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	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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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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(2) I am indebted to Mrs. F. Karush for technical assistance in this investigation.
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