Tiselius electrophoretic apparatus with the Longsworth scanning method. The protein was crystallized three times as prepared by the method of Palmer.[6] Results showed that the material behaved as a single component in acetate buffer of ionic strength 0.10 at *p*H 5.3 and 5.6 with mobilities of -1.4×10^{-5} and -2.5×10^{-5} sq. cm. per sec. per volt at 1.5°, respectively. But, when the same preparation was electrolyzed at *p*H 4.8 and 6.5, it appeared to consist of three components with the following mobilities and relative concentrations:

*p*H	Mobility (10^5), sq. cm./sec./volt	Relative concn., %
4.8	+2.3	68
	+1.9	22
	+1.2	10
6.5	−5.6	48
	−5.2	25
	−4.5	27

Further crystallizations of the protein did not alter this electrophoretic behavior. It may be noted that the relative concentration of each component at *p*H 4.8 is not the same as that for the same components at *p*H 6.5; this disagreement may be caused by certain interactions between the components occurring in the mixture. However, it is clear that the fastest boundary is formed by the protein which is in the highest concentration. From the plot of values of mobility against *p*H's, the isoelectric point of the main component can be shown to be at *p*H 5.1. This value does not differ greatly from that obtained by Pedersen[5] as the isoelectric point of β-lactoglobulin.

Whether or not the demonstrated electrophoretic inhomogeneity of the crystalline β-lactoglobulin depends upon the method of preparation is now being investigated.

(6) Palmer, *J. Biol. Chem.*, **104**, 359 (1934); the author is greatly indebted to Dr. E. F. Jansen who kindly prepared the crystalline protein for these experiments.

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DEGRADATIVE STUDIES ON STREPTOMYCIN

Sir:

Acetylation of ethyl dihydrothiostreptobiosaminide hydrochloride[1] yields a pentaacetate (I): m. p. 116–116.5°, $[\alpha]^{23}D$ −172° (*c*, 1, chloroform).

Anal. Calcd. for $C_{13}H_{19}O_4(C_2H_5S)(NCOCH_3)(OCOCH_3)_4$: C, 50.58; H, 6.62; N, 2.36; S, 5.39; CH_3CO (O-acetyl), 6.74 cc. of 0.1 *N* NaOH per 100 mg. Found: C, 50.69; H, 6.43; N, 2.30; S, 5.42; CH_3CO (O-acetyl[2]), 6.41 cc.

(1) F. A. Kuehl, Jr., E. H. Flynn, N. G. Brink and K. Folkers, THIS JOURNAL, **68**, 2096 (1946).

(2) M. L. Wolfrom, M. Konigsberg and S. Soltzberg, *ibid.*, **58**, 490 (1936).

Hydrogenolysis[3] of I followed by reacetylation yields desoxydihydrostreptobiosamine pentaacetate (II); m. p. 131°, $[\alpha]^{23}D$ −87° (*c*, 1, chloroform).

Anal. Calcd. for $C_{13}H_{20}O_4(NCOCH_3)(OCOCH_3)_4$: C, 51.77; H. 6.61; N, 2.63; CH_3CO (O-acetyl), 7.49 cc. of 0.1 *N* NaOH per 100 mg. Found: C, 51.75; H, 6.56; N, 2.73; CH_3CO (O-acetyl[2]), 7.58 cc.

A refined assay for terminal methyl groups[4] in several derivatives of streptomycin yields (moles terminal methyl per mole): methyl streptobiosaminide dimethyl acetal tetraacetate[5] 5.0; methyl dihydrostreptobiosaminide pentaacetate (III,[3,6,7] 6.0; II, 6.0; didesoxydihydrostreptobiosamine tetraacetate (IV),[3,1] 5.9. The fact that the total number of CH_3-C groups present in the first three is one greater than the known number of acetyl groups, confirms the presence of a CH_3-C group in streptomycin.[3] III is therefore thus established as a pentaacetate. IV shows a preponderance of two CH_3-C groups over those required by its known acetyl content, thus establishing the presence of an aldehyde group in the central moiety of the original streptomycin molecule (C-CHO → C-CH$(SC_2H_5)_2$ → C-CH_3), a finding confirmatory of the work of Fried and Wintersteiner[6] based upon the isolation of an amorphous bromine oxidation product of streptomycin. This aldehyde group must be the one which undergoes thioacetal formation since the mercaptolysis product (I) of dihydrostreptomycin contains only one thioethoxyl group. It must be more than one carbon atom away from the terminal methyl group originally present since otherwise no enhancement of the CH_3-C assay would result by its reduction to the hydrocarbon stage. Moreover, the reducing group liberated on hydrolysis of the streptidine portion is likewise doubtless aldehydic (cyclic hemiacetal) in nature since the two anomeric forms[3] of ethyl thiostreptobiosaminide diethyl thioacetal tetraacetate produce on hydrogenolysis good yields of the same reduction product, R—CH_2—O.

Further proof for the presence of two aldehyde groups other than that of the hexosamine portion is provided by quantitative measurements of hypoiodite oxidation.[8] Although this reagent caused some general oxidation, time curves showed definite breaks at the following consumptions expressed in atoms of oxygen: streptomycin, 1; streptomycin hydrolyzate, 2; dihydrostrepto-

(3) I. R. Hooper, L. H. Klemm, W. J. Polglase and M. L. Wolfrom, *ibid.*, **68**, 2120 (1946).

(4) R. U. Lemieux and C. B. Purves, *Can. J. Research*, in press.

(5) N. G. Brink, F. A. Kuehl, Jr., and K. Folkers, *Science*, **102**, 506 (1945).

(6) J. Fried and O. Wintersteiner, Abstracts of Papers 110th Meeting, Am. Chem. Soc., Chicago, September 9–13, p. 15B (1946).

(7) Q. R. Bartz, J. Controulis, H. M. Crooks, Jr., and Mildred C. Rebstock, THIS JOURNAL, **68**, 2163 (1946).

(8) H. A. Rutherford, F. W. Minor, A. R. Martin and M. Harris, *J. Research Natl. Bur. Standards*, **29**, 131 (1942).

mycin, 0; dihydrostreptomycin hydrolyzate, 1.

The presence of at least two aldehyde groups and a terminal methyl group in the central moiety, $C_6H_8O_5<$, of streptomycin, together with the lack of chemical evidence for a hydroxyl group, indicates that this portion probably possesses two carbon chains linked in a polyacetal type of structure.

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RECEIVED NOVEMBER 20, 1946

THE ELECTRIC MOMENT OF *n*-BUTYLLITHIUM AND THE NATURE OF THE LITHIUM-CARBON BOND

Sir:

Despite the wide interest in the reactions of organolithium compounds, little information is available as to the nature of the metal–carbon bond in these compounds and conflicting opinions have been expressed. Thus, Morton,[1] in a recent review, has chosen to regard all organoalkali compounds as salts, although lithium alkyls and aryls are soluble in non-polar solvents, can be distilled, and conduct poorly in solution in zinc alkyls.[2] We have made some measurements of the molar polarizations of *n*-butyllithium in benzene solutions which provide information on this subject and report them here since the work was temporarily interrupted before it could be extended to include other solvents, other lithium compounds and experimental values of molecular refraction.

Using our value, 40, for the molar polarization of n-C_4H_9Li in benzene, and estimating a value of 1.0 for the atomic refraction of lithium, we calculate a value of $\mu = 0.97D$ for the dipole moment of *n*-butyllithium. This indicates that the Li–C bond must have considerable covalent character, since ion pairs would result in very much higher values of the molar polarization (*e. g.*, $P_\infty = 1309$ for $LiClO_4$, a largely polar compound, in dioxane solution[3]). From the electronegativity difference, 1.5 units,[4] between carbon and lithium, one would predict about 45% ionic character for the Li–C bond and a bond moment of about 1.5*D* (Malone's rule, ref. 4, p. 68). The observed bond moment is 1.37*D*, assuming the C–H bond moment to be 0.4*D* and taking lithium as the positive end of dipole. Lithium alkyls may thus be regarded as covalent compounds, just as organic fluorine compounds are, the rather large amount of ionic character of the Li–C bond being about equal in magnitude to that of the C–F bond.

n-Butyllithium was prepared by stirring a benzene solution of *n*-butyl chloride with an excess of lithium sand for two days. The solution was filtered and transferred to the dielectric constant cell for measurement; the apparatus and technique have been described[5] previously. The density was determined, and aliquots withdrawn and analyzed acidimetrically. All handling was done in a carefully dried, all-glass apparatus, through which dry, oxygen-free nitrogen was continuously passed.

Solutions containing mole fractions 0.06685, 0.03055, and 0.02073 of *n*-butyllithium had densities 0.87098, 0.87035 and 0.87210, and dielectric constants 2.3371, 2.3000 and 2.2946. Extrapolation of the molar polarizations 36.8, 38.0, 39.3 to infinite dilution gave $P_2^\infty = 40.0$. Assuming the atomic refraction of lithium to be 1.0, MR_D was estimated to be 20.6 and μ is calculated to be 0.97 *D*.

(1) A. A. Morton, *Chem. Rev.*, **35**, 1 (1944).

(2) K. Ziegler, F. Crössmann, H. Kleiner and O. Schäfer, *Ann.*, **473**, 1 (1929); Hein, *et al.*, *Z. anorg. allgem. Chem.*, **141**, 161 (1924), and earlier papers.

(3) M. G. Malone and A. L. Ferguson, *J. Chem. Phys.*, **2**, 99 (1934).

(4) Linus Pauling, "Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 2nd ed., p. 64.

(5) Max T. Rogers and John D. Roberts, THIS JOURNAL, **68**, 843 (1946).

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STUDY OF FERTILIZER UPTAKE USING P^{32}

Sir:

A small amount of P^{32} in the form of phosphoric acid was converted to ammonium phosphate and added to an aqueous solution of ammonium phosphate fertilizer. This was used in place of the usual fertilizer in a field experiment using wheat. The wheat was sown with fertilizer in a completely randonized plot on April 29th. The plants grew well and were harvested at intervals. On each occasion, five replicates, consisting of five plants each, were harvested.

The plants were ashed and the fertilizer uptake determined from the measured activity, making due allowance for sample thickness and the decay of P^{32}. The total phosphorus was measured chemically. The difference between the total phosphorus and fertilizer phosphorus gives the phosphorus taken up from the soil. The results are recorded, in part, in Table I.

TABLE I

	Date harvested			
	June 18	July 8	July 29	August 15
% P coming from fertilizer	19.1	13.4	10.8	6.7
% P from soil	80.9	86.6	89.2	93.3
% fertilizer taken up of the total added to the soil	12.9	21.3	23.7	23.6