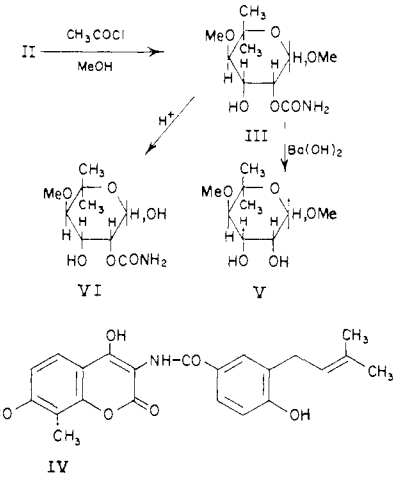
aldose which failed to react with periodate. These findings along with published data[1] permit the assignment of the structure of methyl 2-*O*-carbamyl-4-*O*-methyl-5,5-dimethyl-L-lyxoside to III and show that II is the 2-carbamyl analog of I.



It appears, therefore, that the antibiotic activity of novobiocin is highly dependent on the presence and location of the carbamate group.

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AN INTERPRETATION OF THE PROTON MAGNETIC RESONANCE SPECTRUM OF RIBONUCLEASE

Sir:

Saunders, Wishnia and Kirkwood[1] recently have reported the first proton magnetic spectrum due to a protein, pancreatic ribonuclease, in D_2O. The spectrum consists of four broad peaks, falling roughly into the range between the aromatic and the aliphatic peaks of toluene. The authors have identified the first peak tentatively, corresponding to lower field strength, as due to the aromatic hydrogens and the fourth peak, at highest field strength as due to "aliphatic carbon atoms attached only to other aliphatic carbons," stating that a complete interpretation is not possible at present. However, our studies on the proton magnetic resonance spectra of amino acids and peptides[2,3] together with the amino acid composition of ribonuclease determined by Hirs, Stein and Moore,[4] permit the prediction of a complete n.m.r. spectrum for the protein, which is in excellent agreement with experiment.

There is little reason to doubt that peak I is due essentially to the 18 phenylalanine and 36 tyrosine hydrogens, as suggested.[1] In aqueous solutions these shifted -20 to -30 c.p.s. relative to the aromatic proton of toluene as 0. It probably also includes the 8 CH hydrogens on the imidazole of histidine, found at somewhat lower fields. The total number of non-exchangeable hydrogens in the protein being 697,[4] this will account for 8.9% of the total area under the absorption curve, in agreement with the $9 \pm 1\%$ measured. While in water the guanidino group of arginine gives rise to an additional peak in the same region, this disappears after equilibration with D_2O, so that it would not likely contribute to peak I of ribonuclease in this instance.

A second peak, due to the 123 α-CH protons, of all amino acids the 30 β-protons of serine and 10 β-protons of threonine should be observed at $+90$ to $+110$ c.p.s. relative to toluene if no shifts due to peptide bond formation were to be expected. In addition, the 6 glycine protons and 8 protons of the CH_2 group in arginine are observable at slightly higher fields of $+117$ to $+120$ c.p.s. in a region intermediate between the second and third peaks. Together with the protons of the second peak they would account for 25.4% of the total area, as compared to the experimental estimate of $26 \pm 2\%$. As peptide bond formation causes a displacement of α-CH peaks 15–20 cycles toward lower fields, the maximum of the second peak of ribonuclease, should appear at about $+80$ to $+90$ c.p.s. where it is actually found.

A third peak with a maximum between $+135$ to $+145$ c.p.s. is accounted for by CH_2 groups as follows: 16 from cysteine–cystine, 8 from methionine, 32 from aspartic acid, 20 from lysine (ϵ –CH_2), 10 from proline, 8 from histidine, 6 from phenylalanine and 12 from tyrosine. This amounts to 15.2% of the total area, the experimental estimate for the peak being $18 \pm 3\%$. The 64 CH_2 groups of glutamic acid fall at $+156$ c.p.s., accounting for an additional 9.2% of the area, which could be attributed either to the third or the fourth peak.

It is probable that peak IV is actually a fusion of two broad peaks, one with a maximum at $+180$ to $+190$ c.p.s., the other at $+210$ to $+220$. The first of these is due to aliphatic CH groups of leucine (2 hydrogens) isoleucine (3) and valine (9), CH_2 groups of leucine (4 hydrogens), isoleucine (6), proline (20), lysine (60) and arginine (16) and the CH_3 group of methionine (12 hydrogens), accounting for 19.1% of the area. The second, due to CH_3 groups of alanine (36 hydrogens), valine (54), leucine (12), isoleucine (18), and threonine (30) would respresent 21.6%. The combined area under peak IV, including glutamic acid is 49.9%, closely in agreement with the experimental value of $47 \pm 3\%$. It would be of interest if it were possible to sufficiently average the field seen by the aliphatic protons—perhaps at higher temperature—to actually resolve the fourth peak. It might also be noted, that due to peptide bond formation small and somewhat variable shifts toward lower fields can be expected for hydrogens contributing to peaks III and IV making an exact prediction of their maximum difficult. However, even the foregoing illustrates that NMR spectra

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of amino acid provide a rational basis for the interpretation of patterns which may be attributed to proteins in solution.

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THE ROLE OF DISULFIDE BONDS IN ANTIBODY SPECIFICITY[1,2]

Sir:

The occurrence of disulfide bonds in γ-globulins[3,4] and antibody proteins[5] has led us to suggest[6] that these linkages play an essential role in the maintenance of the specific configuration of the combining region of the antibody molecule. We have obtained evidence supporting this hypothesis from experiments in which the disulfide bonds of purified anti-hapten rabbit antibodies have been reduced and the resulting sulfhydryl groups prevented from re-oxidizing. The effect of such reduction on the specific combination of the antibody with an homologous azohapten provided the basis for our conclusions.

The protein was reduced with 0.1 *M* β-mercaptoethylamine-HCl at *p*H 7.4 in the presence of 0.1 *M* sodium decyl sulfate. The reducing agent was removed by passage of the reaction mixture through a column of Dowex 50-X8(Na^+). The effluent reacted with an excess of iodoacetate by overnight stirring at room temperature. These operations were done under anaerobic conditions by a procedure which will be described in detail elsewhere. The protein solutions were subjected to exhaustive dialysis against 0.001 *M* phosphate buffer *p*H 7.4 for the removal of the detergent. The low ionic strength was necessary to avoid the precipitation of the protein derivative at this *p*H. The test antibody was that specific for the *p*-azophenyl β-lactoside group (anti-Lac)[7] and the control proteins were rabbit γ-pseudoglobulin (RγpG) and antibody specific for the L-phenyl-(*p*-azobenzoylamino)-acetate group (anti-L-I_p).[6]

The ability of the reduced proteins and various control preparations to bind the azohapten *p*-(*p*-dimethylaminobenzeneazo)-phenyl β-lactoside (Lac dye) was measured by equilibrium dialysis at 25° in 0.001 *M* phosphate buffer, *p*H 7.4. The results are shown in Table I in terms of *r* and *r*/*c* where *r* is the average number of dye molecules bound per protein molecule at the free dye concentration *c*. The last column, headed *r*/*c*, provides the most useful measure of the binding affinities. It is evident that detergent treatment alone reduces the specific binding somewhat, about 2-fold, and that the capacity for non-specific binding is acquired by the reduced proteins. When these effects are taken into account the results demonstrate that the specific binding is greatly reduced, to the extent of about 7-fold, when reduction of the disulfide bonds occurs. The residual specific binding observed may be due to the fact that only about 10 disulfide bridges, out of a minimum content of 20,[5] were split in our procedure.

TABLE I

EFFECT OF DISULFIDE REDUCTION ON ANTIBODY BINDING[a]

Expt.	Protein	Treatment	*r*	*r*/*c* × 10^{-4}
A	Anti-Lac	Dialysis	0.74	14.4
A	Anti-Lac	Detergent	.62	7.0
B	Anti-Lac	Detergent	.70	5.6
B	Anti-Lac	Reduction	.22	1.5
B	Anti-L-I_p	Reduction	.13	0.8
B	Anti-L-I_p	Detergent	.06	.3
B	Anti-L-I_p	.....	.00	.0
B	$R\gamma_pG$	Reduction	.14	.9

[a] One ml. of protein solution approximately 2 × 10^{-5} *M* was dialyzed against 1 ml. of 4 × 10^{-5} *M* dye solution. The –SH content of the reduced proteins was measured by the amperometric titration method of Benesch, *et al.*[8] Different anti-Lac preparations were used in experiments A and B.

The additional negative charge acquired by the reduced antibody by reaction of the –SH groups with iodoacetate probably does not play a major role in the reduction of specific binding. Reaction of reduced antibody with iodoacetamide shows the same results but such a preparation is insoluble at *p*H 7.4 and is therefore less useful than the iodoacetate derivative.

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DETERMINATION OF THE SITE OF ^{14}C IN HYDROCORTISONE-^{14}C DERIVED FROM CHOLESTEROL-21-^{14}C INCUBATED WITH BOVINE ADRENAL GLAND TISSUE[1]

Sir:

The biochemical conversion of cholesterol to the C_{21}-steroids of the adrenal cortex was first demonstrated by investigators[2] using cholesterol-3-^{14}C. Later other workers[3] reported the isolation of a labeled six-carbon fragment resulting from the biochemical degradation of cholesterol-26-^{14}C by mammalian tissue extracts. The latter study suggested that the steroid hormones of the adrenal cortex could be derived from cholesterol involving a degradation of the last six carbon atoms of the side chain [$C_{27} \rightarrow C_{21} + (C_6)$].

To obtain further experimental evidence on the

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