rolysis products are formed only at temperatures in excess of 300°C, which can readily occur in foods cooked over open flames. In contrast, the mutagens that we have detected in beef extract are produced at temperatures which do not exceed 105°C, whereas those detected in cooked ground beef are produced at temperatures that do not exceed 200°C. Thus, these mutagens are produced in conditions which occur in common cooking procedures, including the preparation of hamburgers on electrically heated hot plates at conventional cooking temperatures and times.

The mutagens found in beef extract and cooked beef are relatively active compared with a typical mutagen, 2-acetylaminofluorene (AAF), which is also active toward strains TA 1538 and TA 98. Tested on strain TA 1538, 50 μg of AAF (which is in the linear portion of the dose-response curve) yields about 4800 revertants per plate. Active material prepared from a bacterial medium containing 37 percent beef extract yielded 1367 revertants per plate containing 3.85 μg (in the linear part of the dose-response curve) of a preparation partially purified, by successive chromatographic fractionation, from the original methylene chloride extract. Accordingly, the specific activity of the beef extract mutagen is a minimum of about 350 revertants per plate per microgram, compared to 96 revertants per plate per microgram for AAF. On the basis of the estimated 350 revertants per plate per microgram for impure beef extract, a 3.6-g beef bouillon cube contains a minimum of approximately 0.3 μg of mutagen and a 100-g (wet weight) lean-beef hamburger contains approximately 1 to 14 μg of mutagen, depending on the extent of cooking. These figures correspond to concentrations, on a wet weight basis, of 0.1 part per million of mutagen in beef bouillon cubes and from 0.01 to 0.14 part per million in cooked hamburgers.

If, as indicated by the observed correlation between mutagenicity in the Ames test and carcinogenicity, these mutagens—once purified and tested on laboratory animals—are found to be carcinogens, their apparent concentration in some foods may represent an appreciable risk to certain populations. The relatively ordinary circumstances in which these mutagens are formed suggest that they may arise during the course of certain conventional cooking procedures, in addition to the preparation of hamburgers, such as the braising of beef and the evaporation of beef stock in the preparation of stews. However, the sensitivity of the effect of cooking times (see Fig. 2) suggests that it may be possible to modify cooking procedures in ways that reduce the formation of the mutagens.

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5. To avoid confusion, we retain in this report the commercial definition of beef extract, although in a more technical sense it is actually a condensed preparation of beef stock, which is itself an aqueous extract of beef.
6. Whenever a sample derived from biological material appears to induce mutagenic activity in the Ames system it is important to consider the possibility that the effect may be due to the presence of histidine in the sample. (Histidine would enable nonmutant histidine-negative cells to form colonies, giving the appearance of enhanced reverse mutation.) That the effects we describe are not due to the presence of histidine in the samples is shown by the following: (i) Replica plating of test plates show that the colonies are comprised of revertant, histidine-positive cells; (ii) the effect consistently requires the presence of the microsome preparation, a condition not required for the influence of histidine on colony formation; (iii) control experiments show that the extraction and chromatographic procedures which we employ eliminate histidine from the material finally added to the test plates.
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9. The linearity of the dose-response curve also indicates that the material is not significantly toxic to the bacteria over the range of concentrations tested.
10. From thermocouples at the surface of a patty and in its interior, it was determined that the maximum temperature (at the end of the cooking period) at the surface of the patty was 200°C and in the interior, 80°C.
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Unusual Antibody-Induced Modulation of Surface Antigens in the Cell Coat of a Bloodstream Trypanosome

Abstract. *Unlike other eukaryotic cells,* Trypanosoma lewisi *forms caps at 0°C when incubated with rabbit immunoglobulin G (IgG) directed against surface IgG from the rat host. The host IgG, which is specific for parasite antigens, probably does not cause capping of these antigens in vivo, since trypanosomes treated with Fab fragments directed against rat IgG are uniformly labeled and do not cap at 0°C or 37°C.*

The formation of dense aggregates or caps of host immunoglobulin G (IgG), a surface component of the rat parasite *Trypanosoma lewisi*, is not inhibited at 0°C. The diffuse or patchy surface staining typical of labeling with bivalent antibody at low temperatures in the absence of metabolic inhibitors rarely occurs; more than 90 percent of the cells observed are capped immediately after labeling with fluorescein-conjugated rabbit antibody to rat IgG. The caps are usually located along the undulating membrane, or at the posterior of the trypanosomes, or both; bipolar caps are rarely seen. The caps do not reflect the distribution of host antibody in vivo, since trypanosomes are uniformly labeled by Fab fragments directed against rat IgG.

In contrast to *T. lewisi*, the antibody–surface antigen complexes of other eukaryotic cells are observed to first form small aggregates (spots or patches), and later, at temperatures above 0°C, larger, usually polar, dense aggregates (caps) form. After incubation with appropriately labeled antibodies, lymphocyte surface immunoglobulins (*1*) and HLA antigens (*2*) form caps by an energy-dependent redistribution process that is inhibited both by temperatures near 0°C and by metabolic inhibitors. Inhibition of capping at 0°C has also been shown for the protozoan parasites *Leishmania enriettii, L. tropica* (*3*), *L. donovani* (*4*), and *T. brucei* (*5*); in these organisms, the initial fluorescence is uniform and diffuse.

Trypanosoma lewisi has a surface coat composed partially of bound host serum

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components, including IgG (*6*), which it acquires in vivo. The host IgG in the surface coat is specific for *T. lewisi* antigens, since trypanosomes taken from immunosuppressed rats, or from immunocompetent rats infected for less than 3 days, are not labeled by rabbit antibody to rat IgG. After these IgG-negative trypanosomes are incubated in vitro with immune rat serum (but not with normal rat serum), they bind antibody and show positive staining for IgG. As the infection progresses, the intensity of fluorescence increases concomitantly with the ability of host serum to inhibit *T. lewisi* reproduction in vivo (*7*) and in vitro (*8*).

The dense aggregates of host IgG observed after labeling at 0°C (Fig. 1) do not correspond to the distribution of trypanosome antigens in vivo, since labeling at 0°C with fluorescein-conjugated Fab fragments of rabbit antibody to rat IgG, fragments which are incapable of forming caps even at 37°C, results in a uniform, diffuse fluorescence (Fig. 2). Caps are not formed when *T. lewisi* is labeled at 0°C with rabbit antibody to rat IgG in the presence of $3 \times 10^{-1}M$ sodium fluoride or $10^{-2}M$ iodoacetamide, although patches of fluorescence are observed over the entire surface (Fig. 3). It is only in the presence of inhibitor that we observe a patchy distribution; the diffuse staining reported for other hemoflagellates at 0°C (*3–5*) is not seen.

Rat IgG in the surface coat of *T. lewisi* differs in several respects from other eukaryotic cell surface components. First, the rat IgG, although acquired naturally in vivo, is not an autologous constituent of the parasites. Second, the rat IgG is a component of the surface coat, not the cell membrane (*6*). Unlike other cell types that have been studied for capping, trypanosomes possess a cell coat readily discernible by conventional electron microscopic techniques without special staining. The surface coat of *T. lewisi*, however, is loosely organized, diffuse, and filamentous, in contrast to the compact, dense coat of African trypanosomes, such as *T. brucei* (*9*). Third, rat IgG is first detectable in the surface coat in vivo at a time in the infection (day 5) when the only antibody activity present in the blood is that which inhibits parasite reproduction. This antibody, called ablastin (*10*), has been characterized physicochemically as an IgG (*11*).

Most, if not all, of the IgG detected on the trypanosomes may be the reproduction-inhibiting antibody, ablastin, since trypanosomes with adsorbed trypanolytic IgG or IgM are immediately removed from circulation, and are not a significant

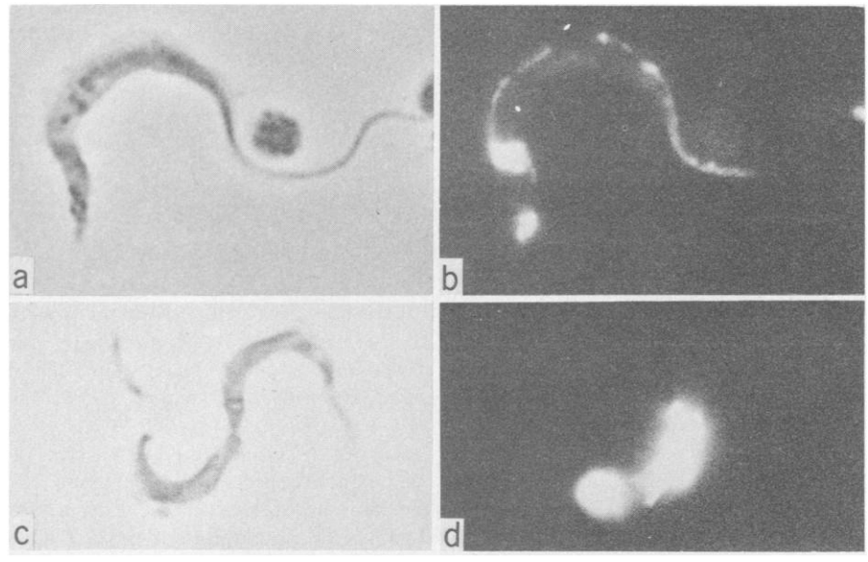

Fig. 1. Trypanosomes were partially purified from infected blood by differential centrifugation, and were washed three times in ice-cold Hanks balanced salt solution with or without 10 percent heat inactivated fetal calf serum. Trypanosomes (10^7 in 0.1 ml) were incubated on ice for 15 to 30 minutes in 0.1 ml of an appropriately diluted fluorescein-conjugated IgG fraction of rabbit antibody to rat IgG (Miles). Labeled trypanosomes were washed three times as described above, and immediately fixed in glutaraldehyde (2.5 percent in 0.1*M* cacodylate buffer, *p*H 7.7). Centrifugation was performed at 1550*g* for 15 minutes at 2° to 4°C (× 6400). (a) Phase-contrast photograph of parasites at 13 days after infection. Host platelets are also seen. (b) Parasites viewed under ultraviolet light, with epi-illumination at 13 days after infection. Note posterior cap and accumulation of antigen-antibody complexes along the undulating membrane. (c) Parasites viewed in phase contrast 8 days after infection. (d) Parasites viewed in ultraviolet light 8 days after infection.

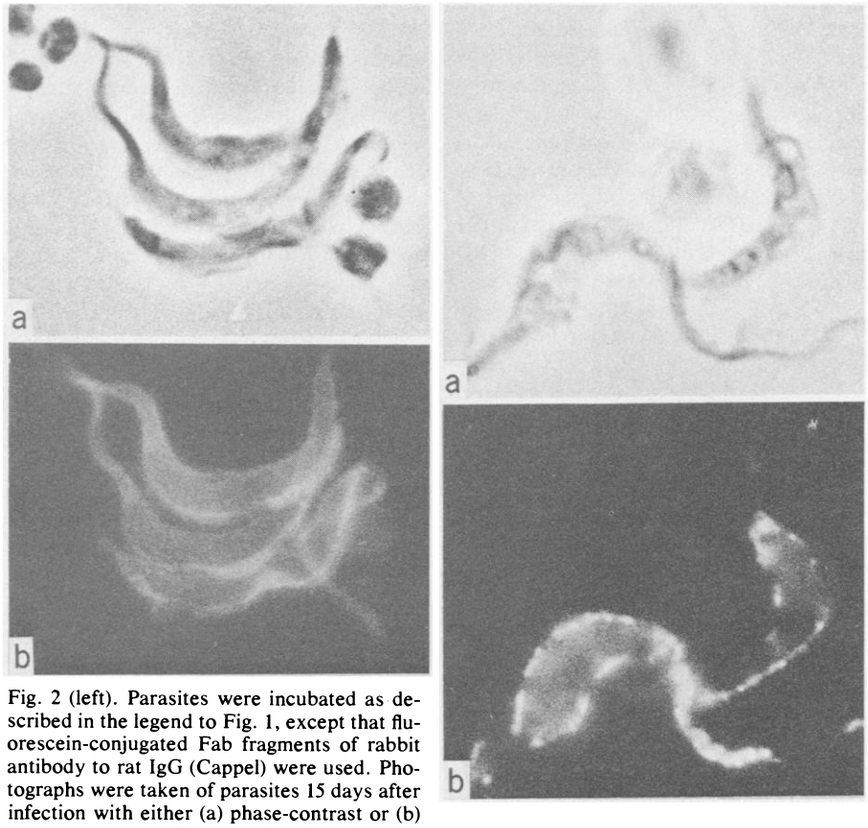

Fig. 2 (left). Parasites were incubated as described in the legend to Fig. 1, except that fluorescein-conjugated Fab fragments of rabbit antibody to rat IgG (Cappel) were used. Photographs were taken of parasites 15 days after infection with either (a) phase-contrast or (b) ultraviolet optics. Fig. 3 (right). Parasites were incubated as described in the legend to Fig. 1, except that all solutions contained 0.01*M* iodoacetamide. Photographs were taken of parasites 9 days after infection with (a) phase-contrast optics, in which two host erythrocytes were also seen, or (b) ultraviolet optics.

proportion of the trypanosome sample. Moreover, when IgG-negative *T. lewisi* cells from immunosuppressed hosts are treated in vitro with immune serum that shows only ablastic activity [after adsorption to remove trypanocidal antibodies (*10, 11*)], they become positive for IgG. Specific, nonablastic antibodies to coat antigens may also occur on circulating trypanosomes, but these have not yet been demonstrated.

Our results suggest that aggregation of surface coat components of *T. lewisi* may require less energy than membrane components of other eukaryotic cells require for capping, since capping occurs rapidly at 0°C. The loose fibrillar coat of the parasite may consist of components that have relatively weak interactions, thus facilitating antigenic mobility.

Others have suggested that antibody-induced capping in vivo might modulate parasite surface antigens, leading to antigenic variation in the causative agents of African trypanosomiasis (*3, 4*), or that it might provide a mechanism for escape from host antibody (*4*). However, host ablastic IgG does not appear to cause capping of *T. lewisi* antigens in vivo; only when this IgG is cross-linked by an additional ligand in vitro does capping occur. Electron microscopic studies of *T. lewisi* in which a double ligand technique with ferritin label is used, provide additional evidence for ligand-induced capping in vitro (*12*). However, in those experiments the primary ligand was multispecific gamma globulin (obtained from recovered hosts and containing ablastin plus trypanocidal antibodies) added in vitro, rather than naturally acquired host IgG. In contrast, our results indicate that specific, ablastic IgG acquired in vivo, despite its effects on cellular metabolism (*13*), causes no detectable modulation of surface antigens.

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Absence of Glycerol Teichoic Acids in Certain Oral Streptococci

Abstract. *Glycerol teichoic acids were not detected immunochemically or chemically in phenol-water, hot saline (Rantz and Randall), or supernatant fluids of disrupted cells of* Streptococcus mitis. *Thus, teichoic acids do not appear to be found in most Gram-positive bacteria, as has been suggested.*

Lipoteichoic acids (LTA) are a class of amphipathic polymers composed of glycerol, phosphate, alanine, fatty acids, and variable quantities of hexoses that are found associated with the cell membranes in Gram-positive bacteria (*1*). In recent studies attempting to develop a serological grouping scheme for *Streptococcus mitis*, an ill-defined group of oral streptococci associated with dental plaque as well as with many cases of endocarditis, I noticed that extracts of many strains did not react with an antiserum against teichoic acids (polyglycerol phosphate or PGP antiserum). The failure to detect teichoic acid was surprising since it has generally been assumed that most Gram-positive bacteria with the exception of certain micrococci, such as *Micrococcus lysodeikticus* (now classified as *M. luteus*), contain these polymers, at least in the membrane-associated form (*1*). Since these teichoic acids have been associated with a number of diverse biologically important activities in Gram-positive bacteria, such as regulation of autolysins, ion transport, immunogenicity, virulence, and adherence of bacteria to mammalian cells and tooth surfaces (*1, 2*), the absence of these polymers in a large group of Gram-positive bacteria could have significant implications for general as well as for oral microbiology.

Although several extensive studies that have provided a better physiological basis for the classification of viridans streptococci have been reported (*3*), many laboratories still use the epithet *Streptococcus mitis* to designate α-hemolytic streptococci that do not fit more easily recognized groups, for example, *S. salivarius, S. mutans*, and *S. sanguis*. Indeed, the most common error appears to be confusion between *S. mitis* and *S. sanguis*. Although these species are similar physiologically, studies have shown that their genomes differ significantly (*4*); one reflection of this difference is the carbohydrate and peptidoglycan composition of their cell walls (*5*). I have observed that one of the simplest means of distinguishing *S. sanguis* from *S. mitis* biochemically is the determination of ammonia from arginine; *S. sanguis* is positive and *S. mitis* negative. Also, *S. sanguis* contains the Lancefield group H antigen whereas it is absent in *S. mitis*.

A large number of strains designated *S. mitis* (*S. mitior*) were assembled from various laboratories for a serological study; in addition, a culture collection of reference strains of *S. sanguis* were available from previous studies. All strains were subcultured on Mitis-Salivarius agar (Difco) in order to ensure purity of the cultures. In some cases, two or more colonial types were observed on this medium but no differences were detected on blood agar, the usual medium used for isolation of these bacteria. Individual colonies were picked for stock cultures, and subsequently biochemical tests were performed in which a modification of the physiological tests for speciation, proposed recently by Facklam, was used (*3*).

Antigen extracts were obtained from whole cells by use of the following procedures. Phenol-water (PW) extraction was carried out at 65° to 68°C with 70 percent phenol; this procedure solubilizes membrane LTA (*6*). The Rantz and Randall (RR) (*7*) method utilizes the supernatants of cells, suspended in 0.15*M* NaCl and heated at 121°C for 15 minutes, as a source of antigens. Studies of *S. sanguis* suggest that this procedure solubilizes primarily cell wall polymers including wall teichoic acids (*8*). Antigens were also obtained from supernatants of cells disrupted in a Ribi press at 55,000 pounds per square inch at 10° to 15°C; these preparations contain an admixture of cell wall, cytoplasmic components, and LTA (*1, 6*). All of the extracts were dialyzed against H_2O and were lyophilized prior to testing by Ouchterlony and

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