HAUSER[8] in a program[9] written for a Hewlett-Packard 9100B Calculator fitted with a 9101A Extended Memory. The computed eigenvalues and singular values are given in the Table.

Comparison of the singular values of the Table with the standard deviation of the matrix elements indicates 4 singular values greater than twice the standard deviation and, therefore, nonzero. The matrix thus appears to be an approximation to a matrix of rank 4, as originally suggested by WALLACE and KATZ[4].

Zusammenfassung. Neues Verfahren zur Bestimmung des Ranges, wonach die Quadratwurzeln der Eigenwerte mit der Standardabweichung der Extinktionsmessungen verglichen werden. Die Zahl der Komponenten aus einem Lösungsgemisch kann aus dem Rang der Matrix der Extinktionskoeffizienten bestimmt werden.

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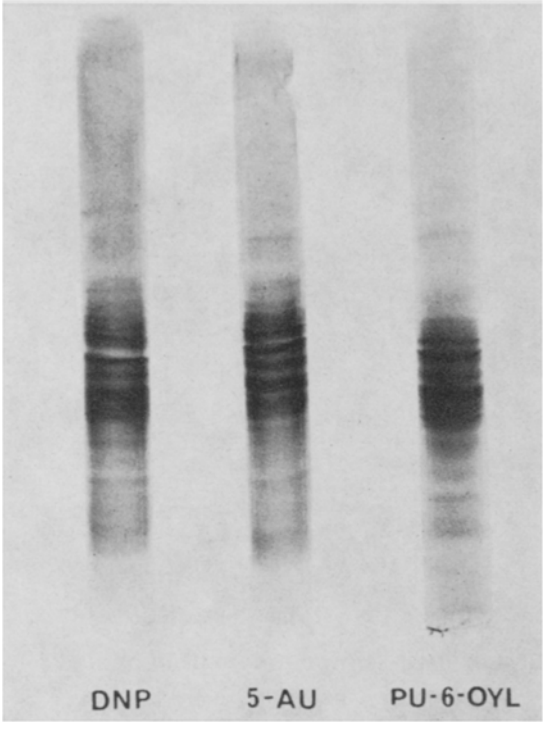
Multiple Binding Functions of Sheep Anti-DNP Antibodies

An immune serum is generally assumed to exhibit a high degree of specificity against the antigen used to induce the immune response. This general rule has become questionable by the recent findings of several workers[1–6] suggesting that individual antibodies may competitively bind several structurally dissimilar haptens. These observations indicate that hapten binding by the antibody might not be an all-or-none function but rather that the antibody combining site might be a polyfunctional region, capable of binding several structurally dissimilar haptens; however, with different affinities.

The present work was undertaken in order to examine this hypothesis in sheep antibodies towards the 2, 4-dinitrophenyl (DNP) determinant and to establish the degree of polyfunctionality of these antibodies.



Isoelectrofocusing pattern of the whole anti-DNP antibody population and of the subfractions isolated from the 5-acetyl-uracil-BSA immunoadsorbent and the purine-6-oyl-BSA immunoadsorbent respectively.

Material and methods. Antibodies were raised by i.m. injection of 5 mg DNP-BGG in complete Freund's adjuvant. In regular 1 month intervals, the sheep were boostered with the same amount of antigen. After 6 boosters the antibody titers reached an average of 0.5–1.0 mg/ml.

Immunoadsorbents consisted of hapten-protein-conjugates mechanically entrapped into polyacrylamide gels according to the method of CARREL[7]. Active protein was desorbed with 0.2 *M* glycine, pH 2.3. 5-acetyluracil-BSA, purine-6-oyl-BSA, nucleotide- and nucleoside-protein-conjugates were synthesized as described by BEISER et al.[8], nitroazidophenyl-BSA by the method of FLEET[9]. DNP-BSA and DNP-BGG were prepared according to LITTLE and EISEN[10]. Equilibrium dialysis was performed as described by EISEN[11], using tritiated ε-N-DNP-L-lysine (New England Nuclear). The relative association constant with various hapten-protein-conjugates were studied by the inhibition of the binding of radioactively labeled DNP-BSA to an insoluble antibody preparation. This method has the advantage that the reaction with the immunogen itself may be studied. Essentially antibody coupled to bromoacetylcellulose[12] was reacted with ^{125}I DNP-BSA. After centrifugation and washing, the radioactivity in the sediment was counted, giving 100% of binding. The relative affinities against the cross-reacting hapten-protein-conjugates were calculated

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from the molarity at 50% inhibition. The reaction with the homologous antigen DNP-BSA was set one. DNP-BSA was labelled with the iodine monochloride method of MCFARLANE[13].

Results. By using DNP-BSA as immunoadsorbent and DNP-BGG as immunogen, only DNP-specific but no carrier specific antibodies were isolated. This antibody population precipitated DNP-BSA as well as DNP-BGG. The average intrinsic association constant (K_0) with DNP-L-lysine was 2.0×10^5. Precipitin reactions were also observed with 5-acetyluracil-BSA and with purine-6-oyl-BSA. By means of immunoadsorbents consisting of 5-acetyluracil-BSA and purine-6-oyl-BSA, it was possible to isolated the fractions cross-reacting with these two hapten-protein-conjugates. The corresponding yields were about 20 to 25% for acetyluracil-BSA and between 15 and 20% for purine-6-oyl-BSA. The two cross-reacting subfractions were compared with the whole anti-DNP population by isoelectrofocusing in polyacrylamide gel, using a pH gradient from 3 to 10. (Figure). As may be seen from the Figure, no significant differences between the whole anti-DNP antibody population and the fractions eluted from the 5-acetyluracil-BSA or the purine-6-oyl-BSA immunoadsorbent respectively were detectable in the isoelectrofocusing pattern, indicating that the isolation of these subpopulations does not lead to a discernible decrease in heterogeneity.

Binding data of the whole anti-DNP antibody population for various hapten-protein-conjugates are given in the Table. As expected, the highest affinity was observed with the homologous antigen DNP. Similar results were obtained with rabbit anti-DNP antibodies (with a K_0 value of 3×10^7), indicating that the obtained results are not species specific.

When the antibody population obtained after immunoadsorption of the fraction crossreacting with 5-acetyluracil-BSA was studied, the same interactions could still be observed. The relative affinity for 5-acetyluracil-BSA, however, was now about 3 orders of magnitude smaller; the relative affinities for the other hapten-protein-conjugates were also decreased but to a lesser extent. The affinity for DNP-L-lysine of this fraction did not differ from that of the whole anti-DNP population.

Discussion. These results indicate that essentially every single anti-DNP antibody shows multiple binding functions with various hapten-protein-conjugates, but that the relative affinities towards these compounds differ within certain subfractions of the whole anti-DNP population.

The relative affinities against the cross-reacting compounds seem to be at least two or more orders of magnitude smaller than for the immunizing antigen. These differences in affinity would still be compatible with a rather high specificity of an individual antibody molecule. Moreover one could argue that the observed reactions only occur in vitro. VARGA et al.[14], however, were able to demonstrate that 2 structurally dissimilar haptens coupled to a carrier may stimulate the production of a immunoglobulin binding both haptens, presumably by activation of the same cell-surface receptor. This suggests that these multiple binding reactions do not only occur in vitro, but that, inspite of the differences in the relative affinities, they play a significant role in the stimulation and probably also the maturation of antibodies. Moreover the existence of polyfunctional regions within the antibody combining site would have the consequence that fewer antibody species would be required since one antibody molecule might bind several structurally different antigens.

On the other hand, it remains to be proved whether the results reported for antibodies elicited by the DNP determinant are also valid for other antigen-antibody systems.

Binding inhibition data

Compound	Concentration at 50% inhibition (M/l)	K (rel.)
2,4-Dinitrophenyl-BSA	3×10^{-10}	1
Nitroazidophenyl-BSA	9×10^{-8}	3.3×10^{-3}
5-Acetyluracil-1-BSA	1.5×10^{-7}	2.0×10^{-3}
Guanosine-BSA	6.5×10^{-7}	4.8×10^{-4}
Purine-6-Oyl-BSA	5.5×10^{-6}	5.5×10^{-5}
Uridine-5′-Monophosphate-BSA	4.0×10^{-5}	7.7×10^{-6}
Adenosine-5′-Monophosphate-BSA	$> 10^{-4}$	
Cytidine-BSA	$> 10^{-4}$	
p-Azobenzenearsenate-BSA	$> 10^{-4}$	
Dansyl-BSA	$> 10^{-4}$	
BSA	$> 10^{-4}$	
RNA	$> 10^{-4}$	
DNA native	> 1 mg/ml	
DNA single stranded	> 1 mg/ml	

Zusammenfassung. Spezifitätsuntersuchungen an anti-Dinitrophenyl-Antikörpern unter Verwendung einer Methode, die eine Bestimmung der relativen Assoziationskonstanten gegenüber verschiedenen Hapten-Protein-Konjugaten gestattet.

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Automatic Assay of the Distribution of ^{3}H-, ^{14}C- and ^{32}P-Labelled Compounds within an Early Chick Embryo by a Semiconductor Detector

Autoradiography[1] has hitherto been used as a single non-destructive assay of the distribution of β-nuclide labelled substances in embryology. The main advantage of the semiconductographic method[2], using a computer-controlled device with a special silicon barrier detector[3], consists in a quantitative determination of the particular β-nuclides in the presence of each other at each location of the differentially labelled sample. Moreover, records may