Flat in situ carcinoma of the bladder: cytological examination of urine in diagnosis, follow up, and assessment of response to chemotherapy

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SUMMARY   Urine cytology was performed for the diagnosis and follow up of flat carcinoma in situ (CIS) of the bladder in a series of 35 patients without associated or previous bladder tumours. Ninety six per cent had positive or suspicious cytology at initial presentation. There were no false positive reports. Cytological diagnosis of malignancy was made before biopsy in 24 patients: CIS in voided urine presents as flat sheets of five to 15 cells with features of high grade malignancy. Development of tumour during follow up was suggested by the appearance of large thick sheets and clusters of 30 or more malignant cells which were large and pleomorphic in high grade tumours and relatively small and closely cohesive in low grade tumours. Eleven of 13 patients with these clusters had bladder or ureteric tumours and two had malignant disease in the prostate. Negative cytological results in the presence of degenerative changes caused by chemotherapy was an unreliable indicator of response to chemotherapy, and there were five patients with false negative reports during treatment, of whom three had developed tumour. Persistence of malignant cells with features similar to those seen in the urine before treatment reliably predicted failure to respond to chemotherapy.

Flat carcinoma in situ (CIS) of the bladder essentially presents in two forms. The commonest is that occurring in patients with transitional tumours when flat CIS is found incidentally in the urothelium adjacent to the tumour, or in random biopsy specimens at sites away from the tumour. These foci of CIS with their potential for tumour development adversely affect the prognosis, and their recognition is important in the future management of the patient.[1] A less common presentation is when patients present with a variety of symptoms and no macroscopic tumour, but histological evidence of CIS in random biopsy specimens or in areas of cystoscopic abnormality. The reliability of the cytological analysis of urine in diagnosing CIS is now well recognised.[2–5] It is of particular value for initial diagnosis in those patients presenting without a history of tumour but with bizarre and misleading symptoms, in whom malignancy might not otherwise be suspected.[2] Regular cytological analysis of urine also plays a valuable part in the management of patients with flat in situ carcinoma and of assessment of response to chemotherapy. Urine cytology was used both for initial diagnosis and to monitor progress and

response to chemotherapy in 35 patients with flat CIS without preceding or associated tumour.

The aims of this study were to describe: (i) the cytological features of flat in situ carcinoma of the bladder in voided urine; (ii) features which could be used to diagnose papillary or solid tumours developing during cytological surveillance of patients with flat in situ carcinoma; (iii) the changes in cytological features induced by chemotherapy in patients with flat in situ carcinoma; and (iv) to assess the reliability of urine cytology in the diagnosis of flat in situ carcinoma of the bladder and its value in follow up and assessment of response to chemotherapy.

Material and methods

A series of 43 patients with flat CIS without preceding or associated tumour were seen in the St Peter's group of hospitals between 1956 and 1987. All patients had regular cystoscopic examinations and multiple biopsies performed. Urine was cytologically examined on 35 of the 43 patients in the series both for initial diagnosis and to monitor progress. Fourteen of these patients were given chemotherapy: the agents used were cyclophosphamide and methotrexate systematically and epodyl, adriamycin, and mitomycin

intravesically. Cyclophosphamide was given at three weekly intervals in doses of 1·5 g intravenously, and methotrexate 100 mg intravenously twice weekly.

Mitomycin was administered intravesically in doses of 30 mg in 50 ml of distilled water and adriamycin in doses of 50 mg in 50 ml of distilled water every month. Epodyl was administered intravesically in a monthly dose of 100 ml of a 1% solution. Voided urines were examined about every three months and before each installation or injection of chemotherapy. Between 1965 and 1975 Papanicolaou stained smears of spun deposits were used for diagnosis. After 1975 these were replaced by Papanicolaou stained Millipore filter preparations (Millipore UK Ltd).

Results

Thirty two of the 35 initial urine samples were suitable for cytological examination. A positive report of malignant transitional cells was made on the first urine sent for examination in 28 of the 32 urines. A suspicious report was given for three patients and a negative in one. Twenty four of the 28 positive reports were issued before the first histological diagnosis of CIS. In 18 patients cytological diagnosis of malignancy was made two weeks to 13 months before biopsy diagnosis of CIS and in six patients immediately before the first biopsy specimen was taken. The negative report was made in a woman in whom the cells in the first specimen were largely polymorphs and in whom two subsequent specimens were largely squamous. Thereafter cytological results were persistently positive.

The cytological features which characterised flat CIS in the voided urines closely reflected those in the biopsy specimens (fig 1, table). The cells showed features of high grade malignancy and were arranged both singly and in small, relatively flat sheets. Nuclei were irregular in shape and one and a half to twice the size of a normal intermediate transitional cell nucleus with abnormal nuclear cytoplasmic (N:C) ratios. They were either vesicular with coarse, granular chromatin, or dark and structureless (figs 2 and 3). Occasional cells contained multiple malignant nuclei. Nucleoli were not prominent. The cytological features characterising dysplasia were essentially similar to those of flat CIS but of a lesser degree. In particular, nuclear enlargement was less prominent and N:C ratio less disturbed (figs 4 and 5).

In 13 patients during follow up the urine contained not only single and flat sheets of malignant cells with the features described above, but also thick sheets and three dimensional clusters of 30 or more malignant cells which had not been present in earlier specimens (fig 6). Of these, 10 were found to have solid or papillary tumours of the bladder, one, a ureteric
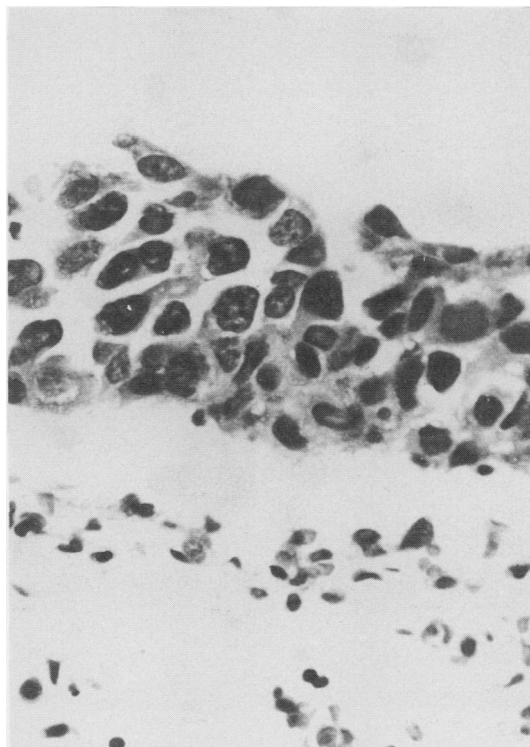

Fig 1 *Carcinoma in situ of bladder: histological section. (Haematoxylin and eosin.)*

tumour, and two, transitional carcinoma affecting the prostatic ducts and associated with extensive carcinoma in situ of the bladder but no solid or papillary tumour. The cytological features in the urine correlated with grade of tumour. The clusters of malignant cells from high grade tumours had larger nuclei than those in CIS, with increased pleomorphism and prominent nucleoli. With tumour of lower grade, the clusters were composed of smaller more closely cohesive cells with less pleomorphism.

Table *Cytological features of CIS in voided urine samples*

Arrangement of malignant cells	Single and sheets
Size of sheets	Average 5–15 cells
Cell size	Same or slightly larger than intermediate transitional cells
Cell shape	Round or polygonal, occasionally irregularly elongated
Nucleus	Usually single, occasionally multiple
Nuclear shape	Often irregular, notched, or pointed
Chromatin	Black and structureless or coarse granular
Nucleoli	Generally absent
Background	Clean
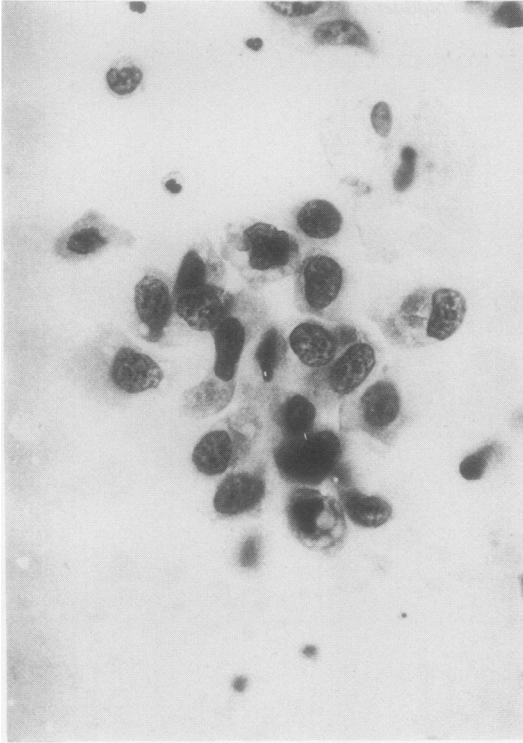
Fig 2
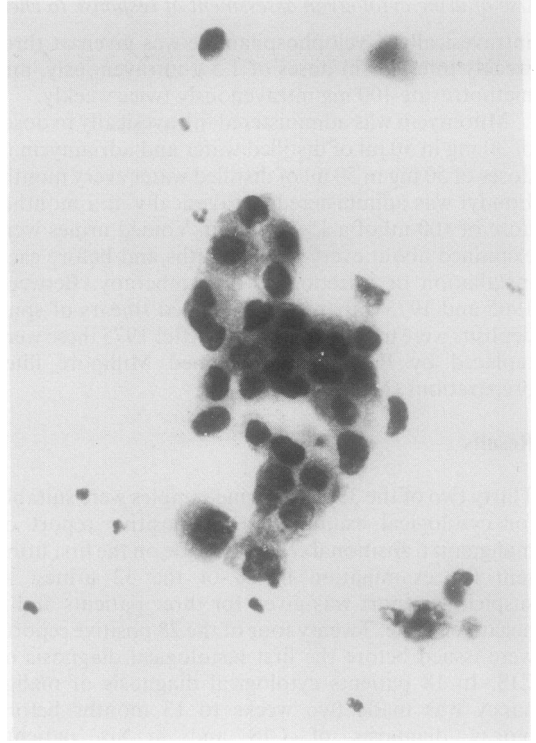
Fig 3


Fig 4
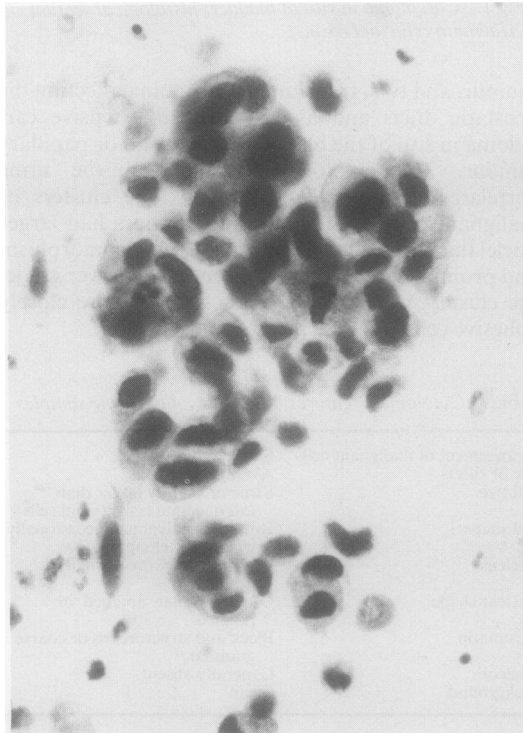
Fig 2   *Voided urine: flat sheet of loosely cohesive malignant transitional cells with vesicular and hyperchromatic nuclei. (Papanicolaou.)*

Fig 3   *Voided urine: flat sheet of loosely cohesive malignant transitional cells with hyperchromatic structureless nuclei. (Papanicolaou.)*

Fig 4   *Dysplasia in voided urine: sheet of transitional cells with irregular, hyperchromatic slightly enlarged nuclei. (Papanicolaou.)*
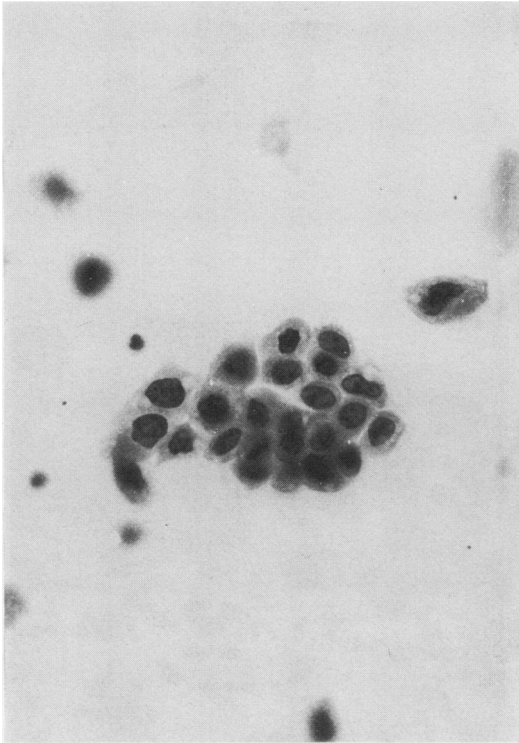
Fig 5 *Dysplasia in voided urine: sheet of cells with irregular, hyperchromatic nuclei, a few enlarged. (Papanicolaou.)*
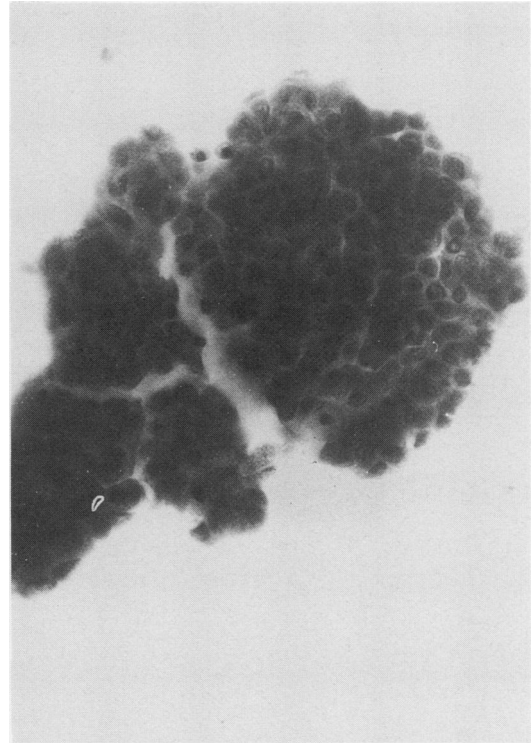
Fig 6 *Cluster of malignant cells in voided urine from patient with CIS of bladder extending into prostatic ducts. (Papanicolaou.)*

The administration of chemotherapeutic agents produced certain characteristic changes in the urine. The duration and severity of these changes varied in each patient. They were not specific for any drug but were most pronounced with intravesically administered mitomycin and adriamycin. These changes were rapid in onset, occurring as early as one week after the first drug dose. They were characterised by a reduction in number, and occasionally, complete disappearance of recognisable malignant cells, together with pronounced degenerative changes affecting both nucleus and cytoplasm of all epithelial cells. There was extensive nuclear pyknosis and karyorrhexis, and cytoplasm became thick and lumpy or vacuolated. In some specimens the deposits consisted entirely of single degenerate cells with normal sized or enlarged misshaped pyknotic nuclei.

A striking feature in these specimens was the appearance of greatly enlarged single cells of bizarre form. They varied from two to six times the size of a normal superficial cell with a proportionately enlarged nucleus, which was either black and structureless or vesicular with lumpy, unevenly distributed chromatin and large nucleoli (fig 7). Although remarkable for

their appearance, they were relatively few in number, rarely exceeding 2% of the total. An attempt to find the source of these bizarre cells in the relevant histological sections proved difficult because of loss of superficial layers of epithelium in many of the biopsy specimens. Where full thickness was intact these cells appeared to originate in the superficial zones of the dysplastic or malignant epithelium (fig 8). The normal urothelium included in the biopsy specimens showed minimal changes. When cytological analysis of urine was used as a means of assessing response to chemotherapy a positive report was issued only when morphologically viable malignant cells with features similar to those seen in the urine specimens before treatment persisted during chemotherapy. As the nature of the large bizarre cells appearing during chemotherapy was not clear they were not used for a diagnosis of malignancy.

To assess the reliability of the cytological examination of urine in the management of CIS, the findings were compared with those of the relevant biopsy specimen taken within three months of cytological diagnosis. Where cytology reports were positive the histological sections showed in situ carcinoma, severe
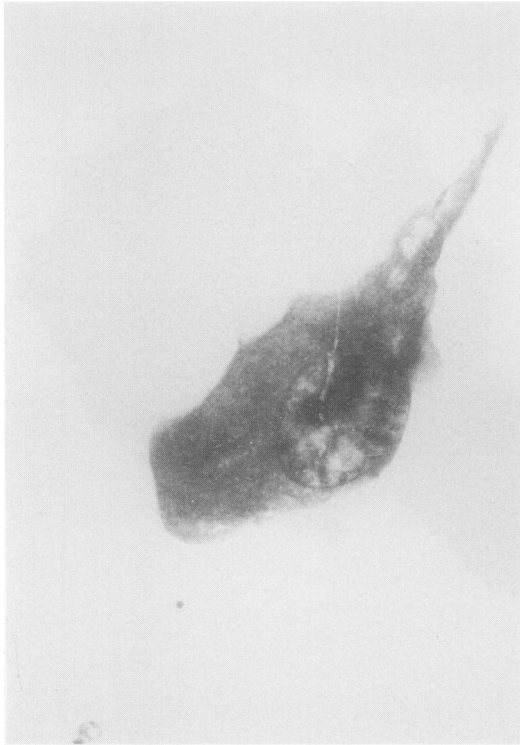
Fig 7 *Effect of chemotherapy on transitional cell: voided urine. (Papanicolaou.)*
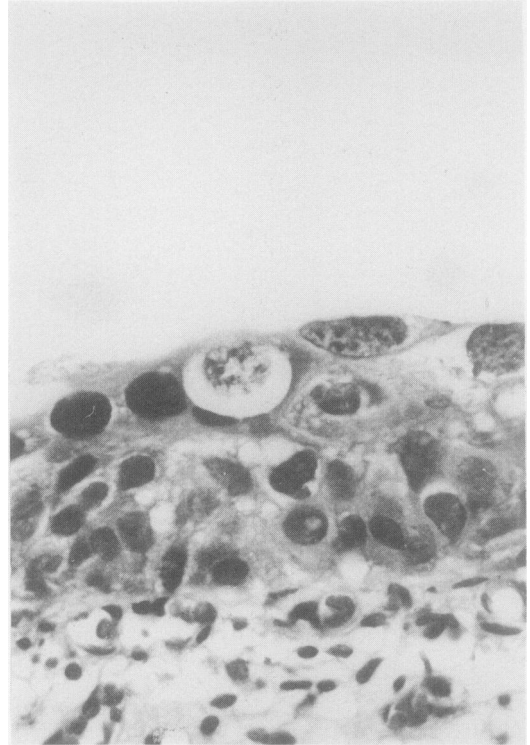

Fig 8 *Effect of chemotherapy on superficial cells of in situ carcinoma: histological section. (Papanicolaou.)*

dysplasia, or papillary or solid tumour. In six instances, however, a positive cytological report was associated with no histological evidence of malignancy. In two of these patients urine cytology was positive when the patient first presented, but histological diagnosis of CIS was established only in a second biopsy specimen taken nine and 13 months after the first positive cytological report. In four patients with positive cytology and negative bladder biopsy specimens during follow up, CIS of the bladder was diagnosed histologically only after repeat biopsies were performed in three, while one patient with negative bladder biopsy specimens had developed a solid anaplastic carcinoma of the ureter. There were therefore no false positive reports in this series. False negative reports were issued in five cases during chemotherapy. All five cases showed severe degenerative changes and absence of the viable malignant cells seen in the urine samples before treatment. In all five cases, however, the relevant histological sections showed persistent CIS or dysplasia, and in three cases, associated papillary or solid tumours.

Discussion

Flat CIS in patients without preceding tumours may pose a diagnostic problem to the clinician because of the bizarre and often long established symptoms which characterise the condition, together with the difficulty of localising areas of histological abnormality at cystoscopy.[6] In this series frequency of micturition, both diurnal and nocturnal, was the predominant symptom associated with penile or peroneal pain in one third of patients. Only 13 had haematuria. There were nine patients with no visible abnormality at first cystoscopic examination.

The role of the cytological examination of urine in the diagnosis and clinical management of flat CIS is now well documented.[2-5] This series, in which 24 of the initial 28 urine samples were positive, and three were suspicious, gave a 96% positive or suspicious result, which was consistent with the results of other reported series.

The poor cohesion between cells and between epithelium and basement membrane accounts for the

readiness with which diagnostic material may be found in the urine, and for the problem of histological interpretation in those biopsy specimens where part or full thickness of the epithelium may be lost. Nine patients in this series reported passing debris in the urine which may well have represented sheets of exfoliated epithelium. The features of high grade malignancy which characterise the exfoliated cells enable them to be readily recognised and reliably interpreted. False positive cytological results are unlikely and there were no false positive reports in this series.

Several authors have indicated the importance of careful follow up in patients with persistent positive cytological results and negative investigations.[4 7-9] The findings in this series support the importance of further bladder biopsies and investigation of the urethra and upper tract where appropriate in patients whose urine is persistently positive without evidence of malignancy.

While defining the cytological features of flat CIS in the urine in this series, features were sought which might enable cytologists to recognise the development of papillary or solid tumours during follow up. While agreeing with reported statements that it is not always possible to distinguish carcinoma in situ from papillary or solid tumours of high grade malignancy during cytological examination of urine,[9-11] the findings suggest that when thick sheets and large three dimensional clusters of malignant cells appear during cytological follow up of carcinoma in situ, there is a strong possibility that papillary or solid tumours have developed. These features may therefore be used as predictors of tumour development during cytological follow up. Increased cellular pleomorphism is another feature suggestive of tumour development as other authors have reported.[9 11] Interestingly, two cases of CIS of the bladder with spread to the prostate but no solid or papillary tumour in the bladder or ureter showed large clusters of malignant cells in their urine, similar to those in patients with CIS and bladder tumours. These findings accord with those reported in a series of six patients with CIS of the bladder, four of whom had malignant disease in the prostatic duct but no bladder tumour.[12]

Cytological examination of urine was used in this series to monitor response to chemotherapy. Reliable cytological criteria of response or failure of response are important, not only as a guide to the oncologist, but also in view of the poor prognostic importance of positive cytology at initial three month follow up in patients receiving intravesical chemotherapy.[13] In this series persistence of those malignant cells seen in the urine samples before treatment, with features unchanged by chemotherapy, was regarded as the only reliable criterion of failure of response. This was confirmed in the follow up biopsy specimens. Positive urine cytology using these criteria is therefore a reliable indicator of failure to respond to chemotherapy. Negative results in the presence of pronounced degenerative changes are not reliable indicators as the findings in five patients proved. Indeed, it was alarming that three of these patients with negative cytological results throughout chemotherapy had developed tumours.

The clinical importance of the large bizarre cells which made their appearance so strikingly during chemotherapy remains somewhat unclear. Cells of similar appearance were found overlying areas of dysplasia and CIS in biopsy specimens taken during chemotherapy. It was not possible to exclude entirely that these large bizarre cells were derived from normal urothelium transformed by chemotherapy as they were identical with those shown in normal female dogs treated with intravesical thiotepa[14] and with those induced by cyclophosphamide and busulphan in patients treated for non-urological malignancy.[11 15] They were therefore not regarded as being reliable for diagnostic purposes.

The following conclusions can be drawn from this study. Malignant cells with characteristic morphology can be seen in the urine at first presentation in virtually all patients with flat CIS. Cytological examination of voided urine is therefore essential in all patients with unexplained symptoms to avoid delay in diagnosis.

Cytological diagnosis of CIS may precede histological confirmation by many months. In the event of persistent positive cytological results with negative histological examination, random biopsy specimens of the bladder should be repeated, the urethra re-examined, and investigation of the upper tract considered.

Development of papillary or solid tumours during follow up is suggested by the presence of thick sheets and large three dimensional clusters of malignant cells which differ in appearance from the smaller relatively flat sheets of malignant cells that characterise CIS. These clusters of malignant cells may originate in tumours in the bladder or ureter. They may also be indicative of extension of CIS into the prostate.

In assessing response to chemotherapy, positive cytological results, as evidenced by persistence of viable malignant cells with similar features to those in urine samples before treatment, are reliable indicators of failure of response. Negative cytological results may occur in the presence of pronounced degenerative changes and cannot be relied on as an indicator of response to chemotherapy.

Harland for urine specimens from patients receiving chemotherapy.

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