Summary

Small biopsy specimens of the colonic mucosa from just above the recto-sigmoid junction have been obtained in 111 instances from 42 patients with ulcerative colitis. A group of 24 patients not suffering from ulcerative colitis and with apparently normal mucosa at sigmoidoscopy have been similarly studied as a control group.

Specimens obtained from the control group were all normal apart from two which showed slight histological changes.

Among 71 specimens from patients with ulcerative colitis in the stage of symptoms, 67 showed inflammation. More than half of the 40 specimens taken from ulcerative colitis patients in clinical remission showed inflammation.

Brief details are given of two patients being studied by serial biopsy in whom histological relapse preceded clinical relapse by some weeks.

We are grateful to Miss Shirley Thomas, who assisted at most of the biopsy examinations; to Mr. Richard Salt, who made the biopsy instrument for our use; to Miss M. Rowe for technical assistance; to Mr. M. Morris for the photomicrographs; and to Sister Houle for her active co-operation.

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ABNORMAL EPITHELIAL CELLS IN ULCERATIVE COLITIS

BY

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[WITH SPECIAL PLATE]

Cytology is playing an increasing part in the early diagnosis of carcinoma, but has not been much used for the study of non-malignant diseases. We have recently made cytological studies on patients with ulcerative colitis and on other patients not suffering from this disease, who form a useful comparison group. In many instances we can relate the cytological findings to the histological appearances met with in a small biopsy specimen, for the present work was proceeding simultaneously with a biopsy study of ulcerative colitis, although it began a few months later.

Methods

The specimen for study has been obtained under direct vision through a sigmoidoscope, using a special tool for wiping the colonic mucosa which has been made for our use by Mr. Richard Salt, chief technician in the Nuffield Department of Anaesthetics. The tool consists of a long metal rod, with a "perspex" head screwed on to it. The perspex head is rectangular, but with smoothed edges. The two flat faces are grooved in a criss-cross manner, so that the face has a slightly abrasive action when it is drawn across a mucosal surface (Fig. A). The tail end of the instrument is fitted with a metal handle heavy enough to act as a counterbalance when the instrument is in use through a sigmoidoscope, so that it can be used with ease and delicacy.

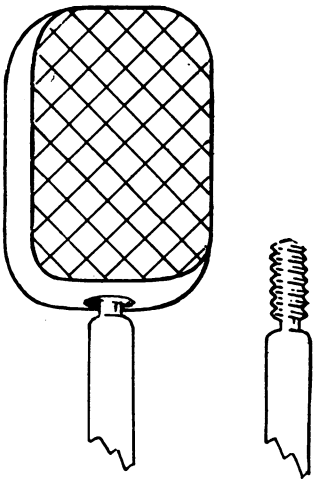

FIG. A.—The "perspex" head of special tool used for taking specimens of colonic epithelium.

When the sigmoidoscope has been inserted, a site for wiping is selected. Whenever possible we have chosen a relatively clean area of mucosa and have wiped it without preparation. When faecal material has prevented this we have first cleaned the bowel by means of the suction irrigator described in a previous paper (Truelove, Horler, and Richards, 1955). If the mucosa is merely coated with exudate, as is frequently the case in patients with ulcerative colitis, we do not irrigate it. We have found that after washing a less satisfactory film is obtained, chiefly because the presence of saline appears to hinder the formation of a good smear. This difficulty can be partly overcome by the use of albumen-coated slides.

When the mucosal surface has been lightly rubbed with the perspex head the instrument is removed from the sigmoidoscope and smears are made on clean glass slides. The flat face of the perspex head makes the spreading of these smears an easy matter. Some of the slides thus smeared are immediately "wet-fixed" in a mixture of ether and absolute alcohol in equal proportions for later staining by Papanicolaou's (1942, 1954) method, which demonstrates the epithelial cells. Other films are rapidly "air-dried" for staining with Romanowsky stains. These slides are useful for the study of any exudate, and particularly for the rapid recognition of eosinophil leucocytes.

Results in Control Group

Twenty-two examinations have been made of 18 patients not suffering from ulcerative colitis or any other organic disease of the gastro-intestinal tract. The specimen obtained in these patients consists of a small amount of mucus, sometimes with faecal debris present, and usually containing scattered clumps of columnar epithelial cells. Goblet cells are sometimes recognized among them. Bacteria are scanty. Occasional squamous epithelial cells are encountered which we think may have been carried up by the sigmoidoscope or may work their own way up the rectum and lower colon from the anal canal.

Results in Ulcerative Colitis

Ninety-four examinations have been made on 31 patients with ulcerative colitis in every stage, from complete remission to grave illness. Those few specimens from patients in complete remission and with normal sigmoidoscopic findings show features similar in every way to those found in the control group. In all other patients the specimen

consists largely of pus and blood, with numerous bacteria and with epithelial cells always present. The epithelial cells are commonly found in the form of large sheets, as opposed to the tiny clumps of cells seen in patients with normal sigmoidoscopic findings. In some cases of ulcerative colitis the epithelial cells appear normal (Special Plate, Fig. 1), but in about two-thirds of the specimens abnormal epithelial cells have been observed. The mildest change is an increase in the size of the epithelial cell, with the nucleus enlarged but otherwise appearing normal. The next stage is the presence of epithelial cells which also are moderately enlarged but, in addition, show obvious variation in size and prominent nucleoli.

Finally, we come to specimens in which many of the cells are grossly enlarged and there is extreme variation in cell size. In the larger examples of the cells the nucleus also is grossly enlarged, with a diameter more than double that of a normal nucleus and with a disturbed chromatin pattern (Plate, Fig. 2). In a few examples of this group we have encountered epithelial cells which are so large as to merit the title of giant cells (Plate, Figs. 3 and 4). These show similar features to the cells of the severe group generally, and differ only in being much larger. The diameter of the nucleus in a giant cell may be as great as 40 μ—that is, five times the diameter of a normal epithelial nucleus. If the nuclei were spherical this would mean that the nucleus of the giant cell was 125 times the volume of a normal nucleus. Nuclei are not spheres, and the true volume is probably much less than this estimate, but, nevertheless, the increase in nuclear size among these giant cells must be very great. The cytoplasm is likewise abundant, but the edges may be poorly defined in places. We regard these giant cells as extreme examples of the changes that are occurring in all the cells classed as severely abnormal.

We are confident that these severely abnormal cells are colonic epithelial cells. In a specimen showing severely abnormal cells it is often possible to trace a range of epithelial cells from those showing only slight changes from the normal up to those which exhibit all the characters we have classed as severely abnormal. By reference to biopsy specimens of colonic mucosa taken from the same patients we have found abnormal epithelial cells *in situ* which appear to correspond with those we have found on cytological examination. Figs. 5 and 6 (see Plate) show examples of these large abnormal cells, in one case situated in the surface epithelium, in another in the wall of a crypt of Lieberkühn. We have not yet made extensive studies of the histological sections to see how closely the finding of abnormal cells on cytological examination is correlated with the identification of abnormal epithelial cells in small biopsy specimens from a neighbouring mucosal site.

The question immediately arises whether the finding of abnormal epithelial cells is related to the activity of the disease process as judged by clinical and sigmoidoscopic criteria : the relevant information is set out in the Table, and also in Fig B, where the results are shown as proportions. The clinical groupings are those used in the biopsy study reported separately (Truelove and Richards, 1956). The cytological grades follow from the description we have already given, except that we have combined into one group (mild to moderate) all those specimens showing moderate increase in cell size, irrespective of whether or not the nuclei stained normally or there was variation in nuclear size.

Cytological Findings in Ulcerative Colitis in Relation to Clinical State

Clinical State	Cytological Findings			
	Normal Appearances	Mild and Moderate Abnormalities	Severe Abnormalities	Total
Remission with normal sigmoidoscopic appearances	5	—	—	5
Remission with abnormal sigmoidoscopic appearances	8	12	2	22
Mild and moderate symptoms	27	20	7	54
Severe symptoms	—	8	5	13
Total ..	40	40	14	94

It will be seen that the few examinations made on patients in clinical remission and with normal sigmoidoscopic findings yielded normal epithelial cells. A larger number of examinations were made on patients who, though in clinical remission, had abnormal sigmoidoscopic appearances, and these patients often yielded abnormal epithelial cells, though these were usually not severely abnormal. Allowing for some variation, due presumably to random sampling, the figure shows a progression in cytological severity which roughly corresponds to increasing clinical severity. Among the 13 examinations made on cases classed as clinically severe, all the specimens showed abnormal cells and nearly half of the specimens showed severe abnormalities.

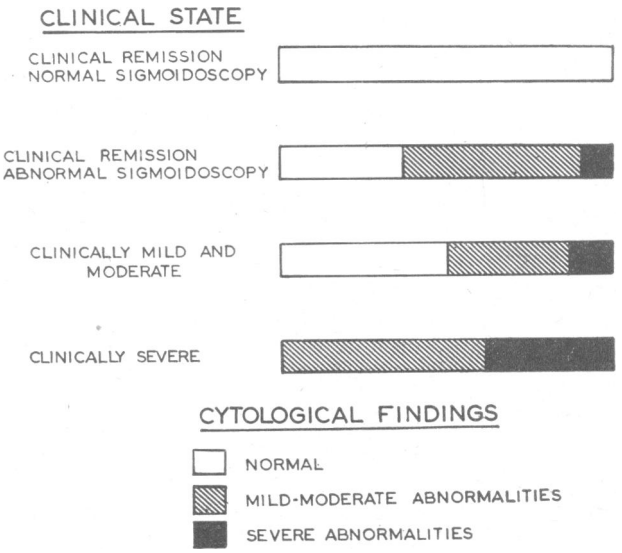

FIG. B.—The cytological findings in ulcerative colitis in relation to the clinical state.

As many of the patients were being treated with cortisone or related hormone therapy, it is necessary to consider whether this form of treatment had any relationship to the occurrence of severely abnormal cells, for it is not inconceivable that cortisone might pervert epithelial development. Severe abnormalities were indeed twice as frequent among patients treated with cortisone, but were not confined to them. The reason for the high incidence of severe abnormalities appears to be that almost all the patients with moderate or severe symptoms were promptly treated with cortisone or corticotrophin. Analysis of our data taking simultaneous account of both clinical severity and corticoid treatment shows that the abnormality of the epithelial cells is related to the severity of the disease rather than to the particular treatment.

Another possibility is that abnormal epithelial cells may be the consequence of long-continued disease of the bowel. We have therefore compared the findings in patients in their first attack of ulcerative colitis with those in patients in a relapse, many of whom had suffered from the disease for at least several years. There is little difference between these two groups of patients except that those in their first attack were more apt to show severe epithelial changes—a finding related to the fact that these patients were often severely ill. However, the more important aspect of the finding is that the abnormal changes we have described can manifest themselves early in the disease and therefore cannot be dismissed as a consequence of long-continued inflammation. In other words, the abnormal epithelial changes are correlated with the severity of the disease rather than with its chronicity.

Eosinophilia

Most of the patients with active ulcerative colitis show a large number of leucocytes in the colonic smear. In many instances neutrophil polymorphs are almost the only white blood cells seen, but in others there are a large number of eosinophils, which in some cases amount to 15–20% of the total polymorphonuclear leucocytes seen. Among 62 specimens in which large numbers of leucocytes were seen, 26 showed 5% or more of eosinophils. There is no close connexion between the severity of the epithelial changes and the occurrence of eosinophilia. Some patients repeatedly show eosinophilia in their colonic smear, and the question arises whether, in them at least, an allergic process is in being.

Discussion

The finding of abnormal epithelial cells in ulcerative colitis is of interest in two connexions.

1. The Nature of the Abnormal Cells

We have given reasons in the text in support of the view that the abnormal cells are colonic epithelial cells. There appear to be three possible explanations for their occurrence :

(*a*) *They are a Normal Feature of Damaged Colonic Epithelium which is Actively Regenerating in an Attempt to Repair Damage.*—Florey (1933) showed that when an ulcer is created in the intestine by bacterial inflammation the neighbouring epithelial cells spread rapidly across the ulcer surface, becoming cuboidal or flattened in the process, while simultaneously increased mitotic activity occurs in the depths of the glands. A traumatic ulcer in the rectal mucosa of man is covered by epithelium in a similar fashion (Lumb and Protheroe, 1955). It is possible that the active migration of cells to repair the damage is a result of chemotactic stimuli created by the injury. The property possessed by cells in spreading themselves thinly over the surface, becoming flattened in the process, is known as *thigmotaxis*. Biopsy specimens from the colon in active ulcerative colitis frequently show areas of surface epithelium in a cuboidal or flattened state. Such epithelial cells would presumably appear to be considerably larger when smeared on a slide than the columnar epithelial cells of a colon not in process of active regeneration. We think it is probable that this circumstance explains the occurrence of epithelial cells showing moderate enlargement of both cytoplasm and nucleus. If extensive damage is occurring in the epithelium as a result of severe inflammation so that epithelial regeneration is extremely active, it is conceivable that most of the appearances we have described among the epithelial cells could be explained on this basis. We do not know of any cytological studies on the human colon damaged by processes other than ulcerative colitis, so that at present our information is insufficient to judge this possibility.

(*b*) *They Represent a Primary Epithelial Disturbance in Ulcerative Colitis.*—With a disease of unknown aetiology it is natural to speculate whether any abnormality discovered is related to the basic cause of the disease. If we assume that the cells we have described as markedly abnormal are indeed abnormal, we must ask why they have become so large, an enlargement which affects both cytoplasm and nucleus. It would be possible to explain the occurrence of such cells on the basis of a maturation arrest of epithelial development. The epithelial cells at the bottom of the crypts of Lieberkühn represent the zone of active proliferation. As cells move up the glands towards the surface they normally differentiate into goblet cells and ordinary epithelial cells, a process which is probably irreversible (Florey, 1932, 1954). Lack of a specific factor could halt such differentiation and permit the emergence of cells which retain juvenile characteristics and continue to grow in size. We are familiar with such a process in the occurrence of megaloblasts in pernicious anaemia through absence of vitamin B_{12}. If such a process were to occur in colonic epithelium it could account for the large epithelial cells, which sometimes attain an impressive size ; by analogy, such cells could be regarded as "epithelial megaloblasts."

(*c*) *The Cells are a Secondary Manifestation of the Disease Process.*—Finally, the abnormal cells could be secondary to an inflammatory change in the lamina propria, with resulting toxic effects upon the epithelial cells. The frequent occurrence of abnormal cells in first attacks of the disease might at first sight appear to be in favour of their being an essential feature of the disease. However, the patients in their first attacks of ulcerative colitis were often severely ill, so the occurrence of abnormal cells can be accounted for as a secondary phenomenon dependent upon the severity of the disease process.

On the evidence at present available it is impossible to say which of these three general possibilities is the most likely explanation of the abnormal cells we have observed, and further study is necessary to settle the issue.

2. Relationship of Abnormal Cells to Carcinoma of Colon Supervening Upon Ulcerative Colitis

It has become generally recognized that ulcerative colitis may terminate in carcinoma of the colon. Bargen has been interested in this association for the last 25 years, and he and his colleagues have published an important study of the subject (Bargen *et al.*, 1954). They obtained follow-up information on 1,564 patients with ulcerative colitis who were seen at the Mayo Clinic between 1918 and 1937, and found that 98 of them had developed carcinoma of the colon or rectum. Among these patients the chance of developing carcinoma was about 30 times that of the general population. Several other recent studies confirm the unduly high risk of carcinoma in ulcerative colitis, particularly when the disease has lasted for 10 years or more (Kiefer *et al.*, 1951 ; Counsell and Dukes, 1952 ; Weckesser and Chinn, 1953 ; Dukes, 1954).

Cytological specimens falling in our "severe" group present many of the features of malignancy, such as marked anisocytosis and enlarged variable nuclei which exhibit prominent nucleoli and coarse chromatin patterns. We have compared these abnormal epithelial cells with neoplastic cells obtained by the same technique from the surface of a carcinoma of the recto-sigmoid junction, and find that the two sorts of nuclei are similar. The neoplastic cells differ chiefly in possessing only a small amount of somewhat necrotic cytoplasm instead of the abundant cytoplasm found in the abnormal cells of ulcerative colitis. In illustration of the fact that some of the "severely abnormal" specimens from ulcerative colitis closely resemble the findings met with in neoplasm, we may mention that an experienced cytologist was confident that the diagnosis must be carcinoma when he was shown one of them containing giant cells.

Summary

Examination of colonic epithelial cells by Papanicolaou's method has revealed that abnormal cells are often present in ulcerative colitis.

In their most pronounced form the abnormal cells are much enlarged and possess large nuclei with prominent nucleoli and a disturbed chromatin pattern. These abnormal cells may be found in any patient with abnormal sigmoidoscopic findings, but are most frequent in patients with severe disease. They are commonly found in patients in their first attack of ulcerative colitis.

There seem to be three possible explanations for the occurrence of these cells : (1) they are to be expected whenever the colonic epithelium is provoked into vigorous regenerative activity by extensive damage, from whatever cause ; (2) they represent a primary epithelial disturbance in ulcerative colitis, as, for example, a maturation arrest due to the absence of a specific factor

necessary for epithelial development; and (3) they are abnormal cells secondary to severe inflammation in the colonic mucosa.

The abnormal cells have many of the features of malignant cells, a point of interest in view of the fact that patients with ulcerative colitis are unduly liable to develop carcinoma of the colon.

We are grateful to Miss Shirley Thomas for her assistance, and to Mr. M. Morris and Miss Rowden for the photomicrographs.

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DIVIDED RENAL FUNCTION STUDIES IN HYPERTENSION

BY

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[WITH SPECIAL PLATE]

The wave of enthusiasm for unilateral nephrectomy as a treatment for hypertension engendered by the animal experiments of Goldblatt *et al.* (1934) and Goldblatt (1937) has been dampened by experience. Goldring and Chasis (1944), reviewing 76 such cases subjected to unilateral nephrectomy, accepted only 9% "successes," while Smith (1951), accepting 47 "successes" out of 242 reported cases (19%), has stated that "the advisability of nephrectomy must rest upon conservative and recognized surgical indications and not upon the hope of reducing the blood pressure."

The disappointing results of unilateral nephrectomy suggest either that hypertension is rarely caused by unilateral renal disease or that when the ischaemic kidney is an aetiological factor in hypertension it rapidly induces irreversible vascular changes in the contralateral kidney; alternatively, it is possible that the majority of patients subjected to unilateral nephrectomy have in fact significant bilateral renal disease.

It is reasonable to advise nephrectomy in a hypertensive patient, with the hope that the blood pressure will fall and remain lowered, when it has been well established that the renal abnormality is unilateral. On occasion, however, concomitant early hypertensive changes may be present in the contralateral kidney: this should not necessarily negate nephrectomy, because restitution may well follow surgical removal of the primary disease.

This paper records our experience of and observations on 13 hypertensive patients referred to us by our colleagues in the Department of Medicine for study and consideration for nephrectomy. Excretion urography had been carried out previously in 12 cases and perirenal air insufflation in five.

Methods

Intravenous pyelography was repeated after a period of 12 hours' dehydration. A few days later clearance studies were made, these being preceded by food and water deprivation for 12 hours, and administration of phenobarbitone, 2 gr. (0.13 g.) by mouth, for sedation. Low spinal anaesthesia to block the sacral segments was used for male patients, and local analgesia applied to the urethra for the females. Cystoscopy and ureteric catheterization was performed and urine obtained from the kidneys with the patients still in a state of dehydration; size 12 radio-opaque whistle-tipped ureteric catheters were used. The cystoscope was withdrawn and a soft rubber No. 8 catheter with five lateral openings was passed into the bladder. The ureteric catheters were manipulated under radiographic control so that the tips were approximately 2 cm. above the pelviureteric junction. After specimens had been collected from each ureter for specific-gravity determinations, patients were given 0.5 litre of water to drink and separate renal function tests were carried out in accordance with the methods of Smith *et al.* (1938). Glomerular filtration rate (G.F.R.) was measured by inulin clearance (C_{IN}) and endogenous creatinine clearance (C_{CR}), effective renal plasma flow (E.R.P.F.) and maximal tubular excretory rate (Tm) by *para*-aminohippurate clearance (C_{PAH}; Tm_{PAH}).

A stabilization period of 60 minutes for the intravenous infusion was required for constant blood levels of inulin and PAH. For the determinations pelvic urine was collected during three or four separate ten-minute periods in each case. At the end of every ten-minute period the bladder was carefully washed out through the indwelling rubber catheter and the washings were kept separately for determination of any leak around the ureteric catheters. Distribution of leak between the two sides may be determined by the formula $C_L(x) + C_R(1-x) = C_B$, where C_L, C_R, C_B are the concentrations of creatinine, PAH, or inulin per ml. of urine collected from the left ureter, right ureter, and bladder respectively during each collection period, and x is the fraction per ml. of the leak contributed by the left ureter. When C_L and C_R are equal the two sides of the equation cancel out and the formula cannot be applied (see footnote† to Table).

All urine samples were collected into 50-ml. flasks and measured by volume differences (Dempster and Graber, 1953). When maximal tubular excretory rate was determined, the intravenous infusion was supplemented by suitable quantities of 20% PAH (Chasis *et al.*, 1945) and the time required for stabilization (30 minutes) was used to carry out retrograde pyelography. Blood samples for plasma blank determinations were taken by forearm venepuncture, and the same needle was utilized for introducing the priming and sustaining solutions of inulin and PAH. Blood samples taken during the urine-collection periods were collected two minutes before the midpoint of each period through an antecubital vein in the contralateral forearm. Inulin estimations in urine and plasma samples were made by the method of Rolf *et al.* (1949), creatinine by the method of Brod and Sirota (1948), and PAH by the method of Smith *et al.* (1945).

Case Histories

Case 1 (Hypertension; unilateral pyelonephritis. Nephrectomy; operative "success").

Male aged 33, admitted December, 1954, with a blood pressure of 220/140 mm. Hg. The heart was not enlarged clinically or radiologically, but there was electrocardiographic evidence of left ventricular hypertrophy. The optic

M. M. BODDINGTON AND S. C. TRUELOVE: EPITHELIAL CELLS IN ULCERATIVE COLITIS

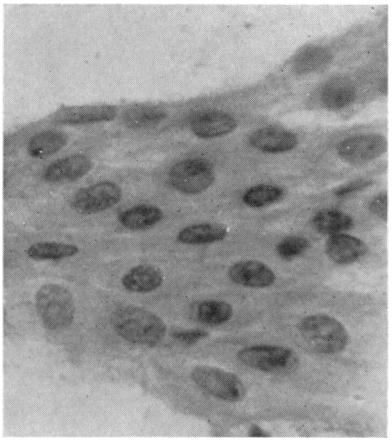

FIG. 1.—Colonic epithelial cells with normal appearances from a mild case of ulcerative colitis. (×525.)

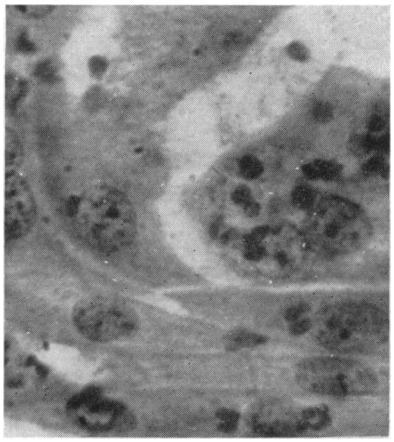

FIG. 2.—Colonic epithelial cells showing general enlargement in active ulcerative colitis. Variation in size not shown in this particular area. (×525.)

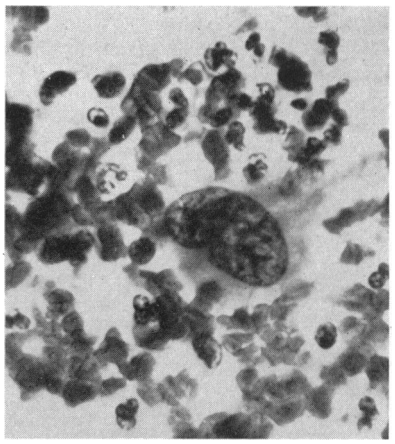

FIG. 3.—Typical giant epithelial cell in ulcerative colitis. (×525.)

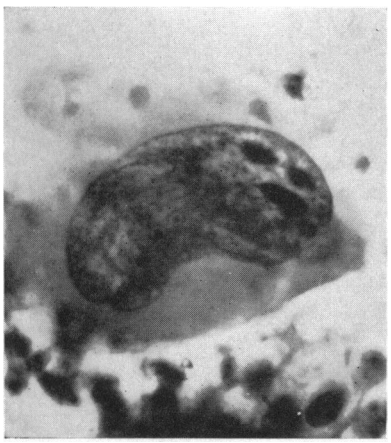

FIG. 4.—Extreme example of giant epithelial cell. In this reproduction the cytoplasm is not well shown, but in reality it is abundant and well defined. (×525.)

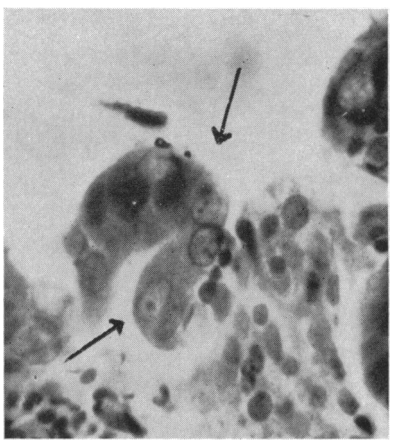

FIG. 5.—Abnormal epithelial cells in surface epithelium of a biopsy specimen. (×525.)

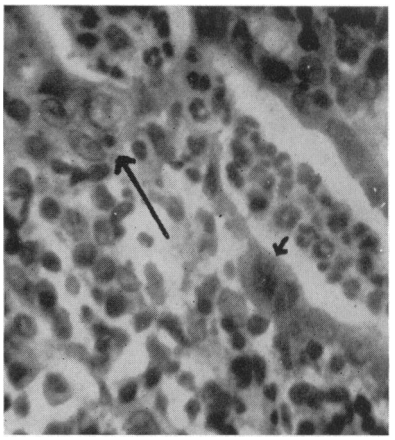

FIG. 6.—Abnormal epithelial cells in the wall of a crypt of Lieberkühn. (×525.)

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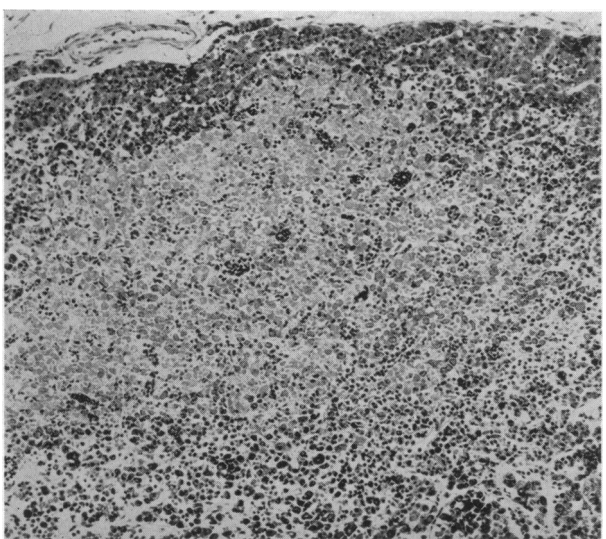

FIG. 1.—Photomicrograph of anterior lobe of pituitary showing a localized area of ischaemic necrosis. (H. and E. ×100.)

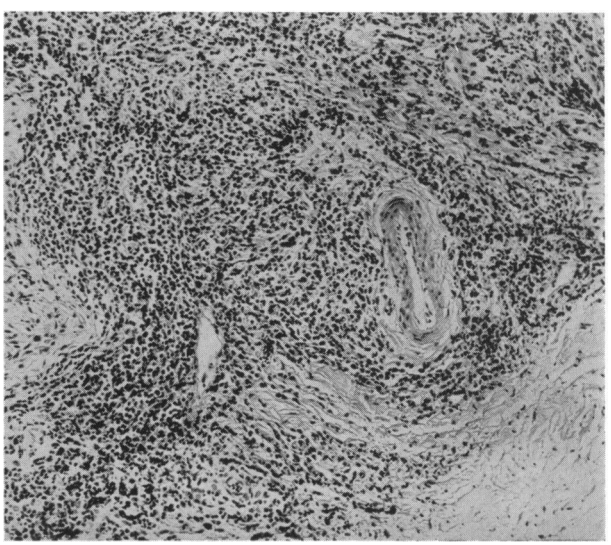

FIG. 2.—Photomicrograph of posterior lobe of pituitary showing extensive leukaemic infiltration. (H. and E. ×100.)