quency (Jones, 1938 ; Read and Asher, 1956 ; Seaton and Goldberg, 1960 ; Hall, 1965).

Koilonychia and dystrophic nail changes of lesser severity have long been regarded as characteristic of chronic iron deficiency (Witts, 1930 ; Davies, 1931), and this has been restated recently by Jacobs (1969). In this study the incidence of koilonychia in patients with iron-deficiency anaemia was much lower than in previously reported series, but it was not possible to compare the incidence of lesser nail changes because of the differing criteria used. In the present series the incidence of nail changes was not significantly different from that in control subjects, some of whom may have been sideropenic. We conclude, therefore, that nail changes, though they may be a feature of sideropenia, are not helpful evidence of iron-deficiency anaemia in a clinical situation.

Vitiligo was found only in anaemic patients, particularly those with deficiency of vitamin-B₁₂ and folate. Its rarity, however, diminishes its importance as a helpful diagnostic sign in anaemia.

In view of the age-dependence of impairment of vibration sense, both in anaemic and in control patients, this sign is of little value in the diagnosis of pernicious anaemia in the elderly patient.

Fever was not useful in differentiating between the various types of anaemia. Frequently there were obvious reasons for the fever in anaemic patients, a point stressed by Jones and Payne (1960).

Many of the previously reported studies of the clinical manifestations of anaemia were made some decades ago, and represent the classic descriptions of severe and long-standing anaemia of varying aetiology. Our observations on the inci-

dence of glossitis and koilonychia in iron-deficiency anaemia differ substantially from these earlier studies. This may reflect a trend towards more prompt diagnosis and more effective treatment of iron-deficiency anaemia at the present time, with a consequent reduction in its chronicity and severity.

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Immunoglobulins and the X-Chromosome

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Summary : Serum levels of immunoglobulins (Ig) G, M, and A were determined in 28 women with an additional X-chromosome (XXX), and in equal numbers of age-matched normal women and men. Mean IgM levels were found to be highest in the XXX group, intermediate in normal women, and lowest in men ; these differences were statistically significant. Mean IgM values obtained from seven XXY and three XXXY cases were almost identical with those of normal women and XXX women respectively. No such sex linkage was observed for IgA and IgG levels. These results support the suggestion that the serum level of IgM is related to the number of X chromosomes present.

Introduction

In an investigation comparing immunological responses of normal persons with those of patients suffering from rheumatoid arthritis we found that mean serum IgM levels of women were significantly higher than those of men among both patients and healthy persons (Rhodes *et al.*, 1969). Higher female IgM levels compared with male have also been reported by Butter-

worth *et al.* (1967), Rowe *et al.* (1968), and others, and the occurrence of this phenomenon in diverse populations suggests that it is due to hereditary rather than environmental factors. Evidence for the genetic control of immunoglobulin levels comes from the work of Cohen *et al.* (1961), who showed that higher rates of synthesis in American Negroes than in Caucasians persisted in different environments. The presence of apparently sex-linked alterations in the level of one immunoglobulin alone has, to our knowledge, not been hitherto reported. The predominant male incidence of congenital agammaglobulinaemia reveals sex linkage, but the antibody deficiency usually relates to low concentrations of IgG and IgA as well as of IgM. Other immunoglobulin-deficiency states such as acquired agammaglobulinaemia and Swiss type agammaglobulinaemia, though genetically determined (Tobler and Cottier, 1958 ; Hitzig and Willi, 1961 ; Kamin *et al.*, 1968) are generally not thought to be sex-linked.

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FIG. 1.—Distributions of levels of immunoglobulins within each group of subjects, with means and standard deviations. Each point represents the mean of duplicate determinations. ○ Age-matched set rejected from IgM figures because of unexplained high value in XXX subject. □ Case of thyrotoxicosis.

If IgM levels were wholly or partially controlled by genes located on the X-chromosome and both X-chromosomes were genetically active during early foetal life, then females would be homozygous for this trait and affected to a greater extent than heterozygous males. In this case one might also expect persons with an abnormally high X-chromosome complement to have higher IgM levels than normal genotype females.

To test this premise we decided to measure the immunoglobulin levels of three genetically distinct groups; (1) women with an additional X-chromosome (XXX), (2) normal women (XX), and (3) normal men (XY).

Materials and Methods

Sera from 28 women (aged 20–74 years) with an additional X-chromosome (XXX) as well as sera from 10 persons with other sex chromosome aberrations (seven XXY and three XXXY) were obtained. Control sera were collected from 28 women and 28 men volunteers whose ages matched those of the XXX subjects within one year. Careful questioning of control subjects about their state of health and family history relating particularly to chronic illness, autoimmune disorders, infectious mononucleosis, and hepatitis revealed freedom from disease in these respects in all. Nuclear sexing had to be carried out on the controls, but the XXX women, who were inmates of institutions, had been investigated previously. Questionnaires sent to their physicians about their state of health revealed that none suffered, at the time of taking the blood sample or during the preceding five years, from any of the above-mentioned diseases, except one who had thyrotoxicosis.

Values of immunoglobulins G, M, and A were determined by radial immunodiffusion (Fahey and McKelvey, 1965), serum samples which had been snap frozen and stored at -20°C . being used. Individual sera were divided before storage to allow estimations to be carried out on samples that had been frozen and thawed once only. Radial immunodiffusion plates and standard solutions supplied by Hyland Laboratories were used. The relative values of the standard solutions were checked by estimations on a series of dilutions of a single serum. The precipitation rings were allowed to develop for 24 hours at 4°C . and their diameters then measured on a mechanical microscope stage, bringing opposite sides of the circle in turn into line with a fixed point on an eyepiece scale. The diameters of the precipitation circles given by the standard solutions were plotted against their concentrations on semi-logarithmic graph

paper and the concentrations of the immunoglobulins in the test sera calculated from these graphs. Estimations of immunoglobulin levels were not carried out until blood samples had been obtained from all the subjects. The order of the specimens was then randomized within each of the three groups, and in each batch of tests equal numbers of sera from each group were included on each immunodiffusion plate. Duplicate determinations were carried out in separated batches.

Results

The individual levels found in our subjects of the three immunoglobulin classes investigated are shown in Fig. 1. Statistical analysis of these data (Fig. 2) revealed a significantly higher mean IgM level in the XXX group ($P<0.01$) compared with normal women, who in turn had significantly higher mean levels ($P<0.05$) than normal men. When the data for normal men and women from this investigation were combined with those obtained from the healthy subjects in our previous study (Rhodes *et al.*, 1969), the sex difference became of higher significance ($P<0.001$). One subject of the XXX group was excluded from the statistical analysis because her IgM value of 1.6 g./100 ml. ($18\times$ S.D. from the mean) fell so far outside the range of the remainder of the group, though no clinical cause for such an increase was found. Although the numbers of XXY and XXXY subjects were small it is nevertheless interesting that the mean IgM level of the XXY group was almost identical with that of the XX group, and that of the XXXY group with the XXX group. Thus it appears that the level of IgM is influenced by the number of X-chromosomes and the presence of a Y-chromosome does not appear to alter this level.

The mean level of IgG in normal men was higher than that in normal women, but the difference was without statistical significance. Nevertheless, on combining the data obtained in this investigation with those of the normal controls in our previous study (Rhodes *et al.*, 1969), the difference between the sexes became significant ($P<0.025$). In the XXX group the levels were significantly higher than in normal women or men.

IgA levels showed no sex difference and a significantly lowered value in the XXX subjects. The mean levels of IgG and IgA in the XXY and XXXY subjects relative to the levels in the other groups also lacked any consistent pattern such as that found in the levels of IgM.

FIG. 2.—Mean levels of immunoglobulins. All statistically significant differences between means are indicated. Numbers of subjects shown at bases of columns. * Data from previous investigation combined with that of present study.

Discussion

These results support our suggestion that IgM levels are influenced by the number of X-chromosomes. Although it is generally thought that in the presence of several X-chromosomes only one remains active (Lyon, 1962), there are many instances in which this hypothesis has to be modified. Repression of genetic expression of the second X-chromosome is thought to occur at a very early stage in embryonic development, but it is possible that an influence on IgM levels is exerted before its deletion. The presence of IgM in the spleen has been detected in 12-week-old human embryos, whereas IgG and IgA do not appear until after the fifth and sixth months respectively (Bulle and Burtin, 1967). Because IgMs are important antibodies concerned with immediate protection from many pathogens, their increased concentration may have been of evolutionary importance in safeguarding maternal survival in an animal with a long gestation period and immature young. The mean levels of the other classes of immunoglobulins differ significantly among the groups of subjects studied, but present no pattern suggesting that they are influenced by the sex chromosomes in any simple manner, and in view of the lack of correlation in the individual between levels of immunoglobulin classes (Allansmith *et al.*, 1967; Rowe *et al.*, 1968) it would be unreasonable to expect to find a single pattern common to all of them.

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