH elution. The two cations choline and tris, as the chloride salts, are more effective in eluting α toxin, as compared with KCl or NaCl. The antichaotrope phosphate effectively elutes α toxin, and as such it is the only anion tested that has a different effect in terms of the general order of elution potency. This may suggest that phosphate primarily modifies the protein per se.

A second variable, the pH of the eluent, has a profound effect on the binding of proteins to CPG; and, in general, increasing pH tends to weaken the binding. Thus, apoBDH adsorbs strongly to CPG in 1M LiBr when the pH is below 7.5, but is eluted in the same salt if the pH is raised to 7.9. Similarly, α toxin in all salts tested is bound to CPG if the pH is below 6.9, but is eluted with 1M phosphate buffer when the pH is raised to 7.5.

The ionic strength is a third significant variable in this procedure, and its effects are not readily predictable. For example, we have found that some proteins elute with 1M phosphate buffer, pH 8.15 (Fig. 1, gel 3), but not with 1.5M phosphate buffer at the same pH.

Finally, a major advantage of using chaotropic salts in the purification procedures for proteins is their capacity to solvate proteins, tending both to stabilize them and to prevent their self-association and aggregation. The extraordinary capacity of CPG to reversibly adsorb proteins in the presence of high concentrations of chaotropic salts provides the basis for the chromatographic procedure described here.

HANS GEORG BOCK

PATE SKENE, SIDNEY FLEISCHER

Department of Molecular Biology,
Vanderbilt University,
Nashville, Tennessee 37235

PAUL CASSIDY, SIDNEY HARSHMAN
Department of Microbiology,
Vanderbilt University

Table 2. Elution of apoBDH and staphylococcal α toxin from CPG using different salts at 1M concentration. For recovery of BDH activity, small columns, containing 0.5 ml of CPG-10-350, were prepared in Pasteur pipets (0.56 cm inside diameter by 2.0 cm), with a glass wool plug to retain the CPG. Separate columns were prepared for each of the elution conditions shown. Purified apoBDH (0.4 to 0.6 mg in 0.2 ml of 1 mM HEPES buffer, pH 7.0, containing 0.4M LiBr and 5 mM dithiothreitol) was applied to each column at 4°C, and the column was washed with 3 ml of 1 mM HEPES buffer, pH 7.0, containing 2 mM dithiothreitol. Under these conditions apoBDH adsorption onto CPG was complete. The columns were then eluted with 0.1M tris-acetate buffer, pH 8.15, containing 2 mM dithiothreitol, and the various salts, at 1.0M. No apoBDH was eluted by 0.1M tris-acetate, pH 8.15, in the absence of these extra salts. Fraction A contained the first 0.5 ml of effluent; fraction B contained the next 1.0 ml of effluent; and fraction C contained the next 2.0 ml of effluent. Fraction D was eluted by 4 ml 0.1M tris-acetate buffer, pH 8.15, containing 2 mM dithiothreitol and 1M LiBr, which eluted any remaining apoBDH activity from each column. The elution efficiency for each salt is shown as the percentage activity recovered per fraction. Total activity recovered is the sum of A, B, C, plus D. The difference between total activity and 100 percent is presumed to represent the loss due to inactivation. The BDH activity was assayed after the addition of mitochondrial phospholipid to the apoBDH. For the recovery of α toxin small CPG columns as in A, above, were prepared in 20 mM tris-HCl, pH 7.5. The α toxin samples (50 μ g of protein in the same buffer) were applied to the columns, and chromatography was performed at 4°C. After the columns were washed with 3 ml of 0.1M tris-HCl, pH 7.5, during which no toxin activity eluted, they were eluted with 0.1M tris-HCl, pH 7.5, in 1M salt as listed. Three separate fractions were collected as described for apoBDH. In this case, the sum of fractions A to C is designated total activity.

Salt	ApoBDH: recovery of enzymatic activity (%)				Total A to D	α Toxin: recovery of hemolytic activity (%)			Total A to C
	Initial elution			D*		Initial elution			
	A	B	C			A	B	C	
NaSCN	35	19	6	0	60	21	52	0	73
NaI	42	13	6	0	61	4	66	42	112
NaNO ₃	15	29	41	0	85	6	32	13	51
KBr	19	27	38	5	89				
NaBr	23	22	35	9	89	0	35	34	69
LiBr	21	24	40	12	97				
KCl	0	5	24	58	87	0	0	19	19
NaCl	0	5	21	70	96	0	5	9	14
Choline-HCl	6	55	6	0	67	22	37	3	62
Tris-HCl						14	49	0	63
Na ₂ SO ₄						0	0	0	0
CH ₃ COONa	0	0	0	81	81	0	1	2	3
Na ₃ PO ₄	0	0	0	100	100	18	48	0	66

*1M LiBr.

References and Notes

1. W. Haller, *Nature (London)* **206**, 693 (1965); *Virology* **33**, 740 (1967); R. C. Collins and W. Haller, *Anal. Biochem.* **54**, 47 (1973); H. H. Gschwender, W. Haller, P. H. Hofschneider, *Biochim. Biophys. Acta* **190**, 460 (1969).
2. H. G. Bock and S. Fleischer, *Meth. Enzymol.* **32**, 374 (1974); *J. Biol. Chem.* **250**, 5774 (1975).
3. S. Fleischer, H. G. Bock, P. Gazzotti, in *Membrane Proteins in Transport and Phosphorylation*, G. F. Azzone, M. E. Klingenberg, E. Quagliariello, N. Siliprandi, Eds. (North-Holland, Amsterdam, 1974), p. 125; P. Gazzotti, H. G. Bock, S. Fleischer, *Biochem. Biophys. Res. Commun.* **58**, 309 (1974); *J. Biol. Chem.* **250**, 5782 (1975).
4. A. W. Bernheimer and L. L. Schwartz, *J. Gen. Microbiol.* **30**, 455 (1963).
5. H. R. Six and S. Harshman, *Biochemistry* **12**, 2672 (1973).
6. Y. Hatefi and W. G. Hanstein, *Meth. Enzymol.* **31**, 770 (1974).
7. W. L. Zahler, B. Fleischer, S. Fleischer, *Biochim. Biophys. Acta* **203**, 283 (1970).
8. Supported in part by NIH grants AM 14632, AM 17026 (Diabetes-Endocrinology Center, Vanderbilt University), AI-11564, and CA-17050 and by NSF grant P3B1190. We are pleased to acknowledge the continued interest and cooperation of Mr. Andrew Hanlein and Electro-Nucleonics, Inc. (Fairfield, N.J.). H.G.B. was supported by Vivian Allen Fund, Vanderbilt School of Medicine, and P.C. is a Vanderbilt biomedical sciences fellow.

8 August 1975; revised 28 October 1975

Crossbridge Attachment, Resistance to Stretch, and Viscoelasticity in Resting Mammalian Smooth Muscle

Abstract. *There exists a calcium-dependent resistance to stretch in resting mammalian smooth muscle that is not caused by depolarization of the cell membrane or release of calcium from intracellular sites. The similarity of the resistance to stretch in the resting state to that in rigor suggests that most, if not all, crossbridges are attached and thus able to resist stretch in noncontracting smooth muscles. When the muscle is stretched the breaking and subsequent reformation of links in nonstrained positions accounts for most of the so-called viscoelasticity, except at extreme lengths.*

When smooth muscles are rapidly stretched they exhibit a sharp rise in tension, which then decays nearly exponentially over a period of time. The initial rise in tension (resistance to stretch) and decay of tension (stress relaxation) have been attributed to a passive viscoelastic property of the muscle (1). In the course of experiments in which the effects of different ions on such relationships as length-tension and force-velocity were examined in various smooth muscles, it was noted that resistance to stretch and the resulting stress relaxation were markedly affected by the presence or absence of calcium in the bathing medium.

Tension was monitored in relation to change in length or stimulation (induced electrically or by potassium) in strips of rabbit taenia coli ($N = 56$), portal vein ($N = 8$), myometrium ($N = 6$), and aorta ($N = 4$), and of guinea pig taenia coli ($N = 8$). The muscles were bathed in a flowing Krebs-bicarbonate solution gassed with 95 percent O_2 and 5 percent CO_2 , which had the following composition (millimolar): NaCl, 118; KCl, 4.7; $MgSO_4$, 1.18; KH_2PO_4 , 1.2; $CaCl_2$, 1.9; $NaHCO_3$, 25; and glucose, 11. Calcium-free Krebs solution contained 2 mM EGTA [disodium salt of ethylene glycol-bis(β -aminoethylether)- N,N' -tetraacetic acid, Sigma] and no added calcium. In sodium-free solutions NaCl was replaced with 118 mM dimethyldiethanol ammonium chloride (DDA, Eastman Organic Chemicals), $NaHCO_3$ was replaced with 25 mM choline bicarbonate, and atropine (1 μM) was added (2). 5-[[3,4-Dimethoxyphenethyl] methylamino]-

2-(3,4,5-trimethoxyphenyl)-2-isopropylvaleronitrile (D600, a gift from Knoll, AG) was used at 1 to 10 μM . For stimulation by potassium, KCl was added to a concentration of 100 mM in the Krebs solution. Solutions for caffeine contractures included either 4 or 8 mM caffeine. Thymol (Fisher

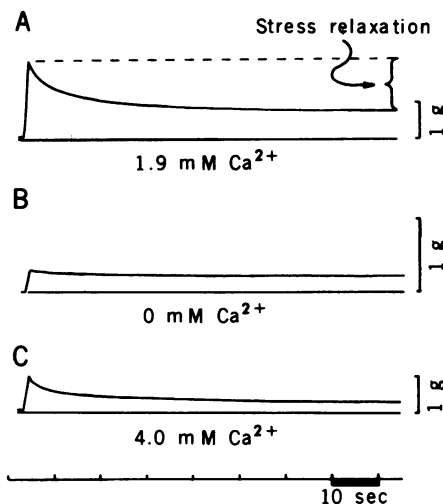


Fig. 1. Tension response to a length change from 3.10 to 3.60 mm at 1.0 mm/sec in the rabbit taenia coli at 21°C. The muscle was bathed in a 1.9 mM Ca^{2+} -Krebs solution (A), then 0 mM Ca^{2+} (2 mM EGTA)-Krebs solution for 0.5 hour (B), and finally 4 mM Ca^{2+} -Krebs solution for 1 hour (C). Note that record in (B) is amplified twice compared to (A) and (C). The muscle gave maximal active tension with tetanic stimulation at a length of 4.8 mm in 1.9 mM Ca^{2+} -Krebs solution. The solid line below each trace represents zero tension. Stress relaxation was calculated as the difference between the peak and the subsequent steady-state tension reached following the stretch.

Scientific) was used at 1 mM in Krebs solution. 1-Fluoro-2,4-dinitrobenzene (FDNB, Eastman Organic Chemicals) was used at 0.38 mM in glucose-free, calcium-free (2 mM EGTA) Krebs solutions gassed with 95 percent N_2 and 5 percent CO_2 .

The muscles used for measurement of adenosine triphosphate (ATP) were frozen by use of a device (Auto-pulverizer, Biochem Instruments) which quickly flattened the muscles between two stainless steel blocks precooled to $-190^\circ C$ with liquid nitrogen. The muscles were extracted as described previously (3). Adenosine triphosphate was assayed using a Varian 4200 Liquid Chromatograph with a Variscan detector and an NH_2 column (Varian Associates). Measurement was accomplished using a mobile phase of 0.35M KH_2PO_4 flowing at 60 ml/hour and by comparing the absorbance of samples and standards at 259 nm.

The apparatus and method used for electrically stimulating the muscles and for determining length-tension relationships was described by Gordon and Siegelman (4). Length changes were made manually with a micrometer drive or with a servomotor (Gould Instruments). Temperature was kept at 21°C to eliminate spontaneous contractile activity of the tissue (4).

Qualitatively similar results were obtained in all of the muscle preparations studied, and Fig. 1A shows a typical tension response to a length change of 0.5 mm at the rate of 1 mm/sec in 1.9 mM calcium-containing Krebs solution. After incubation in calcium-free Krebs solution for 0.5 hour the resistance to stretch and resulting stress relaxation were dramatically reduced (Fig. 1B). The ATP content of the muscle in a calcium-free solution was the same as that of a control muscle in a calcium-containing medium. Reequilibration in 1.9 or even 4.0 mM calcium-containing Krebs solution began to restore the resistance to stretch within 2 minutes. Within 1 hour it had stabilized to 76 ± 3 percent [$N = 7$, mean \pm standard error of the mean (S.E.M.) for all muscle lengths] of its original value (Fig. 1C). Increasing the calcium concentration of the Krebs solution from 1.9 to 4.0 mM had no effect on the passive tension, resistance to stretch, or stress relaxation. Although the passive tension of the muscle, measured as the steady-state force maintenance at a particular length, is slightly greater than the minimum tension reached upon release from longer lengths, neither was affected by the presence or absence of calcium in the bathing medium.

As shown in Fig. 1A, stress relaxation is measured as the difference between the peak tension response and the subsequent steady-state tension achieved after the

Protein purification: adsorption chromatography on controlled pore glass with the use of chaotropic buffers

HG Bock, P Skene, S Fleischer, P Cassidy and S Harshman

Science **191** (4225), 380-383.
DOI: 10.1126/science.1859

ARTICLE TOOLS

<http://science.sciencemag.org/content/191/4225/380>

REFERENCES

This article cites 12 articles, 2 of which you can access for free
<http://science.sciencemag.org/content/191/4225/380#BIBL>

PERMISSIONS

<http://www.sciencemag.org/help/reprints-and-permissions>

Use of this article is subject to the [Terms of Service](#)

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. The title *Science* is a registered trademark of AAAS.

Copyright © 1976 The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original U.S. Government Works.