Neutralization of 59972 virus by Hughes group immune fluids[a,b]. Immune mouse serum (S) or ascitic fluid (AF)

	Hughes (AF)	Sapphire II (S)	Raza (S)	Farallon (AF)	Punta salinas (AF)	Soldado (AF)	Zirqa (AF)
Virus 59972	0.6 / 2.7	0.4 / 3.0	0.5 / 3.0	0.6 / 2.0	1.7 / 5.0	1.3 / 2.8	1.5 / 4.0

[a] Log neutralization index (LNI), rounded off to nearest 0.1 log; average of 3 tests. [b] Numerator = LNI of immune serum with 59972 virus; denominator = LNI of immune serum with its own virus.

colonization (9 male, 13 female). Laboratory-reared larvae were identical to those *O. maritimus* redescribed by HOOGSTRAAL et al. (in preparation). Tick suspensions were inoculated into suckling white mice and Vero cell cultures. Virus isolation and identification procedures were performed as described by THOMAS et al.[3], CLIFFORD et al.[4] and EARLEY et al.[5].

Three virus strains were obtained in Vero cells from 3 pools of 5 female ticks each. Average survival time of suckling mice inoculated intercerebrally with 0.02 ml of tick suspension was 10–11 days. Suckling mouse brain from early passages varied in titer from $10^{1.54}$–$10^{2.75}$ LD_{50}/0.02 ml. Preliminary complement fixation (CF) screen tests with 38 representative grouped and ungrouped arboviruses demonstrated relationships only with viruses of the Hughes group. CF cross reactivity showed the isolate to be most closely related to Soldado virus[6]. We were unable to study this virus in depth by mouse neutralization test (NT) because of low virus titers.

The virus was tested in a one-way plaque NT with immune fluids prepared against 7 Hughes group agents: Hughes (Dry Tortugas), Sapphire II (52301-14), Farallon (Ar 846), Raza (5/18/64), Punta Salinas (Ar 888), Soldado (Tr 52214), and Zirqa (Por 7866). The most significant reduction of plaques was obtained only with the last three (Table). Of these three, proportionately more 59972 virus was neutralized by Soldado immune ascitic fluid than by Zirqa or Punta Salinas immune fluids.

Earlier (1970) we processed 70 nymphal *O. maritimus* collected on Great Saltee Island (G.A.W.). These ticks were negative for virus in suckling mice; however, they had not been tested in tissue culture.

Soldado virus, to which this agent is closely related, was isolated from *O. capensis* group ticks near Trinidad[6]. More recently it has been reported from *O.* (*A.*) *maritimus* on Puffin Island, Wales[7].

Further studies on viruses of the Hughes group are deemed important because at least 2 of these agents, Punta Salinas and Zirqa, have been implicated in human illness[8,9]. Punta Salinas, Zirqa, Soldado and 59972 viruses have all been isolated from closely related species of the subgenus *Alectorobius*. The association of some of these ticks with human illness emphasizes the need for further biological and epidemiological investigation of *Alectorobius* ticks and the viruses they carry.

This is the second recorded tickborne arbovirus in Ireland where, previously, only louping-ill was known to occur[10,11].

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Antibody-Induced Formation of Caps in *Toxoplasma gondii*

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Summary. Trophozoites of *Toxoplasma gondii* from mouse peritoneal exudate move their surface membrane antigens towards one pole of the cell when incubated with antibodies. The phenomenon may be induced in up to 50% of incubated parasites. It is prevented by some metabolic inhibitors and low temperatures (0–4 °C). These properties do not change in parasites subpassaged after repeated incubation with antibodies.

It has been demonstrated that immunoglobulin (Ig) molecules distributed on the cell membrane of B lymphocytes show a dramatic redistribution when subjected to the action of divalent antibodies directed against surface Ig[2–5]. The Ig-molecules first aggregate into patches, which then move towards one pole of the cell to form a 'cap'. Similar redistribution of other surface membrane components takes place in many mammalian cells after reaction with specific antibodies or concanavalin A[6,7], and it has recently been reported that capping also occurs in some parasitic protozoa such as *Leishmania enriettii*[8], *Leishmania tropica*[9] and *Entamoeba histolytica*[10]. The studies described below were aimed at demonstrating cap formation in *Toxoplasma gondii*.

Materials and method. The strain of parasites. The RH strain of *T. gondii* was used for the studies. It was maintained by passage every 3–4 days in the peritoneal cavity of male CFW mice. Peritoneal exudate of the infected animals was diluted for the experiments in TC Medium 199 (Difco) supplemented with 10% heat-inactivated foetal calf serum (Colorado Serum Company, Denver, Colorado). The number of organisms was adjusted to 10^7/ml of the diluting medium.

Antisera. Human antisera were employed in the studies. They showed high levels of anti-*Toxoplasma* antibodies as measured by an indirect fluorescent antibody (IFA) test on formalin fixed parasites; their titres ranged from 1:2560 to 1:10,240. Before being used in the experiments, these sera were heated for 30 min at 56 °C.

IFA-test. This was carried out on live parasites. 10^6 toxoplasmas in 0.1 ml of the medium were added to an equal volume of each of antiserum dilutions and incubated at either 37 °C or 0–4 °C for periods varying from 30 min up to 1 h. The toxoplasmas were then washed in the medium and incubated with fluorescein labelled anti-human globulin for 30 min at 37 °C or at 0–4 °C. Finally, the parasites were washed again in the medium and examined under UV-epi-illumination in Leitz Ortholux microscope equipped with Philips CS 200 lamp. In some experiments, the parasites after incubation with human antisera were fixed with 2% formalin, and then treated as described above for live toxoplasmas.

Influence of metabolic inhibitors. 2 inhibitors were used, namely ,sodium azide (POCH, Gliwice) and iodoacetamide (BDH Chemicals Ltd.). Both were tested at concentrations ranging from 10^{-1} *M* down to 10^{-4} *M*. The inhibitors were mixed with suspensions of parasites just before their incubation with antisera; every initial concentration of the inhibitor was maintained throughout the experiment, up to the time of observation.

Influence of incubation with antisera on the staining pattern of subpassaged parasites. 10^6 parasites were premixed with either 0.1 ml of complement (Complementum lyophilisatum, Biomed, Cracow) or 0.1 ml of the medium and added to 0.1 ml of an antiserum dilution. The mixture was incubated for 1 h at 37 °C, then it was inoculated into mice. After 3 days the infected animals were killed, the parasites collected from peritoneal cavity and examined by the IFA test and re-incubated with the antiserum dilution. The procedure described above was repeated 3 times.

Results and discussion. The parasites incubated with antisera at 0–4 °C and then kept at this temperature for all subsequent manipulations showed uniform fluorescence of the pellicle (Figure A). If the first step of the IFA test

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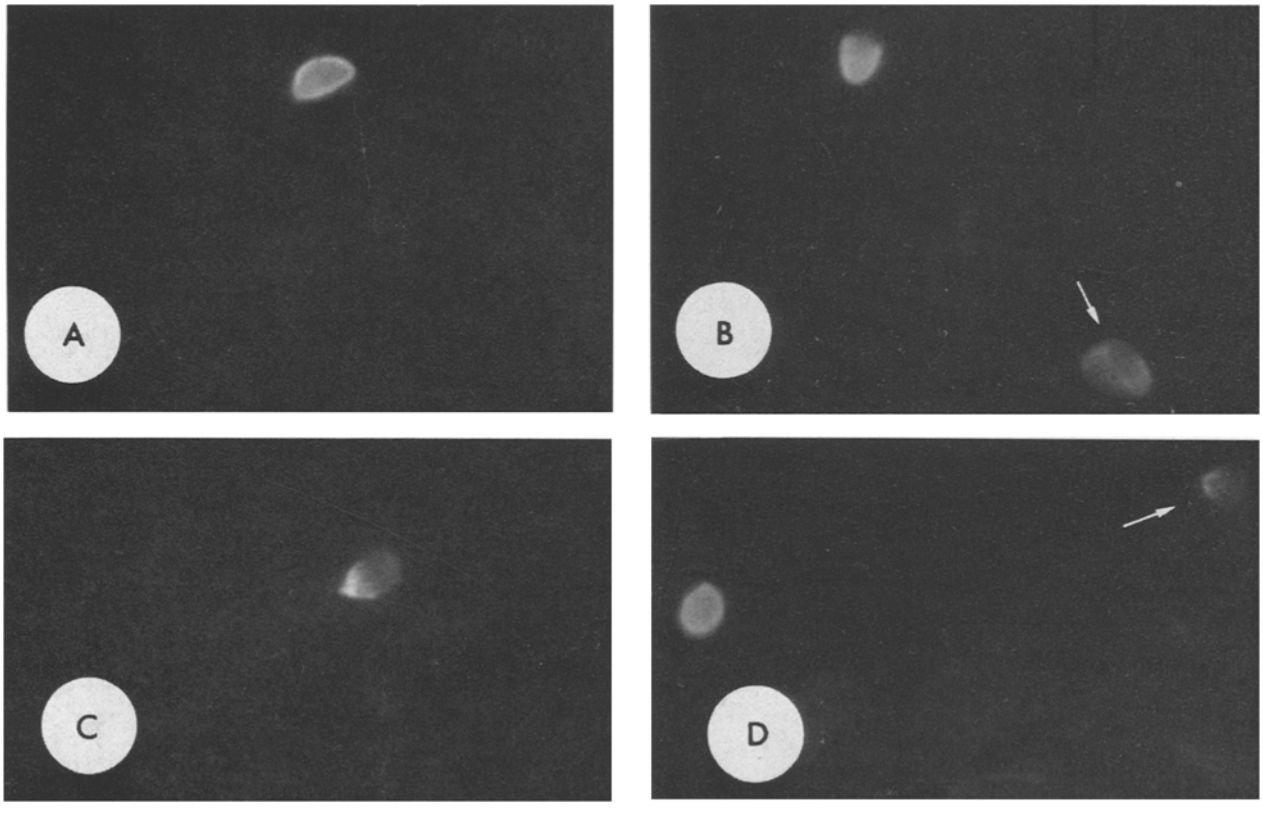

Staining patterns of live *T. gondii* trophozoites incubated with human antisera and fluorescein labelled anti-human globulin. A) staining done at 0–4 °C. B) C) and D) staining performed at 37 °C. Note uniform fluorescence of the parasite pellicle shown on A) and typical caps of fluorescence on C) and D) (arrow). The arrow on B) points a parasite with a patchy staining pattern.

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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