Br. J. Cancer (1973) **28**, Suppl. I, 276

THE TREATMENT OF ADVANCED BLADDER CANCER WITH SENSITIZED PIG LYMPHOCYTES

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Summary.—Sixteen patients with advanced carcinoma of the bladder were treated by infusion of immunized pig lymphocytes into the arterial blood supply of the tumour. Seven patients obtained definite benefit as assessed clinically and pathologically.

THE theoretical basis for the use of sensitized xenogeneic lymphocytes in the treatment of advanced human malignant disease has recently been reviewed (Symes and Riddell, 1973). In brief, this form of immunotherapy is an attempt to transfer adoptive immunity from a pig, immunized against a given tumour, to the patient with that tumour. To this end the patient receives an injection of sensitized lymphocytes, from the pig, into the arterial blood supply of the tumour.

It has been found convenient to use pigs as donors of sensitized lymphocytes, because this animal is easy to handle, is free from infection and has a long continuous mesenteric node chain.

Symes and Riddell (1973) reported a series of 14 patients, including cases of malignant melanomata and carcinomata of the colon, rectum, ovary and urinary bladder, treated with pig cells. Seven of the patients obtained definite clinical benefit. This was defined as symptomatic improvement coupled with objective evidence of tumour regression, clinical, radiological and histological, as appropriate.

The present communication describes the early outcome in a further series of 16 patients with advanced carcinomata of the urinary bladder.

MATERIALS AND METHODS

Patient selection.—All 16 patients had advanced disease, associated with haematuria in 15. In 14 of the 16 the tumour had spread beyond the bladder, a pelvic mass was present and surgical resection was considered inappropriate. All patients had either shown recurrent tumour following radiotherapy or, by reason of their age, were unsuitable for a full course of radiotherapy.

Of the 16 patients, 15 had high or average grade, transitional cell carcinomata according to the histological grading methods described by Brown in Miller, Mitchell and Brown (1969). One patient (Case 13) had a pleomorphic epidermoid carcinoma. All but 2 of the tumours showed invasion of the bladder wall.

Plan of treatment.—On Day − 7 a specimen of tumour was obtained by transurethral resection and multiple fragments were implanted into the mesentery of a pig within 4 hours. At the same time a portion of each tumour was retained for histological examination. On Day 0 the segment of the pig lymph node chain draining the implanted length of mesentery was excised and a cell suspension prepared from it. Biopsies were obtained from this portion of the node chain and from that part of the chain distant from the site of tumour implantation. Comparative histological examination of these 2 specimens confirmed the immunization of that portion of node chain draining the tumour fragments in every case. Also on Day 0 two tumour fragments, selected at random in each case, were removed from the mesentery and examined histologically to determine the cellular events, at 7 days, in the response of a pig to human tumour.

Further details of the method of tumour implantation into the pig mesentery, and the preparation of lymph node cell suspensions are given by Symes and Riddell (1973).

TABLE I.—*Synopsis of Patients with Bladder Carcinoma Treated by Intra-arterial Injection of Sensitized Pig Lymphocytes*

	Patient no.	Age and sex	Clinical features	Treatment	Response	Length of survival
(A)	1	61 ♂	9/12 history of haematuria Non-function left kidney Filling defect in bladder Large pelvic mass 10 × 7 cm Radiotherapy 4/12 previously High grade T.C.C.	1. Day 0 $2{\cdot}5 \times 10^9$ pig lymphocytes 2. Day 181 7.25×10^9 bull lymphocytes	Tumour necrosis—1, 2 Regression for 4/12 } 1 Return of renal function }	322 days. Necropsy showed primary necrotic, massive recent hepatic secondaries
(C)	2	64 ♂	3 year history 5/12 profuse haematuria Previous resection and radiotherapy Multiple tumours, average grade T.C.C.	1. Day 0 $5{\cdot}75 \times 10^9$ pig lymphocytes 2. Day 21 total cystectomy	Nil	52 days. Cystectomy specimen total replacement of bladder mucosa with non-invasive carcinoma
(B)	3	74 ♂	$2\frac{1}{2}$ year history haematuria Pelvic mass 3 × 2 cm Previous resection and radiotherapy Average grade T.C.C.	1. Day 0 $4{\cdot}8 \times 10^9$	Regression and necrosis 3/12 Tumour growth recommenced 6/12	> 366 days
(A)	4	62 ♂	1/12 history perineal pain and haematuria Pelvic mass 3 × 2 cm High grade T.C.C.	1. Day 0 $5{\cdot}05 \times 10^9$ 2. At 6/12 4000 rad in 1/12	At 5/12 tumour increased vascularity At 9/12 total regression	> 359 days
(A)	5	73 ♂	3/12 history haematuria Right kidney nephrogram only Palpably thickened bladder wall Average grade T.C.C.	1. Day 0 $6{\cdot}4 \times 10^9$	Remission 8/12 Return of renal function Small discrete low grade tumours excised at 3/12 and 7/12 F.B. giant cells present	> 268 days
(A)	6	73 ♀	8/12 history haematuria Mass 3 × 2 cm right lateral wall High grade T.C.C.	1. Day 0 $1{\cdot}4 \times 10^9$	Remission 1/12 Massive tumour necrosis 3/12 hepatic secondaries	189 days. No necropsy
(A)	7	70 ♂	3/52 history haematuria I.V.P. left nephrogram Pelvic mass 5 × 5 cm High grade T.C.C.	1. Day 0 3×10^9 2. At 1/12 4000 rad	At 3/52 tumour oedematous and part necrotic with cellular infiltrate At 4/12 almost total regression	> 216 days
(C)	8	83 ♀	2/12 history haematuria Pelvic mass 6 × 5 cm Fixed to left pelvic wall High grade T.C.C.	1. Day 0 $2{\cdot}15 \times 10^9$	At 3/52 massive tumour necrosis Subsequently patient became incontinent	147 days. Died—no necropsy
(A)	9	76 ♂	1/12 history haematuria Large fixed mass 9 × 9 cm in left bladder wall and pelvis High grade T.C.C.	1. Day 0 $3{\cdot}10 \times 10^9$	At 3/52 tumour oedematous with some necrosis and marked infiltration with eosinophils and F.B. giant cells At 4/12 pelvic mass smaller and more mobile	> 127 days

TABLE I.—*Continued*

	Patient no.	Age and sex	Clinical features	Treatment	Response	Length of survival
(C)	10	55 ♂	3 year history haematuria Multiple low-grade transitional cell carcinomata	1. Day 0 $3{\cdot}03 \times 10^9$	At 3/52 some tumour necrosis 2/12 tumour growth recommenced. Recommended for radiotherapy	> 120 days
(C)	11	62 ♂	1 year 4/12 history haematuria Previous radiotherapy Mass 5 × 4 cm left lateral wall of pelvis High grade T.C.C.	1. Day 0 $2{\cdot}13 \times 10^9$	At 3/52 superficial tumour necrosis only 3/12 ascites and hepatic enlargement	> 99 days
(C)	12	82 ♂	1/12 history profuse haematuria, Hb 38% Fixed mass 6 × 5 cm left pelvic wall Pleomorphic epidermoid carcinoma	1. Day 0 $1{\cdot}37 \times 10^9$	At 2/12 tumour growth uninterrupted Large suprapubic mass	69 days. Necropsy showed tumour in bladder cavity necrotic. However, massive total involvement of bladder wall and aortic nodes with viable tumour. Metastases also in bones and liver
(C)	13	75 ♂	5/12 history dysuria 2/12 haematuria I.V.P. large filling defect in bladder Mass 5 × 4 cm left lateral wall of pelvis High grade T.C.C.	1. Day 0 $2{\cdot}3 \times 10^9$	Day 64 superficial necrosis Histology no definite change For radiotherapy	> 72 days
(B)	14	71 ♂	1/12 history—emergency admission with haematuria I.V.P. non-functioning left kidney Mass 6 × 8 cm left wall of bladder Average grade T.C.C.	1. Day 0 $2{\cdot}65 \times 10^9$	At 3/52 superficial tumour necrosis Pelvic mass a little smaller Histology some necrosis—no giant cells For radiotherapy	76 days. Necropsy—tumour in bladder extending right through the wall, bilateral hydronephrosis. Some enlarged para-aortic nodes
(A)	15	67 ♂	3/12 history dysuria and nocturia Tumour 3 × 4 cm left base of bladder Average grade T.C.C.	1. Day 0 $3{\cdot}00 \times 10^9$	Day 16 tumour oedematous; increased necrosis with F.B. giant cells and eosinophils	> 65 days
(B)	16	59 ♂	2 year history. Previous radiotherapy Recurrence ♂ haematuria 5/12 before immunotherapy Non-function right kidney Fixed mass 4 × 5 cm right base of bladder High grade T.C.C.	1. Day 0 $2{\cdot}70 \times 10^9$	Day 22 tumour more localized and part ulcerated; histology some necrosis	> 44 days

Assessment of the patient response.—This was carried out at four levels: (i) Symptomatic with particular reference to haematuria; (ii) review cystoscopy and bimanual pelvic examination under anaesthesia at intervals. The first such examination was usually carried out on Day 14 or 21; (iii) histological examination of biopsy material obtained by transurethral resection during (ii). The histological appearance in comparison with pretreatment biopsies was noted; (iv) periodic radiological examination of the urinary tract where appropriate.

RESULTS

A synopsis of the 16 patients treated and the outcome are presented in Table I. It may be seen that the cases divide themselves into 3 groups: (A) Cases in which assessment showed beneficial change, nos. 1, 4, 5, 6, 7, 9 and 15; (B) cases where the outcome was equivocal, nos. 3, 14 and 16; (C) patients in whom there was no evidence of tumour regression, nos. 2, 8, 10, 11, 12 and 13.

Case Reports

The following case reports are illustrative of patients in the Groups A and C. It is too early to assess the outcome of the patients in Group B.

Group A

Case 1.—This patient has been reported previously (Symes and Riddell, 1973). He had a 9-month history of high grade transitional cell carcinoma of the bladder, which had failed to respond to radiotherapy given 4 months previously. A large left sided pelvic mass was present and no function in the left kidney was seen on I.V.P.

On Day 14 after cell infusion, the tumour showed marked necrosis and round cell infiltration at cystoscopy and biopsy. By Day 48 the regression of the tumour had reached a stage where radiological evidence of return of left kidney function was obtained. On Day 83 a small recurrence was excised from the bladder, otherwise the patient remained well.

On Day 154, due to massive tumour regrowth, the patient received a second injection, this time of bull cells, to avoid the risk of hypersensitivity to pig antigens. This was followed by further marked tumour necrosis, seen at cystoscopy on Day 195. However, when seen on Day 240 following the initial therapy with pig cells, the patient showed a rapid deterioration in condition. He died on Day 322 and at post mortem examination, although the primary tumour was necrotic, the liver showed massive replacement by secondary tumour of apparently recent origin.

Case 5.—This patient presented in April, 1972 with a 2-month history of haematuria. An I.V.P. showed obstruction to the right kidney so that a nephogram only was obtained on this side (Fig. 1).

He was found to have a very extensive papillary tumour, involving the right wall of the bladder which was palpably thickened. Histology showed the tumour to be of average grade (Fig. 2).

On Day 14, after infusion of pig cells, there was marked necrosis of tumour remnant at cystoscopy. The tumour, on biopsy, was now low grade and showed marked necrosis associated with the presence of foreign body multinucleated giant cells (Fig. 3, 4, 5). At this time, an I.V.P. showed a return of right renal function (Fig. 6).

Small discrete histologically low grade tumours were excised from the bladder at 3 and 7 months after therapy. Otherwise the patient has remained well at 268 days post treatment.

Case 7.—This man had a 3-week history of painless haematuria associated with the passage of clots. An I.V.P. showed a nephrogram only in the left side with a filling defect on the left wall of the bladder. At cytoscopy in June 1972 there was an extensive solid tumour involving the left wall of the bladder. A pelvic mass was present, measuring 5 × 5 cm which was fixed to the left pelvic wall. Biopsy showed a high grade invasive tumour.

On Day 14 following cell infusion, the bladder wall, at cytoscopy, appeared oedematous and there was considerable tumour necrosis with lymphocytic infiltration. The pelvic mass was more mobile. However, as the oedema appeared likely to obstruct both ureteric orifices, the patient was given 4000 rad in August 1972.

When seen 2 months later (4 months after cell infusion) regression of both the intra- and extra-vesical tumour was almost complete.

Case 9.—This patient presented in June 1972 with a history of dysuria and initial

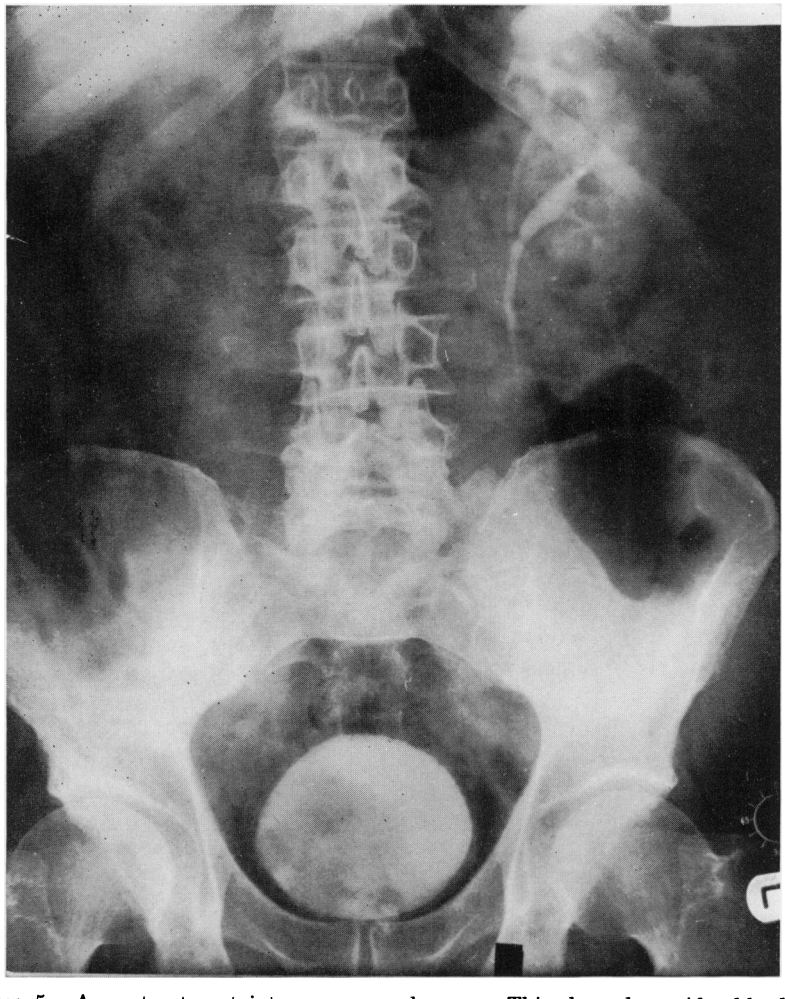

FIG. 1.—Case 5. A pre-treatment intravenous pyelogram. This showed considerable delay in the function of the right kidney and a large filling defect in the bladder.

haematuria on several occasions. At cystoscopy there was a solid tumour mass involving the whole of the left bladder wall and a pelvic mass, which was fixed and measured approximately 10 × 10 cm. Biopsy showed a high grade invasive tumour. Pig cells were infused and on Day 21 there was marked oedema and some necrosis of the tumour on the left bladder wall. Histology showed tumour necrosis and in other areas infiltration with eosinophils and giant cells. On Day 123 the patient was well with only slight intermittent haematuria.

Case 15.—This patient presented in September 1972 with a 3-month history of dysuria and nocturia. He was found in November 1972 to have an average grade papillary tumour involving the base of the bladder on the right side.

On Day 16, after injection of pig cells, the tumour appeared oedematous and histology confirmed the presence of increased necrosis again associated with giant cells and a marked eosinophilic cellular infiltrate.

This patient is to receive radiotherapy.

Group C

Case 12.—This man, aged 82, was admitted as an emergency due to haematuria with clots in October 1972. At cystoscopy there was an

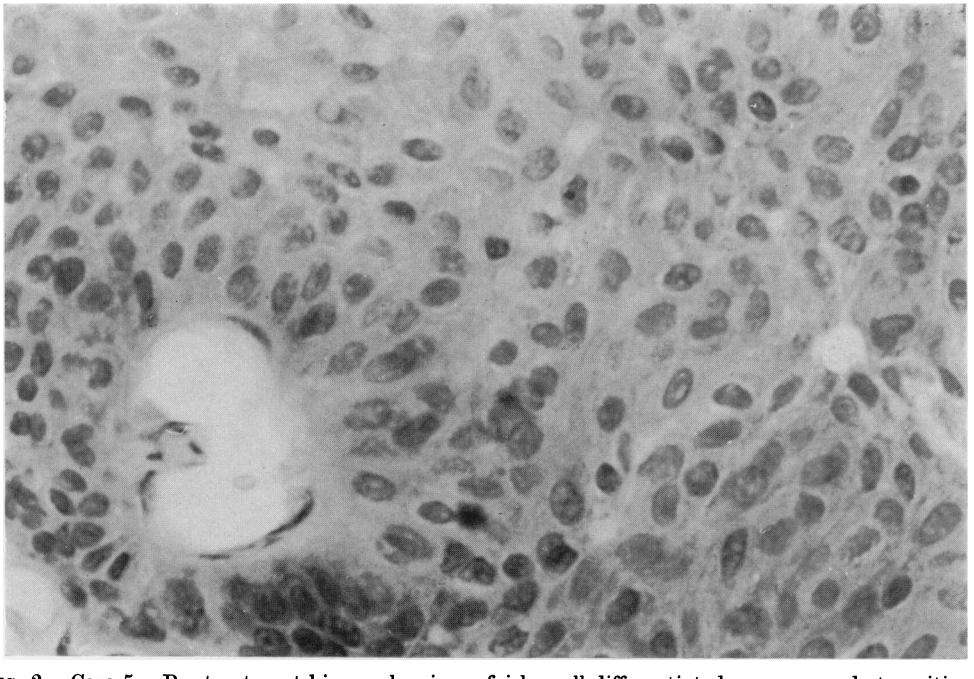

FIG. 2.—Case 5. Pre-treatment biopsy showing a fairly well differentiated average grade transitional cell carcinoma. H. & E. × 328.

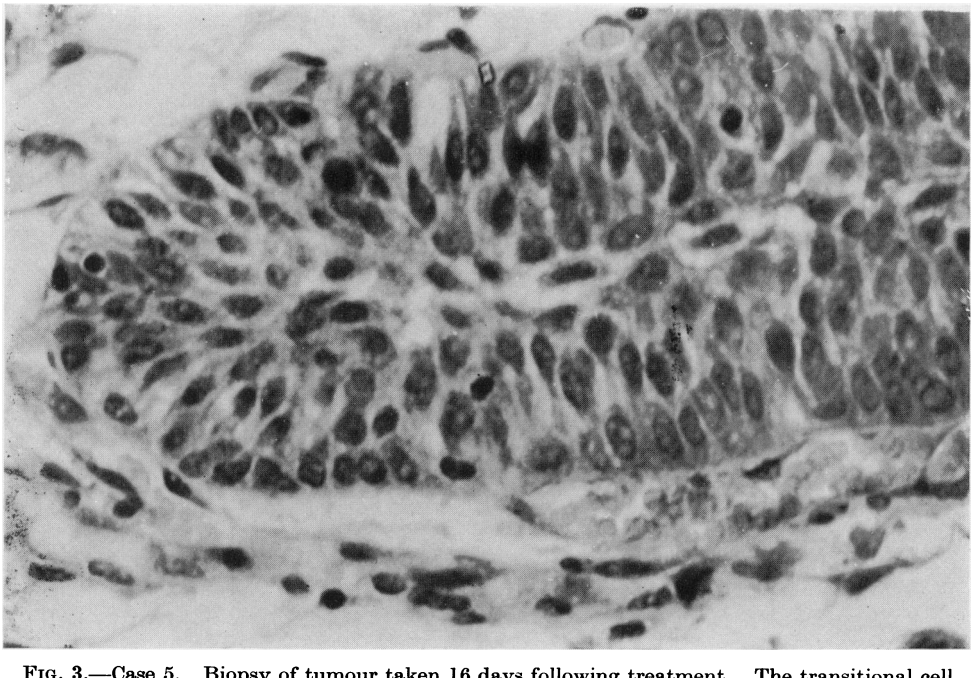

FIG. 3.—Case 5. Biopsy of tumour taken 16 days following treatment. The transitional cell carcinoma is now of low grade malignancy. H. & E. × 328.

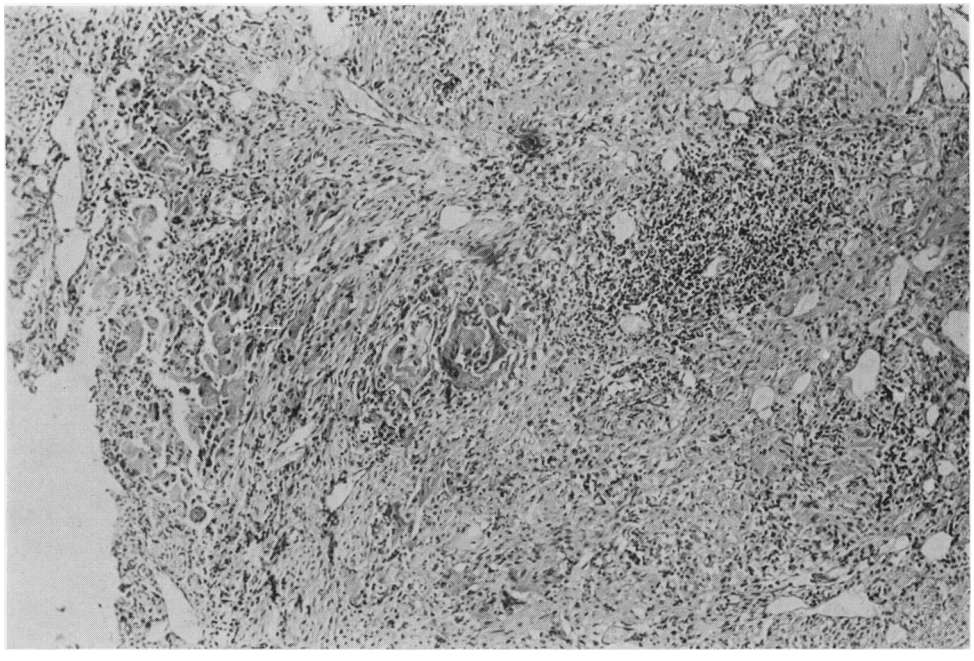

FIG. 4.—Case 5. Another area of the biopsy illustrated in Fig. 3. This low power picture shows extensive lymphocytic infiltration and foreign body multinucleated giant cells in a region not containing viable tumour. H. & E. × 52.

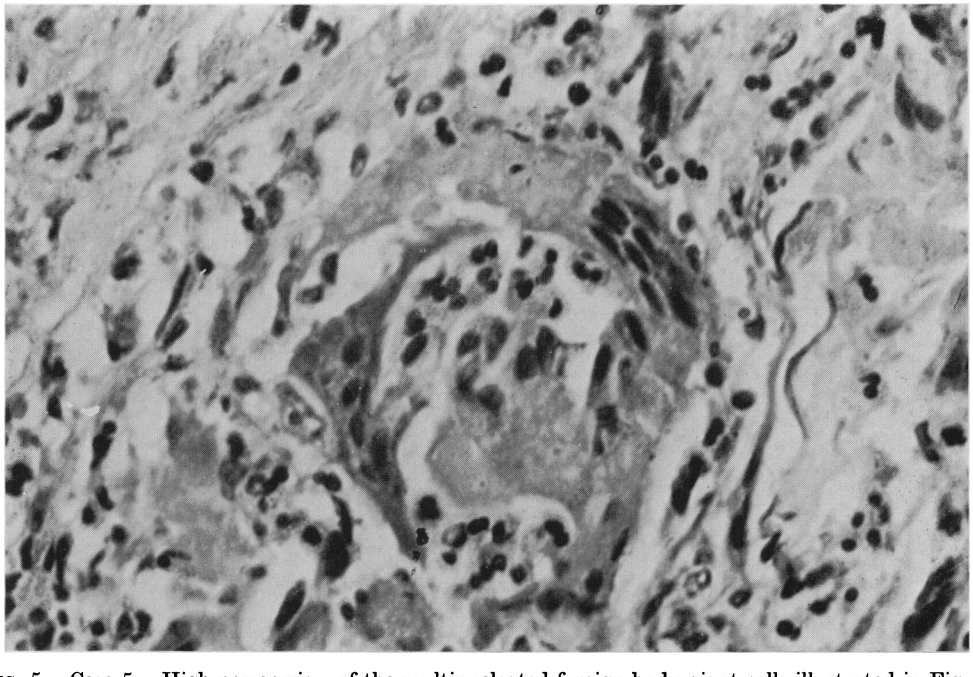

FIG. 5.—Case 5. High power view of the multinucleated foreign body giant cells illustrated in Fig. 4. Similar cells were also prominent in Cases 9 and 15. H. & E. × 328.

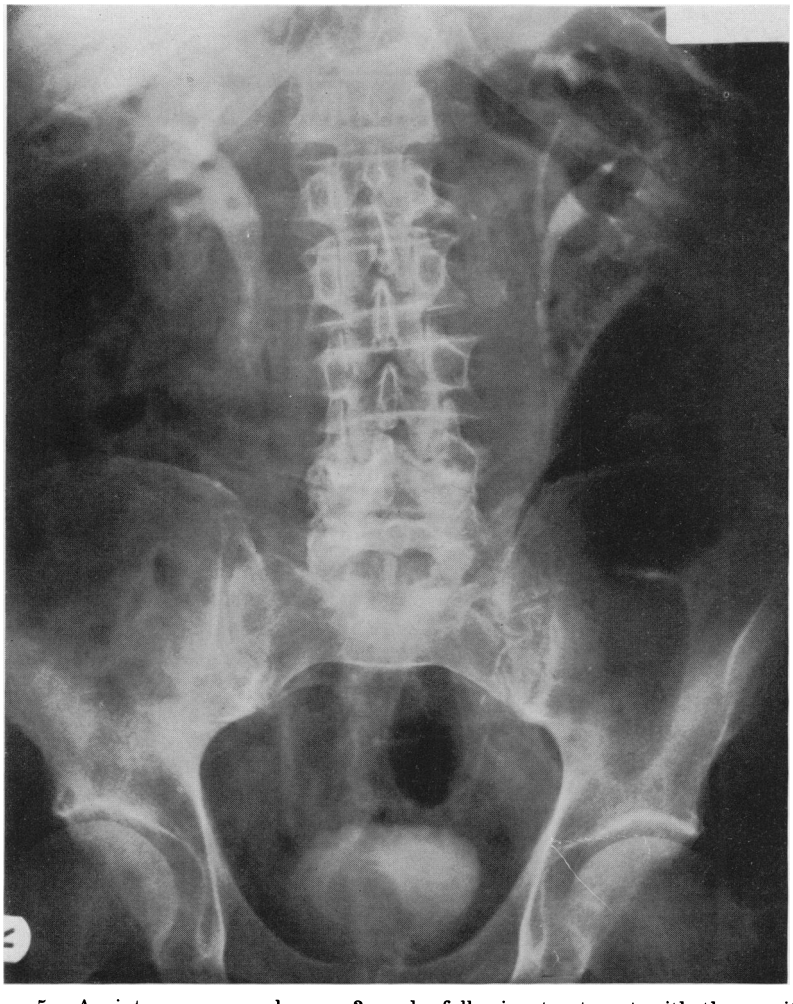

FIG. 6.—Case 5. An intravenous pyelogram 3 weeks following treatment with the sensitized pig lymphocytes. This shows excretion from the right kidney with filling of the pelvicalyceal system and ureter. The filling defect in the bladder is reduced in size. A balloon catheter is seen in the bladder.

area of tumour approximately 6 × 5 cm fixed to the left bladder wall. Histologically at this stage the tumour was thought to be an average grade transitional cell carcinoma. He received pig lymphocytes on November 14, 1972 (Day 0).

At review cystoscopy on Day 56, the tumour was much larger. In addition there was a palpable suprapubic extension of about 6 cm diameter. However, his haematuria had ceased. He died on Day 69 and at post mortem examination, whilst the tumour within the bladder cavity appeared necrotic there had been a massive spread to involve the wall of the bladder *in toto*, with additional extension to the para-aortic lymph nodes. Histology confirmed the replacement of the bladder wall by pleomorphic epidermoid carcinoma and only occasional muscle bundles were seen amid the tumour. Tumour deposits were also found in the bones and liver.

One further general point may be noted. Of the 15 patients who had suffered from haematuria before treatment with pig cells, 8 had no recurrence of this symptom and this improvement was unrelated to the overall outcome noted above.

DISCUSSION

Seven of the 16 patients treated have to date obtained definite benefit. In 5 cases this was associated with injection of pig cells alone and in 2 the addition of radiotherapy was a contributory factor.

In 5 of the above 7 patients tumour necrosis was associated with marked lymphocytic and/or eosinophil infiltration and in 3 of these cases with the obvious presence of foreign body multinucleated giant cells. The nature of the latter remains in doubt, but they were also seen in all tumour biopsy fragments removed from the pig 7 days after implantation. However, they were not seen in any biopsy taken before treatment. In some cases there were in the latter category 2 biopsies taken at an interval of 3 weeks, just before treatment. Thus, it seems unlikely that the giant cells were a surgically induced artefact.

Cessation of haematuria, noted in 8 of the patients, may have been associated with vascular thrombosis which, being characteristic of the second set homograft reaction, might be expected to occur in the course of tumour rejection mediated by immunized cells.

In 2 patients, nos. 4 and 7, the tumour showed abnormal sensitivity to radiotherapy, *i.e.* total or near total regression following 4000 rad as opposed to the usual dose of 6000 rad. This may have been due to increased vascularity resulting from the host response to necrotic tissue.

This prediction was confirmed histologically in Case 4 and with this in mind Cases 9, 13, 14 and 15 are to receive radiotherapy at the reduced dose level of 4000 rad.

In the only patient with a low grade tumour (Case 10) treated with pig cells, no regression of the tumour was noted. It is possible that the more anaplastic tumours, with their less organized blood supply, are more susceptible to the vascular occlusion associated with the second-set homograft reaction.

It has been assumed that the tumour necrosis reported is due to an immune reaction mediated by the sensitized pig lymphocytes. However, there are two other possibilities. Firstly, the host immunologically competent cells, attracted to the tumour during rejection of the injected pig cells, may also react against tumour associated antigens with resultant damage to the tumour. But, in the majority of patients treated, immune responsiveness was depressed (Rees, personal communication). Secondly, injection of pig cells may have caused occlusion of the tumour blood supply due to their aggregation with consequent formation of microemboli. Further evidence on this point could be obtained by comparing the effect of non-immunized and immunized pig cells.

We are indebted to Miss Teresa Lai for expert assistance, to Mr G. W. Sweetnam and his staff for their continued advice on matters of animal welfare, and to Miss Lesley Pugsley for preparing the typescript.

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