remaining two cases all we can say is that thyroid function, perhaps already inadequate in Case 2, deteriorated acutely with the onset of infection, while in Case 3 no previous history was available but the patient became rapidly comatose with the development of pneumonia.

A final point of interest was the contrast between the relative lack of symptoms and the evidence of extensive consolidation in four of our five patients. We think that the silent character of these severe infections is a matter of significance which needs stressing, and that attention should be drawn towards the possible presence of myxoedema when faced with pneumonia with minimal symptoms.

Summary

Five cases of myxoedema of acute onset, including one new case of coma, are reported. The stress factors were severe pneumonia and exposure to cold. It is suggested that in all these cases there was a sudden breakdown of function in potentially inadequate thyroid glands, already working at maximum capacity to meet normal requirements. As the pneumonia caused few symptoms in all our patients it was liable to be overlooked.

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The Second British Congress on the History of Medicine and Pharmacy was held in London from September 27 to 29. It was organized by the Faculty of the History of Medicine and Pharmacy of the Worshipful Society of Apothecaries of London with the co-operation of the Chemical Society. The subject chosen for discussion was "Chemistry in the Service of Medicine," and papers were read at the Apothecaries' Hall, at the Royal College of Physicians, at the Royal College of Surgeons, and at the Chemical Society, Burlington House. At a brief ceremony during the Congress the chairman of the Faculty, Dr. W. S. C. Copeman, conferred the Faculty's honorary fellowship on Professor K. J. Franklin, F.R.S., distinguished physiologist and medical historian, and on Dr. Ernst Wickersheimer, president of the Société Internationale d'Histoire de la Médecine and a leading historian of medicine in France. During the Congress a dinner was held in the hall of the Worshipful Society of Apothecaries. The guest of honour was Sir Howard Florey, P.R.S., and the president of the Chemical Society, Sir Alexander Todd, F.R.S., gave an address. Sir Alexander discussed the long-standing relation between chemistry and medicine, and stressed that medicine and the search for health had provided a great stimulus to chemical research. He foresaw the day when the accepted separation of the basic sciences would have to be broken down. At present the range of skills for tackling a research problem comprehensively could probably only be found in research institutes, but research could best be carried out in the universities, which must use the constant influx of young minds to cross the barriers between the sciences.

NUCLEAR SEXING WITH ACETO-ORCEIN

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Nuclear sexing, which stems from the discovery of sexual dimorphism in interphase nuclei (Barr and Bertram, 1949), is now accepted as a routine clinical diagnostic test (Lennox, 1956; Davidson, 1960). The oral-mucosal-smear method (Moore and Barr, 1955; Marberger, Boccabella, and Nelson, 1955; Klinger and Ludwig, 1957) has gained wide popularity because specimens may be obtained with minimal inconvenience to the patient. The present paper describes the application of the aceto-orcein squash technique to oral mucosal smears. It is just as accurate as other methods but much more rapid, and it has the additional advantage that fine details of nuclear structure are more readily revealed by squashing and flattening the freshly obtained cells.

Materials and Methods

Stains

Orcein may be used with or without lactic acid.

Stock Solution.—Synthetic orcein, 1 g.; glacial acetic acid, 45 ml. Boil, cool, and filter.

Simple Aceto-orcein.—Dilute 45 parts stock solution with 55 parts distilled water and filter. Periodic filtration is necessary.

Lacto-aceto-orcein.—Dilute 50 parts stock solution with 50 parts of 70% lactic acid and filter.

Fresh Preparations

An oral mucosal smear is obtained by scraping the inside of the patient's cheek with a wooden spatula. Several short vertical strokes are made with firm pressure, and the desquamated cells and saliva are then scooped up on the end of the spatula and spread on the surface of a clean dry slide. One drop of stain is quickly placed on the centre of the smear and a thin coverglass— $\frac{3}{4}$ in. (22 mm.) square or $\frac{1}{8}$ by $1\frac{1}{2}$ in. (22 by 38 mm.) rectangle—is laid over it. One or more layers of filter paper are placed on top and the coverglass is pressed down by drawing the thumb across from one end of the slide to the other. To avoid moving the coverglass on the slide the filter paper is firmly held down over one corner of the cover with the left hand while the right hand moves across from left to right. The filter paper absorbs excess stain expressed from below the coverslip. With insufficient pressure the nuclear membrane is wrinkled: with correct pressure the nuclei are evenly flattened. Extrusion of the nuclei from the cell is not disadvantageous provided that the nuclear membrane remains intact. With simple aceto-orcein they should be stored in a covered tray in a refrigerator and fresh stain added to the edge of the coverglass to prevent drying out. The hygroscopic lactic acid in lacto-aceto-orcein

prevents drying out, and experience has shown that the slides may be kept at room temperature for as long as six weeks.

Scrutiny

Simple aceto-orcein stains the nucleus and cytoplasm more rapidly than lacto-aceto-orcein, but temporary preparations of either type may be scrutinized immediately. With both stains, contaminating bacteria are not prominent at first but become more obtrusive with prolonged staining. Although other methods may give preparations in which bacteria are relatively unobtrusive there is considerable loss of cells in processing. With orcein, however, there is an abundance of cells from which to select ideal nuclei. The orcein smear can be readily scanned with a 1/7-in. oil-immersion objective, which gives a large field and depth of focus. An optical glass filter of peacock blue (Type OB2), which is complementary in colour to orcein red, is advantageous.

Permanent Preparations

To make permanent preparations the slides are inverted in a ridged dish in a 3:1 mixture of absolute alcohol and glacial acetic acid until the covers separate. As cells adhere to the cover and slide both are given three two-minute washes in absolute alcohol, cleared in "euparal essence," mounted in "euparal," either separately or recombined, and left on a warm plate to dry. Pretreatment with silicone prevents adhesion of cells to coverslip but precludes immediate scrutiny of the temporary preparation with an immersion lens, since the silicone on the upper surface of the cover prevents even spreading of the immersion oil.

Surveys

A small survey of normal subjects was carried out in a mixed class of over 100 students. Oral mucosal smears were obtained, aceto-orcein preparations were made on coded slides, and scrutiny of each was carried out without knowledge of the sex of the subject.

A larger survey of all the inmates in an institution for oligophrenic mental defectives was carried out. The material was collected in a few hours by a team of two, one obtaining the smears and the other making the preparations. In one group of 100 subjects who were lined up for examination the smears were obtained and the preparations made at a rate of more than 20 every five minutes, the slides and coverslips having been cleaned and numbered beforehand. In the oligophrenic subjects contamination with bacteria and food particles or other debris was a frequent cause of unsatisfactory smears, and about 20% of the preparations had to be repeated. The nuclear sex was recorded as chromatin-negative if no structure resembling sex chromatin was

seen in 30 consecutive suitable nuclei. In preparations with sex chromatin present a minimum of 30 consecutive nuclei with sex chromatin were scrutinized. The nuclear sex was recorded as chromatin-positive if there was no suggestion of multiple sex chromatin bodies in any of these nuclei. In smears where a proportion of the nuclei showed more than one body (chromatin-double-positive), or in doubtful or difficult smears, new preparations were obtained, and in these at least 100 suitable nuclei were scrutinized.

Results

Normal Subjects

In every case sex was diagnosed correctly. With simple aceto-orcein, staining of the sex-chromatin body is so extremely rapid that a smear may be obtained, stained, and photographed all in less than a minute. Sex chromatin is seen with equal clarity in permanent preparations. In obtaining oral mucosal smears it was found necessary to exert firm pressure to secure suitable cells, for patches of cells with different types of nuclei occur. The patches are apparently derived from three different epithelial strata. (1) The superficial layer contains pyknotic degenerating nuclei which are often heavily contaminated by bacteria. (2) The intermediate layer provides nuclei suitable for sexing. These have a smooth nuclear membrane and finely granular nucleoplasm in which the sex-chromatin body, if present, is deeply stained. (3) The deep layer has nuclei with prophase chromosomes and prominent nucleoli.

Oligophrenic Subjects

Preparations from 245 apparent males were scrutinized. Two smears were found to be chromatin-positive and 243 were chromatin-negative. Both subjects with chromatin-positive nuclei were found to have the typical clinical and hormonal feature of medullary dysgenesis (Stewart *et al.*, 1959). Smears from 240 apparent females were scrutinized. One subject was found to have double-positive nuclei and was apparently an example of the triplo-X syndrome (Stewart and Sanderson, 1960). Smears from the other 239 female subjects were chromatin-positive and none was chromatin-negative.

Nuclear Morphology

Orcein-stained nuclei exhibit fine details of nuclear morphology. A chromatin-negative nucleus (Fig. 1) has a finely granular nucleoplasm and a well-defined nuclear membrane with no sex chromatin. A chromatin-positive nucleus (Fig. 2) is similar but exhibits sex chromatin adjacent to the nuclear membrane. In some nuclei from normal females the sex-chromatin body is

FIG. 1

FIG. 2

FIG. 3

FIG. 4

FIG. 5

FIG. 1.—Typical chromatin-negative nucleus from a normal male. FIG. 2.—Typical chromatin-positive nucleus from a normal female. FIG. 3.—Degenerating nucleus unsuitable for sexing. FIG. 4.—Chromatin-positive nucleus from a male with medullary dysgenesis. FIG. 5.—Chromatin-double-positive nucleus from a triplo-X female.

apparently connected to a nucleolus by a fine chromatin thread. The sex-chromatin body may appear flattened, convex, tent-shaped, or diplococcoid at the nuclear membrane. It may also lie in the centre of the nucleus, but it cannot always be distinguished from a nucleolus, and such centrally placed chromatin bodies are therefore not suitable for enumeration. Nuclei which are unsuitable for sexing include those which are pyknotic and degenerating (Fig. 3), crumpled and crenated, or contaminated by bacteria, although these may be eliminated by careful focusing.

In males with medullary dysgenesis and chromatin-positive nuclei (Fig. 4) the sex chromatin body is exactly comparable to that found in a normal female. In the triplo-X female some nuclei have two such chromatin bodies (Fig. 5). These are positioned at random on the nuclear membrane, opposite, adjacent, or closely adjacent to each other. In some chromatin-negative nuclei derived from normal (XY) males a slim heterochromatic crescent is discernible at the nuclear membrane. A similar structure is present together with sex chromatin in some chromatin-positive nuclei derived from male subjects with medullary dysgenesis (XXY) but apparently absent from nuclei derived from normal (XX) or from triplo-X (XXX) females.

Discussion

Orcein has several advantages for nuclear sexing. These include ease of preparation, rapidity of sexing, and the revelation of fine details of nuclear structure.

Ease of Preparation and Rapidity of Sexing.—Orcein preparations may be made with extreme ease and rapidity, and scrutiny is facilitated by squashing, which enlarges the nuclei. Simple aceto-orcein is better for rapid sexing since it stains so very quickly. Lacto-aceto-orcein is recommended for survey work, as temporary preparations may be made with great facility at the rate of 250 an hour but may be scrutinized at leisure because they do not deteriorate for several weeks. The value of the technique for survey work has been demonstrated by our results, which are consistent with recent large surveys of male (Barr *et al.*, 1959) and female (Fraser *et al.*, 1960) oligophrenic patients. Our results also provide some additional data of value in establishing the incidence of sex-chromosome anomalies in such populations.

Revelation of Fine Detail.—Squashing of the nucleus, with subsequent enlargement, permits study of its fine structure. The fine chromatin thread which apparently connects the sex-chromatin body to a nucleolus was first recognized by Reitalu (1957) in foetal tissue, and was subsequently described by Serr and his co-workers (Serr *et al.*, 1958; Lennox *et al.*, 1960) in tissue-culture material. In view of the difficulties of sexing encountered in foetal tissue (Park, 1957) and in tissue culture (Miles, 1960) the recognition of this structure in oral mucosal smears is of value. The importance of this structure with respect to the nature of sex chromatin is discussed elsewhere (Stewart, 1961). The discovery of a slim heterochromatic crescent in male nuclei stained by orcein (Sanderson, 1960) has been confirmed by other workers (Ohno and Makino, 1961). Evidence is as yet insufficient to correlate it with heterochromatin derived from the Y chromosome, although this is the most likely explanation of its presence in males (XY and XXY) and its absence in females (XX and XXX).

Summary

The sex-chromatin body is readily recognizable in oral mucosal smears stained with orcein. Simple aceto-orcein is recommended for rapid nuclear sexing, and lacto-aceto-orcein is recommended for survey work. In a survey of 245 males and 240 females in an institution for oligophrenic mental defectives two cases of medullary dysgenesis and one triplo-X female were found.

The aceto-orcein method reveals fine details of intranuclear structure, including a structure found in the nuclei of subjects with a Y chromosome and perhaps related to heterochromatin derived from this chromosome.

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Medical Memoranda

Traumatic Rupture of the Heart

Rupture of the heart after myocardial infarction has long been recognized as a dramatic and rapidly fatal complication. The incidence of rupture of the ventricle subsequent to acute myocardial infarction is given as between 1 and 10% of the post-mortem cases of myocardial infarction (Kohn, 1959).

A rather less common cause of rupture of the heart is that following trauma to the body, in which the chest wall or pericardium remains intact (Crane, 1952). The diagnosis of this condition is difficult, as the following case report shows. After trauma to the body, in which the chest wall or pericardium remains intact, rupture of the healthy myocardium can only conceivably occur as a result of profound changes in intrathoracic and intracardiac pressure.

Cardiac trauma produced by contusive and concussive forces may lead to disruption of the cardiac musculature (Bright and Beck, 1935). Cardiac tamponade, with an ultimately fatal issue, will occur after a varying lapse of time (Pollock *et al.*, 1952). Recovery may occur after