control groups at the beginning and the end of series was studied in the same way. 9 days after irradiation and injection of the peripheral blood cells, the spleens of the recipient mice were fixed in Bouin's solution and the colonies were counted.

The results are given in the Table. A depression of the CFU content of peripheral blood was observed 2 days after infection. The same phenomenon had been found previously in the bone marrow and the spleen[5]. After 5 and 13 days an increase of CFU's/ml blood occurred. The peripheral blood cell count of the donor mice varied between 4,000 and 7,300 nucleated cells/mm³ without more than 2% erythroblasts, but with an increasing percentage of lymphocytes. The animal 23 days after infection had severe erythroblastosis and a 50-fold rise in CFU's/ml blood.

These experiments are being extended in current studies in the laboratory with special reference to the leukemic or normal status of these CFU's, using the F_1-hybrid system[6].

Rauscher virus leukemia
CFU's in the peripheral blood

Days after infection	Injected blood volume (ml)	Average colonies counted per spleen	CFU/ml blood
Control I	0.3	2.6 ± 0.3[b]	7.8 ± 1.0
2	0.35	0	0
5	0.2	16.6 ± 1.7	83 ± 8.5
13	0.2	20.2 ± 0.8	101 ± 4
23[a]	0.04	18.6 ± 1.2	465 ± 30
Control II	0.3	2.8 ± 0.4	8.4 ± 1.3

[a] Blood cell count 54,000 cells/mm³ (83% erythroblasts). [b] ± SEM.

Zusammenfassung. CBA Mäuse zeigen nach der Infektion mit dem Rauscher Virus einen starken Anstieg hömopoetischer Stammzellen («colony-forming units») im peripheren Blut. Nach 21 Tagen wird im Zustand der peripheren Erythroblastose eine 50-fache Zunahme gefunden.

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21 December 1972.

[6] S. THOMSON and A. A. AXELRAD, Cancer Res. *28*, 2105 (1968).
[7] Supported by the Deutsche Forschungsgemeinschaft.

Interactions of Anti-DNA Antibodies with Dinitrophenyl- and other Hapten-Protein-Conjugates

Several myeloma proteins in the BALB/c strain of mice[1,2] and in man[3] have been reported to react with dinitrophenyl (DNP) and with compounds, which seem to be structurally unrelated to DNP, such as 5-acetyluracil, purine-6-oyl and DNA. Conventionally induced anti-DNP antibodies have been shown to give the same unexpected crossreactions[1,4–6]. The crossreactivity of antibodies to DNA with DNP, however, has not yet been investigated.

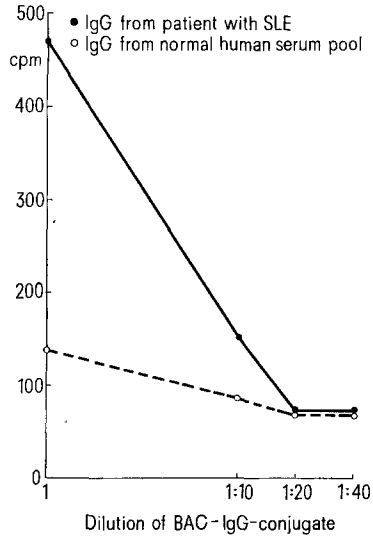

Fig. 1. Binding of ^{125}I-DNP-BSA to IgG-BAC from a patient with SLE (filled circles) and to IgG-BAC from a normal human serum pool.

The purpose of the present report was therefore to study the interactions of antibodies to DNA as found in patients with systemic lupus erythematosus[7,8] (SLE) with DNP- and various other hapten-protein-conjugates, including 5-acetyluracil, purine-6-oyl, nucleosides and nucleotides coupled to bovine serum albumin (BSA).

As some of the previously mentioned myeloma proteins with anti-DNP specificity seemed to react only with DNP-protein-conjugates, not with the free haptens[3], we used a radioimmunoassay in order to demonstrate crossreactions of anti-DNA antibodies. This method is applicable to a measurement of the relative binding affinities of antibodies for complete antigens as well as for haptens.

Material and methods. Sera of 4 patients with SLE were investigated. The patients IgG isolated by ion-exchange chromatography was coupled to bromoacetylcellulose (BAC) according to ROBBINS et al.[9]. DNP-BSA was labeled with ^{125}I by the iodine monochloride method described by MCFARLANE[10]. The reaction of the insolubilized IgG-BAC with ^{125}I-DNP-BSA was taken as 100%

[1] D. SCHUBERT, A. JOBE and M. COHN, Nature, Lond. *220*, 882 (1968).
[2] D. SCHUBERT, A. ROMAN and N. COHN, Nature, Lond. *225*, 154 (1970).
[3] W. RIESEN and A. MORELL, Immunochemistry *9*, 979 (1972).
[4] M. A. SMITH and M. POTTER, Fedn. Proc. *28*, 819 (1969).
[5] B. J. UNDERDOWN and H. N. EISEN, J. Immun. *106*, 1431 (1971).
[6] W. RIESEN, S. CARREL, V. CASTEL and A. MORELL, Abstr. Commun. Meeting Fed. Eur. Biochem. Soc. *8*, 688 (1972).
[7] M. SELIGMANN, C.r. Acad. Sci., Paris *245*, 243 (1957).
[8] W. C. ROBBINS, H. R. HOLAMN, H. DEICHER and H. G. KUNKEL, Proc. Soc. exp. Biol. Med. *96*, 575 (1957).
[9] J. B. ROBBINS, J. HAIMOVICH and M. SELA, Immunochemistry *4*, 11 (1967).
[10] A. S. MCFARLANE, Nature, Lond. *182*, 53 (1958).

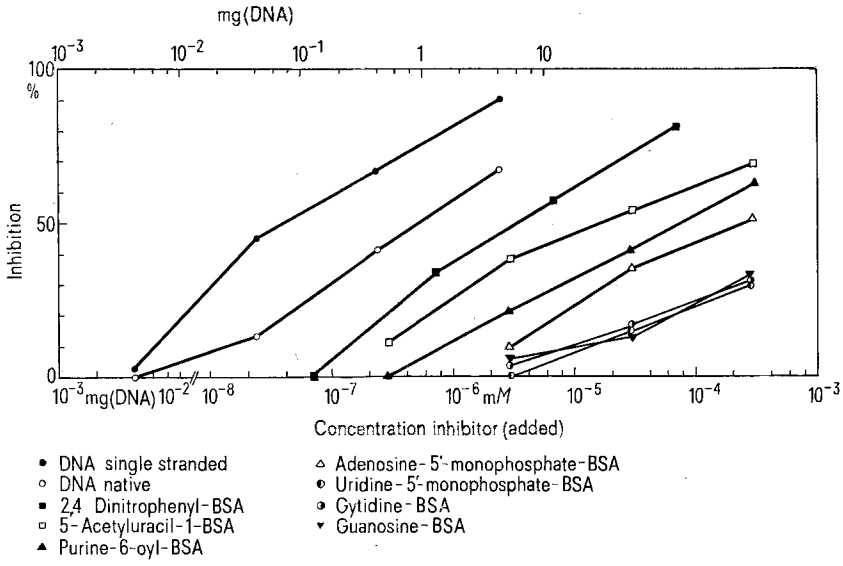

Fig. 2. Inhibition of binding of ^{125}I-DNP-BSA to IgG-BAC from a patient with SLE by various inhibitors.

binding. The relative affinities for the crossreacting compounds were expressed as percent inhibition of binding of ^{125}I-DNP-BSA at different concentrations of unlabeled inhibitor and plotted on a semilogarithmic scale.

The binding inhibition system had to be referred to the crossreacting DNP-protein-conjugate, not the homologous antigen DNA, because radioactive DNA was not available in sufficient quantities for this assay. DNA prepared from *M. lysodeiktus* was obtained from Miles-Seravac (Maidenhead/England). Hapten-protein-conjugates were synthetized according to the procedures of other authors and outlined by us elsewhere[3].

Results. Figures 1 and 2 illustrate typical binding and binding-inhibition data. The binding curve obtained with the IgG-BAC-conjugate from a patient with antibodies against DNA up to a dilution of 1:50 and radioactive DNP-BSA is shown in Figure 1 (filled circles). The corresponding experiment done with IgG isolated from a normal human serum pool served as a control in order to estimate the rate of unspecific binding of DNP-BSA (open circles). Figure 2 illustrates the binding inhibition curves obtained with various inhibitors. The relative affinities expressed as molarity or concentration respectively at 50% inhibition are summarized in the Table.

Binding inhibition data

Compound	Concentration at 50% inhibition[a]
DNA single stranded	0.07 mg/ml
DNA native	0.9 mg/ml $\sim 9\times10^{-7}M/l$
2,4-Dinitrophenyl-BSA	$3\times10^{-6}M/l$
5-Acetyluracil-BSA	$1.5\times10^{-5}M/l$
Purine-6-oyl-BSA	$7\times10^{-5}M/l$
Adenosine-5'-monophosphate-BSA	$2.1\times10^{-4}M/l$
Guanosine-BSA	$1.2\times10^{-4}M/l$ at 25% inhibition
Cytidine-BSA	$1.2\times10^{-4}M/l$
Uridine-5'-monophosphate-BSA	$2\times10^{-4}M/l$

[a] Less than 10% inhibition up to a molarity of 5×10^{-4} was observed with the following compounds: Nitraozidophenyl-BSA *p*-azobenzenearsenate-BSA, BSA.

The binding of ^{125}I-DNP-BSA to the insolubilized IgG of patients with anti-DNA antibodies could be inhibited by the following antigens or hapten-protein-conjugates respectively: single stranded DNA, native DNA, DNP-BSA, 5-acetyluracil-BSA, purine-6-oyl-BSA and adenosine-5'-monophosphate-BSA. Weak interactions were observed with guanosine-BSA, cytidine-BSA and uridine-5'-monophosphate-BSA. No inhibition was observed with other benzenoid compounds, such as nitroazidophenyl- or *p*-azobenzenearsenate-BSA, nor with BSA alone.

No precipitation could be detected when the sera containing anti-DNA antibodies were reacted with DNP-BSA or DNP-BGG, nor with the other hapten-protein-conjugates in the double diffusion technique in agar.

Discussion. The results of the present study show that antibodies to DNA, as detected in patients with SLE, crossreact with DNP-BSA and with other hapten-protein-conjugates such as 5-acetyluracil-, purine-6-oyl- and some nucleoside- and nucleotide-BSA-conjugates. As expected, the relative affinity for the putative antigen DNA is stronger than for DNP. However, the difference of about one order of magnitude is smaller than would be expected. One possible explanation for this finding could be the fact that the inhibition system was referred to the crossreacting DNP-compound, not the homologous antigen DNA. Such an effect has indeed been observed with antibodies to DNP when the crossreacting compound 5-acetyluracil was radioactively labeled[11].

Previous results of other authors[5] have shown that antibodies elicited with 5-acetyluracil-protein-conjugates crossreact with DNP and that antibodies to DNP crossreact with 5-acetyluracil. In addition Butler et al.[12] and Tanenbaum and Beiser[13] described crossreactions of antibodies to purine-6-oyl and 5-acetyluracil with single stranded DNA. In view of these findings, our results are not surprising. The chemical or biological basis for the reported crossreactions is, however, not yet understood. The possibility exists that the stereochemical behaviour,

[11] W. Riesen, unpublished results.

[12] V. P. Butler, S. M. Beiser, B. F. Erlanger, S. W. Tanenbaum, S. Cohen and A. Bendich, Proc. natn. Acad. Sci., USA *48*, 1597 (1962).

[13] S. W. Tanenbaum and S. M. Beiser, Proc. natn. Acad. Sci. USA *49*, 662 (1963).

the electrostatic charge or the hydrophobicity of the compound DNP may be such that it fits to a number of antibodies which are not related to DNP. Indeed PARKER and OSTERLAND[14] have shown that there is a hydrophobic region on the Fab fragment of a variety of immunoglobulins, which is capable of reacting non-specifically with benzenoid ligands. Confirming these reports, we observed weak interactions, when IgG isolated from a normal human serum pool was reacted with DNP-protein-conjugates by our technique. On the other hand, we could show that besides this unspecific binding the reaction of antibodies to DNA with DNP-protein-conjugates is much stronger.

Among the nucleoside- and nucleotide-protein-conjugates, adenosine-monophosphate-BSA and to a much lesser extent guanosine-, cytidine- and uridinemonophosphate-BSA reacted with the anti-DNA antibodies. It may not be excluded, however, that the weak interactions observed with the last three compound might be due to unspecific quenching, which occasionally occurs when an inhibitor is added at high concentrations.

The results reported might be of some relevance to the relatively frequent findings of myeloma proteins precipitating DNP-protein-conjugates and crossreacting with DNA[1–3], as they add further evidence to the speculations that the specificity of these myeloma proteins might be directed in fact against DNA or degradation products of DNA, instead of DNP.

Zusammenfassung. Kreuzreaktionen mit Dinitrophenyl-, Pyrimidin-, Purin- sowie Nucleosid- und Nucleotid-Protein-Konjugaten konnten bei Antikörpern gegen DNS, wie sie im Serum von Patienten mit Lupus erythematodes auftreten, nachgewiesen werden. Diese Resultate sind möglicherweise von Bedeutung für die Interpretation der auffällig hohen Frequenz von Paraproteinen, welche mit Dinitrophenylverbindungen reagieren.

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[14] G. W. PARKER and C. K. OSTERLAND, Biochemistry *9*, 1074 (1970).

[15] Acknowledgments. The authors wish to thank Miss E. ISCHI for her excellent technical assistance, Dr. H. W. BAENKLER (Erlangen) and Prof. P. H. LAMBERT (Geneva) for sera of patients with SLE.

[16] This investigation was supportec by the Swiss National Foundation for Scientific Research.

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Immunological Reactivity During Pregnancy in the Mouse

It is generally accepted that the placental filter is not an absolute barrier for foetal antigens, which can frequently stimulate the lymphatic system of the mother. This challenge results in the production of leucoagglutinins[1] and anti-HL-A antibodies[2] directed against foetal leucocytes antigens. In contrast, cell-mediated immune reactions against foetal antigens seem to be depressed in the mother. Recent interpretations of such a discrepancy are based on the depression or even abrogation of cell-mediated immune reactions by blocking antibodies, capable of coating the transplantation antigens of foetal cells[3] and/or those of maternal lymphocytes[2]. Alternatively the depression of cell-mediated immunity could be due to the particular hormonal balance during pregnancy[4]. These 2 different interpretations however do not exclude each other, because the proposed subtle interplay between humoral and cellular immunity during pregnancy[2] could hypothetically be favoured or even induced by some hormones secreted in that condition. In order to decide between these possibilities cell mediated immune reactions (contact allergy) and antibody response (PFCs capacity) have been quantitatively evaluated at different periods of pregnancy in the mouse.

Materials and methods. Inbred C3H and outbred Swiss albino young adult mice have been used. Virgin females of both strains have been mated with syngeneic or allogeneic males for a 24 h period. In order to get identical experimental conditions in pregnant and in virgin females, time of sensitization, matings and tests were clearly defined. Contact allergy has been induced[5] by painting the shaved abdomen of mice with picryl chloride (BDH, Chemicals, Poole, England) dissolved in absolute ethanol; the reaction has been quantitatively evaluated by measuring with a Panter micrometer the ear thickness before, at 24 and 48 h after a second application with the sensitizing agent dissolved in pure olive oil. Animals were either sensitized before pregnancy and then tested at different stages of pregnancy or sensitized at 10 days of pregnancy and tested 20 days after delivery.

Antibody response has been evaluated by measuring the number of plaques-forming cells (PFCs) against sheep erythrocytes (SRBC) according to the method of JERNE et al.[6] Animals were immunized by an i.p. injection with 0.1 ml of a 20% suspension of SRBC in physiological saline and sacrificed 4 days after immunization.

Results. C3H female mice sensitized to picryl chloride before pregnancy and then tested during pregnancy show a clear depression of the allergic reactions both at 24 and 48 h after challenge (Table). The group tested 30 days after delivery shows a complete normalization of the reaction. On the other hand C3H females sensitized the 10th day of pregnancy and tested 20 days after delivery react in a normal way (Table). In contrast to these findings on cellular immunity, the antibody response increased during pregnancy both in C3H and in Swiss Albino mice (Figure). The increments are statistically significant only in the groups sacrificed at 7, 11 and 14 days of gestation. In the groups sacrificed at 17, 19 and 21 days after conception (the last group was tested 1 day after delivery), the number of PFCs per spleen drops to the level present in non pregnant females. We have observed a 5–10 fold increase in pregnant C3H mice at 14 days of

[1] R. PAYNE and M. R. J. ROLFS, Clin. Invest. *37*, 1756 (1958).

[2] R. CEPPELLINI, *Progress in Immunology* (Academic Press, N.Y. 1971), p. 1573.

[3] K. E. HELLSTRÖM, I. HELLSTRÖM and J. BRAUN, Nature Lond. *224*, 914 (1969).

[4] S. KASAKURU, Proc. 6th Leucocyte Culture Conference (Ed. M. R. SCHWARZ; Acad. Press, N. Y. 1972), p. 711.

[5] G. L. ASHERSON and N. PTAK, Immunology *15*, 405 (1968).

[6] N. K. JERNE, A. A. NORDIN and C. HENRY, in *Cell-Bound antibodies*, Wistar Institute Symposium Monograph No. 3, Ed. V. DEFENDI; Wistar Institute Press, Philadelphia 1963), p. 109.