was done at 37 °C and the second step at 0–4 °C, or at 37 °C but after fixation of parasites with 2% formalin, then most toxoplasmas were stained as described above and only some of them displayed uneven shining of the pellicle.

When both steps of the IFA test were performed at 37 °C, or the first step at 0–4 °C and the second step at 37 °C, the pellicle of the majority of toxoplasmas showed irregular staining, i.e. the staining pattern of parasites was patchy (Figure B) and up to 50% of toxoplasmas formed caps (Figures C and D).

With all antisera used in the studies, a pronounced prozone phenomenon was observed; the sera did not stain the parasites at the highest concentrations and each of them had its optimum dilution for capping, which was usually somewhat higher than that required for maximum fluorescence. Both of the metabolic inhibitors tested had an adverse effect on capping: iodoacetamide prevented capping at all concentrations applied, while sodium azide did so at 10^{-1} *M* and 10^{-2} *M*. The parasites after incubation with different, capping and non-capping, dilutions of antisera did not change their original staining properties, even when re-incubated with antisera after each of 3 consecutive passages; addition of complement to the incubated parasites did not alter these results.

The results clearly demonstrate that toxoplasmas from mouse peritoneal exudate are able to move their surface antigens when incubated with antibodies. The antigen-antibody complexes accumulate in some parasites on one pole of the cell forming a cap. Application of the second layer of antibodies in the IFA test had a crucial effect on cap formation. The phenomenon occurred in metabolically active parasites and was prevented by some metabolic inhibitors.

All these findings are analogous to those already reported by DOYLE et al.[8] for *Leishmania*. However, we were unable to induce caps in more than 50% of the parasites, which figure is considerably lower than that found for *Leishmania*. Our finding may be relevant to that described by JONES et al.[11] who reported that approximately 50% of the toxoplasmas taken up by mouse macrophages are destroyed within first 6 h. It seems very likely that in the surviving half of the toxoplasmas there are only the individuals capable of forming caps.

The reason for cap formation in *T. gondii* is obscure. It has been suggested by DOYLE et al.[8] that capping of *Leishmania* amastigotes may represent an early stage of modulation of parasite membrane antigens by antibodies. In our experiments we were unable to show antigenic changes in parasites subpassaged after repeated incubation with antibodies in capping and non-capping concentrations. However, the procedure adopted to this end might have been too simple and limited, for instance, we did not use human accessory factor while incubating parasites with antibodies. There is a possibility that surface antigens are bound to antibodies and then removed in the process of capping exposing another layer of antigens with different specificity. Such an attractive hypothesis should not be discarded too hastily.

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Adsorption of Hepatitis B Surface Antigen to Matrix-Bound Long Chain Hydrocarbon Structures

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Summary. The binding of hepatitis B surface antigen (HB_sAg) to various matrix bound long-chain hydrocarbon structures has been studied. It was found that HB_sAg was strongly bound to straight hydrocarbon chains with more than seven carbon atoms. The adsorbents can probably be used for removal and/or detection of hepatitis B infectious material.

The risk of transmitting hepatitis has long been a major problem in blood transfusion and in the use of certain blood products. With the discovery of the Australia antigen, later found to be the coat component of the hepatitis B virus, it became possible to trace a hepatitis-related component. Testing of the blood donations by the various methods developed to detect hepatitis B surface antigen (HB_sAg) made it possible to reject many potentially dangerous donations. But despite the great progress that has been made, the risk of hepatitis is still one of the most serious problems in blood transfusion and in the use of certain blood products.

In connection with studies on the application of affinity chromatography techniques to plasma fractionation, it was decided to study whether it was possible to find some gel adsorbent that could selectively bind HB_sAg. The lipoprotein character of the HB_sAg caused us to focus our attention on hydrophobic affinity adsorbents. Adsorption to matrix-bound antibodies against HB_sAg was tried earlier for selective hepatitis virus removal[2], but due to certain disadvantages this method has not found any practical use so far.

The gel adsorbents were prepared either by direct coupling of the alkylamine to Sepharose 4B gel using the CNBr method[3,4] or by first coupling a linear diamine such as ethylene- or hexamethylenediamine as a spacer, followed by coupling of the carboxylic acid derivate we intended to study, using either water-soluble or -insoluble carbodiimide[5,6]. Depending on the solubility of the hydro-

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HB_sAg-binding to hydrophobic gels

Ligands coupled to Sepharose 4 B	μmol Ligand per ml adsorbent	HB_sAg titre of supernatant in	
		ID	Ausria II®
Hexylamine	2.8	1:32	
Octylamine	10.5	Absent	1:1024
Decylamine	10.8	Absent	1:512
Dodecylamine	8.0	Absent	1:128
Octadecylamine	2.7	Absent	1:32
5-Amino-nonan	2.8	1:32	
Glycyl-norleucine	8.2[a]	1:32	
Cykloheptylamine	5.3	1:32	
Hexamethylenediamine	12.8	1:32	
Hexamethylenediamine-benzoic acid	15.8	1:32	
Hexamethylenediamine-heptanoic acid	8.6	1:16	
Ethylenediamine	—[b]	1:32	
Ethylenediamine-succinic acid	2.5	1:32	
Ethylenediamine-octylsuccinic acid	8.9	Absent	1:128

[a] Determined by amino acid analysis. [b] Trinitrobenzenesulphonic acid reaction showed that the coupling functioned.

phobic ligand, we sometimes used organic solvents like ethanol, dioxane, dimethylformamide or solvents mixed with various amounts of water instead of buffered aqueous solutions. The amounts of ligand coupled was determined by NMR after hydrolysis of the gel adsorbent in concentrated formic acid or 6 *M* hydrochloric acid.

The adsorption experiments were performed by adding 2 ml of gel adsorbent to a test tube containing 2 ml of plasma having a HB_sAg titer of 1:64 in double immunodiffusion tests (ID). The tube was gently shaken for 15 min at room temperature. The gel was then removed by centrifugation and the supernatant was tested for the presence of HB_sAg. The determination of HB_sAg was performed by ID in agarose plates containing 2% dextrane to increase the sensitivity[7]. A rabbit HB_sAg-antiserum was used as precipitating agent. In some experiments, a sensitive radioimmuno assay method (Ausria II®) was used.

A number of different gel adsorbents were tested, and the results are shown in the Table. It is only the adsorbents that contain a straight hydrocarbon chain with more than seven carbon atoms that do bind HB_sAg. The other gel adsorbents do not show any affinity for HB_sAg, with the possible exception of heptanoic acid-hexamethylenediamine-Sepharose. The binding was very strong. The adsorbed material could not be eluted by changing pH or ionic strength of the eluting buffer. Elution with 6 *M* urea or 70 m*M* caprylate was not effective. As can be seen from the results in radioimmuno assay, there appears to be a correlation between the size of the straight hydrocarbon chain and the effectiveness of the adsorption of HB_sAg. The longer the hydrocarbon chain, the more effective the binding. The amount of coupled ligand seems to be of minor importance compared to the length of the hydrocarbon chain. It is established that albumins do bind to these types of gel adsorbents, but, despite this fact, HB_sAg binds to a very large extent to these gel adsorbents in the prescence of large amounts of albumin. This indicates that the competition of albumin for the affinity of HB_sAg is of minor importance.

The strong binding of HB_sAg to these gels suggests that they can be used for the removal of hepatitis virus from blood and blood products. It is fairly probable that the intact virus shows a similar behaviour to that of its coat component, but is cannot be ruled out that there may be a difference. Adsorption studies on the intact virus will answer that question. Another possible use of these adsorbents is in connection with HB_sAg experiments. For example, the amount of bound HB_sAg can be measured by incubating the gel with labelled HB_sAg-antibodies followed by suitable detection. The concentrating effect of the gel adsorbent would make such a test extremely sensitive.

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4-Isothiocyanato-4′-Nitrodiphenylamine (C 9333-Go/CGP 4540), an Anthelminthic with an Unusual Spectrum of Activity Against Intestinal Nematodes, Filariae and Schistosomes

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Summary. 4-isothiocyanato-4′-nitrodiphenylamine was found to possess activity against intestinal nematodes in mice, against schistosomes in various hosts including primates and against two filarial species in the mongolian jird. Upon administration in a single oral dose it is equally effective against *S. haematobium*, *S. mansoni* and *S. japonicum*.

Most of the known anthelminthics are active either against gastro-intestinal helminths or against systemic helminths. In our investigations with isothiocyanato compounds, we found substances displaying activity against both intestinal nematodes and systemic nematodes and trematodes. A 'broad-spectrum' anthelminthic would be of immense value in countries still in the throes of economic development, where intestinal helminthiasis, schistosomiasis and filariasis are not only highly endemic but often found simultaneously in one and the same