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

(5) National Research Council Fellow.
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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	RγpG	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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(2) I am indebted to Mrs. F. Karush for technical assistance in this investigation.
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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

(1) This investigation was supported by the John J. Morton Cancer Fund and by a fellowship (HF-6137) from the National Heart Institute of the Public Health Service.
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pathway of the biochemical conversion of cholesterol to hydrocortisone, cholesterol-21-^{14}C was prepared[4] and gave, on incubation with bovine adrenal gland tissue, hydrocortisone-^{14}C.[5] A part of the isolated hydrocortisone-^{14}C (5.15 mg.; specific activity, 159 d.p.m./mg.) was diluted with an equal amount of non-labeled hydrocortisone, and then degraded by periodate oxidation according to a procedure previously employed for similar reactions.[6] From the oxidation mixture, 7.5 mg. of neutral material was recovered and was found to contain no hydrocortisone after paper chromatographic analysis. From the acidic fraction (9.7 mg.), 11β,17α-dihydroxy-3-oxo-4-etiocholenic acid[7] was isolated and identified by comparison with an authentic sample, by mixed paper chromatography,[8] and by its ultraviolet absorption spectrum in concentrated sulfuric acid.[9] This etio acid was found to contain no radioactivity.[10] Carbon atom 21 of the hydocortisone-^{14}C was obtained as formaldehyde which was isolated as its dimedone derivative, 4.6 mg., m.p. 191.5–192°. A mixture of this derivative and an authentic reference sample (m.p. 192-193°) melted at 191.5-192° (melting points not corrected). The dimedone derivative had a radioactivity[10] of 26 d.p.m./mg.[11]

This evidence demonstrates that carbon atom 21 of cholesterol is maintained during the biochemical conversion of cholesterol to hydrocortisone and that the biosynthesis of hydrocortisone from cholesterol in the adrenal cortex involves the elimination of carbon atoms 22–27 of the original cholesterol side chain.

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(10) Radioactivity determinations by New England Nuclear Corporation, Boston, Massachusetts.

(11) The amount of the dimedone derivative obtained was in excess of the expected amount and the radioactivity of the sample was lower than the calculated value of 98 d.p.m./mg. However, it was found that the elution of two filter paper blanks yielded 23.2 and 34.3 mg. of residue which gave upon analogous periodate oxidation[6] and reaction with dimedone, 2.4 and 3.5 mg. respectively of the dimedone derivative of formaldehyde. This would account for the lower radioactivity obtained in the experimental sample.

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A DIFFERENTIAL ULTRACENTRIFUGE TECHNIQUE FOR MEASURING SMALL CHANGES IN SEDIMENTATION COEFFICIENTS[1]

Sir:

With the recent adaptation of the Rayleigh interferometer to the ultracentrifuge,[2] it has become possible to perform differential experiments whereby very small changes in sedimentation coefficients can be measured accurately. This communication deals with such experiments and some potential applications.

The technique involves a comparison, at conjugate levels, of the refractive indices of two solutions contained in separate compartments of a double-sector ultracentrifuge cell. If both solutions are identical the Rayleigh pattern consists of a series of parallel, straight interference fringes. For the fringes to be straight even in the presence of concentration gradients, the two boundaries must migrate and spread at the same rate so that the refractive index difference at conjugate levels throughout the cell is constant. When one boundary moves faster than the other, the fringe pattern at the boundaries is warped to produce curved fringes whose shape resembles the tracings produced by schlieren optical systems.[3] Depending upon which compartment contains the faster moving species, the pattern will show a maximum or a minimum. With transport equations the difference in sedimentation coefficients can be expressed directly in terms of the change with time of the first moment of the area defined by the curved fringes.

To test the method small amounts of D_2O were added to different solutions of bushy stunt virus, and the reduction in sedimentation coefficient of the virus was measured by the differential technique. For decreases of 0.82% and 1.65% (determined by interpolation of data from conventional ultracentrifuge gives 0.78% and 1.61%, respectively. Comparable precision is realized in experiments with proteins.

The sensitivity and accuracy of the method even at this early stage of development are sufficient to commend it for many experiments involving small differences in sedimentation coefficients. These changes may result from reduction in the buoyancy term as with D_2O or from a change in the molecular weight or frictional coefficient of the macromolecule. The latter is illustrated by preliminary results from a study of the binding of small ions to serum albumin, in which the sedimentation coefficient decreased by about 0.5% despite an increase of 4% in molecular weight. The technique also provides valuable data for the analysis of equilibrium systems involving rapid reactions between a small ion, A, and a protein, P, to form complexes, PA_i, with i varying from 0 to n. For such systems equation (1) gives the equilibrium concentration of free A component

$$[A] = \frac{[\bar{A}]\{\bar{s}_P - \bar{s}_A - (1/n)(\bar{s}_P - s_P)\} + [\bar{P}](\bar{s}_P - s_P)}{\bar{s}_P - s_A - (1/n)(\bar{s}_P - s_P)} \qquad (1)^5$$

$[\bar{A}]$ and $[\bar{P}]$ are the total concentrations and s_A and s_P are the sedimentation coefficients of the pure components, and $\bar{s}_A$ and $\bar{s}_P$ are the constituent

(1) This work has been supported in part by a grant from the National Science Foundation.

(2) This optical system is available commercially from the Spinco Division, Beckman Instruments, Inc., Palo Alto, California.

(3) Svensson[4] obtained similar fringe patterns with the Rayleigh interferometer during examination of two identical diffusing boundaries which were slightly displaced from one another.

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