

From these results, it appeared that chlorpromazine and Me^{++} : (1) activated serum cholinesterase of pigs at concentrations lower than those required for inhibition; (2) activated by reducing the stability of the (ES) complex and inhibited by increasing its stability; (3) bound at a ratio of drug or Me^{++} to active site which at inhibitory concentrations was twice that ratio observed at activating concentrations; (4) competed for the same site on the enzyme.

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¹ Hofstee, B. H. J., *J. Pharmacol.*, **128**, 3, 299 (1960).

² Oliver, W. T., and Funnell, H. S., *Amer. J. Vet. Res.*, **24**, 98, 82 (1963).

³ Oliver, W. T., and Funnell, H. S. (to be published).

⁴ Dixon, M., and Webb, E. C., *Enzymes* (Longmans, Green and Co., London, 1958).

⁵ Van der Meer, C., *Nature*, **171**, 78 (1953).

H-Antigen in HeLa Cells and H-Antibodies in Anti-HeLa Sera

In 1959 Kelus *et al.*¹ were able to demonstrate H-antigen in HeLa cells by means of mixed agglutination using extracts from *Ulex europaeus* as anti-H agglutinin. At that time in our laboratory the species-specific antigenicity of cultivated mammalian cells was being investigated by means of a haemagglutination test which involved guinea pig anti-cell sera and erythrocytes of various species². The species-specificity of this test was established, partly by excluding blood-group specific reaction. This finding was supported by the work of Högman³, who verified that human cell cultures lose blood group A and B antigens in the early stages of cultivation. Apparently, this does not occur with regard to H-antigen according to the observation of Kelus *et al.* mentioned here. Yet, H-specific reaction was found not to interfere in species-specific haemagglutination. This conclusion was reached indirectly by demonstrating species-specific cell haemagglutinogens and erythrocyte receptors to be proteins in contrast to the carbohydrate nature of blood-group substances⁴. A more direct examination of this problem was made possible when Bombay blood erythrocytes (lacking H-antigen) and Bombay blood serum (containing anti-H isoagglutinins) became available to us. Various experiments were carried out.

First, the finding of Kelus *et al.*¹ demonstrating H-antigen in HeLa cells was confirmed. Positive mixed agglutination was obtained between ficin or trypsin-treated HeLa cells and human erythrocytes of any blood group. However, only *Ulex*-agglutinins mediated the reaction whereas Bombay anti-H serum (with a haemagglutination titre of 1:20) did not mix-agglutinate HeLa cells and erythrocytes. The *Ulex* mixed agglutination reaction was, nevertheless, considered H-specific on grounds of negative control results with Bombay erythrocytes.

Secondly, Bombay anti-H serum (containing four haemagglutination units per reaction volume for blood group O erythrocytes) was absorbed with HeLa cells. This resulted in the removal of all measurable anti-H isoagglutinins. So, the presence of H-antigen in HeLa cells was verified by a direct reaction of the cells with anti-H isoagglutinins. (In this experiment it was noticed that HeLa cells seem to possess higher capacity to absorb anti-H isoagglutinins than species-specific haemagglutinins, that is, much larger amounts of cells are necessary for absorbing an equal proportion of species specific haemagglutinins under comparable conditions.)

Third, guinea pig anti-HeLa hyperimmune sera were repeatedly absorbed: (a) with group O (H-positive) erythrocytes; (b) with Bombay (H-negative) erythrocytes. Absorbed and unabsorbed serum samples were titrated in agglutination tests with: (a) O-erythrocytes; (b) Bombay-erythrocytes. It was found that H-negative Bombay-erythrocytes were unable to remove a small fraction of agglutinins which specifically reacted with erythrocytes containing the H-antigen. This fraction amounted to no more than 1 per cent of human-specific haemagglutinins present in the unabsorbed serum samples. These results showed that sera of guinea pigs hyperimmunized against HeLa cells may contain anti-H agglutinins, though in proportions too minute as to interfere with species-specific haemagglutination of any human erythrocytes including Bombay blood.

In these experiments, which confirmed the presence of H-antigen in HeLa cells, use was made of methods and materials not previously applied to the study of this particular problem. The persistence in human cell cultures of the H-antigen, in contrast to blood group A and B antigens, appears to be an interesting phenomenon with respect to the nature and relationship of blood group antigens. Work on these problems is being continued in this laboratory as well as further systematic investigations on the fate of the various blood group antigens in carcinomatous cell populations.

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¹ Kelus, A., Gurner, B. W., and Coombs, R. R. A., *Immunol.*, **2**, 262 (1959).

² Brand, K. G., and Syverton, J. T., *Proc. Amer. Assoc. Cancer Res.*, **3**, 8 (1959); *J. Nat. Cancer Inst.*, **24**, 1007 (1960).

³ Högman, C. F., *Exp. Cell Res.*, **21**, 137 (1960).

⁴ Brand, K. G., and Syverton, J. T., *Fed. Proc.*, **20**, 151 (1961). Brand, K. G., *Exp. Cell Res.* (in the press).

High-density Lipoprotein Concentrations in Men and Women

AN extensive literature is available dealing with the comparison of serum lipoprotein and lipid concentrations between men and women^{1,2}. The present concept is that normal pre-menopausal women have greater amounts of high-density (or α -) lipoproteins than men of a comparable age. This difference has been ascribed to sex hormone secretion, especially higher oestrogen-levels in women.

We report here that when the high-density lipoproteins, that is, those with densities greater than 1.0635 g/ml., are sub-fractionated, this sex difference prevails in only one of the several high-density lipoproteins characterized at present, the HDL₂ (or S₇₄₋₀) component (1.125, sodium chloride).

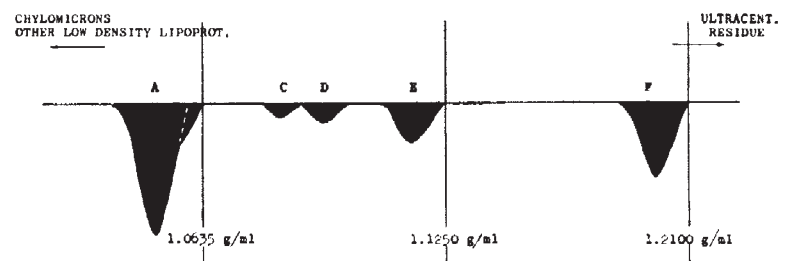


Fig. 1. Schematic diagram of high-density lipoproteins in normal human serum