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PART I.

ORIGINAL COMMUNICATIONS.

ART. IV.—*An Investigation into the Clinical Value of Staining Urinary Deposits.* By EDWARD SANDWITH JOHNSON, M.D. Univ. Dubl. ; Senior Assistant Medical Officer, Sheffield Union Hospital.

THE paucity of work done on this subject and the great prospects which it appeared to afford of throwing light on "morbid conditions of the urogenital tract," by helping in diagnosis, prognosis, and determining progress in these cases, were the features which called me to investigate this question.

I have stained the deposits of nearly 500 urines, over 100 of which were specially selected from patients suffering from urogenital diseases, which yielded almost every pathological possibility.

The only literature on this subject is to be found in *Deutsche medizinische Wochenschrift*, of July 4th, 1912 (p. 1271). This article contains the procedure followed and results obtained from the use of six stains which, up to that time, had been used for staining urinary deposits.

It was delivered at a Medical Clinique at Vienna University, entitled "Methods of Staining Urinary Deposits," by Drs. A. Edelmann and L. Karpel.

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I will proceed by giving a translation of this article.

M. Kohn introduces a sudan-hæmatoxylin stain.

The urinary sediment placed on a slide is dried in air and treated with 10 per cent. formalin for ten minutes, and afterwards washed with water. The preparation, so fixed, becomes stained after ten minutes' submersion in a concentrated solution of sudan in 70 per cent. alcohol, and rinsed afterwards from half to one minute with 70 per cent. alcohol. After this re-stain with hæmatoxylin and mount in glycerine.

The fat takes on a red colour.

The cell-bodies a violet colour.

This process is suitable for staining fatty casts.

Leibmann's method is to apply a solution of 2 grains methylene blue in 100 c.cm. 10 per cent. formalin to the urinary deposit, and let this stand for ten minutes. Then wash with water

The hyaline casts stain light blue.

The waxy casts, dark blue.

The cell bodies and bacteria, dark blue.

This method gives no differentiation between cells, but is very suitable for discerning the individual casts.

According to E. Wolff, the deposit is fixed with 10 per cent. formalin and placed in 90 per cent. alcohol for twenty-four hours for further fixation. On the next day restore by means of fresh alcohol ten to fifteen minutes, and apply the stain consisting of hæmatoxylin-eosin for twenty-four hours, wash with water until no discoloration remains, add 5 to 6 drops of concentrated alcoholised eosin solution and after some minutes 90 per cent. alcohol. Mount in Canada balsam.

Cannata washes the deposit with physiological saline, and fixes for two days in "Folscher's solution."

Folscher's solution	{	Chromic acid 1 per cent., 25 c.cm.
		Osmic acid 1 per cent., 2 c.cm.
		Acetic acid 1 per cent., 16 c.cm.
		Distilled water, 263 c.cm.

The fluid is then centrifuged, the residue washed with physiological saline, placed on a slide, dried in air, and stained according to "Ziehl."

A. Schott disclosed a method using two staining solutions.

Solution 1.—5 per cent. aniline blue (water free) in distilled water.

Solution 2.—2 per cent. eosin in glycerine with 5 per cent. phenol.

Put 5 drops of solution No. 1 and 6 to 8 drops of No. 2 in the centrifuge tube nearly full of urine, shake well and centrifuge.

The leucocytes stain violet-blue, the nuclei showing up well.

The erythrocytes, brown to eosin red.

Epithelium stains blue.

Nuclei, red.

An accurate cell differentiation is not obtainable, although it yields a good general survey.

Senator's method gives the most useful results.

A drop of urine sediment is spread on a slide, dried in air, and fixed in the Bunsen flame. On this place a drop of Ehrlich's triacid stain for ten minutes and wash with water and then with alcohol. Dry and mount in Canada balsam.

The hyaline casts and protoplasm of the cells becomes stained violet.

Nuclei, blue to blue-green.

Erythrocytes, orange colour.

Although this method is, as stated, the most useful for clinical purposes, the results are not entirely satisfactory. As Senator himself states in the "Encyclopædia of Microscopic Technique, 1910"—"The granulation of the protoplasm and the chromatic structure of the nucleus is not discernable."

All the above methods have taken the chemical state of the urine too little into account. The reaction of the urine is nearly always acid, and this is bound to influence

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the staining capacity and shape of the elements in the deposit. A factor worthy of consideration is the precipitation of crystalline and amorphous salts which collect the stain, and not only prevent a good even stain but cloud the field of vision. The washing with physiological saline does not completely obviate this.

The precipitate is not dissolved, and, as far as our experience goes, many of the morphological characteristics are lost.

Proceeding on this assumption, we treated urine with a solution of sodium bicarbonate until the reaction was weakly alkaline.

We proceed as follows :—

Fresh urine is treated with a few drops of bicarbonate of sodium and centrifuged. Then by means of a thin pipette a little sediment is placed upon a clean slide and dried. The air-dried preparation can either be fixed by heating in a copper oven to 100° (Ehrlich's method), or fixed, according to von Jagie, in an acetone bath for ten minutes.

Von Jagie's method is certainly worthy of recommendation for clinical purposes.

The preparations so fixed can be stained with Ehrlich's triacid stain for ten to twenty minutes, wash cautiously in water, dry with filter paper, and mount in neutral balsam.

Thus we obtain good pictures in which the separate elements can easily be distinguished.

The leucocytes stain green.

The eosinophil granules, copper red.

The neutrophil, reddish violet.

The protoplasm of epithelium cells, light red.

The nuclei, green to dark blue.

Red corpuscles, orange.

Spermatozoa—heads, red ; tails, bluish.

This method is especially useful for distinguishing the blood cells and for recognising kidney epithelium, which usually is very difficult.

Weigert and von Jagie agree that it is difficult, if not

impossible, to say for certain what is kidney epithelium in a urinary deposit.

When Romanowski's stains are used the urine should give a neutral, not alkaline, reaction.

Acetone is the best method of fixation for these stains.

Leishman's stain is the one we recommend. Fix for five minutes in acetone and stain for five minutes with Leishman's stain. Then add a double quantity of water, and allow to stand for about two minutes, wash in water, dry with blotter, and mount in Canada balsam. In this manner good pictures are obtained.

The protoplasm of the epithelial cells stain red.

The nuclei of the epithelial cells dark blue.

(The chromatic structure is well marked.)

Kidney epithelium stains deeper blue than bladder epithelium.

Hyaline and epithelium casts stain a good blue.

Spermatozoa and bacteria are also stained.

I commenced to work on this subject by using these stains. I used specially selected urines—that is, urines containing specimens in their deposits which the stain was supposed to show specifically.

The difficulties I experienced, and how I overcame them, I will relate later. Here it is my wish to give a short account of the results I obtained by using the above stains.

1. *Kohn's Sudan-Hæmatoxylin Stain.*

This method is only of use to demonstrate fat, and though the ingredients of urinary deposits are usually undergoing one or other process of degeneration, fatty degeneration is seldom seen. The fat in fatty casts is hard to demonstrate, and this is not the best stain for showing it. Then again the procedure is lengthy and tedious.

2. *Leibmann's Methylene Blue Stain.*

This has the advantage of being easy and short, fixation of the deposit and staining going on concurrently with one application, and only implies one washing, which allows for fixation inefficiencies.

This stain gives a good survey, staining every ingredient of a urinary deposit. The only differentiation between cell-body and nucleus is that the latter is stained slightly deeper. I could not demonstrate its stated quality of staining amyloid material in casts deeper blue. What I found was that casts stained in three degrees with this stain :—

1. Light blue—translucent.
2. Dark blue—translucent (not amyloid).
3. Wedgwood blue solid flakes here and there in casts—sometimes as a striation (probably amyloid).
3. *Schott's Eosin and Methylene Blue.*

This is an excellent stain for the cellular elements, especially blood cells.

The procedure is simple and takes a very short time, since staining and centrifuging are concurrent. Washing the deposit is obviated, the inefficiencies and difficulties of which I will mention later.

Blood cells show as characteristically and completely as in a blood-film stained by Jenner's or any of the Romanowski stains.

All cells, such as bladder, kidney, or tumour cells, stain in direct ratio to their integrity or degeneracy, as the case may be.

4. *Senator's Triacid Stain.*

This method has the obvious drawbacks of needing alkalisation of the urine and neutral balsam if mounting is desired.

Then again the procedure of washing the stained deposit with alcohol incurs the risk of over-decolorising.

But the results obtained are undoubtedly better with this stain than with any of the above stains.

Every structure in urinary deposit is stained by this method with the fair differentiation between nucleus with its chromatic structure and cell protoplasm; but, as Edelmann and Karpel say, "much is yet to be desired."

I found that—

Nuclei stained blue to purple.

Cell-protoplasm stained dull red.

Red blood cells stain orange colour.

Casts stain pink to cerise.

Leucocytes stain red with purple nuclei and granules—not green as in literature, which differs somewhat from the author's account.

5 and 6. *Cannata's and Wolff's Stains.*

The results I obtained from the use of these stains only compared unfavourably with those obtained from the previous stains, none of which justify a three-day procedure.

The most striking, and at the same time detrimental, feature (and one which cannot be obviated) in the results I have obtained, is the degeneracy of all ingredients of urinary deposits.

It is due to their degeneracy that they are there, and the medium in which they have to abide is not conducive to prolonged integrity. I have seen examples of every stage and type of cellular degeneration in cells from the bladder and kidney, &c.

Cloudy swelling, including karyolysis and karyorrhexis.

Fatty degeneration is demonstrable on almost every slide.

Hyaline and colloid degeneration (that is masses of cells which have grown together into a homogeneous mass).

The cells lose their salient points, shape, and shape of nucleus; then their staining capacity generally goes—a swollen appearance is the first feature, this occurs especially in alkaline urine.

I experienced great difficulty in getting rid of *débris*, crystals (phosphates and urates, &c.), albumen and mucus deposits, around which the stain hangs, obliterating a good view of the cells and important structures in the deposit.

I found the following *modus operandi* fairly successful in surmounting these difficulties.

Use an absolutely fresh specimen. If this is not possible, the integrity of the individual members of the deposit is moderately well preserved for twenty-four hours by adding a few drops of formalin to the urine, which should be kept covered.

If phosphates are present clarify by acidulation.

If urates are present, alkalinise the urine by adding a few drops of sodium bicarbonate which dissolves the urates.

If albumen, mucin, or *débris* is abundant, centrifuge some urine from the bottom of the specimen glass for a few minutes, then pour off the urine, add a few c.cs. of normal saline and centrifuge again (thus washing the deposit).

It is best not to fix by heat if albumen is present since it coagulates and holds the stain.

Another good way of getting rid of *débris* is to wash the fixed deposit with $\frac{1}{2}$ per cent. acetic acid.

A very important item is that the slide should be clean and devoid of grease; this can be obtained by washing the slide with ether or alcohol, perhaps best of all is a weak lysol solution (lysol 2 per cent.).

Filter all stains immediately before use.

The fixation of the film is, perhaps, the greatest difficulty. Fixation by heat damages the cells and fixes the mucus, albumen, and other undesirable ingredients of urinary deposits—thus, when the slide is stained, it looks dirty and a good view of the cells and casts, &c., is prevented.

Fixation by alcohol, acetone, or formalin is not very satisfactory; after a twenty minutes' application part of the deposit is washed off even when the slide is rinsed in standing water.

These formidable difficulties, one or two, at least, of which are present on every occasion, together with the laborious methods prescribed and the indefinite and incomplete nature of results obtained, almost made me condemn and cease to work on this subject.

But there still appeared to be hope along two channels, namely :—

1. To find a specific stain for each and every ingredient present in urinary deposits. Stains to show up amyloid and fatty degeneracy respectively in casts. Stains to show striation and encapsulation of bacteria. Stains to empha-

sise the salient features of the cellular elements found in urinary deposits.

2. To find a quick and easy *modus operandi*, one stain, if possible, which would give a useful broad survey of the ingredients of a urinary deposit, as a help in diagnosis and prognosis of urogenital cases and one which would demonstrate whether or no the more exact procedure of Article I. was desirous or necessary. In other words—a *régime* in staining urinary deposits which might be useful to Clinical Medicine as a routine.

I proceeded along these lines and used all the known stains in the search.

I. (a) For the differentiation of *Casts* I used the following stains :—

- | | |
|-------------------------------|---|
| 1. Methylene Blue. | Rough survey. |
| 2. Leibmann's stain | } Specific stains for
lardaceous matter. |
| 3. Methyl violet | |
| 4. Picro-carmin | |
| 5. Iodine | |
| 6. Scharlach | } Specific stains for
fat. |
| 7. Nile blue | |
| 8. Osmic acid | |
| 9. Sudan iii. or Kohn's stain | |
| 10. Leishman | For blood casts and
cellular casts. |

Casts, since they are the most degenerate structures in urinary deposits stain very freely but none the less very characteristically.

The great majority of casts stain as pale, translucent, filmy, structureless cylinders, varying greatly in length, thickness and shape.

Granular Casts differ only in that their contour is less even, disintegration being in process, and they are studded indiscriminately with dots which have stained a deeper shade.

The granulations vary in size and number per cast. High-power one-sixth objective is essential to see the details of casts.

Fatty Casts are identical with these, except that the dots are hollow bubbles unstained unless some special stain is used to demonstrate the fat present in the vacuoles.

Some casts are almost completely composed of epithelial cells of the kidney tubules which appear to be tubules shed intact, and these cells stain perfectly.

There are some casts which have one or two such cells still in their position and retaining their staining capacity.

There are solid casts, composed of blood, and other cells and all kinds of *débris* which have obviously been formed as plugs in kidney tubules.

Then, lastly, there are some which are evidently too degenerate to take up the stain at all—they appear unstained as transparent films.

Amyloid Casts.—Though patients have obviously extensive amyloid disease, in which the kidneys are involved, yet it is far from always possible to demonstrate waxy casts in their urinary deposits.

Even when methyl-violet or picro-carmin, the specific stains for lardaceous matter, is used for staining so-called amyloid casts, very variable and uncertain results are obtained. The whole cast and all casts take on the violet or pink colour, according to the stain used, in varying degrees of intensity (this is probably due to the degeneracy or colloid character of the material of which casts are composed). This faint staining comes out on the application of weak acetic acid, instead of turning blue as it should do.

There are some casts and areas in others which take on the true violet colour of amyloid matter, but these are few and far between. The high-power one-sixth objective shows these best.

When iodine is used much the same happens. All casts present take on a pale orange colour—a few casts and areas in others take on the deep red-brown colour of amyloid.

With Leibmann's methyl-blue stain—a few casts,

stained in bulk light-blue like their neighbours, present non-translucent areas of wedgwood blue. Thus looking like a tube of cloudy amber with clear and cloudy areas alternating, coloured, of course, light-blue and wedgwood respectively.

These areas that I have demonstrated by all the above stains are possibly areas of amyloid matter.

But it is my opinion that there is no such thing as an amyloid cast. If there be such it is, at any rate, impossible to recognise it in a urinary deposit.

The three outstanding features of the casts present in the urinary deposits of the five cases of amyloid disease of the kidney, on whom I have studied, were :—

1. The great number of casts present—the deposit was, in each case, a veritable mass of casts—the film had to be very thin in order to separate the casts enough to see them individually.

2. The extraordinary length of the casts—chiefly three times as long as tube-casts found in any other morbid condition.

3. The similarity of the casts in length, shape, construction, type and staining capacity—nearly every cast present was a true cast of a kidney tubule, long and curled and perfect in shape. The great majority were hyaline casts, just a few were granular or fatty.

These three features present in any one case will always make me think of amyloid disease of the kidney, and they should be of help even if they do not make the diagnosis certain.

Fatty Casts.—Do not fix by heat—use either formalin or 70 per cent. alcohol.

When scharlach is used and the slides are washed with 50 per cent. alcohol after staining, the small fat globules, numerously dotted about these casts, stain a brilliant translucent red colour.

When Nile blue, followed by sulphurous acid, is used, the casts appear as pale-blue films studded with deep-blue dots (these should be mauve).

Osmic acid and sudan stain the fat black and deep-orange respectively.

Though the other stains are more modern and stain fat in all forms, viz., neutral fats, fatty acids and soaps, and give a prettier picture, yet osmic acid, which stains only oleic acid and oleates, is the most certain stain for fat as present in casts, and this is not reliable.

My experience of fatty casts calls for only one remark, namely—that they are far less common than is usually taught or understood. Though a certain number of casts (when unstained) appear to contain fat globules, remarkably few take up fat stains. In my opinion it is more a case of vacuolation and disintegration than fatty degeneration.

Fatty casts occur in many types of urinary deposits, and are generally accompanied by other tube-casts, thus it is probable that they have no pathognomonic significance. If this is the case then it is of no clinical value to distinguish them from other casts.

I found many types of casts besides those mentioned above—solid casts of the kidney tubules, such as blood casts, pus casts, and *débris* casts—solid casts, moulds of the ducts of the prostate, seminal vesicles and Cowper's glands composed of mucus, pus and spermatozoa, &c.—these latter I will describe under the subject of shreds.

It is my opinion, if I may say so, that casts need fresh nomenclature and classification, and are probably deserving of new significance. Though the differentiation of casts by staining is not effective as I had hoped, yet a great deal more can be ascertained with greater certainty by the help of the above stains than by microscopic examination in the ordinary way.

Let me here give instances which go to prove this.

In medicine, a "large white body" connotes "large white kidney"—each of my five cases of amyloid disease of the kidney had not only the typical large white body (even those with concomitant phthisis) but all the other signs and symptoms of chronic parenchymatous ne-

phritis, of which the large white kidney is pathological and pathognomonic.

Repeated examinations of the casts present in a urinary deposit (using the above methods and stains) tell us of the progress of the disease or the progress of the patient, as the case may be, and thus aid in the prognosis of all forms of nephritis.

I. (b) For differentiation of *Bacteria* present in urinary deposits, I used the following stains :—

- | | |
|--------------------------|--|
| 1. Methylene blue | For a general survey. |
| 2. Fuchsin | |
| 3. Gram's stain | For classification purposes. |
| 4. Ziehl Neelsen's stain | For tubercle bacilli. |
| 5. Pughes' stain | } For diphtheria bacillus
(K.L.) and the diph-
theroids. |
| 6. Löffler's stain | |
| 7. Neisser's stain | |

As large a quantity of urine as possible should be centrifuged at least 10 c.c.

All utensils—specimen glasses and centrifuge barrels, &c.—should be sterilised before use. The best method of doing this is to boil them. This is unnecessary if only a rough estimate is needed as to the bacteria present.

Urine can be obtained aseptically from the bladder by using a sterilised catheter, after previously irrigating the anterior urethra with sterile boric acid solution.

Since fixation is best obtained by heat it is necessary to get rid of any albumen present; it is also very necessary that there should be as little *débris* or crystalline matter as possible present in the deposit, if a satisfactory survey of bacteria is to be made, so it is almost always necessary to wash the deposit with normal saline, sterile in this case, and centrifuge again before making slides.

The best procedure is to make at least three slides. Stain slide No. 1 with methylene blue to ascertain if bacteria are present, and, if so, whether bacilli, cocci, or both, including their shape and mode of clustering, &c. Stain slide No. 2 with Gram's stain to ascertain if the

bacilli or cocci present, as the case may be, are Gram-positive or negative. We are now usually in a position to say what specific stain to use in the case of slide No. 3. If diphtheroids are suspected use Pughes' or Neisser's stain, or if tubercle bacilli use Ziehl Neelsen's method.

The one-twelfth objective with oil-immersion is always used for this work.

I found that bacilli or cocci, and commonly both, were present in a large percentage of urines. Thus it appears that there are non-pathogenic bacteria which normally inhabit the urinary tract.

Then again there appear to be bacilli and cocci present in urinary deposits which have not yet been named or classified—at least their morphological characteristics, &c., do not coincide with any known microbes.

E.g.—There are bacilli and cocci occasionally present in urinary deposits which react indefinitely to Gram, their indecision is apparently two-fold—some of these organisms partially retaining the violet colour of the carbol-gentian violet—some retain it at one time and not at another (they are very easily decolourised).

Then again there are large bacilli peculiarly grouped, *e.g.*, in chains, &c., and many peculiarly striated bacilli, probably diphtheroids, though differing from any I have ever seen.

In my work on this section I have identified microscopically the following cocci and bacilli in urinary deposits :—

Staphylococci	<i>B. coli communis</i>
Gonococci	<i>B. typhosus</i>
Streptococci	<i>B. subtilis</i>
Pneumococci	<i>B. tuberculosus</i>
Unknown cocci	<i>smegma bacillus</i>
	diphtheroid bacilli
	(very rarely Klebs-Löffler)
	unknown bacilli

The majority of these are just as easily demonstrated microscopically from urine, as from such *media* as pus

from an abscess or membrane from a throat, &c. So I need not waste much space in relating the individual cases with the respective bacteria found.

I should like just to state that the difficulty in the case of tubercle bacilli can be greatly lessened by getting rid of the pus present in the urine, which prevents satisfactory centrifugalisation of the organisms and obliterates them from view when the slide is made. This can be done by pouring off superfluous urine and adding one-quarter as much antiformin (30 per cent.) as there is pus, shake this for a few minutes and leave till the pus dissolves, then dilute and centrifuge. This also destroys all bacilli except tubercle, including smegma, thus obviating the decolorisation with alcohol.

Pughes' stain is the best for differentiating the diphtheroids, the striation or polar bodies, as the case may be, are well shown and the *modus operandi* is very simple, viz., to a fixed smear add a few drops of the stain for a few minutes (3-5), then wash off and dry between blotting paper.

By far the commonest cause of primary bacilluria and spontaneous cystitis, which are exceedingly common complaints among senile and prematurely senile patients of whom we see so many among the poorer classes, is the *Bacillus coli communis*.

Other organisms, however, may be responsible for precisely similar lesions and symptoms, and some may easily be mistaken for *Bacillus coli communis* if only a casual investigation is made into their nature.

An instance of this forms the basis of a paper entitled "Infection of the Urinary Tract by the *Bacillus lactis aerogenes*," by J. A. Luetscher, M.D., in the Johns Hopkins Hospital Bulletin for 1911, page 361, in which two cases are described. Case 1 was a woman suffering from cystitis which was at first thought to be due to *Bacillus coli communis*. Case 2, a man who had a urethritis, with yellow discharge, which might easily have been put down to gonococci. But in each of these cases

the *Bacillus lactis aërogenes* was found in pure culture in several consecutive examinations.

The condition lasted for four weeks with a marked tendency to recurrence whenever urotropin was omitted. Later cultures were negative. Case 2 was so seriously ill, yet physical examination revealed nothing, that septicæmia or enteric fever was suspected, but blood culture and Widal gave no help. It was only on full bacteriological examination of the urine that the diagnosis was made, the treatment instituted and the cases were cured.

The organism was a small encapsuled bacillus with rounded ends Gram-negative and non-motile.

When such cases are correctly diagnosticated and put on urinary antiseptics they usually clear up within a month, when not correctly diagnosticated such cases soon become chronic and daily run the risk of the infection spreading and producing pyelitis, &c.

I found borovertin or urotropin the best drug for this purpose. Though it is not always possible to say for certain *viâ* the microscope what bacillus from the morphology is causing the trouble, yet I hope I have shown by what I have said that a great deal can be learned by such means which is both instructive and useful.

Culture of organisms and inoculation of guinea-pigs can be resorted to if necessary.

For many years it has been customary to examine urine microscopically in suspected cases, for gonococci—*B. typhosus*, and the tubercle bacillus. Why should these three be separated from their many equally injurious and common colleagues? The answer is that the value of staining urinary deposits has not yet been properly emphasised.

I. (c) *Shreds in Urine*.—Before I could do any work on this branch I had to seek a definition and a classification of shreds. The shortest and most graphic and recent literature I could find was an article by L. G. Gunn, F.R.C.S.I., in the *Medical Press* of April, 1912.

I will give a short *résumé* of it here—just those parts

that were of help to me. Shreds in muddy urine are usually from the kidney, ureter or bladder, and it is chiefly due to the disintegrated shreds that the urine is not clear.

Shreds in clear urine are casts formed in or about the urethra, and carried out with the stream of clear urine.

There are five possible places in the urethral tract for such shreds to form.

1. The surface of the mucous membrane of the urethra. Two kinds of shreds are formed from this—(a) large blobs with tails of muco-pus floating from them if the amount of pus is great; (b) moulds of the shallow folds if the amount of pus is small.

Microscopic examination shows their composition to be epithelium, mucus, pus and bacteria.

2. Seminal vesicles and ducts thereof. Five different types of shreds are formed here. Three of these are found in normal urines, skins, sugar and sago bodies respectively, composed of columnar epithelium, mucin and spermatozoa.

The other two forms are abnormal—(a) the so-called casts like bunches of grapes; (b) vesicle shreds—long and thin from the ejaculator ducts containing pus-cells and bacteria.

3. Prostate and its numerous ducts. Here two kinds of shreds are formed, both indicative of disease—(a) a large coarse pus thread formed from ducts altered by acute inflammation; (b) comma-shaped threads very common and indicative of the last stages of chronic prostatitis.

4. Cowper's glands.

5. Glands opening into the spongy part of the urethra. There are two types of shreds formed here, both equally common—(a) thin shreds made of flat rolls of epithelium, pus and non-pathogenic bacteria—these shreds occur in normal urine; (b) comma-shaped shreds like those from the prostate formed of pus, mucus and bacteria.

I confined my work on shreds to the abnormal variety, and since their abnormality seems to be in direct ratio to

the bacteria they contain I commenced by a search for bacteria in shreds.

I worked with urines of cases of gonorrhœa of all stages. I found shreds in about sixty per cent. of the cases, most prevalent in the chronic cases and the recent cures.

The commonest bacteria present in shreds are :—

1. *Staphylococcus aureus*.
2. Diphtheroid bacilli.
3. Gonococci.
4. *B. coli*.
5. A large Gram-positive coccus.
6. A doubtfully reacting diplococcus.

From what I have said it is obvious that our knowledge on the subject of shreds has been greatly augmented by staining them before examination, and if any further insight into the formation, composition and significance of shreds is possible, it is *viâ* stains we shall derive that knowledge.

I. (*d*) For the differentiation of *Cells* present in urinary deposits, I used the following stains :—

1. Methylene blue.
2. Fuchsin.
3. Jenner's stain.
4. Giemsa's stain.
5. Leishman's stain.
6. Triacid stain of Ehrlich.
7. Schott's stain.

Nos. 1 and 2 stain every cell present in a urinary deposit, the nuclei staining deeper than the protoplasm, so a good general survey is obtained.

If the differentiation is not sufficient one can at least say whether or no it is desirous to proceed with other stains.

Nos. 6 and 7 I have already described in the early part of this work.

Nos. 3 and 4 are excellent stains and give as good results as is possible under the circumstances.

No. 5—Leishman's stain—is much the best stain for the cellular elements in urinary deposits. The procedure is quick, easy, and the results are better than those obtained by any other stain.

The differentiation between nucleus and cell protoplasm is good—the former stains a damson colour, the chromatic structure showing up well, the latter staining amethyst. By this method kidney epithelium is stained a deeper shade than bladder epithelium, which is a very useful feature. Casts stain blue. Blood cells are beautifully shown and easily distinguished. Spermatozoa and bacteria also show up well.

Most of the cells in the deposit of a fresh urine are as easily and characteristically stained as the cells of paraffin sections, &c.

Blood and tumour cells give no chance of being mistaken, and after a little practice one gets an estimate of the different epithelium cells possible in a urinary deposit, and it becomes quite easy to say from what part of the urogenital tract any epithelium cell has come.

I will say more about the cellular elements in urinary deposits when summing up.

II. *The wet method*.—During my work on this subject I used almost every known stain in the search for a simple, quick and effective method of staining urinary deposits adaptable as a routine.

The stain which most adequately fulfilled these conditions was a stain composed of equal parts of methylene blue and polychrome methylene blue (chromatised old methylene blue), and the method I have called the “wet method” proceeds as follows:—Take up with a pipette some sediment from the bottom of the specimen of urine for examination. Apply a drop of this to a slide, which has previously been washed with a 20 per cent. lysol solution, spread over the slide with the flat of the pipette or

with the edge of another slide, then dry in air and fix in the flame or with ten per cent. formalin.

If the sediment is small in quantity then centrifuge for a few minutes.

If abundant with crystals, *débris* or albumen, centrifuge a few c.cs. of urine taken from the bottom of the specimen glass, pour off the urine, wash the deposit with normal saline, and centrifuge again.

If urates are present, add a little sodium bicarbonate.

If phosphates are present, a trace of acetic acid should be added, washing with normal saline. Make a film and fix as mentioned above.

When the film is dry apply a few drops of the stain and cover immediately with a clean cover glass and press between blotting paper. Then the slide is ready for immediate examination under the microscope with either low power or high power.

The following are the six specially valuable features of this method :—

1. The whole procedure takes but a few minutes (10-20) inclusive of centrifuging (which is far from always necessary), and the microscopic examination.

2. It stains everything in the deposit which will stain by any method used in this work.

3. It is the neatest *modus operandi*, with least labour ; there is no washing of the film after staining, thus the fixation difficulties are obviated.

4. It enables the oil immersion lens (one-twelfth objective) to be used, which is essential if bacteria are to be seen or accurate estimates made of types of cells present or any specificity of staining to be appreciated.

5. It gives at least as good, if not the best, differentiation between nucleus, with its chromatic structure, and cell-protoplasm and cell-wall.

6. By this method the ingredients of the deposit are magnified—this feature being especially useful in bacterio-

logical examination of urinary deposits, *e.g.*, it greatly helps in showing up the shape and capsules of bacilli and the striation of the diphtheroid group if a drop of 1 per cent. acetic acid is allowed to run under the cover glass (whilst the examining eye is still at the eye-piece), the acetic acid is seen to dissolve the stain from the bacillus sooner than from the striations, and this intensifies the latter.

Thus after a fifteen minute procedure one is able to name, with a fair amount of accuracy, the ingredients present in a urinary deposit; or one is, at least, in a position to say whether or no anything further could be ascertained by using some of the specific stains which I have described in the early part of this paper.

For instance, if there are cells present which suggest a tumour of the tract use Senator's or Leishman's stains. Or if there are microbes present, of which it would be desirable to know more of the morphology, then it would be necessary to resort to Gram's stain and sterile vessels, &c.

Since polychrome methylene blue is not a stable stain to keep made up, ordinary methylene blue may be used as described above with as good results, except that there is not so marked a differentiation between cell-body and nucleus.

As a summary on my work I wish to state a few cases and give a few instances which go to prove the desirability, if not the necessity, of staining urinary deposits.

Let me first take the subject of casts. As I have already mentioned, it appears to me that casts need new nomenclature and are deserving of more and probably new significance, but this we must leave to the future.

Casts of all kinds are a great deal more common in urine than is at present generally understood. I found casts in about seventy per cent. of the urines I examined, which included a large number of normal or so-called normal urines.

Here may I ask, what is a normal urine? The answer is—A urine of normal colour and specific gravity, con-

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taining no albumen, pus or other abnormal ingredient which would be found by the present routine method of examining urine.

This is not correct. A urine cannot be designated as normal unless the deposit has been stained.

Let me give some instances to prove this. The urines of convalescent typhoid cases appear normal enough and give no reaction to any of our routine urine tests. It is bacteriological examination which reveals them teeming with *B. typhosus*.

In my own work I had four striking instances of this fallacy. In each case the urine was reported as normal by the Sister in charge who tests the urine on admission and each week following.

Three of these cases, though they had comparatively no symptoms, had serious urogenital diseases, which turned out to be :—

- (1) Cystic kidney.
- (2) Stone in the kidney.
- (3) Late tubercular kidney, respectively.

In the last case the diseased kidney had ceased to functionate and had become partially shut off.

It was only after staining the urinary deposits and finding a few casts, pus and blood cells, and ureter epithelium, &c., that I was able to locate the disease and ultimately to diagnosticate the cases.

The fourth case was one of phenol poisoning, and though the urine was never characteristically green, yet by staining I found abundant casts in the urine from the third day for many weeks.

Then, again, there are casts of all kinds present in the urine of athletes after any strenuous event, especially efforts of long duration, and though these clear up in thirty-six hours, yet it is important to recognise their presence, and this is best done by staining the urinary deposit.

Lastly, a few words on the cellular elements found in urinary deposits.

Take cases of hæmaturia—these are common and vary widely in the cause thereof.

A good microscopic picture of the urinary deposit (such as is obtained by staining) is very useful and instructive in these cases and often determines the diagnosis of the case. For instance, where the hæmorrhage is due to cancer or some other tumour of the tract, not only are tumour cells present in the deposit, but also specific cells from the locality of the growth are liberated by the local inflammation.

Where toxic agents or poisons are the cause, cells from all parts of the tract are present. There were epithelial cells from ureter, urethra, bladder and kidney in the case of phenol poisoning which I mentioned earlier in this paper.

Parasitic causes of hæmaturia are usually easily demonstrated in urinary deposits, *e.g.*, *filaria sanguinis hominis*, *echinococcus*, ova of *bilharzia*.

Then again, where only blood cells are present, one has to think of the cases Klemperer and Harris described and called “renal epistaxis.”

Examination of the urinary deposit immediately differentiates between hæmaturia, hæmoglobinuria and lymphuria.

The last-named cases are rare, generally occurring in children as intermittent albuminuria with constipation. These cases are usually diagnosticated by the fact that there is no globulin in the urine, but the urinary deposit is very characteristic—an abundance of young lymphocytes are present with only here and there a red cell or polymorphonuclear leucocyte. These show up perfectly when stained.

A patient under my care, suffering from chronic cystitis, suddenly developed a swinging temperature and rapid pulse, and as I suspected pyelitis had set in I did daily

examinations of his urinary deposit, and it was instructive to see the different cells appear as the infection spread up the tract. No longer the monotonous pus cells with here and there a degenerate bladder cell, but fresh epithelial cells from ureter, pelvis of the kidney, and kidney, blood-cells, and solid pus casts of kidney tubules showed themselves as the case became one of pyelonephritis.

Another memorable case of mine was a man who was obviously suffering from malignant disease somewhere. The question was, where? His urine was scanty and he passed it frequently in small amounts, occasionally there was a little blood in it, but these are common signs. After frequent examination of his urinary deposit, one day I demonstrated tumour cells. A month later there was a palpable growth, which turned out *post-mortem* to be malignant, situated in the fundus of the bladder and only fungating over a small area of the roof on the inner surface of bladder. It was in a difficult place to see cystoscopically and would have been diagnosticated late by this method.

In conclusion I can only say that to see such pathological evidence of disease of the urinary tract as I have mentioned, whilst the patient still lives, is often as conclusive as pathology after a *post-mortem*, and in my opinion the cases of urogenital disease discovered or correctly diagnosticated for the first time in the *post-mortem* room would be greatly lessened if urinary deposits were stained before examination.

Examination of the urine of all patients has been routine for centuries. Since now, however, the microscope is so universally used, staining urinary deposits should become routine in clinical medicine.