## Occurrences in Normal Human Sera of 'Incomplete' Forms of 'Cold' Auto-Antibodies

THE existence of 'incomplete' forms of antibodies active against human erythrocytes and detectable by means of the Coombs test<sup>1</sup> is now widely recognized. Rh antibodies are commonly present in this form, and so are some at least of the recently recognized rarer immune antibodies<sup>2</sup>, such as anti-Kell and anti-S. The fact that the iso-antibodies anti-A and anti-Bmay similarly exist in incomplete forms is also being increasingly realized<sup>3</sup>.

The possibility that 'cold' auto-antibodies might also exist in incomplete forms seems, however, scarcely to have been considered. Although Levine and Wigod<sup>4</sup> mentioned that 'cold' agglutinins might be a cause of confusion in carrying out the Coombs 'developing' test, they gave no details of the phe-nomenon. The purpose of the present note is to record that incomplete 'cold' antibodies active against corpuscles of all groups do, in fact, exist, and that they may be found in most, if not in all, sera from apparently healthy persons, as may 'cold' panhæmagglutinins at low titres.

The presence of these normally occurring incomplete 'cold' antibodies can often be demonstrated if defibrinated blood is chilled at 2-5° C. for 1-2 hr., and the corpuscles then exposed to the action of antiglobulin rabbit serum, after repeatedly washing them in saline warmed to 37° C. In these circumstances. although the 'cold' hæmagglutinins are rapidly eluted into the warm saline, the incomplete antibodies appear to remain adsorbed on to the corpuscles. A stronger reaction can be obtained if a weak (0.5-5 per)cent) suspension of corpuscles is made in their own serum and dealt with as described above. Sometimes with weak suspensions a positive Coombs test will be obtained in this way, even if the sensitization is carried out at room temperature.

There is evidence that the presence of fresh serum is necessary for the adsorption of the antibody, for corpuscles suspended in serum previously heated to 56° C. for 5-30 min. fail to give a positive reaction after chilling. The addition of a small amount of absorbed fresh serum to heat-inactivated serum restores in part, however, its power of sensitizing corpuscles. Heparin (0.5 mgm. per ml.), sodium citrate (6 mgm. per ml.) or Wintrobe's oxalate mixture (2 mgm. per ml.) added to serum also inhibit sensitization to a greater or less extent, depending upon the titre of the antibodies and the potency of the anti-globulin rabbit serum. Another pointer to qualitative differences between incomplete 'cold' antibodies and incomplete anti-Rh is that antiglobulin rabbit sera are not necessarily equally effective in revealing sensitization by the two kinds of antibodies. Some sera, for example, although highly potent in revealing sensitization by anti-D, give weaker reactions with corpuscles sensitized by incomplete 'cold' antibodies, and vice versa.

J. V. DACIE

Department of Pathology Postgraduate Medical School of London, London, W.12. March 2.

- <sup>1</sup> Coombs, R. R. A., Mourant, A. E., and Race, R. R., Brit. J. Exp. Path., 26, 255 (1945).
  <sup>2</sup> Leading article, Lancet, July 16, 1949.
  <sup>3</sup> Witebsky, E., Blood, special issue No. 2, 66 (1948).
  <sup>4</sup> Levine, P., and Wigod, M., first meeting of the International Society of Hematology, Buffalo (1948).

## Heteropycnosis in Human Chromosomes

INVESTIGATORS of human chromosomes noticed many years ago the presence of two chromatic masses or chromocentres in resting nuclei. On the other hand, Schultz<sup>1</sup> was able to analyse in male human material the structure of a couple of elements pairing at pachytene during spermatogenesis, each spatially connected with a nucleolus, and, therefore, called nucleolus organisers.

The question arises whether there is a relationship between nucleolus-organising chromosomes seen in the spermatocytes, and the two chromocentres appearing in resting nuclei of different tissues<sup>2</sup>. Suitable material is provided by the epithelial cells flaking off from the vaginal mucosa. Collecting the vaginal fluid at the vaginal ostium, the cells are numerous and in a living condition, some of them showing mitotic stages. The usual squash technique with Acetocarmine staining is sufficient to give good preparations. Nearly four hundred nuclei have been analysed, from eight women.



Fig. 1a Fig. 1 Fig. 1. Nucleus with two nucleoli. The nucleolus-organising chromosomes are very thin

Fig. 1a. Drawing of the nucleus in Fig. 1, showing nucleoli and nucleolar chromosomes only



Fig. 2 Fig. 2a Fig. 2. Nucleus with one chromocentre, and one nucleolar chromosome clearly stained

Fig. 2a. Drawing of the nucleus in Fig. 2



Fig. 3. Nucleus with two chromocentres. Nucleoli not visible. (Magnification nearly  $1,000 \times$ )

Such cells have nuclei showing all stages, from the nucleolus-organising chromosomes with the same structure as that described by Schultz to two chromocentres: these two extreme cases are connected with a continuous chain of intermediate stages, where one of the chromosomes is contracted (chromocentre) and the other appears in thread-like form (see Figs. 2 and 2a). This provides evidence that the nucleolus-organisers are present also in the