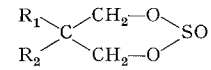
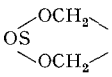
Table I. Central nervous system effects in mice



Compound	R_1	R_2	LD_{50} Route	LD_{50} mg/kg	CD_{50} Route	CD_{50} mg/kg	HD_{50} Route	HD_{50} mg/kg	Sleep time Dose mg/kg i.g.	Sleep time Ratio drug/control
I	CH_3	CH_3	i.p.	9.3 (8.8–9.9)	i.p.	< 10			10	0.63
II	CH_3	C_3H_7	i.g.	140 (100–196)	i.g.	47.0 (31.1–70.8)			25	0.67
			i.p.	52.0 (34.2–79.0)					50	1.17
III	CH_3	C_6H_{13}	i.p.	> 2000			i.p.	> 2000	100	1.00
									200	1.55
IV	C_2H_5	C_2H_5	i.p.	30.5 (27.0–35.0)	i.p.	7.3 (5.8–9.1)			25	0.61
									50	1.55
V	C_2H_5	C_3H_7	i.g.	310 (244–394)	i.p.	45 (27.1–74.7)			20	1.81
									50	0.90
									100	0.67
VI	C_2H_5	C_4H_9	i.p.	880 (746–1038)			i.p.	265 (221–318)	200	1.06
			i.g.	> 1600					400	1.50
VII	C_2H_5	C_6H_5	i.p.	~ 1100	i.p.	~ 500				
VIII	C_2H_5	C_6H_{13}	i.p.	> 1600					50	0.97
			i.g.	> 1600					100	2.53
									200	1.65
IX	C_4H_9	C_4H_9	i.p.	> 1500			i.p.	> 1500	50	1.19
									100	1.95
									200	2.65
X	CH_2OH	CH_2OH	i.p.	> 1000					200	0.66
XI			i.g.	15.2 (12.5–18.5)	i.g.	12.9 (10.3–16.1)			1	1.28
									4	0.72

All tests performed 1 h after drug administration; i.p. = intraperitoneal administration; i.g. = oral administration; LD_{50} = lethal dose for 50% mice (95% confidence limits); CD_{50} = convulsant dose for 50% mice; HD_{50} = hypnotic dose for 50% mice.

pentobarbitalized dog, all compounds were found to produce transient slight vasodepression which was unaltered by atropine.

Résumé. Une étude de 2-2-dialkyl-1-3-propanediol esters cyclique d'acide sulfureux constatait des filiations structure activité très interessantes. Avec un petit ralongant de la chaîne alkyl il est changé d'un stimulant système nerveux central (dimethyl, diethyl, et le pentaerythritol disulfite) à un dépressant système nerveux central fort modérément.

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Molecular Weight of Bovine Growth Hormone

The molecular weight of bovine growth hormone has been measured by several workers[1–6].

The results obtained at pH around 9 with different buffer solutions, vary between 39,300 and 45,000; at pH 7.4 using a Sephadex gel filtration procedure, ANDREWS and FOLLEY[6] found a molecular weight of 20,000 with a preparation obtained from the National Institutes of Health. LI and PEDERSEN[5] have carefully studied the sedimentation behaviour of the hormone prepared by LI, EVANS, and SIMPSON[1,7] under various conditions: at pH 9.93 the results suggested the presence of monomeric and dimeric forms of the hormone in solution, whereas at pH 2.32 and 11.50 it behaved like a monomeric protein. The molecular weight at these two extreme pHs differed radically: 50,000 was found at pH 2.32 and 29,000 at pH 11.50.

[1] C. H. LI, H. M. EVANS, and M. E. SIMPSON, J. biol. Chem. *159*, 353 (1945).
[2] C. H. LI, Ann. Rev. Biochem. *16*, 291 (1947).
[3] C. H. LI, J. phys. coll. Chem. *51*, 218 (1947).
[4] E. L. SMITH, D. M. BROWN, J. B. FISHMAN, and A. E. WILHELMI, J. biol. Chem. *177*, 305 (1949).
[5] C. H. LI and K. O. PEDERSEN, J. biol. Chem. *201*, 595 (1953).
[6] P. ANDREWS and S. J. FOLLEY, Biochem. J. *87*, 3P (1963).
[7] C. H. LI, H. M. EVANS, and M. E. SIMPSON, Science *108*, 624 (1948).

These findings can be interpreted in terms of an association-dissociation equilibria in solution, very difficult to analyse theoretically[8]. In addition to the abovementioned uncertainties, the hormone preparations used were not of the maximum possible purity, since DELLACHA and SONENBERG[9] were able to obtain a hormone with more growth-promoting activity than all previous ones, monodisperse by electrophoresis and ultracentrifugation at pH 9.5 and 4.0. The availability of this purified hormone prompted us to reinvestigate its molecular weight.

DELLACHA, PALADINI, and ENERO[10] have found that bovine growth hormone has the lowest sedimentation coefficient and the highest linear rate of escape through cellophane membranes[11] when it is dissolved in glycine-HCl buffer 0.1 *M*, pH 3.6; accordingly, all measurements in the present work were performed with solutions of the hormone in this buffer.

The molecular weight was obtained both by sedimentation velocity and diffusion methods and by Sephadex gel filtration.

Sedimentation analyses were done in the Spinco model E analytical ultracentrifuge equipped with an RTIC temperature control system, at 20°. Experiments were carried out at 42,040 rpm in a double sector cell. A single symmetrical peak was observed at all concentrations in the range 1.3% to 0.3%; the value obtained for the sedimentation coefficient was $S^{\circ}_{20,w} = 2.14$. This parameter showed very little concentration dependence as indicated by the expression relating both variables: $S_{20,w} = 2.14 + 0.01\ C$ (C = mg/ml).

Diffusion analyses were performed in the Spinco electrophoresis-diffusion apparatus, model H. Diffusion coefficients were computed from Rayleigh interference patterns at several initial protein concentrations between 0.7 g% and 0.3 g%. The value obtained was $D^{\circ}_{20,w} = (10.64 \pm 0.16) \cdot 10^{-7}$ cm² sec⁻¹.

The partial specific volume was calculated from the densities of the protein solutions dialysed against the buffer and their diffusates. The mean of three determinations gave $\bar{V}_{20} = 0.765$ ml/g in good agreement with an earlier value obtained by LI[3].

From these data, a value of 20,800 ± 400 was calculated for the molecular weight of bovine growth hormone.

Sephadex G-100 columns (2.4 · 50 cm) were calibrated for molecular weight estimation using the technique described by ANDREWS[12,13] with cytochrome C (horse heart, type III, Sigma), ribonuclease (4 times cryst., Worthington Biochem. Corp.), trypsin (twice cryst., Sigma), α-chymotrypsin (recryst., Sigma), pepsin (twice cryst., Worthington Biochem. Corp.) and ovalbumin (Sigma) dissolved in the glycine-HCl buffer 0.1 *M*, pH 3.6. All gel filtration experiments were performed between 2 and 4° at a flow rate of 40 ml/h. The ratio elution volume/void volume (Ve/Vo) was the same, in different experiments, for each protein. For growth hormone it was 2.70 ± 0.02. When this value was referred to the calibration curve obtained with the standard proteins (Figure), the molecular weight for bovine growth hormone in the experimental conditions used was found to be 19,800 ± 700.

Thus the molecular weight values obtained by the two independent methods in this work are in good mutual agreement and confirm the result of ANDREWS and FOLLEY[6,20,21].

Zusammenfassung. Das Molekulargewicht des Rinderwachstumshormons wurde mittels Gelfiltration in Sephadex, Sedimentationsgeschwindigkeit und Diffusionsmessungen bestimmt: 19,800 ± 700 und 20,800 ± 400 bzw.

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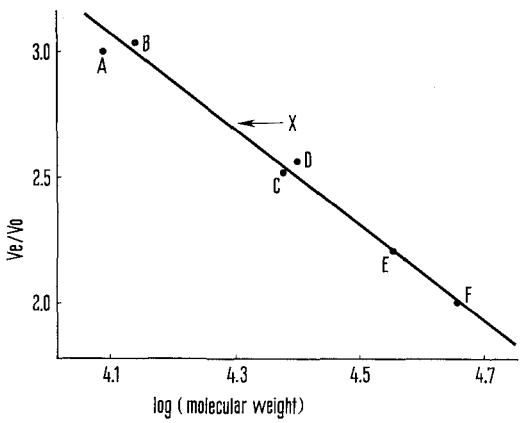

Plot of elution volume/void volume against log (molecular weight) for the proteins listed below:

Protein	M.W.	Reference
A – Cytochrome C	12,400[a]	MARGOLIASH[14]
B – Ribonuclease	13,700	HIRS et al.[15]
C – Trypsin	23,800	CUNNINGHAM[16]
D – α-Chymotrypsin	25,100	WILCOX et al.[17]
E – Pepsin	35,500	BOVEY et al.[18]
F – Ovalbumin	45,000	WARNER[19]
X – Bovine growth hormone		

[a] Including the hem group.

[8] H. K. SCHACHMAN, Brookhaven Symp. Biol. *13*, 49 (1960).
[9] J. M. DELLACHA and M. SONENBERG, J. biol. Chem. *239*, 1515 (1964).
[10] J. M. DELLACHA, A. C. PALADINI, and M. A. ENERO, Proceedings of the VIth Latin American Congress of Physiological Sciences, Vina del Mar (Chile), November 1964.
[11] L. C. CRAIG, in *Analytical Methods of Protein Chemistry* (Ed.: P. ALEXANDER and R. J. BLOCK; Pergamon Press, New York-London-Oxford-Paris 1960), vol. 1, p. 103.
[12] P. ANDREWS, Biochem. J. *91*, 222 (1964).
[13] P. ANDREWS, R. C. BRAY, P. EDWARDS, and K. V. SHOOTER, Biochem. J. *93*, 627 (1964).
[14] E. MARGOLIASH, J. biol. Chem. *237*, 2161 (1962).
[15] C. H. W. HIRS, S. MOORE, and W. H. STEIN, J. biol. Chem. *219*, 623 (1956).
[16] L. W. CUNNINGHAM JR., J. biol. Chem. *211*, 13 (1954).
[17] P. E. WILCOX, E. COHEN, and W. TAN, J. biol. Chem. *228*, 999 (1957).
[18] F. A. BOVEY and S. S. YANARI, in *The Enzymes* (Ed.: P. D. BOYER, H. LARDY, and K. MYRBÄCK; Academic Press Inc., New York 1960), vol. 4, p. 63.
[19] R. C. WARNER, in *The Proteins* (Ed.: H. NEURATH and K. BAILEY; Academic Press Inc., New York 1954), vol. 2, Part A, p. 435.
[20] Acknowledgment: The authors thank Dr. A. C. PALADINI for helpful discussion and encouragement.
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