THE CELLS OF NORMAL HUMAN URINE
A QUANTITATIVE AND QUALITATIVE STUDY USING A NEW METHOD OF PREPARATION

BY

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Despite the many reports of the finding of malignant cells in various body fluids, dating back to Beale, of London, in 1860, the use of exfoliative cytology as a diagnostic procedure did not find much application until the publication in 1943 of the monograph by Papanicolaou and Traut on the diagnosis of uterine cancer by examination of the vaginal smear. Following this classical work the principle has been applied to many regions of the body and now finds widespread acceptance, particularly in the United States, as an aid to diagnosis and for screening in cancer-prevention clinics.

In a survey of the status and future trends of exfoliative cytology Papanicolaou (1952) emphasized the need for more basic studies of the normal cell population in the various body fluids. Of the diverse types and several origins of the cells in urine relatively little is known, although pathological samples have been examined on a large scale. Papanicolaou's paper in 1947 on the examination of samples from 240 clinical cases was followed by several others (Schmidlapp and Marshall, 1948, 1950; Chute and Williams, 1948; Harrison, Botsford, and Tucker, 1951), and in 1952 Crabbe, reporting from this laboratory, gave the results of using the Papanicolaou technique as a screening procedure for 1,000 workers in the dyestuffs industry who may at one time or another have been exposed to bladder carcinogens.

The Papanicolaou technique consists essentially of smearing the sediment from centrifuged urine over an albuminized slide, and, after fixation and staining, the whole smear, which covers most of the slide, is examined under the microscope. The latter process is both laborious and time-consuming owing to the wide dispersion of the cells. A 50-ml sample of normal urine produces a quantity of deposit on centrifugation requiring about five to 10 slides to accommodate it when smeared.

It is apparent that if the cells could be concentrated on a slide within an area of a few square millimetres the examination could be carried out in a very short time. In addition, the diagnosis would be strengthened by the fact that it would be based upon virtually the whole cell content of the sample rather than a fortuitous small fraction of the whole represented by one or two smears.

In the present paper a method for the concentration of the cells of urine is described and the results of its application to quantitative and qualitative study of the cell population of normal male urine are given.

Technique

The sediment which is deposited on centrifuging normal urine consists largely of mucus.* Removal of this mucus permits free suspension of cells and enables a high degree of concentration to be achieved by centrifugation. A simple procedure is described below by which this may be effected: it depends upon the fact that adding absolute alcohol to the sediment results in fixation of cells without irreversibly precipitating the mucus. It then becomes possible to dissolve the mucus by diluting with water without fear of disrupting the cells.

Determination of the Rate of Cell Loss into the Urine.—(1) About one and a half hours' output of urine in a 70-ml centrifuge tube marked as shown (Fig. 1) is adjusted to pH 6 by adding N/2 HCl or N/2 NaOH and centrifuged for 10 minutes at 1,750 g.+ (2) The liquid is gently drawn off and 15 ml absolute alcohol added and mixed thoroughly. After five minutes distilled water is added slowly with shaking to fill the tube. (3) After centrifugation for 10 minutes at 1,750 g. the liquid is drawn off and about 1 ml benzidine/hydrogen peroxide solution is added to

* The term "mucus" is used throughout this paper to mean the whole of the mucilaginous material of the normal urinary sediment, and is broadly justified by the protein reactions of the cell-free sediment and by the action of 33% acetic acid on an aqueous solution of this sediment (see Harrison, 1947).

† Centrifugal force is expressed throughout as a multiple of normal gravity (g.).
"stain" erythrocytes, and well mixed. The centrifuge tube is stoppered and left for half an hour. (4) Dilution with about 30 ml. 20% alcohol is followed by centrifugation for 10 minutes at 1,750 g. and the liquid drawn off to leave about 1 ml. (5) Cell clumps are broken up by drawing the suspension vigorously in and out of a pipette connected to a hypodermic syringe. (6) The suspension is now centrifuged again for five minutes at 1,750 g., the liquid is drawn off to either 0.05 or 0.1 ml., and cell counts are carried out using a Neubauer chamber.

Areas of 1 to 4 sq. mm. are then counted for large cells, small cells, and erythrocytes. An arbitrary value of 15 μ diameter is taken to define large and small cells: in practice the sizes of most of the cells are found to fall conveniently far from this. The benzidine treatment shows erythrocytes as black discs.

The calculation is thus: Cells counted = \( \frac{V}{VT} \) loss in thousands/hour, where \( V \) = volume of suspension in ml. (0.05 or 0.1), \( V = \) volume of suspension actually counted in c.mm., and \( T = \) period of secretion of urine in hours. The time taken for two determinations in parallel is about three hours.

**Preparation and Staining of Cellular Contents of Urine.**—Stages 1 and 2 given above are carried out. Then (3) crystals and the grosser "dirt" particles (and unavoidably a few cells) are deposited by centrifuging for one minute at about 280 g., and are then removed with a pipette. (4) After centrifugation for 10 minutes at 1,750 g. most of the liquor is drawn off, and one half of the remaining volume of a pectin adhesive solution is added and mixed. After a further centrifugation for two minutes at 1,750 g. liquid is removed until a volume roughly equal to that of the sediment remains. (5) After mixing, the whole is transferred by pipette to a grease-free microscope slide. The diameter of the droplet thus deposited is usually about 2 to 10 mm. As evaporation proceeds, a mat, slightly glistening spot remains on the slide which is placed horizontally in 1:1 alcohol/ether fixative.

It is sometimes convenient to make two preparations, one of the small cells (less than 15 μ in diameter) and the other of the remaining cells. The separation is carried out by means of a sintered glass filter. If this separation is desired, the following should be carried out after stage 3:

The cleaned cell suspension is poured into a sintered glass filter funnel, pore size 2, the filtrate from which contains only the small cells; this is collected in a 70 ml centrifuge tube. Meanwhile large cells have been accumulating on the filter; when only a small depth of liquid remains, and before it falls to zero, the filter funnel is inverted over another centrifuge tube, as shown in Fig. 1. A little 20% alcohol is then blown through the filter transferring the cells on it to the centrifuge tube.

Pectin is effective as an adhesive in minute concentration, and being a carbohydrate is relatively refractory to stains.

**Staining.**—From alcohol/ether the preparation is taken through the following sequence (10 seconds or longer at each stage unless otherwise stated):—

Seventy per cent. alcohol: benzidine-H₂O₂ solution, 10 minutes; 70% alcohol: 50% alcohol; Harris' haematoxylin, 10 minutes; 50% alcohol: 70% alcohol containing 1% NH₄OH, one minute; 70% alcohol: orange G., one minute: absolute alcohol, irrigate to remove all surplus orange G.; light green, one minute: absolute alcohol, one second: xylol. The preparations are mounted in balsam.

**Small-cell Preparations.**—Haematoxylin 5 minutes: counterstains at half strength, or use 0.5% eosin.

**For Screening for Malignancy.**—Counterstains at half strength.

The time taken to make the general preparation of six urine samples in parallel is two and a half hours. To make separate small-cell and large-cell preparations of each of six samples, about four hours are required.

**Composition of Solutions Employed.**—The following solutions are employed:

- **Pectin Adhesive.**
  - Pectin...
  - Glycerin...
  - in water

- **Benzidine-Hydrogen Peroxide.**
  - Benzidine...
  - Acetic acid...
  - in 96% alcohol

To 50 ml. of this stock solution 1 ml. 20-volume hydrogen peroxide is added. After 24 hours the mixture is centrifuged.

**Haematoxylin.**

- Harris haematoxylin
- Alcohol 70%
THE CELLS OF NORMAL HUMAN URINE

Orange G... 5% in 50% alcohol
Light Green... 0.2% in 96% alcohol

The Rate of Cell Loss into the Urine

Using the method described above, the rate of cell loss has been determined on 12 men of ages varying from 18 to 47, none of whom was aware of any previous history of kidney, bladder, or prostatic complaints or of macroscopic haematuria. Part or all of a two-hour sample was used, secreted during the period 7.30 a.m. to 9.30 a.m. in each case. Subjects were asked to ensure that the urine stream did not come into contact with the prepuce. Table I shows the rates of cell loss obtained.

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Squamous, Transitional, and Degenerate Cells</th>
<th>Small Epithelial Cells and Leucocytes</th>
<th>R.B.C.s (1,000/hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6-2</td>
<td>54-6</td>
<td>2-8</td>
</tr>
<tr>
<td>II</td>
<td>31-1</td>
<td>67-6</td>
<td>13-0</td>
</tr>
<tr>
<td>III</td>
<td>10-1</td>
<td>54-6</td>
<td>3-7</td>
</tr>
<tr>
<td>IV</td>
<td>33-0</td>
<td>177-1</td>
<td>1-5</td>
</tr>
<tr>
<td>V</td>
<td>14-8</td>
<td>46-7</td>
<td>0-1</td>
</tr>
<tr>
<td>VI</td>
<td>74-8</td>
<td>88-3</td>
<td>0-8</td>
</tr>
<tr>
<td>VII</td>
<td>148-9</td>
<td>165-0</td>
<td>3-7</td>
</tr>
<tr>
<td>VIII</td>
<td>31-6</td>
<td>62-3</td>
<td>1-1</td>
</tr>
<tr>
<td>IX</td>
<td>45-2</td>
<td>28-2</td>
<td>1-2</td>
</tr>
<tr>
<td>X</td>
<td>21-6</td>
<td>136-6</td>
<td>1-0</td>
</tr>
<tr>
<td>XI</td>
<td>17-3</td>
<td>56-6</td>
<td>0-1</td>
</tr>
<tr>
<td>XII</td>
<td>12-2</td>
<td>206-6</td>
<td>2-8</td>
</tr>
</tbody>
</table>

It appears from this limited series that the output of blood cells and exfoliation proceed at very different rates in different individuals. In the same individual, however, the output appears to be constant from day to day. The variation in total cell output as indicated by a repeat determination on another day for each of the 12 subjects was from 0.5 to 20.5%.

The results of a preliminary assessment of diurnal changes in the rate of cell loss in one individual is shown in Figs. 2a and 2b. The red cell output appears to be increasing with the day's activities. The peaks occurring in Fig. 2b for the period 6–8 a.m. might well be due to sloughing off of cells which have accumulated in the upper layers of the epithelium during the night.

The Normal Urine Cell Picture

Samples were obtained from the same subjects. All urines were produced between 7.30 a.m. and 9.30 a.m. By means of the technique described, the cells were separated from extraneous matter and segregated into two groups, those greater than about 15 μ diameter (mainly squamous and transitional) and those less than about 15 μ diameter.

Figs. 2a and 2b.—Diurnal changes in the rate of cell loss from the urogenital system. In this preliminary experiment the changes occurring in one individual on two days (broken line and continuous line) are indicated. Fig. 2a refers to red blood cells. Fig. 2b refers to large epithelial cells and to small epithelial cells and leucocytes.

They were then mounted and stained. Figs. 3 and 4 show typical fields of these large-cell and small-cell preparations.

The Large Cells.—Examples of the main types of cells to be found in the large-cell preparation are shown in Fig. 5. Table II shows that the proportions of the various kinds of large cells exfoliated vary greatly between individuals, although, so far as this has been studied, there appears to be remarkable consistency in the cell picture of urine from the same subject at different times.

The figures in Table II were obtained indirectly by carrying out differential counts on stained preparations of the large cells of normal male urine and applying these proportions to the directly obtained rate of large-cell loss for each individual given in Table I, column 2. About 250 cells were
counted in each case. The last two columns show the reaction to staining with 5% orange G followed by 0.2% light green.

The individuality of the cell picture is evident in one or more of the following: (i) the total quantity of cells present (see Table I); (ii) the occurrence of multinucleate cells (see Fig. 5); (iii) the relative proportions of squamous, transitional, “early degenerate,” and “late degenerate” cells (see Fig. 5); and (iv) the reaction to staining with 5% orange G followed by 0.2% light green (see Table II).

All of these differences invite speculation, not least the reaction to orange G and light green. For example, Subject II shows 91.9% of the cells stained green, Subject XI shows 76.0% stained yellow. The cells which stain yellow are mainly the degenerated type: a count of 973 yellow-staining cells from 20 normal male subjects shows that 2.1% are squamous, 3.7% are transitional, 16.8% are “early degenerate” cells, and the remainder, namely 77.4%, are “late degenerate” cells. These figures are in accord with the preferential staining of cornified tissues by orange G. The
common occurrence in some subjects of apparently undegenerate squamous and transitional cells which nevertheless give a strong yellow reaction could perhaps be taken to show that this reaction is a more sensitive indication of degenerative changes in these types of epithelium than structural criteria. A further point of interest is the occurrence of groups consisting of about six or more structurally identical cells which have developed together and peeled off en masse, in which one or two cells show a strong yellow reaction, although all their fellows stain strongly green. It is tentatively suggested that there is a relation between the state of degeneration of the cells and their affinity for the yellow stain, but signs of degeneration may be difficult to recognize morphologically in some cases.

The Small Cells.—Examples of the main types of small cells are shown in Fig. 6. Table III gives some indication of the rates at which they are lost into the urine: it is evident that there is great variability between individuals.

The figures in Table III were obtained indirectly by carrying out differential counts on stained preparations of the small cells of normal male urine and applying these proportions to the directly obtained rate of small-cell loss for each individual given in Table I, column 3. The average number of cells counted in each case was 280. Rates for loss of red cells are repeated from Table I.

![Figure 6](http://jcp.bmj.com/)
The following criteria were used in counting. Cell type i: a very round granular nucleus and high nuclear/cytoplasmic ratio; cell type ii: no nucleus visible, granular cytoplasm, sometimes vacuolated. The cell types designated iii, iv, and v in Fig. 6 have not been found to be sufficiently characteristic to permit individual classification: they have therefore been counted together, along with the various intermediate types which occur.

Counts of cells of 8 to 30 μ diameter to be found in fresh urinary sediment have been carried out for many years by Addis (1948), who gives 650,000/24 hours as the normal rate of loss of epithelial and white blood cells, and assumes that most of the epithelial cells are derived from the kidney. In the present study a preliminary attempt has been made to indicate the origin of the cell types referred to above by making routine preparations (Section II) of the following material: (1) macerated normal human kidney tissue suspended in cell-free urine and set aside for an hour; (2) urine removed from a human ureter at operation; (3) normal female urines. In addition (4) smears were made of prostatic fluid of three subjects. In (1) and (2) most of the cells were similar to the cells referred to above as type i. A few of these were found in the female urines and none in the prostatic smears. It thus seems reasonable to consider that the type i cells of urine have originated in the tubules of the kidney.

The type ii cells are very probably degenerate forms and are often found to be breaking up. The numbers in which they occur are not connected with deterioration of the urine sample, as when fresh samples are used the output is consistent from day to day at the level characteristic.
for the individual. The fact that they hardly ever
stain preferentially with orange G indicates that
the cells of which they are degenerate forms do
not originate from a region in which cornification
occurs.

The invariable presence of red cells in all indi-
viduals is of interest (see Table III), particularly
as the figures given represent the minimum num-
ber present. Ghosts and broken red cells were
not included. All those counted were unmistak-
ably identified by the benzidine reaction. The
mode of entry of blood cells into urine presents
an interesting problem. The greater ability of
polymorphs to pass from the blood into urine is
demonstrated by the change of the R.B.C./poly-
morph ratio from about 1,000/1 in blood to about
1/30 in urine.

Application to Cytological Diagnosis

Mention has been made of the ease with which
the whole cellular contents of a sample of urine
may be examined, and of the possibility of im-
proved accuracy in diagnosis thus presented. Figs.
7a and 7b give some idea of the appearance of
preparations made from the same sample by the
smear technique and by the method described
above.

It seems possible that the principle of fixation
of cells followed by solution of extraneous material
may be applied with advantage to other body
fluids or washings used for cytological diagnosis.
The use of mechanical segregation by means of a
filter may also find application where it is desired
to examine cells of a particular size occurring in
small proportions, and would be of value where
pus, blood, or bacteria are crowding out the cells
under examination.

With regard to renal diagnosis, Foot and Papan-
icolaou (1949) have shown that single cells of
the tubule epithelium become malignant, though
their fellows appear normal and the lumen remains
patent. If, as is now submitted, the cells of the
small-cell preparation contain identifiable renal
cells, there seems a reasonable chance that its use
might enable early identification of malignancy in
the kidney.

Summary

A method is described whereby the number and
type of cells in a sample of urine may be accu-
rately determined. The method depends upon the
solution and removal of mucus after the urine
sediment has been treated with absolute alcohol.
The cells may then be concentrated by centrifu-
gation and counted by means of a standard blood
counting chamber, or they may be segregated
mechanically according to size and mounted and
stained for microscopic examination.

The use of pectin as an adhesive is described,
and staining procedures are given.

The method was used to investigate the rates of
exfoliation of epithelial cells and of the loss of
leucocytes and erythrocytes into the urine in 12
normal males. The results of a preliminary ex-
periment on the diurnal changes in the rate of cell
loss are also given.

An indication of the normal urinary cell picture
has been established by differential counts on
stained preparations of the cells from the same
12 subjects.

Evidence is submitted suggesting that the origin
of certain of the small cells of the urinary sediment
is the epithelium of the kidney.

The possible application of the method to cyto-
logical diagnosis is briefly discussed.

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