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# Lipoproteins as Substitutes for Serum in Mycoplasma Culture Medium

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Crude lipoprotein-containing fractions obtained from sera of three different animal species were tested, in combination with bovine serum albumin, as substitutes for serum in *Mycoplasma pneumoniae* culture medium. All sera yielded at least one lipoprotein-containing component which was considerably more effective in promoting mycoplasma growth than the unfractionated serum sample from which it was derived. The very low activity of certain whole-serum samples tested in this investigation suggests that toxic substances may be present in whole serum which are not contained in the lipoprotein preparations. The greatest activity appeared in the high-density lipoprotein-containing components of bovine and horse sera and the low-density lipoprotein-containing component of human serum. The high degree of growth-supporting activity of these crude lipoprotein-containing serum components suggests that they may be useful as serum substitutes in mycoplasma culture media.

The concept that serum lipoproteins can act as carriers of lipid growth factors in mycoplasma culture media was advanced by Smith et al. in 1954 (13). However, the possibility of using serum lipoproteins as serum substitutes has not been extensively explored. Recently, Slutzky et al. reported that human high- and low-density lipoproteins (HDL and LDL) can act as cholesterol donors to *Mycoplasma hominis* and *Acholeplasma laidlawii* (11). Additionally, in a recent investigation we showed that certain lipid- and lipoprotein-containing components of a commercial bovine serum fraction (BSF) in combination with bovine serum albumin can effectively substitute for serum for *Mycoplasma pneumoniae* and *Mycoplasma arthritidis* (14). In the present study we compared the growth-supporting activities of serum lipoproteins obtained from three animal species and examined their value as possible serum substitutes in *M. pneumoniae* culture medium.

Commercial calf and horse sera were obtained from Flow Laboratories, fresh adult bovine serum was from The Ohio State University Veterinary Hospital courtesy of Glen Hoffsis, and fresh human serum was from a 28-year-old male volunteer at approximately 1 h postprandially. These sera were fractionated by a sequential ultracentrifugation procedure designed to separate serum lipoproteins by their densities (3, 5). Five components, including the pellet remaining at the bottom of the tube at the end of the procedure, were collected from each sample; the first four were designated components 1 to 4. Components 3 and 4 and the pellet were dialyzed extensively against 0.15 M NaCl containing 0.001 M ethylenediaminetetraacetic acid and 500,000 U of penicillin per liter. All components were stored at 4°C and dialyzed against 0.15 M NaCl for 2 to 3 h at room temperature before use.

The lipoprotein, cholesterol, and total protein contents of these components are listed in Table 1. Lipoproteins were detected by polyacrylamide gel electrophoresis (2), cholesterol was detected by the method of Rudel and Morris (9), and protein was assayed by the Lowry method (6). Cholesterol was detected only in the lipoprotein-containing components. Beta or LDL were found primarily in component 3, and alpha or HDL were primarily in component 4 of each serum. Chylomicrons and very low-density, or pre-beta, lipoproteins (VLDL) were found only in components 1 and 2 from human serum.

Mycoplasma cultivation and assessment of growth-supporting activities of serum components were carried out as described in a previous report (14). Briefly, serum components obtained by density ultracentrifugation were substituted for BSF in SSR2 broth medium, and their growth-supporting activities for glass-grown *M. pneumoniae* strain Cl-8 were quantitated by measuring the protein content of the glass-adherent mycoplasmas. SSR2 broth prepared with components 1, 2, 3, or 4 was also supplemented

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TABLE 1. *Lipoprotein, cholesterol, and total protein content of serum components isolated by density ultracentrifugation*

| Component[a] | Lipoprotein | Cholesterol[b] (mg/100 ml) | Protein[b] (mg/ml) |
|---|---|---|---|
| 1 and 2 (density, <1.007) | | | |
| Calf serum | None | <20 | ND[c] |
| Fresh bovine serum | None | <20 | ND |
| Horse serum | None | <20 | ND |
| Human serum | Chylomicrons, pre-β | 24 | 0.56 |
| 3 (density, 1.007-1.063) | | | |
| Calf serum | β, trace α | 38 | 0.48 |
| Fresh bovine serum | β, trace α | 39 | 0.42 |
| Horse serum | β | 42 | 0.32 |
| Human serum | β | 248 | 2.30 |
| 4 (density, 1.063-1.21) | | | |
| Calf serum | α | 83 | 1.90 |
| Fresh bovine serum | α | 170 | 2.40 |
| Horse serum | α | 106 | 2.40 |
| Human serum | α, trace β | 106 | 5.20 |
| Pellet (density, >1.21) | | | |
| Calf serum | None | <20 | ND |
| Fresh bovine serum | None | <20 | ND |
| Horse serum | None | <20 | ND |
| Human serum | None | <20 | ND |

[a] Serum components were obtained by sequential density ultracentrifugation as described by Hatch and Lees (3).

[b] Cholesterol and protein values for calf serum components represent averages of values from three samples; values for other serum components were obtained from one sample each.

[c] ND, Not done.

with 0.4% (wt/vol) crystalline fatty acid-free bovine serum albumin (14).

Both lipoprotein species (LDL and HDL, contained in components 3 and 4, respectively) obtained from calf serum supported significant levels of mycoplasma growth (Fig. 1). More activity was observed in the HDL (component 4) than in the LDL (component 3) fraction. Interestingly, component 4 also showed considerably more activity than the unfractionated serum. Increasing the concentration of the intact serum stepwise from 3 to 20% (vol/vol) in broth had no effect on its growth-supporting activity (data not shown).

Similar results were obtained with fresh adult bovine and commercial horse sera (Fig. 2). As with calf serum, components 1 and 2 and the pellet were almost entirely inactive and are not included in the figure. In this experiment unfractionated bovine serum was tested at concentrations of 12 and 20% in broth and showed little activity at either concentration. Concentrations below 12% (data not shown) were equally ineffective. Whole horse serum, which is often used in mycoplasma culture media at a concentration of 20%, also proved to be considerably less active at this concentration than its HDL-containing component at 5%. Concentrations of horse serum below 12% (data not shown) were even less effective.

Human serum components showed a different pattern (Fig. 3). A limited amount of whole serum was available; therefore, only concentrations of 0.75 and 3% in broth were tested. These

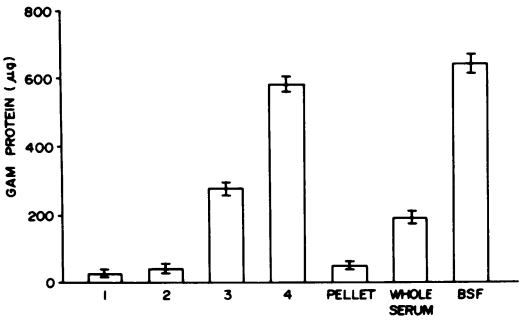


FIG. 1. *Effect on M. pneumoniae growth of calf serum and its components (1 to 4 and the pellet) isolated by density ultracentrifugation. All components and whole calf serum were incorporated into broth at a final concentration of 3% (vol/vol). Mean glass-adhering mycoplasma (GAM) protein and standard error values were calculated from triplicate cultures. Growth in whole calf serum and its components is compared to growth in medium containing 3% (vol/vol) commercial BSF.*

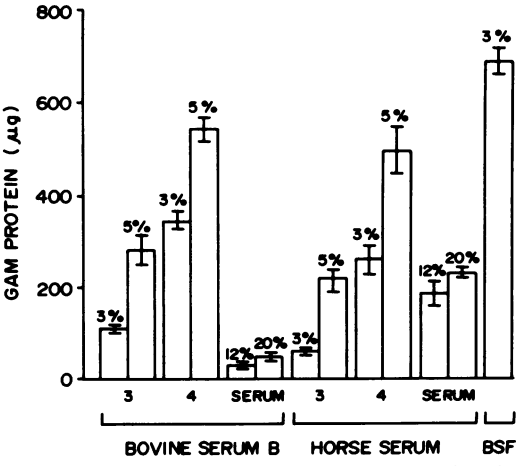


FIG. 2. *Effect on M. pneumoniae growth of bovine and horse sera and components 3 and 4 isolated from them by density ultracentrifugation. Components 3 and 4 were each tested at final concentrations in broth of 3 and 5% (vol/vol) and whole sera at 12 and 20% (vol/vol). One set of cultures grown in medium containing commercial BSF (3%, vol/vol) was included for comparison. Mean glass-adhering mycoplasma (GAM) protein and standard error values were calculated from sets of four replicate cultures.*

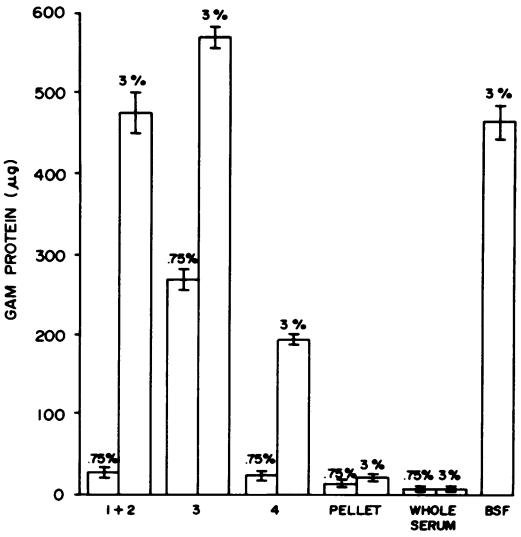


FIG. 3. *Effect on M. pneumoniae growth of human serum and human serum components 1, 2, 3, and 4 and the pellet isolated by density ultracentrifugation. All components and whole serum were incorporated into broth at final concentrations of 0.75 and 3% (vol/vol). Growth in these components is compared to growth in 3% (vol/vol) commercial BSF. Mean glass-adhering mycoplasma (GAM) protein and standard error values were calculated from sets of four replicate cultures.*

levels were unable to support mycoplasma growth. However, all three lipoprotein species from this serum sample exhibited considerable activity. Unlike the other sera tested, the most active component of human serum contained LDL (component 3).

Not surprisingly, the most active component in each serum sample tested contained the major serum lipoprotein of the animal species from which the sample was obtained. Thus, HDL, contained in component 4, carry over 70% of the total serum lipids of cattle (15) and horses (8), but only 35% of the total lipids of human serum (1). Humans also have considerably higher levels of VLDL and chylomicrons than do cattle or horses (7, 8, 15), and human VLDL can carry a significant portion of the total serum lipids, although levels vary considerably depending upon physiological conditions (7, 15). This may explain why our 1-h postprandial VLDL- and chylomicron-containing human serum component exhibited such high growth-supporting activity. In contrast to our observations, Slutzky et al. reported that human VLDL inhibit the growth of some mycoplasma species, although *M. pneumoniae* was not among those tested (12). Differences between mycoplasma species, medium formulations, or lipoprotein preparations may explain this discrepancy.

We found it interesting that whole sera tested at several different concentrations were always markedly less nutritionally effective than the most active lipoprotein-containing components derived from them. This suggests that inhibitory substances were present in whole serum, possibly anti-*M. pneumoniae* antibodies in the case of human serum, and that these toxic substances were separated from the lipoprotein-containing components of serum during the fractionation process.

The BSF control used in this investigation exhibited very high growth-supporting activity. However, as Hughes et al. discovered, different commercial lots of BSF are not consistent but vary widely in quality (4). Indeed, only two other lots of BSF out of seven screened in our laboratory over a 2-year period had comparable activity (unpublished data). Variability has also been noted for commercial whole serum preparations (10). In the present investigation we tested four sera from three different animal species, and at each trial we obtained at least one serum component which showed activity considerably greater than whole serum and comparable to our highly active lot of BSF. This suggests that crude lipoprotein-containing components of serum such as those described in this report may prove to be more reliable sources of essential serum nutrients than either commercial BSF or whole serum and that further investigation into their use as possible whole-serum substitutes for the more fastidious mycoplasmas is warranted.

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