THE PATHOLOGY OF BRACKEN-ASSOCIATED TUMOURS IN CATTLE

BY

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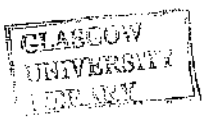

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Pauline E. McNeil, May 1984.

SUMMARY

This thesis describes the pathology of UAT carcinoma and urinary bladder neoplasia in cattle in the West of Scotland. The UAT tumours are squamous carcinomas which occur as verrucose protruberances or ulcerative downgrowths. In a survey of 100 cases, predilection sites were the tongue, soft palate and oropharynx, the oesophagus, the reticular groove and the dorsal sac of the rumen; sites where viral papillomas were also commonly found. The microscopical appearance of the tumours is detailed and a grading system is proposed, based on the degrees of cellular atypia and anaplasia, which correlates well with the incidence of secondary tumours.

Examination of 60 cases of urinary bladder neoplasia revealed vascular, epithelial or fibrous tumours. A striking feature of most cases was the variety and multiplicity of lesions in each bladder. A spectrum of changes was seen ranging from epithelial hyperplasia and dysplasia, capillary proliferation and ectasia and stromal hyperplasia to frank neoplasms which occasionally infiltrated widely and in a few cases produced secondary deposits. The bladder tumours were indistinguishable from those described by other authors in association with CEH and presumed to be due to long term ingestion of bracken fern.

A high incidence of bovine UAT carcinoma has been described by workers in Kenya, but only in Brazil has the co-incidence of UAT and urinary bladder neoplasia in cattle been reported previously. Brazilian authors attributed the occurrence of tumours at both sites to the oncogenic action of bracken fern. Epidemiological studies of cases in the West of Scotland have also implicated bracken fern as a possible aetiological agent.

Chapter II of the thesis describes a preliminary experiment in which four cattle received intra-ruminal inoculations of bovine papillomavirus and were fed bracken fern, intermittently, for up to eight years. Only minimal haematological changes were observed and

the acute toxic effects of the plant were avoided, yet three animals which survived more than five years developed haematuria and had bladder tumours at necropsy. No UAT carcinoma was found and the results do not clarify the possible role of papillomavirus in the development of bovine cancer. This work does confirm the major aetiological role of bracken fern in bovine bladder neoplasia but evidence for its role in the aetiology of UAT carcinoma remains circumstantial.

LIST OF ABBREVIATIONS

ad lib.	ad libitum; at pleasure
BPV	Bovine papilloma virus
CEH	Chronic enzootic haematuria
cf.	confer; compare
et al.	et alia; and others
GUVS	Glasgow University Veterinary School
H&E	Haematoxylin and eosin
PBS	Phosphate buffered saline
UAT	Upper alimentary tract

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THE PATHOLOGY OF BRACKEN-ASSOCIATED TUMOURS IN CATTLE

GENERAL INTRODUCTION

A high incidence of certain tumours has been found in cattle in the West of Scotland. All such geographical areas with a high incidence of disease are significant because localised aetiological agents may be identified. In the system under consideration an environmental carcinogen and an oncogenic virus have been recognised; their possible interaction as causal agents is of particular interest. Also, several distinct neoplasms are involved and the possibility of a common aetiology is important not only in this instance but also in its implications for cancer research in general.

This thesis describes the pathology of some of the tumours found in cattle in the high incidence area. It also includes preliminary experimental work designed to investigate the possible roles of bracken fern and bovine papilloma virus in the aetiology of bovine alimentary tumours.

CHAPTER 1

A HIGH INCIDENCE OF CERTAIN TUMOURS IN CATTLE

CHAPTER 1: A HIGH INCIDENCE OF CERTAIN TUMOURS IN CATTLE

INTRODUCTION

MATERIAL AND METHODS

SECTION A: SQUAMOUS CELL CARCINOMA OF THE UPPER ALIMENTARY TRACT

SECTION B: TUMOURS OF THE URINARY BLADDER

INTRODUCTION

Approximately 300 cattle are submitted for clinical and pathological examination at Glasgow University Veterinary School (G.U.V.S.) every year. Over a number of years, it was noted that there was a high incidence of tumours in cattle from upland regions of the West of Scotland. In particular, tumours of the upper alimentary tract (i.e. mouth, pharynx, oesophagus and forestomachs) and of the urinary bladder appeared to be prevalent. Consequently, an intensive investigation was begun and a detailed pathological study of numerous animals was made in an attempt to establish the type and relative incidence of tumours found and to select animals for retrospective epidemiological studies.

MATERIALS AND METHODS

Veterinarians in general practice in the West of Scotland were contacted, the interest in bovine tumours was explained and their co-operation was requested. Subsequently, individual animals with signs suggestive of neoplasia were referred to G.U.V.S. by those practitioners and were subjected to clinical examination by members of the medicine department and to full post-mortem examination as detailed below. In addition, any animals culled from farms in the area of high incidence were acquired for necropsy whenever feasible.

Other bovine cases of cancer of the upper alimentary tract (UAT) or urinary bladder were found by reviewing the pathology files of G.U.V.S. Cases selected were those derived from farms in the West of Scotland and with detailed descriptions of the pathological findings.

Animals available for necropsy were examined as soon after death as possible. Live animals were killed by exsanguination, via incisions in the great vessels of the neck and anterior thorax, after stunning by captive-bolt pistol and destruction of the medulla oblongata and cervical cord by a flexible pithing rod. The necropsy routine involved macroscopic examination of all major organs and detailed examination of the whole alimentary tract. Representative samples were taken from any lesions found. Tissues for histological examination were treated by standard processing and staining techniques. Blocks were fixed in 10% neutral-buffered formol saline then trimmed and post-fixed in formol-sublimate. Dehydration was effected by passage through a series of alcohols and tissue blocks were then cleared in xylene and embedded in paraffin wax. Sections were cut at 4µm and stained routinely with haematoxylin and eosin (H&E). Special stains were employed for individual sections as required:

Alcian blue (pH 1.0 and pH 2.5) for mucins;
Carbol-chromotrope for eosinophils;
Gordon and Sweet's silver impregnation method for reticulin;
Gram-Twort method for bacteria;
Heidenhain's haematoxylin for prickle cells;
Mallory's or Masson's trichrome methods for connective tissue;
Periodic-acid Schiff method (PAS) for mucus and basement membranes and, with amylase treatment, for glycogen;
Toluidene blue for mast cells;
van Gieson's method for connective tissue;
Verhoeff's method for elastic tissue.

Culling, Handbook of Histopathological Techniques, 1976.
Disbrey and Rack, Histological Laboratory Methods, 1970.

SECTION A: SQUAMOUS CELL CARCINOMA OF THE UPPER ALIMENTARY TRACT

Introduction and Review of the Literature

Results

Macroscopic appearances of tumours

Microscopic appearances of tumours

Discussion

SECTION A. SQUAMOUS CELL CARCINOMA OF THE UPPER ALIMENTARY TRACT.

Introduction and Review of the Literature.

It has been found that the West of Scotland has a high incidence of UAT carcinoma in cattle (Jarrett, 1973). On individual farms this incidence has been as high as 10% in a two year period (Grimshaw, unpublished results). Two other geographical areas with a high incidence of squamous cell carcinoma of the UAT in cattle have been reported previously.

In 1955, Plowright described 3 cases of carcinoma in cattle from the Nasampolai valley, a small locality of the Masai reserve in Kenya. At that time, it was suspected that the animal mortality due to UAT carcinoma might be as high at 10%. By 1971 a minimal incidence rate of 2.5% had been established for ruminal cancer of cattle in that locality (Plowright et al., 1971); three of the 18 cases described had squamous cell carcinoma of the oesophagus in addition to carcinoma of the rumen.

Dobereiner et al., (1967) described a high incidence area of UAT carcinoma in cattle in the Brazilian Highlands. Cases of squamous cell carcinoma of the pharynx, of the oesophagus or of the rumen were recorded in 21 animals, 10 of which were subjected to full post-mortem examination by the authors. Those authors also quoted Curiel (1964) who published data on 9 cases of bladder neoplasia in cattle from Parana State, Brazil; six of those animals had cancerous or precancerous lesions in the pharynx. In 1969, Tokarnia et al., gave details of a further 23 cases of UAT carcinoma from the State of Santa Catarina; of a total 44 cattle, 22 had carcinoma of the pharynx, 4 were said to have precancerous pharyngeal lesions, 26 had carcinoma of the oesophagus and 11 had carcinoma of the rumen. The Botucatu region of São Paulo State was studied by Neto et al., (1975) by means of a questionnaire.

The histopathological lesions found in 17 cases of UAT carcinoma were recorded; oropharyngeal cancer was present in 10 animals and the cardia was involved in 9 animals.

Outwith Kenya and Brazil, squamous cell carcinoma of the UAT in cattle is regarded as a rarity. Collections of tumours in cattle (Davis et al., 1933; Monlux et al., 1956; Plummer, 1956; Cotchin, 1960; Brandly and Migaki, 1963; Misdorp, 1967; Anderson et al., 1969; Vitovec, 1976; Dukes et al., 1982) generally included only a few, if any, tumours of this type. In particular, a large abattoir survey of tumours in British domestic animals revealed only one carcinoma of the upper alimentary tract in 302 tumours from a population of 1.3 million slaughtered cattle (Anderson et al., 1969).

Smith and Jones (1966) compiled a table of neoplasms in domestic animals from results published by various authors from 1936 onwards. They arrived at a total of 1371 bovine tumours which included 2 unspecified carcinomas of the mouth, one squamous cell carcinoma of the oesophagus and one squamous cell carcinoma of the rumen.

The general view that carcinoma of the UAT in cattle is uncommon has not been altered by reports of individual cases. Wood et al., (1957) recorded one case of squamous cell carcinoma of the rumen and Pirie (1973) recorded the "unusual occurrence of squamous carcinoma of the upper alimentary tract in cattle in Britain". The cases described by these authors were derived from the high incidence area in the West of Scotland and those that underwent full pathological examination at G.U.V.S. are included in this thesis.

However, Feldman (1932) reviewed the earlier literature on neoplasms of domestic animals and although he included only 15 well-documented cases of UAT carcinoma in cattle, he noted that the majority of carcinomas were observed in older animals and cited Trotter's survey as an example. Trotter (1911) examined 300 cattle

with malignant disease of which 297 were aged animals largely derived from Ireland with some from the Scottish Highlands. Twenty-five cases of ruminal tumour were recorded in that series, an exceptional incidence which may be the earliest indication of the type of situation that this thesis describes.

Results

A series of 100 cattle with squamous cell carcinoma of the UAT was compiled. Ninety-six of those animals were examined post mortem in the pathology department of GUVS; 49 were destroyed immediately before examination, 24 died and in 23 cases the manner of death was not recorded. Four cases were submitted as pathological specimens for histological examination.

The distribution of lesions in 100 cases is shown in Table 1. The predilection sites of UAT squamous carcinoma proved to be: the lateral areas of the dorsum of the tongue; the soft palate; the oropharynx; the oesophagus; the reticular groove of the rumen including the cardia; and the dorsal sac of the rumen (see Figs. 2 to 11). Tumours occurred in variable numbers at these sites and in over half the cases (59) more than one site was affected. The organ most frequently affected was the oesophagus, closely followed by the rumen and groove; in fact, 96 of the cases involved the oesophagus, groove or rumen, either singly or in combination with each other. Forty-one cases had involvement of one site only and again most of these solitary tumours were found in the oesophagus or rumen. The typical location of ruminal lesions was dorsal and caudal to the reticular groove in the adjacent non-papillated, non-pigmented region (Figs. 7,9); only rarely were lesions found on the ruminal pillars (Fig. 10) or involving the ventral sac. Only 27 animals had oropharyngeal cancer and tumours of the palate and pharynx were only found along with involvement of one or more of the other sites.

Metastases were recorded in 40 cases of UAT carcinoma; the findings are summarised in Table 2. The liver was affected more frequently than the lungs and the kidneys and heart were only rarely involved. Half of the cases involved only the regional lymph nodes (Fig. 12) and only two animals had metastases without lymph node involvement; these both had large ruminal lesions with spread to the liver, possibly by direct extension. Transcoelomic spread was present in a number of the animals with large ruminal

lesions (Figs. 13, 33) and involvement of contiguous tissue at various sites by direct infiltration was notable in 9 cases.

Co-existent papillomas of the UAT, bladder tumours and proliferative intestinal lesions were found in many of the cases. The occurrence and significance of UAT papillomas in cattle has been described elsewhere (see Jarrett et al., 1978; Jarrett, 1981) but it is pertinent to note that the papillomas occurred at the same predilection sites as the carcinomas. The numbers and types of bladder tumours found are detailed in Section B of this chapter. Intestinal lesions were found in 56 animals with UAT carcinomas and included adenomatous plaques and polyps and occasional adenocarcinomas (Figs 14, 15). These lesions are to be described in detail elsewhere. Table 3 lists the various neoplasms found in addition to UAT carcinoma in 100 cattle.

The recorded age of cattle with UAT carcinoma ranged from 6 years to 18 years and the distribution is illustrated in Figure 1. The peak incidence appeared to be at the age of 12 years although age was not recorded in 6 cases and 23 animals were designated "aged" that is, they were known to be over 8 years of age. In all, 97% (91/94) were known to be 8 years or older.

All the major beef breeds and crosses were represented and the distribution is given in Table 4. The total is greater than the number of cases because the breed of both dam and sire was recorded in a few instances. Most of the animals appeared to be first generation crosses rather than pure bred, particularly Aberdeen Angus or Highland crosses; this is a reflection of the type of farm from which most of the cases were derived: hill farms where hardy beef-type cows with a reasonable milk yield are retained to raise calves year after year. The management practices also dictate that only female calves are kept into middle or old age so it is not surprising that no males are included in this series.

Macroscopic appearances of tumours

The squamous carcinomas found in 100 cattle can be broadly divided into two types: (a) cauliflower-like, fungating masses ranging in size from less than 1.0cm to over 15.0cms in diameter (Figs. 3,8,17) and (b) ulcerative plaques which range from less than 1.0cm to almost 50.0cms diameter (Figs. 9,10,11,18). Many lesions infiltrated widely and provoked a scirrhous reaction so that the borders of a tumour were detected as fibrous thickening within the affected tissues. Necrosis was invariably associated with established lesions so that the nodular tumours had a friable surface from which foul-smelling debris was easily detached and the plaques often had crateriform centres. Section of lesions characteristically revealed a scirrhous, almost hyaline, white stroma with scattered yellow foci of keratinisation or necrosis (Fig. 12).

Oral and pharyngeal tumours were commonly excavating lesions with extensive destruction of local tissues (Fig. 2). Many of the oesophageal lesions were early cancers appearing either as small ulcers with brown crateriform centres and raised, rolled edges or as papillomatous nodules (Figs. 5,35,36). Larger lesions caused marked distortion of the organ (Fig. 4) and highly scirrhous tumours produced stricture with functional consequences (Fig. 6). The largest lesions were found in the rumen where extensive plaques were located in the dorsal sac (Fig. 9) and were frequently associated with local adhesive peritonitis. Early cancers or benign papillomas often occurred as satellite lesions scattered around the larger tumours (Fig. 4) and were occasionally closely associated with the base of a large mass (Fig. 8) or the margins of a crater.

There appeared to be a relationship between the size of lesions and the incidence of metastasis so the main tumours of each case were divided into five groups on the basis of their longest diameter; the results are summarised in Table 5. Lesions of less than 2.0cms diameter were considered to be early carcinomas and no

metastases were found in cases with tumours of this type. Metastasis occurred in approximately one fifth of cases with tumours that were over 2.0cms but under 10.0cms in size whereas more than half of the cases with tumours of between 10.0 and 20.0cms had metastases and those with large tumours of over 20.0cms diameter had a metastasis rate of 77%.

Microscopic appearances of tumours

Most of the tumours were composed of easily-recognised squamous cells arranged in large or small nests, columns or cords (Fig. 16). Keratinisation was a prominent feature of most lesions either as so-called pearls, i.e. concentric arrangements of flattened cells around a small area of keratin; or as moderate to large masses of amorphous material that appeared brightly eosinophilic by routine staining methods (Figs. 16,17,18,22,23). All layers of normal, stratified squamous epithelium were recognised and tumour cells could be divided into three main classes; small basal cell type, larger squamoid cells and flattened keratinising elements (Figs. 20,21). Some tumours contained representatives of all layers, others were predominantly of one type and some were a mixture of two types.

Tumours of basal cell type were characterised by large numbers of small, polygonal or fusiform cells arranged in small nests or narrow cords. The nuclei were ovoid with scattered chromatin and the cell border was often distinct with prominent intercellular bridges. The typical squamous cell type of lesion contained larger cells with more open nuclei but paradoxically the prickles were often rather less prominent (See Figs. 27, 28). Keratinisation was a characteristic feature of this type of lesion but it also occurred sporadically in the basal cell type. The third type of lesion in which flattened keratinising elements predominated, was typified by large areas of keratin surrounded by a narrow rim of cells which, in some cases, was difficult to distinguish from the surrounding stroma and occasionally was absent due to apparent pressure necrosis and destruction by foreign-body

giant cells (Figs. 23,25). Central necrosis of the smaller keratin masses in these lesions often imparted a pseudoglandular appearance (Fig. 24).

A degree of inflammation was always present in the tissues surrounding the tumours; this was usually represented by scattered lymphoid and plasma cells (Figs. 16,23,31) but occasionally consisted of a heavy infiltrate of mononuclear cells together with polymorph and eosinophil leucocytes. There was an associated fibroplastic stromal reaction in most cases and this was often sufficiently marked to justify the designation scirrhous carcinoma (Fig. 19).

The degree of differentiation of the tumours was assessed as good, moderate or poor by preliminary examination. However, as the study progressed, the growths with intermediate morphology were further subdivided and a final histological grading system was devised based on four major divisions.

Grade 1 was restricted to those tumours with well-differentiated, well-ordered epithelium, closely resembling the normal, stratified squamous epithelium of the bovine UAT. All layers were represented, there were only moderate numbers of mitotic figures, most of which appeared to be normal and there was little or no cellular or nuclear pleomorphism apart from that which occurs as part of the normal process of differentiation (Fig. 27).

Grade II tumours were less well-differentiated with some pleomorphism and, in particular, loss of polarity. There were increased numbers of mitoses but still less than 5 per high power field and giant cells were rarely seen (Fig. 28). Tumours in this group were often predominantly basal cell type or squamous cell type; it should be noted that the frequency of mitoses tends to be higher per high power field in basal cell type since the cells are smaller and, if true to type, are more likely to be active.

Grade III tumours were pleomorphic with poor organisation of the cells so that there was little resemblance to normal epithelium although the cells were recognisably squamous (Fig. 29). Prickles were not conspicuous although they were frequently present. The mitotic rate was high with over 6 mitoses per high power field and many abnormal figures. Some uninucleate giant cells were present.

Grade IV was reserved for poorly-differentiated tumours, often difficult to recognise as being of squamous cell origin. The cells were fusiform, polygonal or rounded and prickles were difficult to find. Nuclei were often large and markedly pleomorphic with prominent nucleoli (Fig. 30). There were frequent mitoses, most of which were abnormal, and giant cells were a common finding (Fig. 31).

The presence or absence of keratin was not used as a differential feature in determining the grade of a given tumour but keratinisation was common in Grade I and frequent in Grade II. Keratinisation occurred in many of the Grade III tumours but was frequently parakeratotic or dyskeratotic with keratinistion of individual cells (Fig 29). Grade IV tumours contained little or no keratin.

Grading was based on the overall appearance of the major lesion in each case. Five of 100 cases were not graded because the histological sections were either not available for review or were not considered to be representative of the described lesions. A further two cases showed such variable differentiation on all the sections examined that it would have been unrealistic to try to categorise them. Table 6 indicates the distribution of 100 cases according to histological grade and the number in each category with metastases.

Twelve cases were considered to contain only early carcinomas in that neoplastic cells were limited to the epithelium or immediately subjacent connective tissue (see Figs. 34,35); two such cases showed no evidence of invasion beyond the basement

membrane and were thought to represent true intraepithelial carcinomas or carcinoma in situ. One of these cases had lesions in the palate, oesophagus, groove and rumen, the largest of which were described as ulcers approximately 3.0 x 2.0cms in area. Microscopy revealed epithelial dysplasia with attenuation, branching of epithelial pegs and loss of polarity, adjacent to true carcinoma with intraepithelial pearl formation. The degree of anaplasia was roughly equivalent to Grade II as described for invasive lesions. There was very little keratinisation, no fibroplastic reaction and only a few scattered mononuclear cells beneath the lesion. The other case contained an ulcer of 1.0cm diameter in the oesophagus. Microscopy revealed elongation, broadening and branching of the epithelial pegs with intraepithelial pearl formation. This lesion was encroaching on the subepithelial connective tissue but the basement membrane appeared to be intact on the sections examined. There was no scirrhous reaction and a negligible inflammatory response.

Thirty cases of squamous cell carcinoma were ascribed to the histological Grade I; of these, 6 were early lesions and 9 showed metastasis to other organs. Grade II tumours comprised 31 cases of which 4 were early lesions and 8 showed metastases. Sixteen (73%) of 22 Grade II tumours had metastasised whereas only one of 8 Grade IV tumours had not. The microscopic appearance of secondary tumours mimicked the primary in most cases (Fig. 32) although there was occasionally more keratin or less keratin, a more marked or less marked scirrhous reaction and areas of better or poorer differentiation.

The scirrhous reaction associated with the squamous carcinomas varied from mild to marked but there was no obvious relationship between the amount or density of the connective tissue and the site, size or histological grade of the lesion.

The presence or absence of necrosis or inflammation in a given tumour was apparently not related to its histological grade.

Necrosis was present in the majority of lesions ranging from central necrosis of keratinised cell nests to widespread areas of cell death in the larger growths. General bacterial colonisation of necrotic areas was frequently seen. Polymorph and eosinophil leucocytes were usually found in greater numbers near areas of superficial necrosis and ulceration and some oropharyngeal lesions were associated with a heavy infiltrate of mononuclear cells, particularly lymphocytes and plasma cells. A granulomatous appearance was sometimes produced by necrotic masses of keratin surrounded by mononuclear cells, polymorphs and occasional foreign-body giant cells (Fig. 25). An incidental finding in two cases was the presence of infective granulomas on the same sections as the carcinomas (Fig. 26); one in the rumen and one in the pharynx. These lesions had the characteristic appearance of actinobacillosis and special stains confirmed the presence of colonies with clubbed borders and central foci of Gram-negative coccobacilli.

As has been noted earlier, there were often multiple tumours at a given site in many of the animals included in this series. In addition, a variety of changes were found in the epithelium adjacent to major lesions. In the majority of cases there was a degree of epithelial hyperplasia with increased depth of the spinous layer often accompanied by hyperkeratosis and parakeratosis (Fig. 34). Some cases showed attenuation of the epithelium and in others there was epithelial dysplasia with hypercellularity, increased mitotic activity and loss of polarity which merged into areas of frank carcinoma. Carcinoma in situ or early invasive carcinomas were often present as satellite lesions along with papillomas or papillomatous hyperplasia. Transforming papillomas were seen in a number of cases (Fig. 36); infiltrating cords and columns of cells extending from the base of such papillomas were associated with increased numbers of mitoses, loss of polarity and pearl formation within the epithelium.

TABLE 1: Site distribution of squamous carcinoma in 100 cattle

Tongue	19
Palate	9
Pharynx	17
Oesophagus	64
Oesophageal groove	37
Rumen	59

TABLE 2: Site distribution of metastases in 40 cases of UAT carcinoma

Regional lymph nodes	38
Liver	13
Lungs	10
Kidneys	2
Heart	1

TABLE 3: Additional neoplasms found in 100 animals with UAT carcinoma

Type of tumour	Number of cases
Upper alimentary tract	
UAT papillomas	89
ruminal lipomas	1
Lower alimentary tract	
intestinal carcinoma	7
adenomatous intestinal lesions	54
Urinary system	
renal cortical adenoma	2
urinary bladder tumours	21
Genital tract	
uterine carcinoma	1
uterine leiomyoma	1
vaginal carcinoma	1
Endocrine system	
phaeochromocytoma of adrenal	5
thyroid tumour	1
Liver and Gall bladder	
hepatoma	1
gall bladder/cystic duct adenoma	3
Other	
melanoma	1

TABLE 4: Breed distribution in 100 cases of UAT carcinoma

Aberdeen Angus	22
Ayrshire	7
Blue Grey	10
Galloway	14
Hereford	3
Highland	24
Shorthorn	15
Unknown	9
TOTAL	104

TABLE 5: Size of tumour in 100 cases of squamous cell carcinoma and incidence of metastasis

Category	Number	Number with metastases	Percentage
less than 2.0cms	11	0	0
2-5cms	13	3	23
5-10cms	23	5	22
10-20cms	32	18	56
over 20cms	17	13	77
unclassified	4	1	-
TOTAL	100	40	

TABLE 6: Histological grades in 100 cases of squamous cell carcinoma and incidence of metastasis

Category	Number	Number with metastases	Percentage
Grade I	30	9	30
Grade II	31	8	26
Grade III	22	16	73
Grade IV	8	7	88
Carcinoma in situ	2	0	-
Variable differentiation	2	0	-
Unclassified	5	0	
TOTAL	100	40	

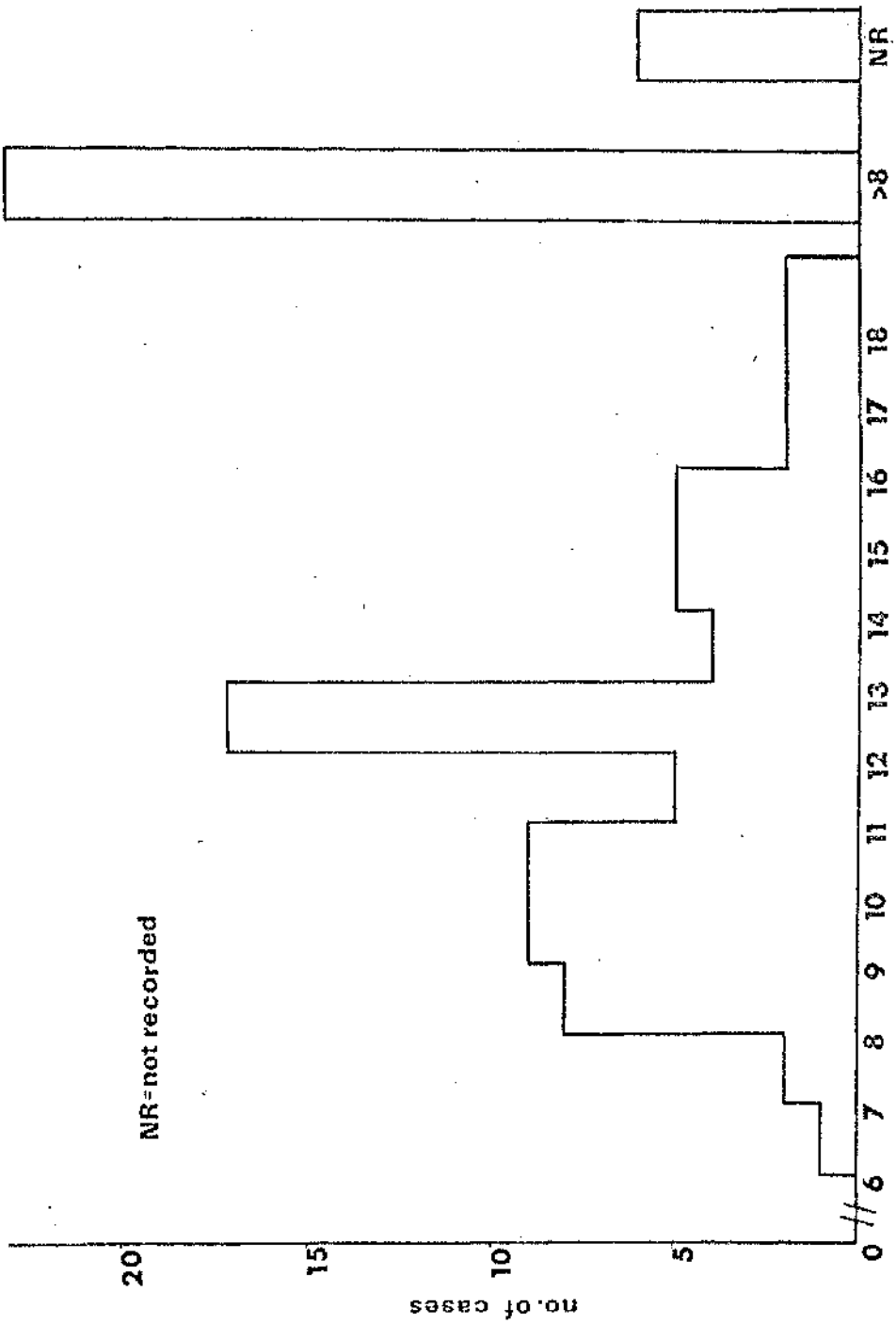


FIG. 1: Age distribution of 100 cattle with UAT carcinoma.

SECTION A: SQUAMOUS CELL CARCINOMA OF THE UPPER ALIMENTARY TRACT

FIGURES 2-13: Macroscopic appearances of tumours

FIG. 2: Squamous cell carcinoma of the tongue. An excavating ulcer with a thickened margin (arrow) is present on the left lateral aspect of the dorsum.

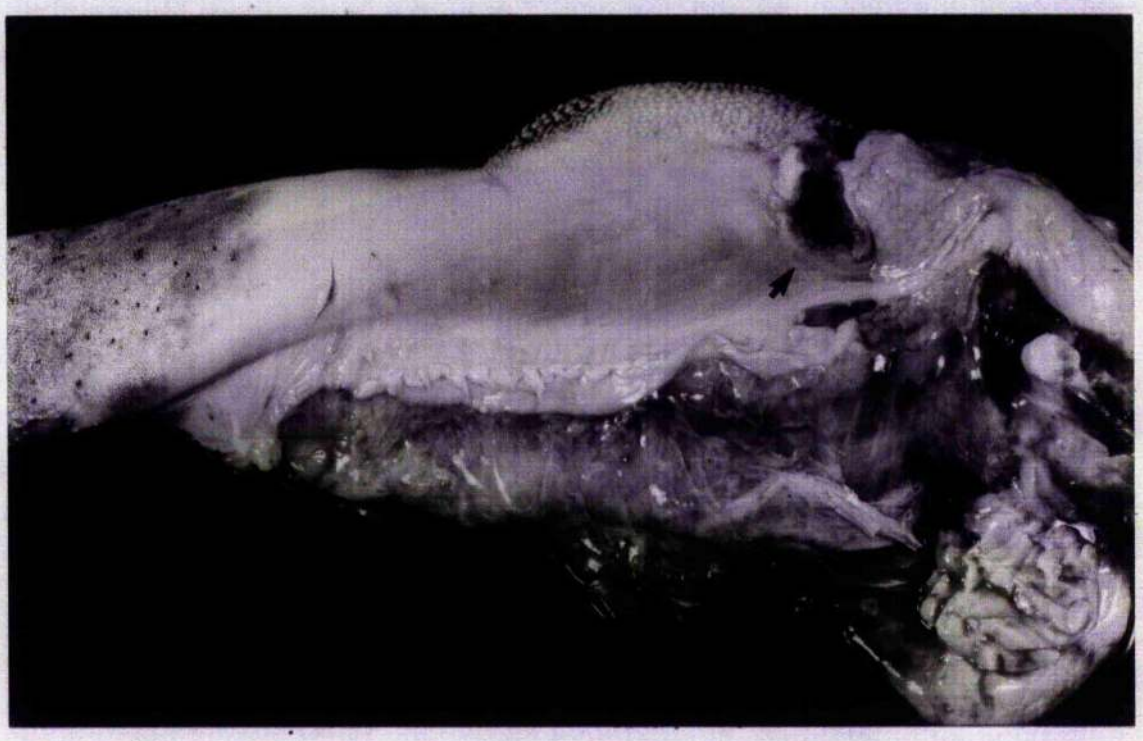


FIG. 3: Squamous cell carcinoma of the oropharynx. A nodular mass extends to involve the margin of the soft palate and the root of the tongue (centre field). Superficial, irregular ulcers (arrows) indicate the extent of the infiltration.

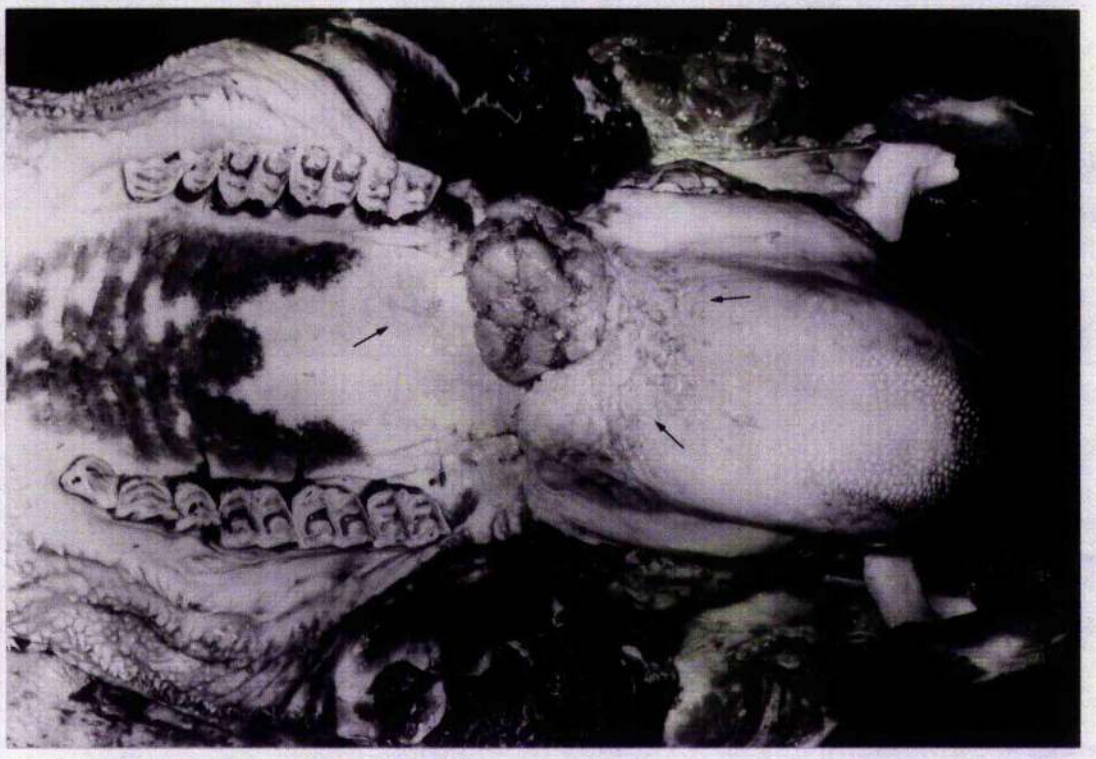


FIG. 4: Squamous cell carcinoma of the oesophagus. Multiple tumour masses have distorted the mucosa with nodules of infiltrative cancer producing a crab-like appearance (arrow). Papillomatous nodules and raised ulcerative lesions are also present. The intervening epithelium appears smooth and folded or roughened and granular.

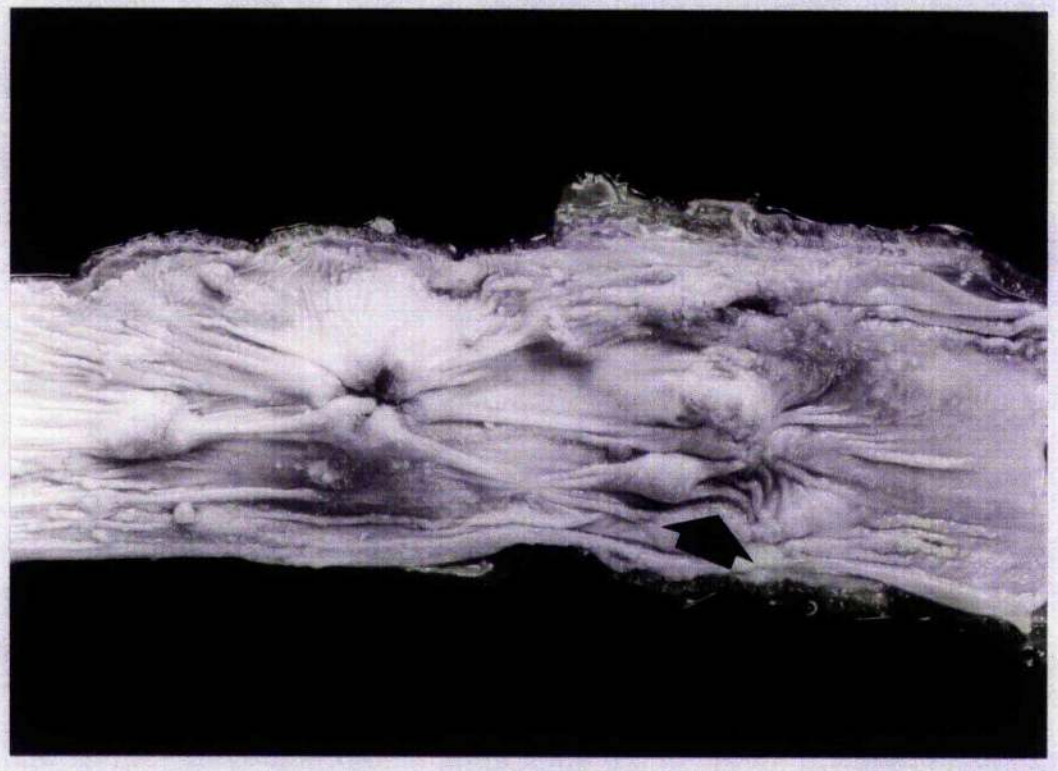


FIG. 5: Multiple oesophageal tumours. The mucosa shows a variety of lesions including early carcinoma (long arrow), transforming papilloma (short arrow) and simple papilloma (arrow head).

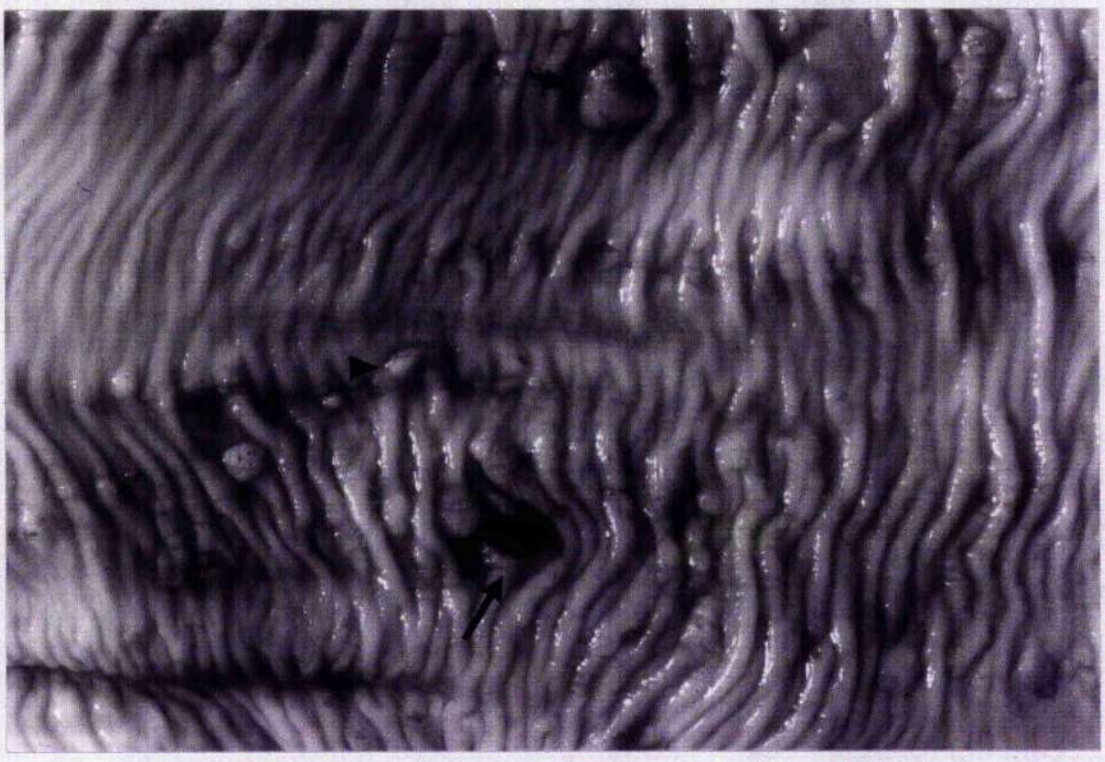
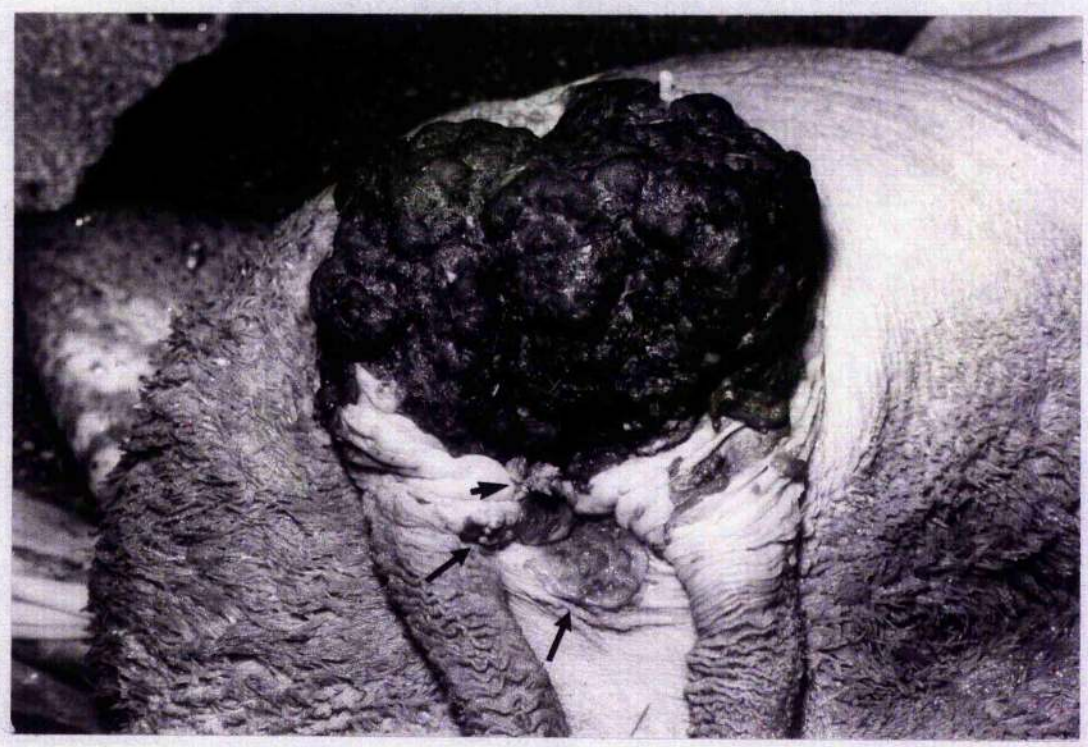


FIG. 6: Squamous cell carcinoma of the oesophagus with stenosis. A nodular and infiltrating tumour in the distal third of the oesophagus has induced contracture of the underlying tissues (arrow).

FIG. 7: Squamous cell carcinoma of the cardia, reticular groove and rumen. An irregular plaque of tumour can be seen in the dorsal non-pigmented region and nodular masses are present at the dorsal margin of the reticular groove (straight arrow) and at the cardia (curved arrow).

FIG. 8: Squamous cell carcinoma of the cardia. A large cauliflower-like mass lies at the dorsal margin of the reticular groove. Satellite lesions including papillomas (short arrow) and carcinomas (long arrows) can be seen.

FIG. 9: Squamous cell carcinoma of the rumen. A large, ulcerated plaque with thickened, rolled edges is present in the dorsal non-pigmented region.



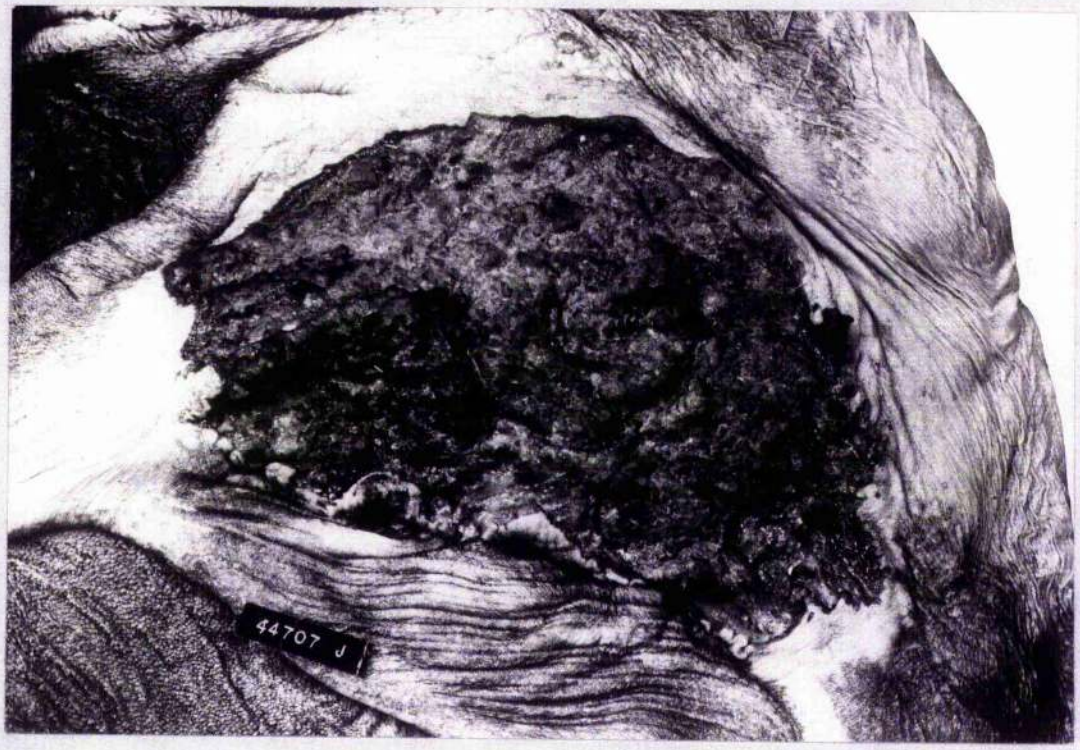

44707 J

FIG. 10: Squamous cell carcinoma of the rumen. An ulcerative plaque with thickened edges is present on the anterior pillar and extends into the ventral sac.

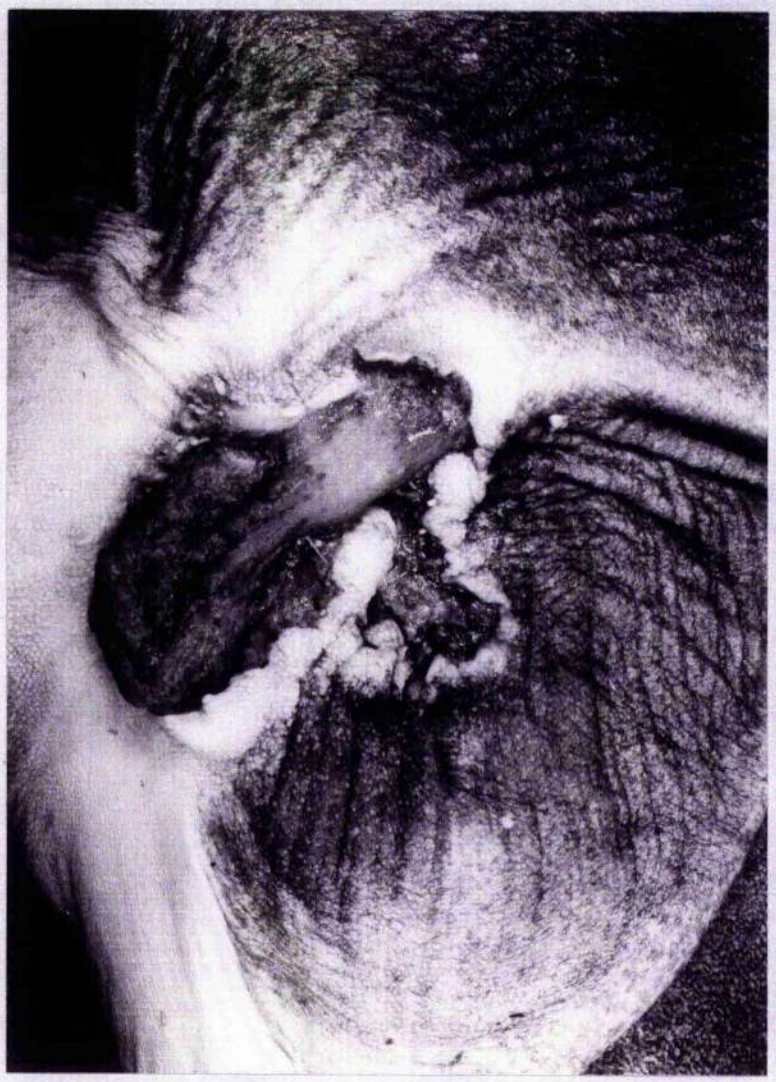


FIG. 11: Squamous cell carcinoma of the rumen. Early lesions are seen as small craters and raised, ulcerated areas within pale, featureless epithelium.

FIG. 12: Secondary tumour deposit. A granular focus of squamous cell carcinoma is present in a lymph node. Within the medulla there is a fibrous reaction to the presence of the tumour (arrow).

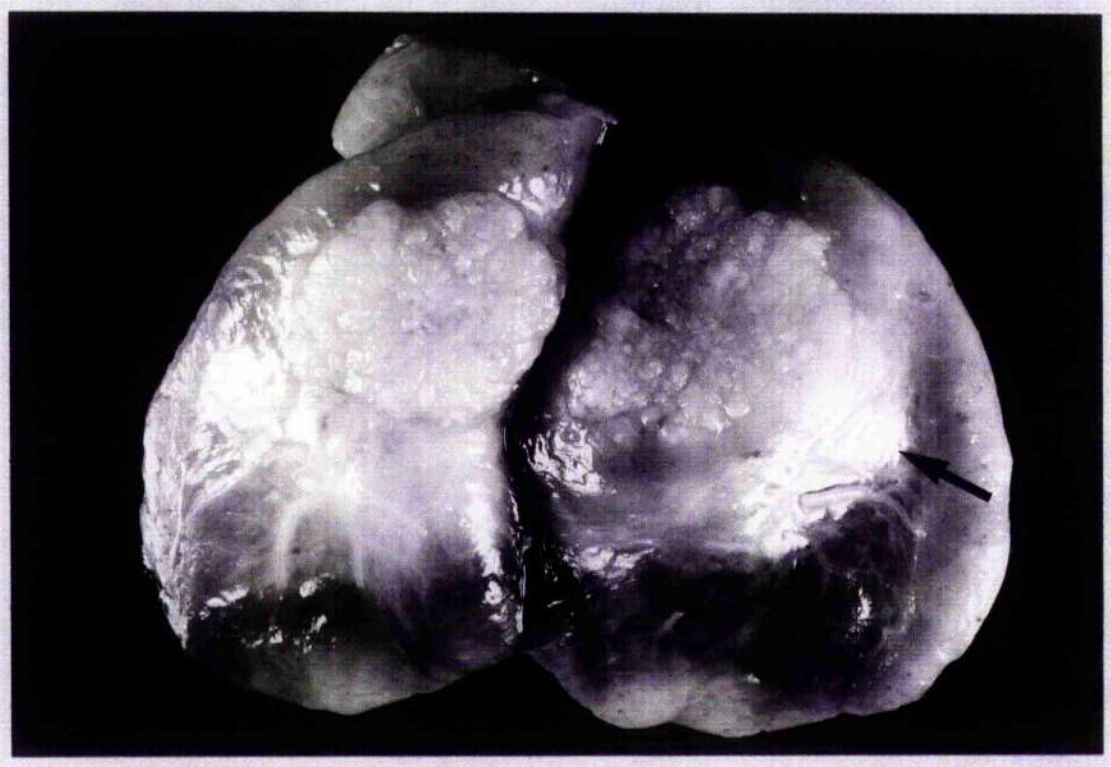
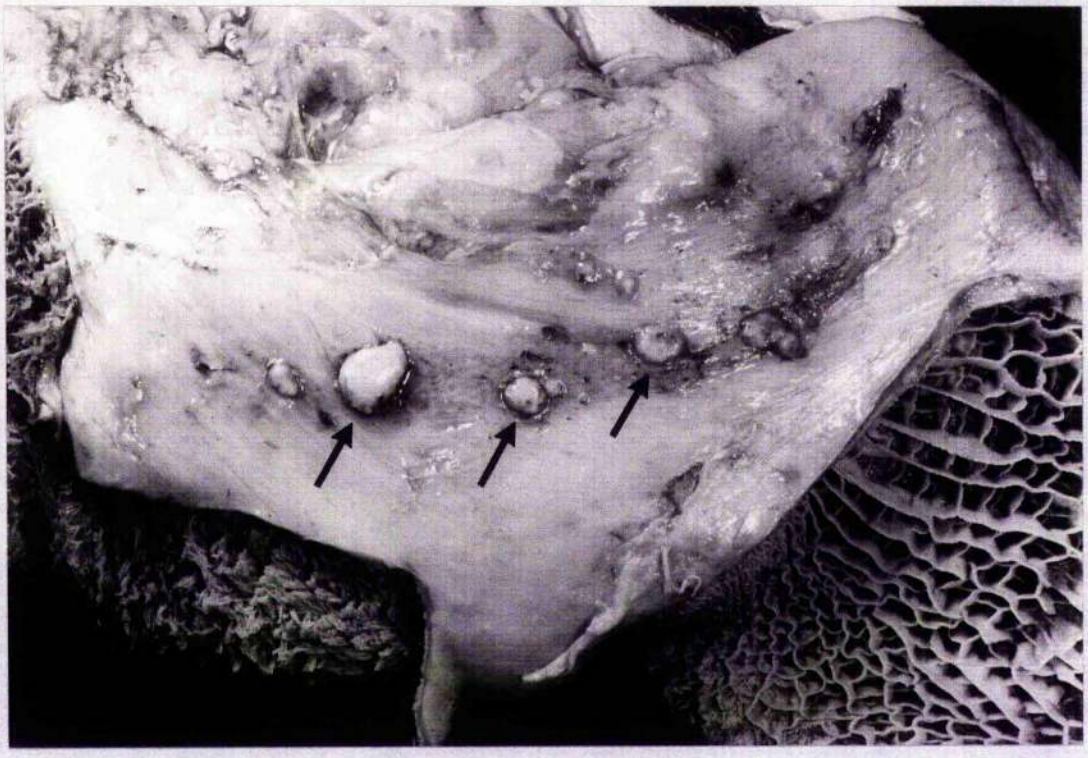


FIG. 13: Secondary tumour deposits. Nodules of squamous cell carcinoma (arrows) are present on the serosal surface of the rumen.

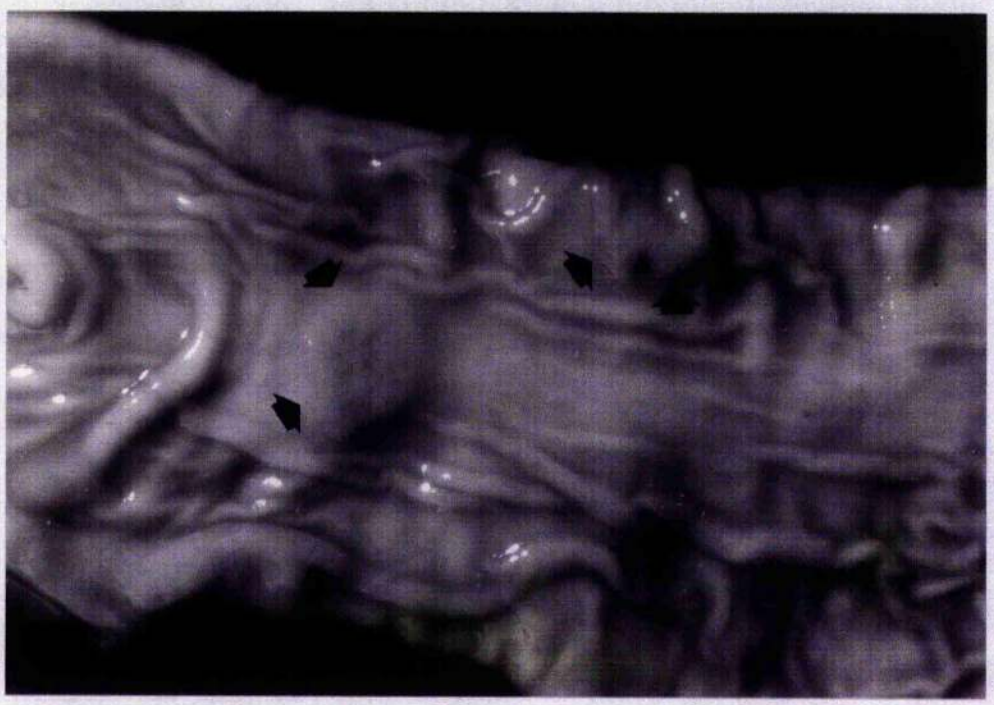


FIGURES 14 and 15: Intestinal lesions found in association with UAT carcinoma.

FIG. 14: Adenomatous lesions of the small intestine.

a) Adenomatous plaques appear as slightly raised, roughened areas (paired arrows) which are not easily distinguished from the adjacent mucosa.

b) Adenomatous polyps (arrows) project from the surface of the intestinal mucosa.



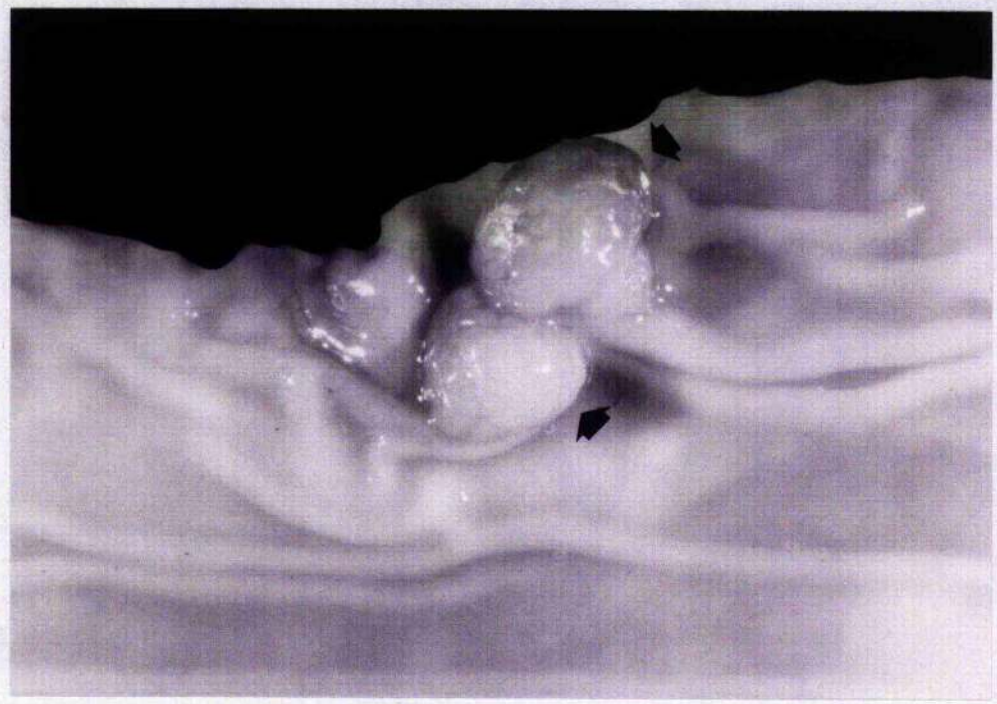
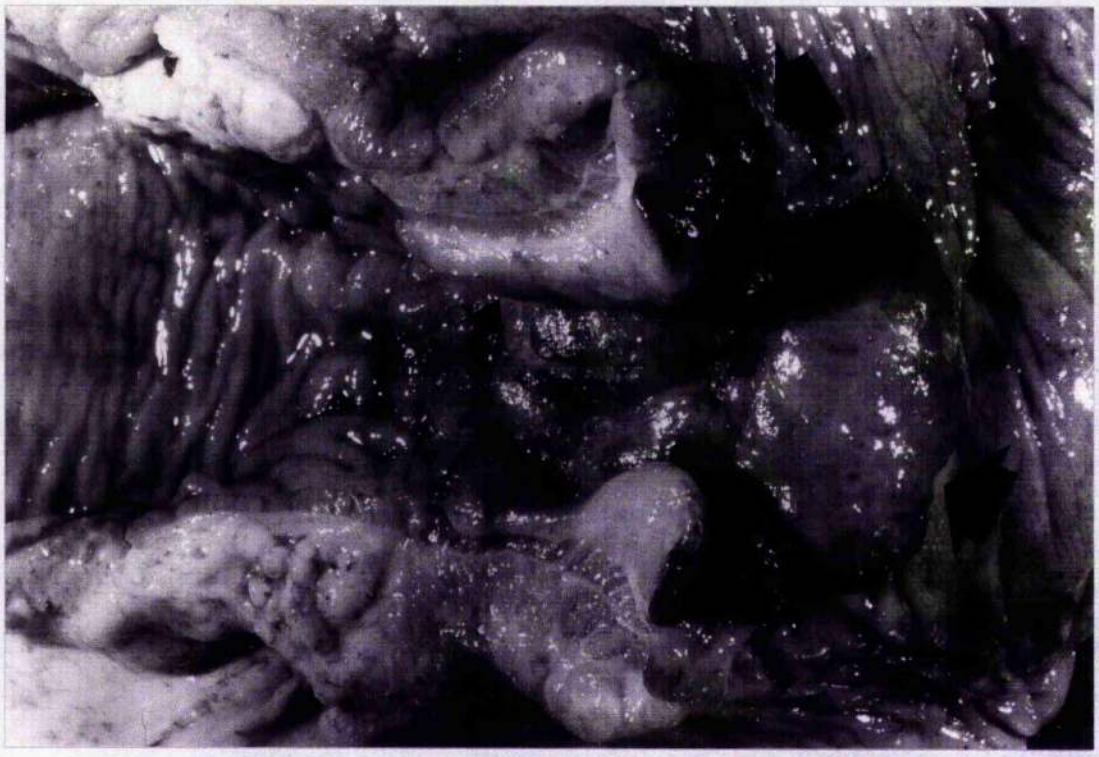


FIG. 15: Adenocarcinoma of the terminal ileum. The ileo-caecal valve is thickened and nodular masses protrude into the caecum (large arrows). The cut surface reveals thickening of the intestinal wall due to the presence of white, hyaline tissue (small arrow).



SECTION A: SQUAMOUS CELL CARCINOMA OF THE UPPER ALIMENTARY TRACT

FIGURES 16-36: Microscopic appearances of tumours.

FIG. 16: Characteristic features of squamous cell carcinoma (H&E x 110). Irregular islands of lightly stained squamous cells (sc) show central keratinisation (k). The islands of tumour cells are surrounded by a connective tissue stroma which contains scattered lymphoid and plasma cells.

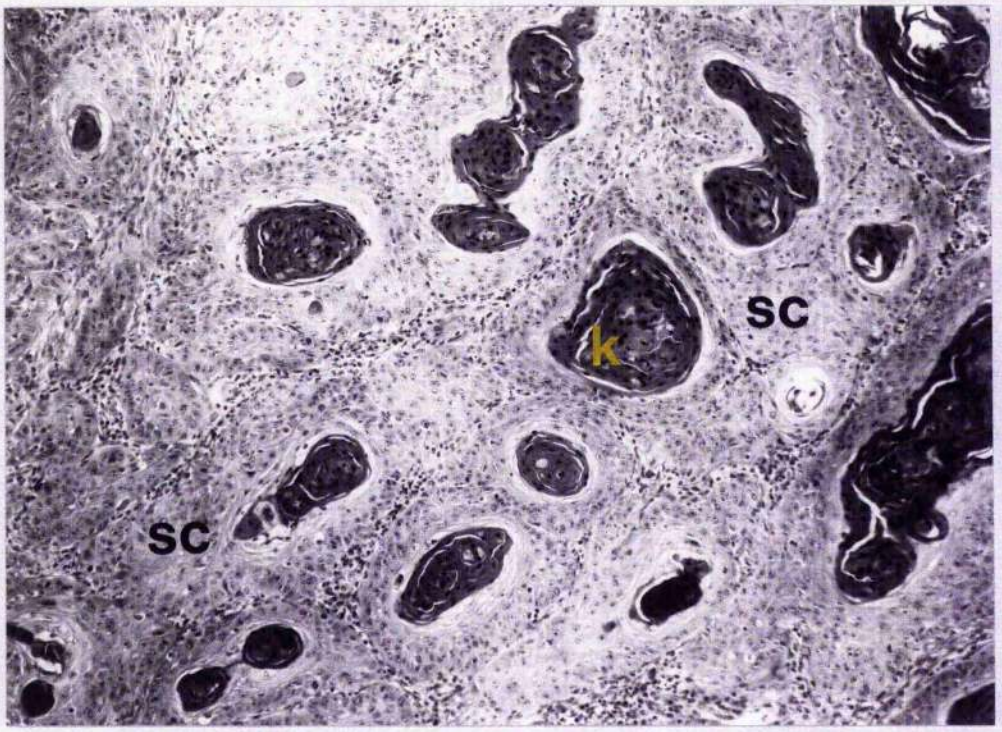
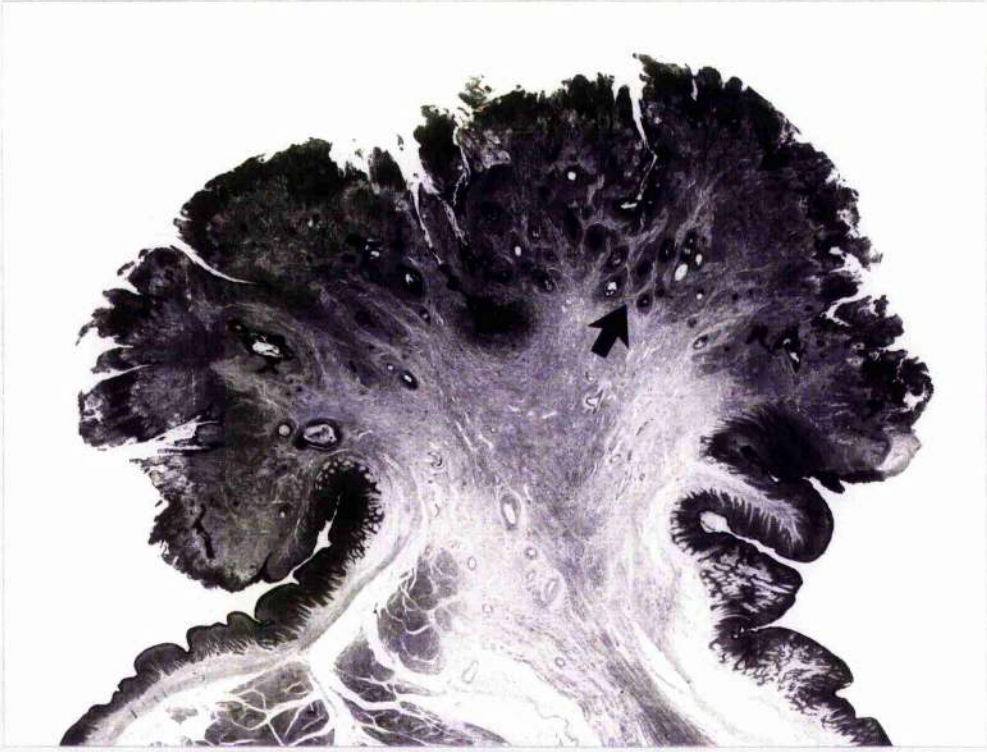

SC
k
SC

FIG. 17: Verrucose ruminal carcinoma (H&E x 6). The tumour forms a pseudopapillomatous outgrowth with transformed epithelium covering a connective tissue stalk. The surface of the lesion is hyperkeratotic and islands of squamous cells with central keratinisation extend into the underlying stroma.

FIG. 18: Ulcerative ruminal carcinoma (H&E x 15). The down-growth comprises irregular masses of darkly stained keratin surrounded by squamous cells embedded in a connective tissue stroma (arrows). Adjacent epithelium (e) is hyperplastic and the surface of the tumour is necrotic.



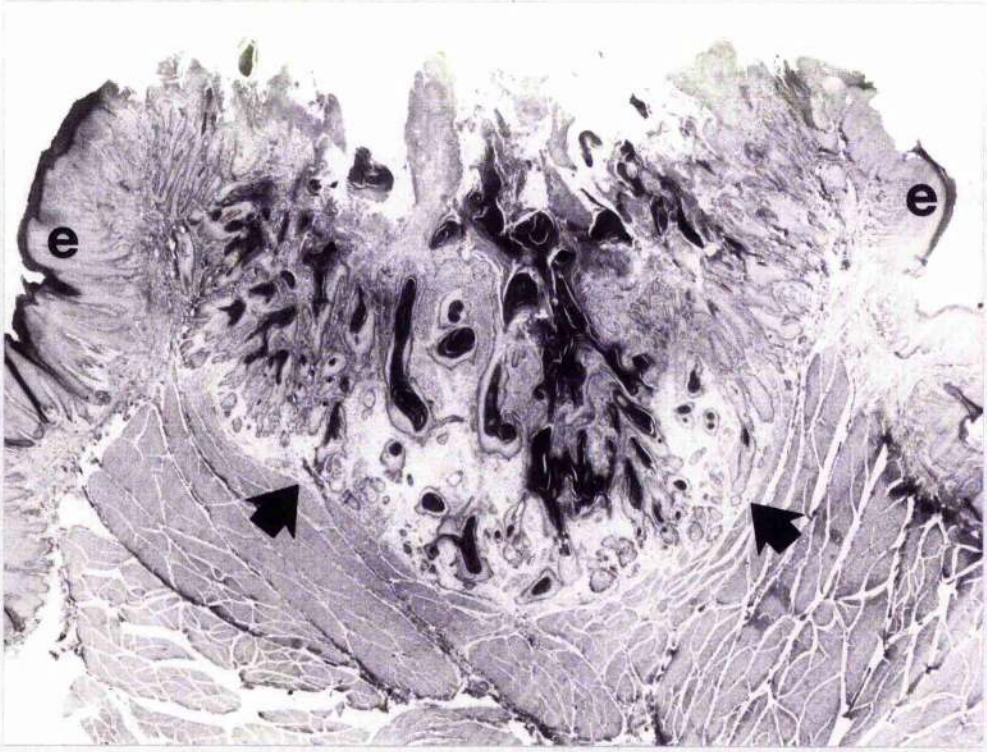

e
e

FIG. 19: Scirrhous carcinoma (H&E x 110). Small islands of squamous cells are embedded in a dense connective tissue stroma (CT).

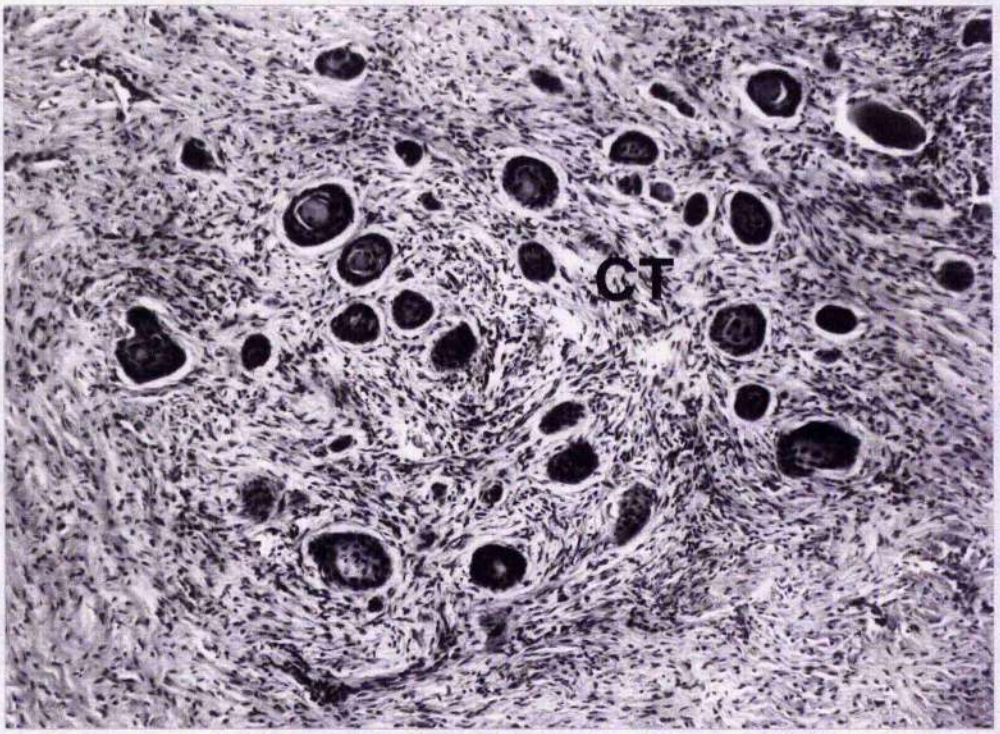
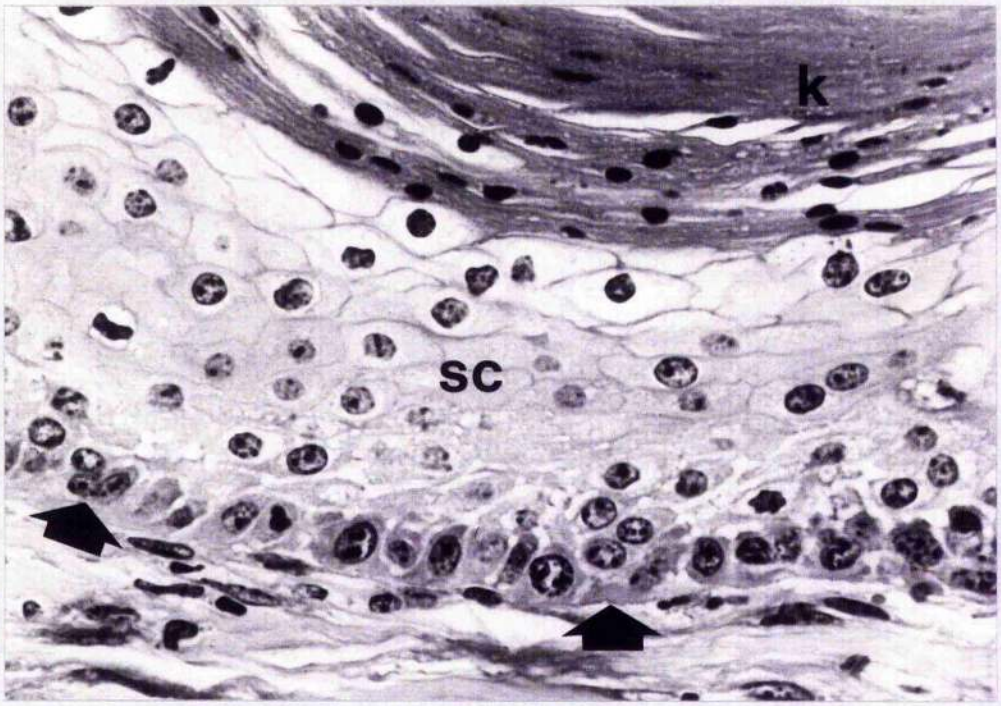

CT

FIG. 20: Cell layers in squamous cell carcinoma (H&E x 450). Basal-type cells (arrows), squamous cells (sc) and keratinised elements (k) may be seen in pleomorphic carcinomas as well as in well-differentiated tumours.

a) Well-differentiated squamous cell carcinoma (Grade I). There is orderly progression from basal-type cells through squamous cells to flattened, keratinising elements.

b) Pleomorphic squamous cell carcinoma (Grade III). Basal-type cells form an irregular but distinct layer. Flattened, keratinising elements are easily recognised although they are not clearly demarcated from the underlying squamous cells.


k
sc

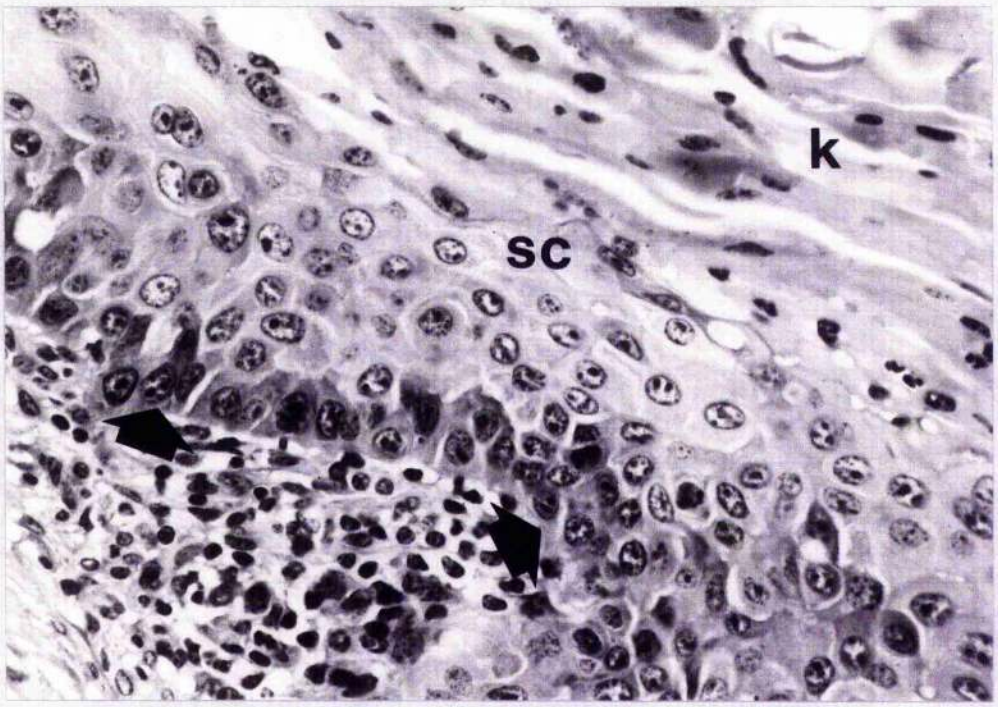
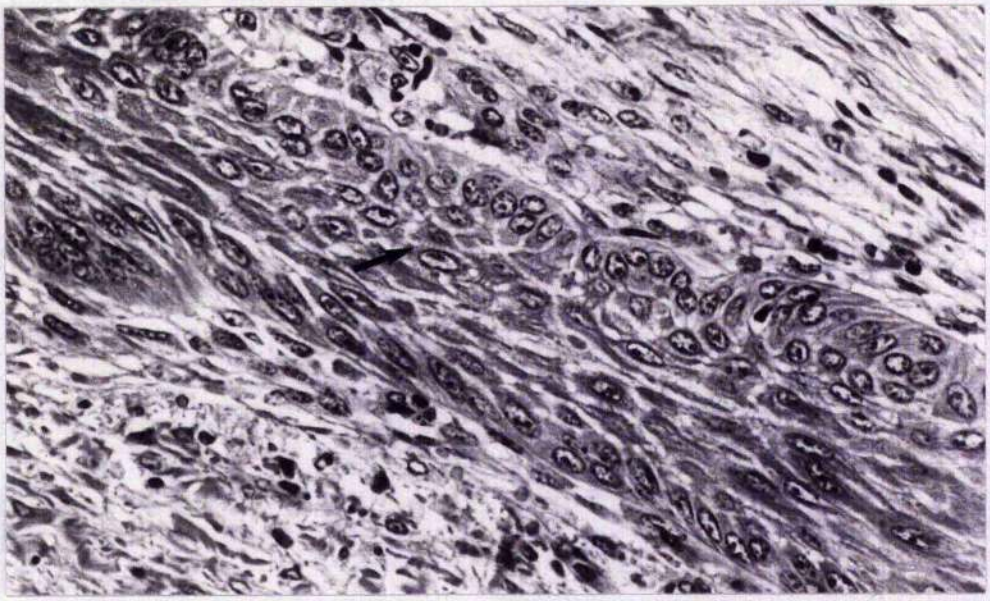

k
sc

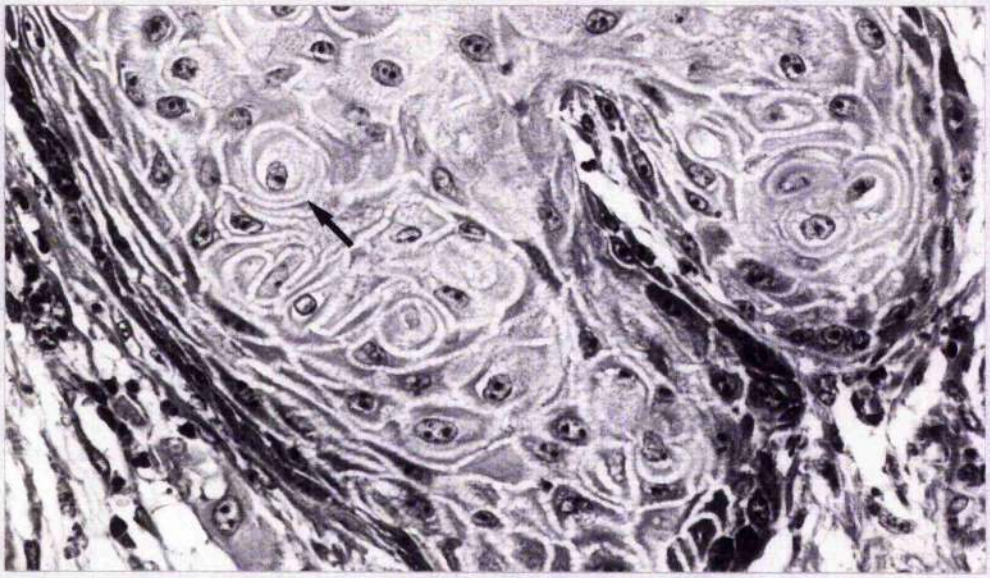
FIG. 21: Cell types in squamous cell carcinoma (H&E x 400).

a) Basal-type cells. Polygonal and fusiform cells resemble the basal layer of normal stratified squamous epithelium. The presence of prominent inter-cellular bridges (arrow) distinguishes the tumour cells from normal basal cells.

b) Squamous cells. The bulk of the tumour comprises large cells with abundant cytoplasm and open nuclei. In this case, the intercellular bridges are unusually prominent (arrow). The periphery of the cell mass is formed by a narrow rim of basal-type cells.

c) Keratinised cells. Layers of flattened keratinised elements (k) are surrounded by a narrow rim of basal-type cells. There are no large squamous cells evident.





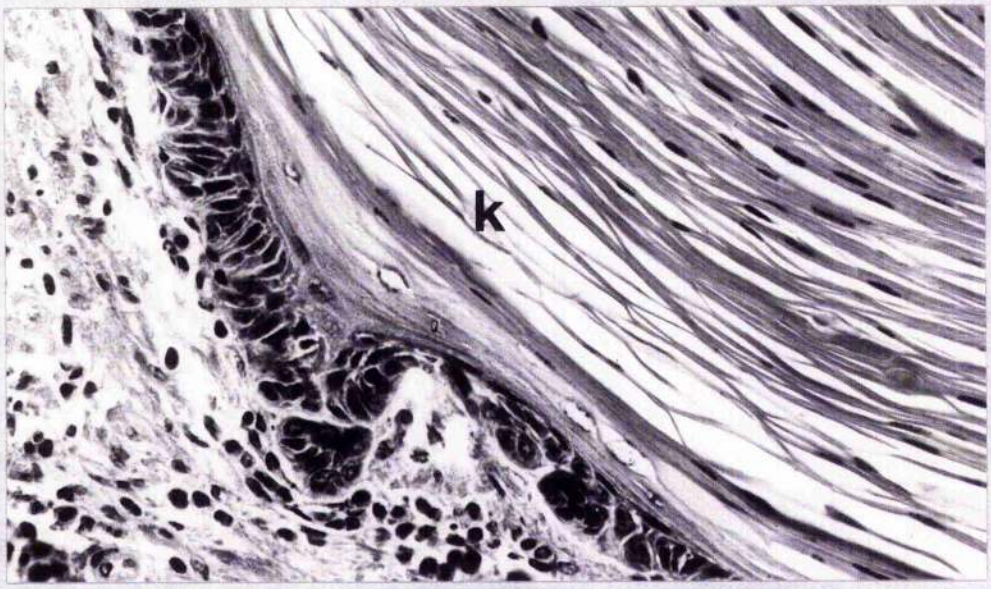

k

FIG. 22: Intraepithelial keratin "pearl" (H&E x 420). A small focus of keratin (k) is surrounded by a whorl of flattened squamous cells.

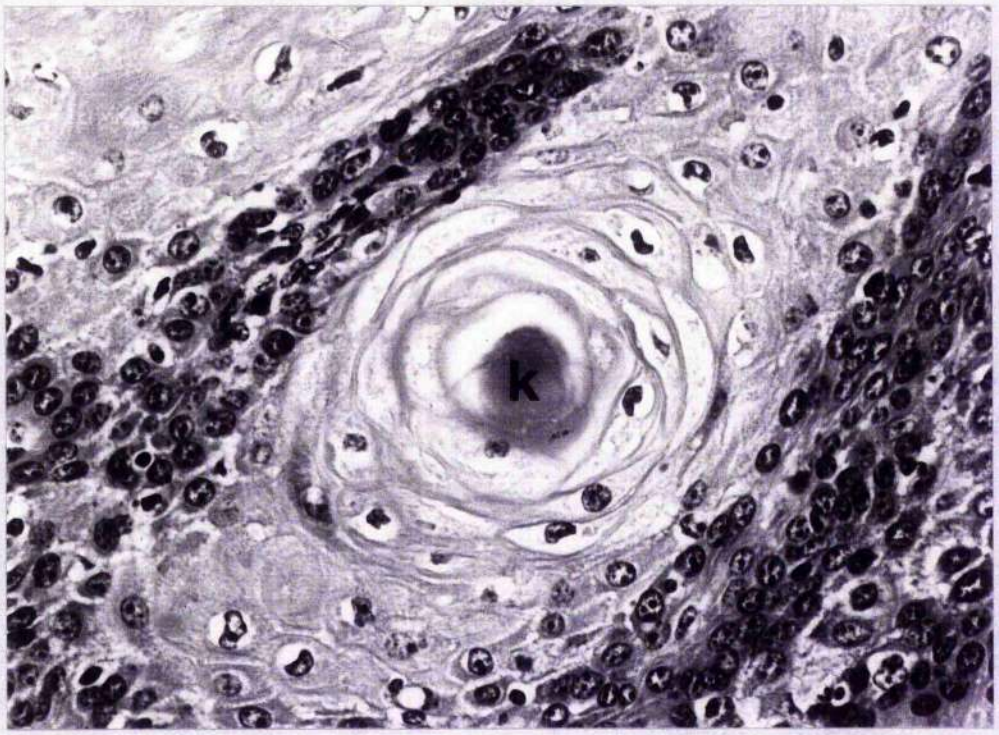
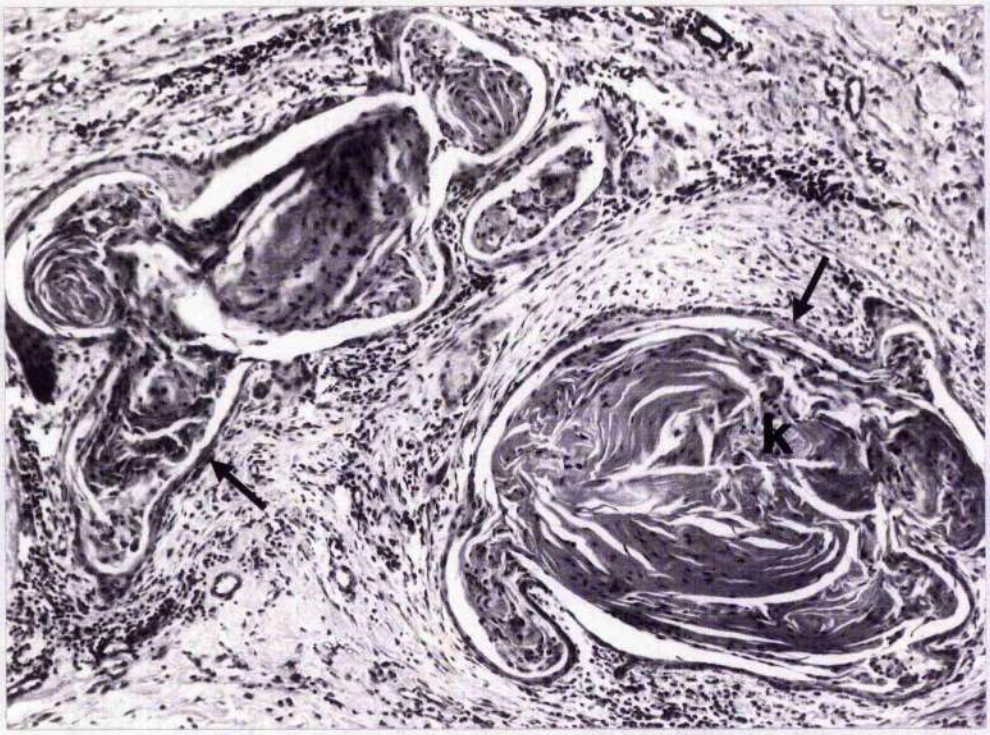

k

FIG. 23: Heavily keratinised squamous cell carcinoma (H&E x 110). Lamellated masses of keratin are surrounded by a narrow rim of basal-type cells and flattened squamous cells. The intervening fibrovascular stroma contains scattered lymphoid and plasma cells.

FIG. 24: Pseudoglandular appearance of keratinised squamous cell carcinoma (H&E x 110). Keratinisation and central necrosis of cell nests (N) imparts a pseudoglandular appearance. In contrast, true pharyngeal mucous glands can be seen adjacent to the tumour (top right).


K

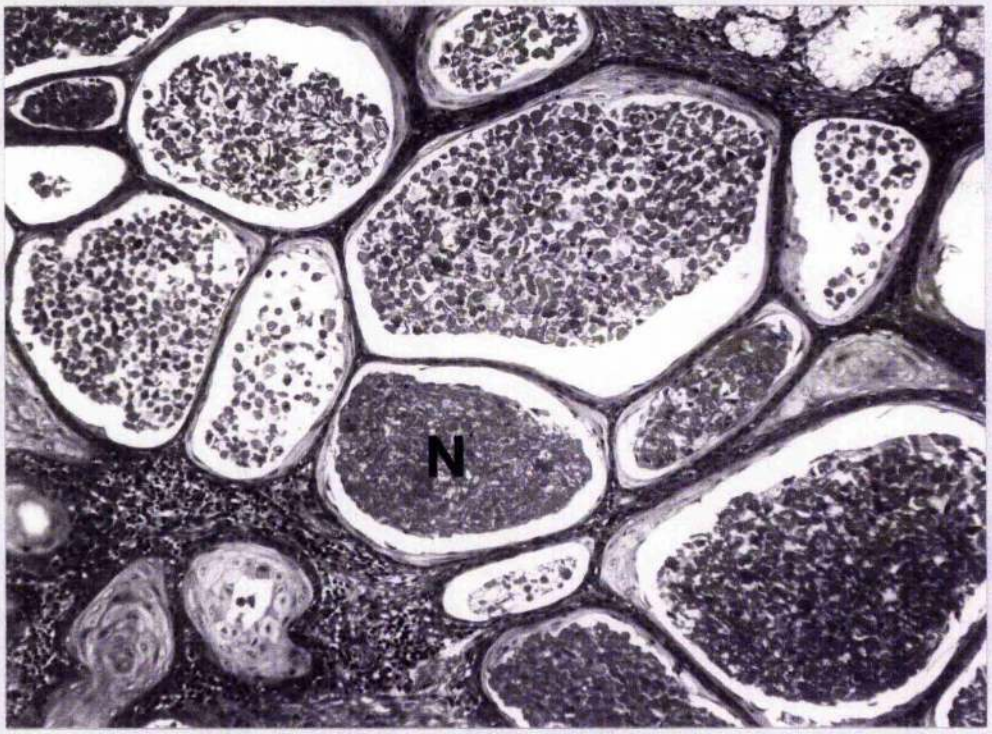

N

FIG. 25: Foreign body giant cells adjacent to keratin (H&E x 250). A granulomatous inflammatory reaction, including foreign body giant cells (arrows), is present in the connective tissue adjacent to an island of keratin (k).

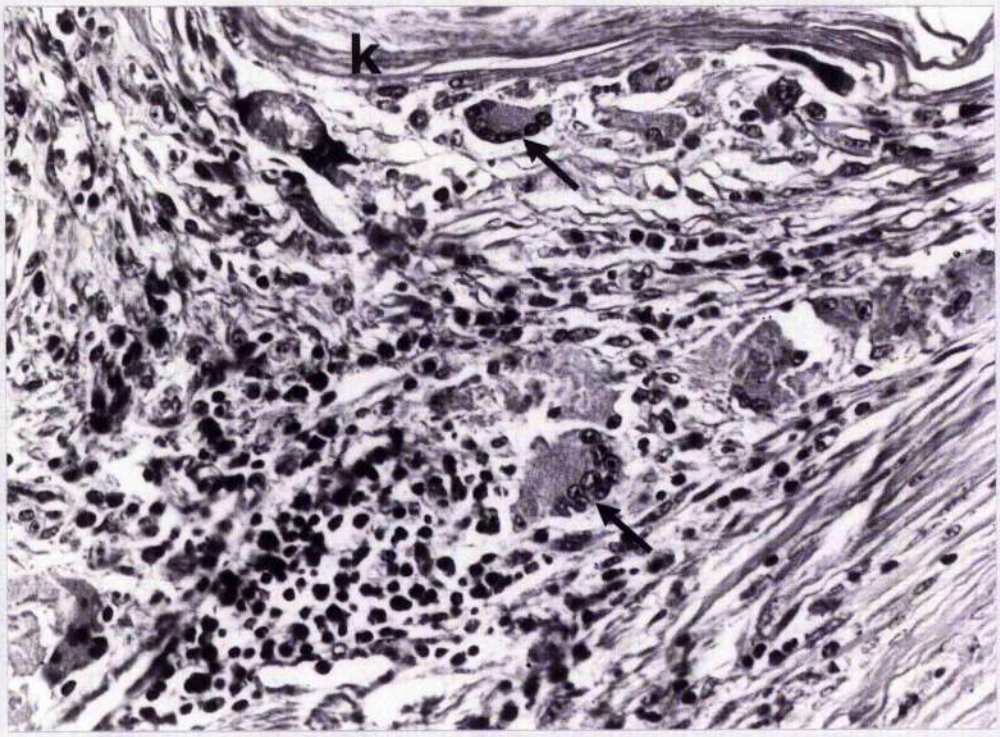

k

FIG. 26: Co-existent actinobacillosis and squamous cell carcinoma (H&E x 250). An island of tumour cells with central keratinisation (k) lies next to a granuloma with a central colony of organisms (c).

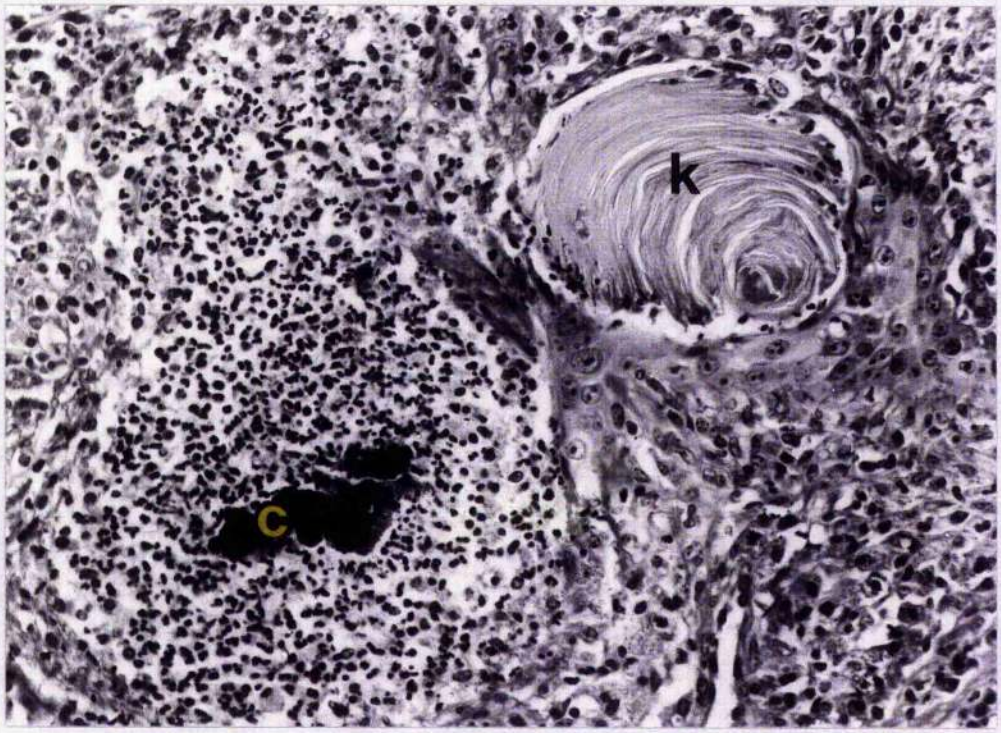
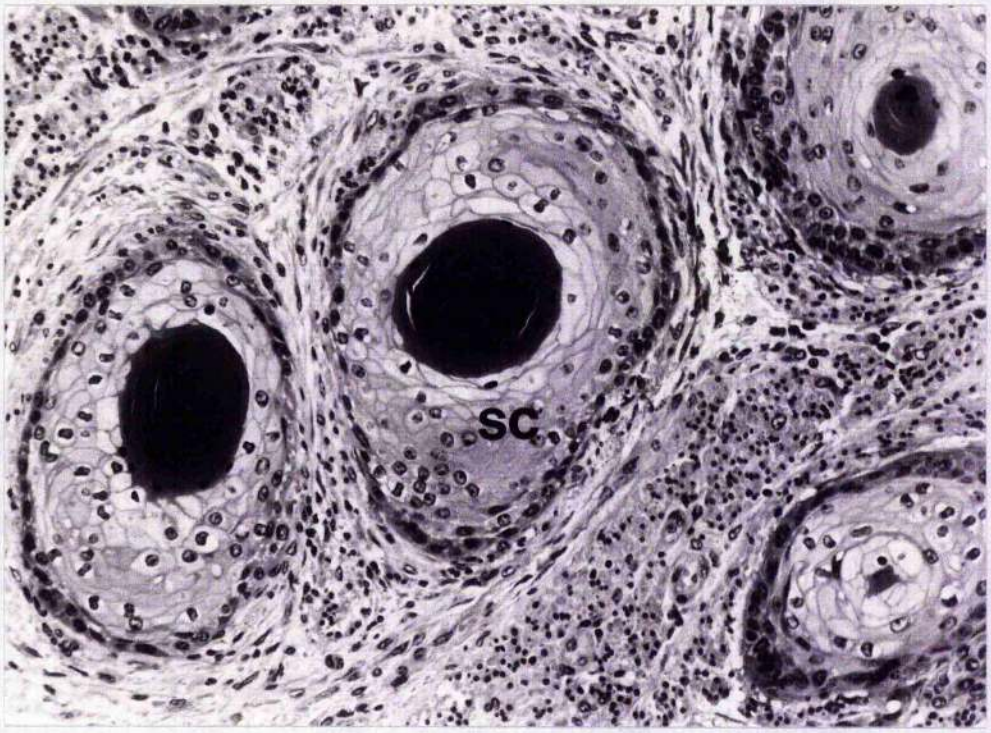

k
c

FIG. 27: Squamous cell carcinoma, Grade I (H&E x 250). Islands of tumour cells show peripheral basal-type cells, large squamous cells (sc) and central keratinisation.

FIG. 28: Squamous cell carcinoma, Grade II (H&E x 250). Islands of squamous cells (sc) show central keratinisation but there is some loss of polarity and nuclear pleomorphism.


SC

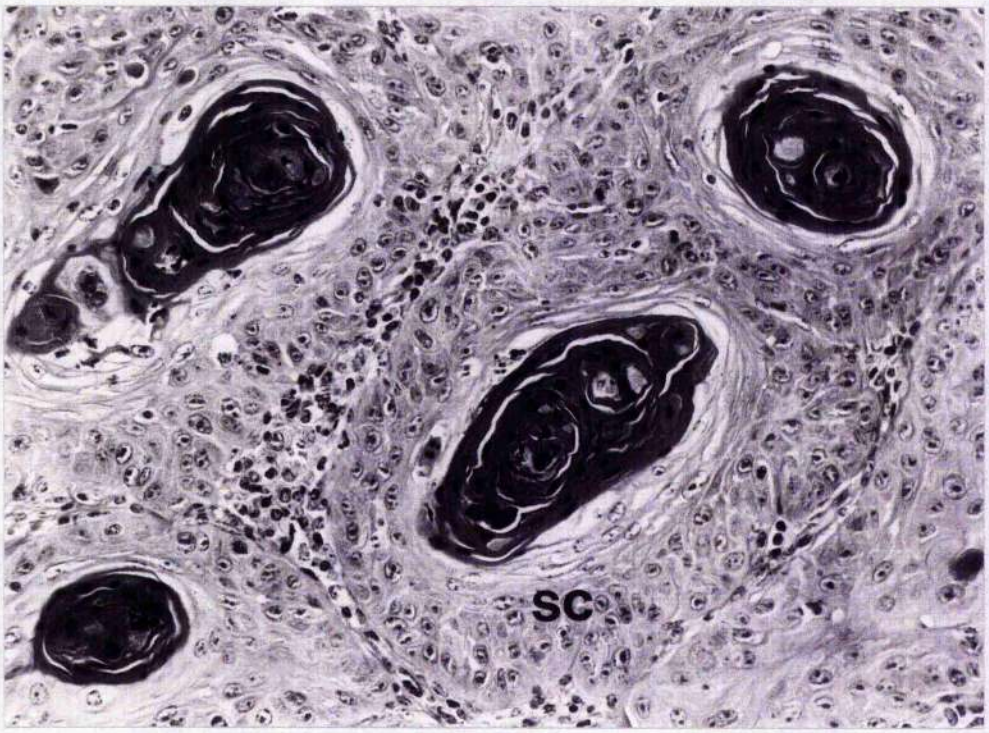
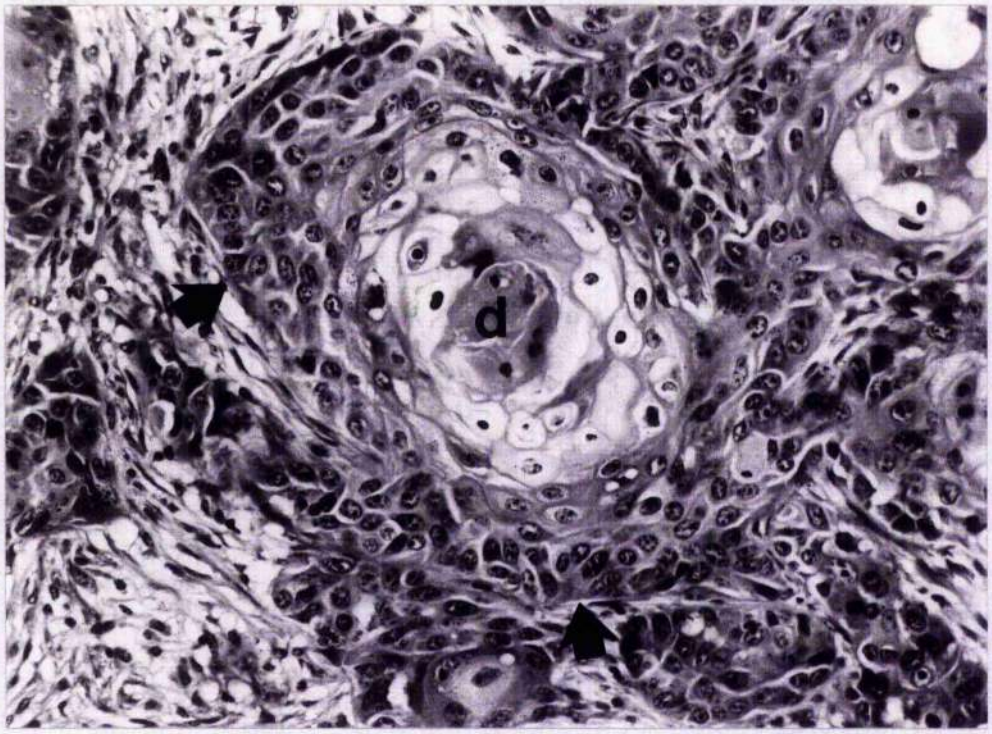

SC

FIG. 29: Squamous cell carcinoma, Grade III (H&E x 250). An irregular island of tumour cells (arrows) shows peripheral loss of polarity and central dyskeratosis (d). There is nuclear and cellular pleomorphism.

FIG. 30: Squamous cell carcinoma, Grade IV (H&E x 250). Irregular islands of tumour cells show marked nuclear and cellular pleomorphism. Uninucleate (small arrow) and multinucleate (large arrow) tumour giant cells are present and a giant mitotic figure can be seen (arrow-head, bottom centre). There is little evidence of keratinisation although a few dyskeratotic cells are associated with central necrosis of a tumour island (N).


d

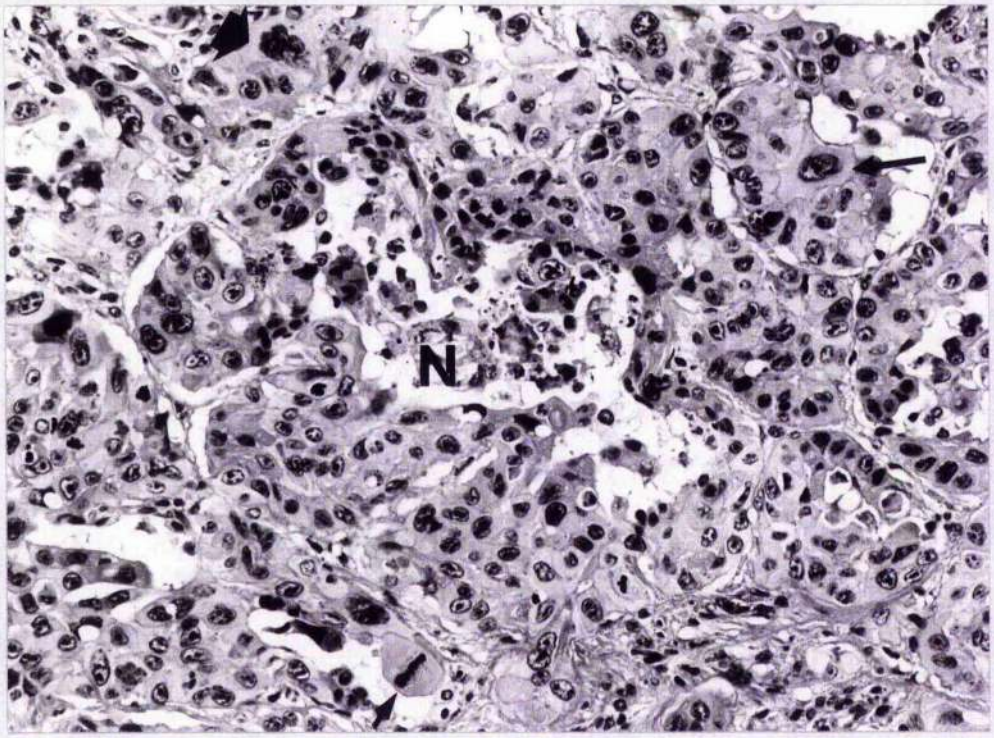

N

FIG. 31: Pleomorphic squamous cell carcinoma (H&E x 400). Islands of pleomorphic squamous cells contain mitotic figures (arrows) and a tumour giant cell (top centre). The groups of tumour cells are surrounded by an infiltrate of lymphoid and plasma cells.

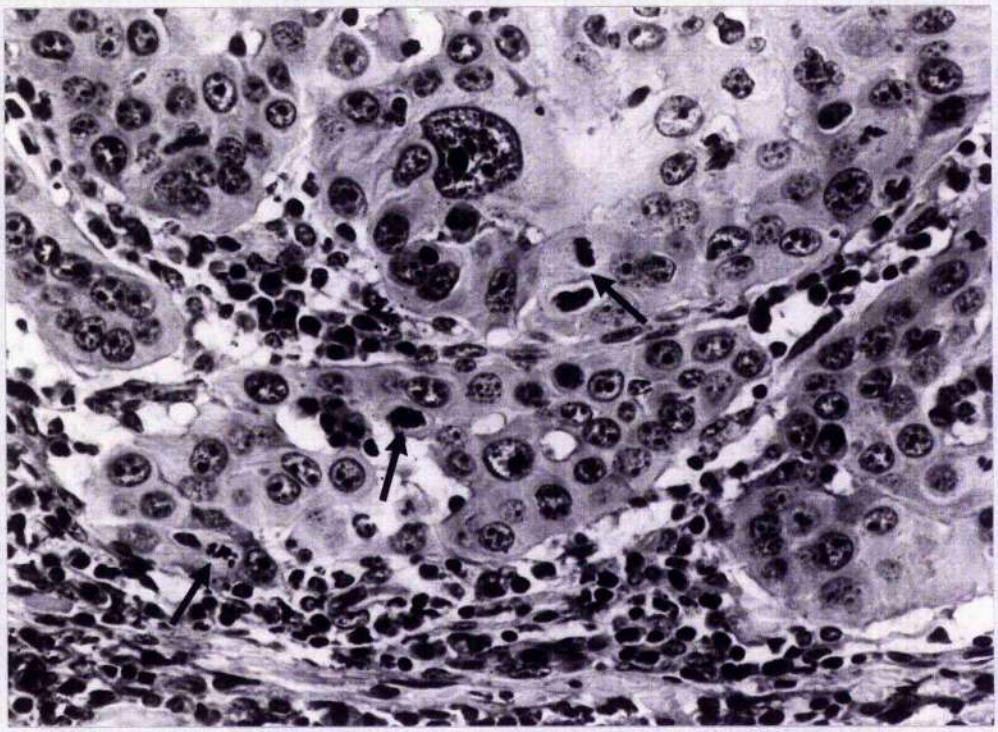


FIG. 32: Secondary squamous cell carcinoma in a lymph node (H&E x 35). Tumour cells form an irregular epithelium (E) which lines a cystic space (S) within the cortex of a lymph node (L).

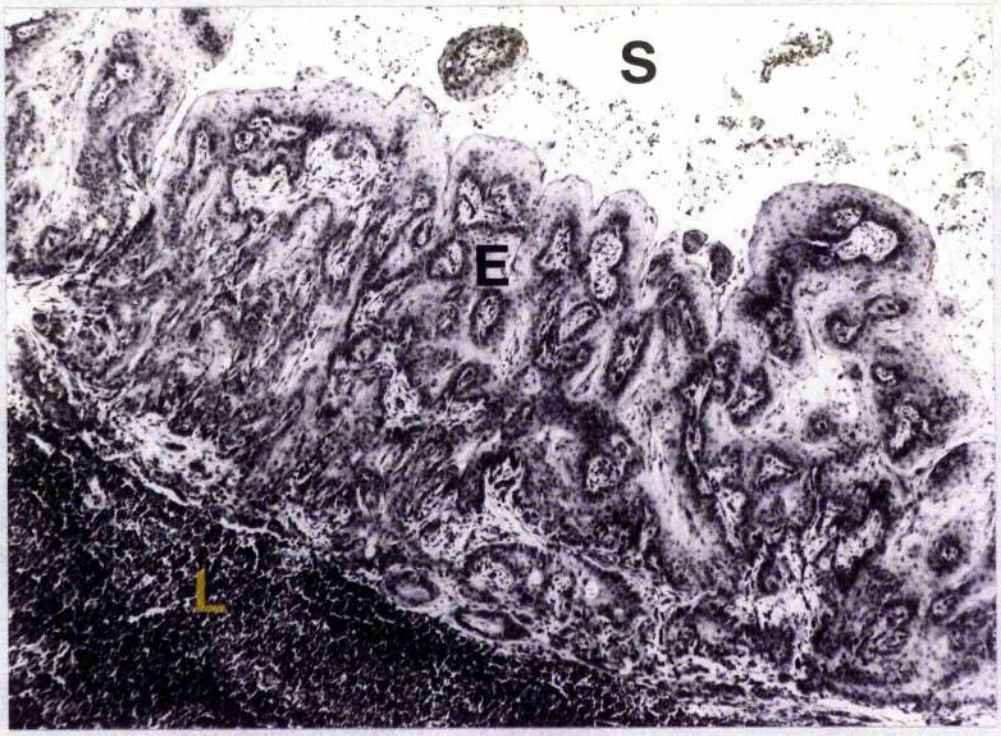

S
E
L

FIG. 33: Secondary squamous cell carcinoma in an intestinal lymphatic (H&E x 110). A clump of poorly-differentiated squamous cells (arrow) lies within a dilated lymphatic vessel in the intestinal wall.

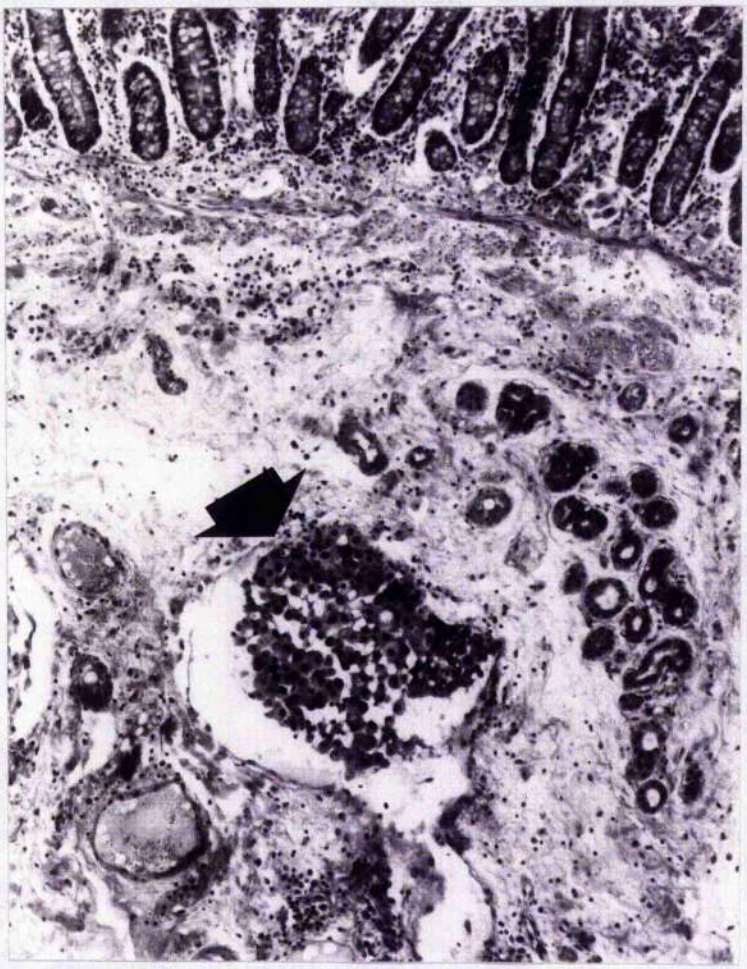
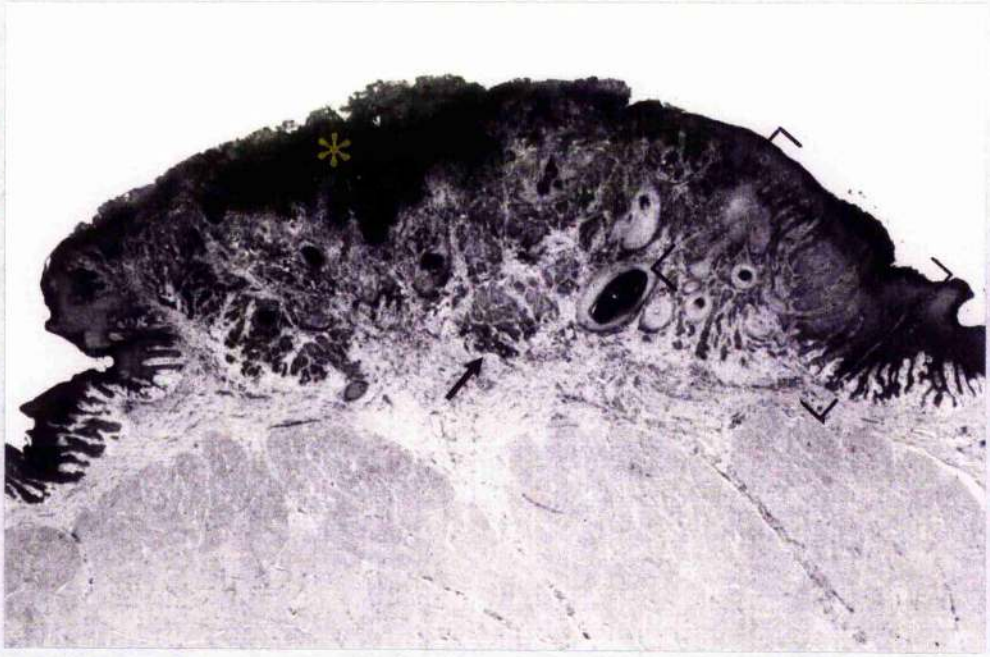


FIG. 34: Early squamous cell carcinoma of the rumen.

a) Low power view (H&E x 30). A small, raised plaque of tumour shows infiltration of the underlying connective tissue (arrow) and superficial necrosis (asterisk). The adjacent epithelium is hyperplastic.

b) High power view of area indicated (H&E x 110). This illustrates the junction of carcinoma (left) and hyperplastic epithelium (right). The carcinoma comprises irregular epithelial pegs (p) showing loss of polarity and intraepithelial pearls (arrows). There is parakeratotic hyperkeratosis (k) of the hyperplastic epithelium which is under-run by the carcinoma.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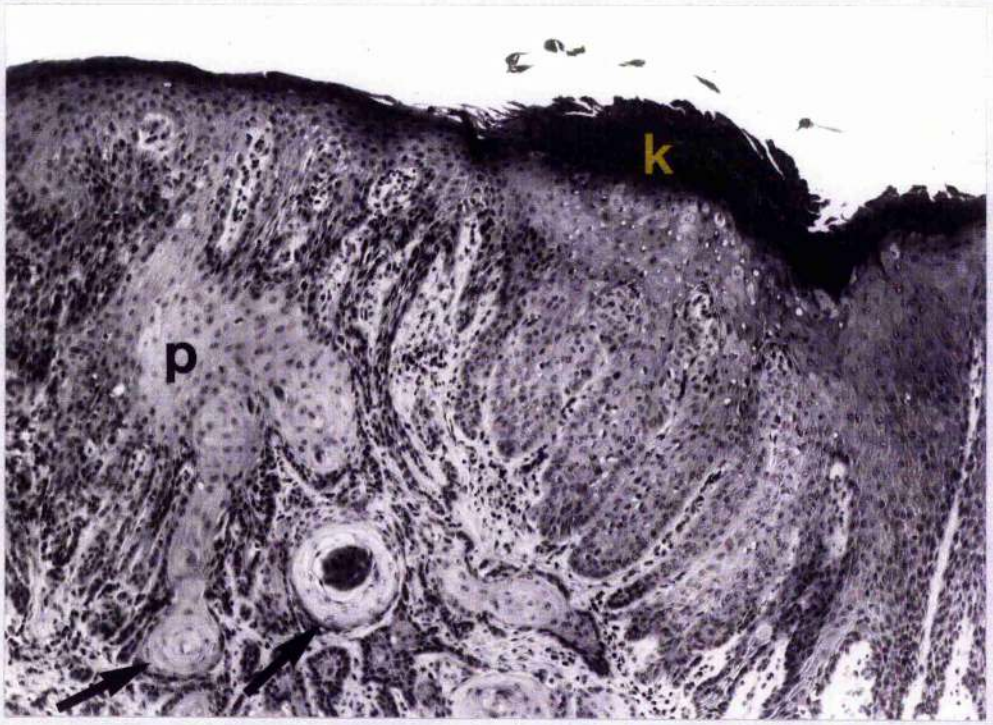
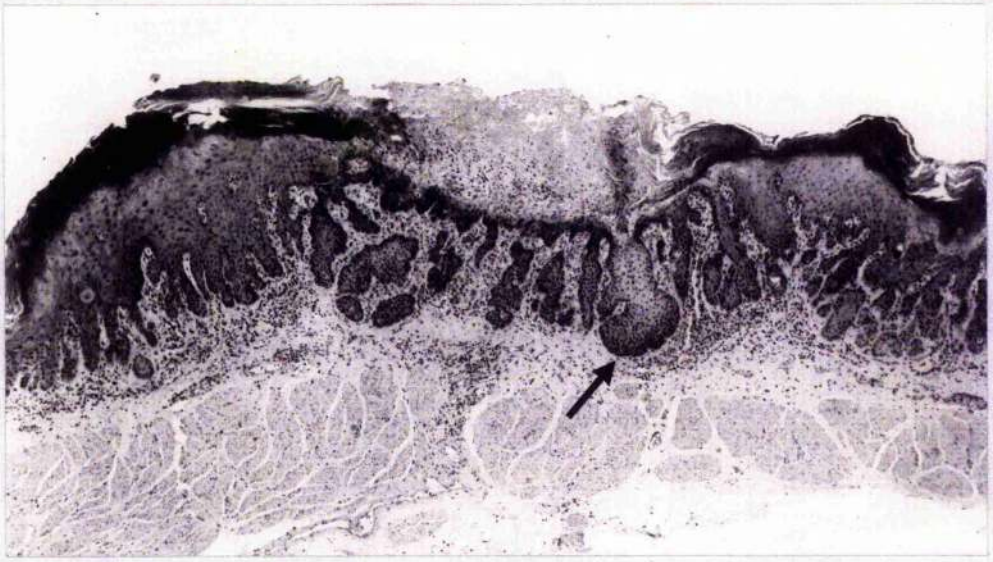

k
p

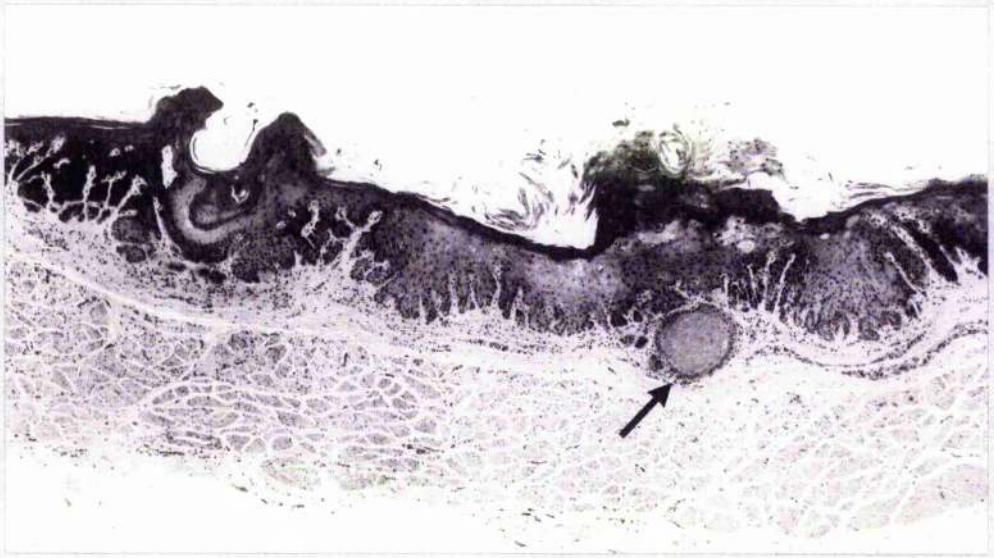
FIG 35: Squamous cell carcinoma of oesophagus showing a variety of early lesions (H&E x 35).

a) There is irregular epithelial hyperplasia with a central area of erosion. Below the erosion there is broadening and branching of epithelial pegs (arrows). In isolation this might be considered to be pseudocarcinomatous hyperplasia.

b) There is irregular epithelial hyperplasia and variable hyperkeratosis. A pseudopapillomatous nodule overlies a focus of squamous cell carcinoma (arrow). The island of tumour cells is situated in the subepithelial connective tissue and shows some loss of polarity.

c) There is irregular epithelial hyperplasia and dysplasia with formation of intraepithelial pearls (long arrow). Irregular islands of squamous cell carcinoma (short arrow) lie within the subepithelial connective tissue.





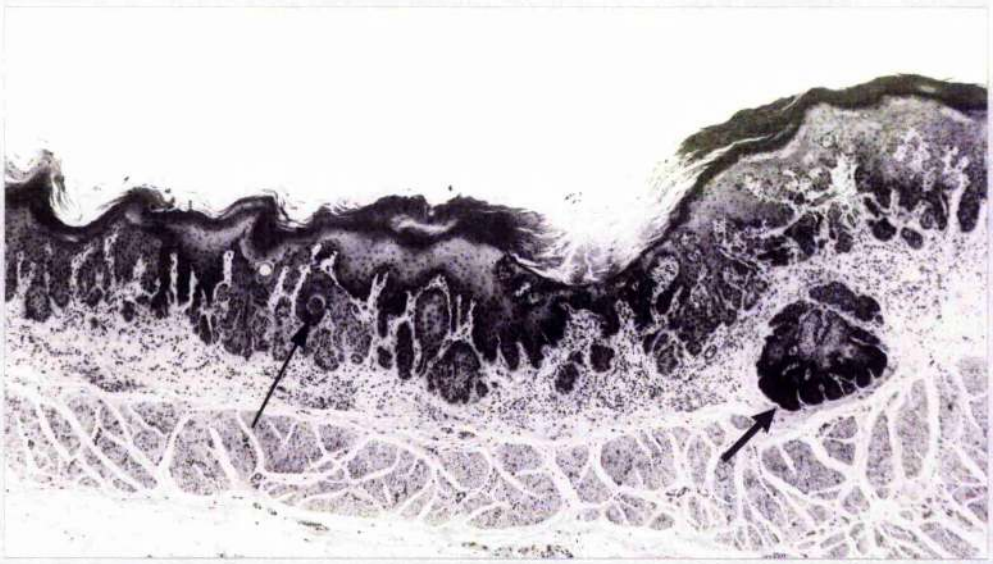
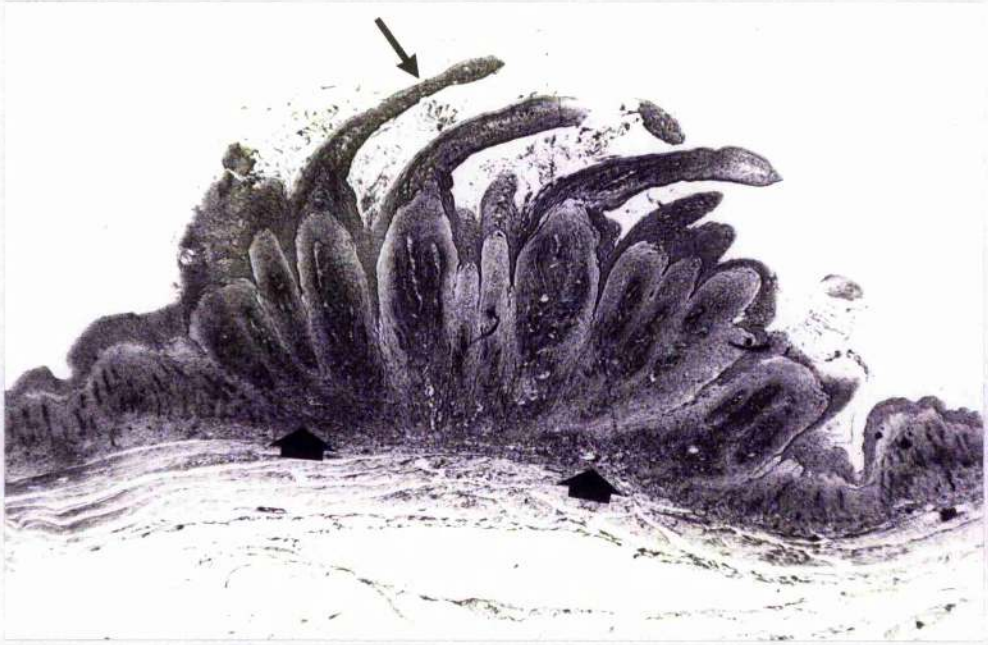
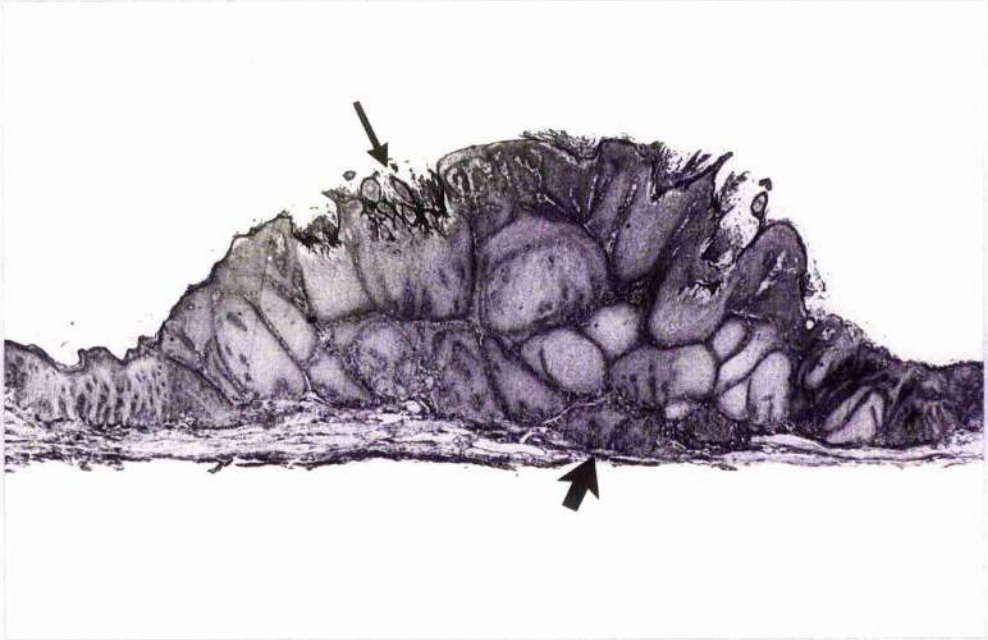


FIG 36: Oesophageal tumours (H&E x 18).

a) Simple papilloma. Hyperplastic and hyperkeratotic fronds of epithelium (thin arrow) are supported by delicate cores of connective tissue. The base of the lesion is flat although there is a cellular infiltrate (thick arrows). The adjacent epithelium is hyperplastic.

b) Transforming papilloma/early carcinoma. The surface of the lesion has a papillomatous appearance (thin arrow) but the bulk of the nodule comprises broad tongues and islands of epithelial cells separated by bands of connective tissue. The base of the lesion is irregular with early infiltration of the underlying connective tissue (thick arrow). The adjacent epithelium also appears to be transformed.





Discussion

In a series of 100 cattle the predilection sites of UAT squamous cell carcinoma were found to be the mouth and pharynx, the oesophagus, the cardia and reticular groove and the dorsal sac of the rumen. The functional consequences of these tumours largely depended on the site; large lesions in the dorsal sac of the rumen were associated with few specific clinical signs compared to lesions at other sites, for example, involvement of the cardia or groove frequently resulted in ruminal tympany. Grimshaw (unpublished results) has defined four clinical syndromes related to the main lesion of functional significance in each case: (1) an oropharyngeal syndrome characterised by halitosis, excess salivation and chronic cough; (2) a cud-dropping syndrome generally due to oesophageal lesions and associated with cervical swelling, halitosis and diarrhoea; (3) chronic ruminal tympany and (4) cows showing only wasting and diarrhoea.

Our findings confirm and amplify the reports of other authors. In our animals, the majority of tumours were found in the oesophagus or rumen with the oesophagus being involved in 64% of cases and the rumen in 59%. This parallels the findings in Brazil, where Tokarnia et al., (1969) recorded oesophageal tumours in 59% (26/44) of cases, and in Kenya, where all cases described had tumours in the rumen (Plowright et al., 1971). There was a contrast in the relative incidence of oropharyngeal lesions; in this series from the West of Scotland only 27% of cases involved the mouth or pharynx whereas the Brazilian workers included 22 cases of pharyngeal carcinoma and a further 4 cases with precancerous pharyngeal lesions in their total of 44 (Tokarnia et al., 1969). Plowright et al., (1971) noted only small papillomas in the mucosae of the pharynx or soft palate and those only in a few cases. There seems to be no obvious reason for this difference between the areas.

Another difference appeared to be in the incidence of metastases; 40 cases with secondary tumours were found in the

present series whereas Tokarnia et al., (1969) noted only 2 cases and Plowright et al., (1971) found only 4 cases. There are two possible explanations for this discrepancy, one is that many of the animals examined at Glasgow were presented at a late stage in the course of the disease, the other is that Brazilian and Kenyan post-mortem examinations may have been less exhaustive; which of these explanations is applicable is by no means clear.

The ages of cattle with squamous cell carcinoma appeared to be similar in the three countries. The youngest animal of known age from the West of Scotland was 6 years old; in Brazil, Tokarnia et al., (1969) included several of 5 years and in Kenya, Plowright et al., (1971) recorded three animals as 4-5 years of age. The ages recorded for the Scottish cattle were those at death or slaughter but since squamous cell carcinoma was not the main cause of mortality or morbidity in every case, those ages do not give an accurate indication of the age at which bovine UAT carcinoma occurs. Nevertheless, the age distribution as shown in Figure 1. does indicate a peak incidence at 12 years of age and a general trend of increasing incidence with advancing age. This is comparable to the situation in man where carcinomas in general are considered to be diseases of the middle-aged or elderly (Willis, 1967a). Murray (1908) noted that "the condition for discovering a considerable number of malignant new growths in animals is still that a sufficiently large number of aged individuals should be carefully examined". It is almost certain that the majority of cattle in countries with extensive farming systems are killed before the latent period of UAT carcinoma has been traversed and this partly accounts for the generally accepted view (Jubb and Kennedy, 1970; Cohrs, 1967) that such tumours are uncommon.

The macroscopic appearance of the squamous cell carcinomas found in 100 cattle broadly resembled that described by other authors and in particular the series recorded in Kenya and Brazil (Plowright, 1955; Dobereiner et al., 1967; Tokarnia et al., 1969; Plowright et al., 1971). The two main forms of the tumour, namely an excavating ulcer or a cauliflower-like growth, have long been

recognised particularly in German descriptions of ruminal lesions (e.g. Joest, 1926, quoted by de Kock and Fourie, 1928; Cohrs, 1967). However, it is possible that the true nature of such lesions has not always been recognised in the past since their appearance superficially resembles that of tuberculous growths or actinomycotic granulomas, both of which were once common in British cattle. One author clearly high-lighted the difficulties when he wrote "little or no reliance can be placed in the writings of the older veterinary authors on the subject of cancer, owing to the fact that diagnosis was generally based solely on clinical and macroscopical characters. For example, the cases of so-called cancer of the tongue of the ox recorded even in comparatively modern veterinary text-books were probably without exception actinomycotic lesions". (Fadyean, 1899).

The situation is further compounded by the occasional coexistence of carcinoma and actinobacillosis which was found in two animals in the West of Scotland and in one in Brazil (Dobereiner et al., 1967). The possibility of confusion between these two conditions has probably contributed to the paucity of reports of UAT cancer in cattle.

Results from this survey of 100 cases of squamous cell carcinoma in cattle revealed a relationship between the size of tumours and the incidence of metastasis. Large tumours, particularly those with a diameter of over 10.0cms had a high incidence of metastasis and there was no evidence of secondary tumours in animals with only small lesions. This feature is well known to pathologists and is often utilised in clinical or pathological staging systems which estimate the extent of cancer in an attempt to assess the prognosis in individual cases. Wahi et al., (1971) explain that a well-differentiated carcinoma less than 1cm. in diameter is less dangerous than a larger one that is comparable in all other respects. It may be assumed, that, in general, a small tumour has a shorter history than a large growth. It appears that most of the lesions found in the Kenyan and Brazilian cattle (Plowright, 1955; Dobereiner et al., 1967;

Tokarnia et al., 1969; Plowright et al., 1971) were relatively small, so it is possible that cattle in our series had a higher incidence of metastasis because more animals had large tumours of comparatively longer duration although Plowright et al., (1971) mention that wide infiltration of the ruminal wall was common in their animals.

The general microscopic features of UAT cancer in 100 cattle were typical of squamous cell carcinoma as described in classic pathology texts (see Ashley, 1978a; Willis, 1967b) and closely resembled those described by Plowright (1955), Dobereiner et al., (1967) and Tokarnia et al., (1969). One well known characteristic of squamous cell carcinoma is their variable histological appearance; both between tumours and in different areas of the same tumour. In this series, some were largely basal cell type, others contained numerous squamoid cells and others comprised large masses of keratin surrounded by flattened cellular elements while many were a mixture of two or three cell types. A characteristic feature of the majority of the tumours was the presence of intercellular bridges or prickles; these were often most prominent in areas of basal cell type and their presence along with the absence of definite palisading served to distinguish such growths from true basal cell tumours.

As well as the variation in the type of squamous cell carcinoma the range of differentiation or anaplasia both between and within tumours is remarkable. It was certainly a feature of the tumours in this series and two cases showed such variable differentiation that any attempt to classify them would have been futile. Nevertheless, it is generally considered useful to divide such tumours into three classes; poorly-differentiated, moderately-differentiated and well-differentiated (e.g, Oota and Sobin, 1977). Poorly-differentiated lesions show a high degree of anaplasia although their squamous cell origin is demonstrated in some areas; well-differentiated lesions closely resemble normal, stratified squamous epithelium and moderately differentiated lesions are of intermediate morphology. More detailed systems of

histological grading have been employed and Ashley (1978a) notes that the system advocated by Broders (1932) has been widely accepted although Willis (1967c) is scathing in his criticism of such procedures. Grading systems are generally used in an attempt to establish some relationship between the histological appearance of a tumour and its biological behaviour in the hope that a more precise prognosis can be given. Willis (1967c) is probably right when he states that "For individual prognosis, no scheme of grading can attain greater accuracy than the general principle affords", the general principle being that the most anaplastic lesions are the most malignant.

The criticisms and difficulties of grading systems are related to the variable appearances of tumours (particularly relevant in cases of squamous cell carcinoma) so that samples are not truly representative and to the fact that grading is, almost of necessity, subjective and hence susceptible to a degree of bias on the part of the examiner. With these problems in mind, it was nevertheless decided to attempt to grade the main lesions in each of 100 cases partly as an aid to description, and partly to determine if there was any relationship between histological appearance and incidence of metastases and partly as a response to a plea in the veterinary literature (Head, 1976). A scheme employing four grades was finally used because the majority of tumours seemed to fall in the broad category of moderate differentiation. Although it has been postulated that the biological behaviour of a tumour is related to its most poorly differentiated component (Oota and Sobin, 1977); since prognosis was not an issue, grading was based on the overall appearance of predominant features of the tumours. However, the instruction of the aforementioned authors, that grading should not be made at the growing edges of a tumour or at portions directly adjacent to ulcerative or inflammatory processes, is valid, because such areas often show more activity and greater anaplasia than the main body of the tumour.

The results from this series of 100 cases showed that three-quarters or more of the Grade III and IV tumours had metastasised

whereas less than one third of the Grade I and II tumours had done so and therefore confirmed Willis's general principle (1967c). Whether this system would be of use as an aid to prognosis in squamous cell carcinomas of other animals is doubtful but the author considers that its value as a descriptive aid justifies its inclusion in this thesis. One interesting feature was the incidence of keratinisation; grading systems employed for human tumours (Wahi et al., 1971; Oota at Sobin, 1977) imply that the degree of keratinisation is inversely related to the degree of anaplasia. This author contends that if grading is based on cellular and nuclear atypia as used here then keratin may be abundant in Grade II or even occasionally in Grade III tumours and Grade I tumours, particularly when small, may show little evidence of keratinisation. However, keratinisation of individual cells did appear to be associated with a greater degree of anaplasia and Ashley (1978a) notes that it is regarded as evidence of defective differentiation and a sign of a more malignant growth.

The scirrhous reaction associated with squamous cell carcinomas is a well recognised phenomenon (Willis, 1967d). Cohrs (1967) notes that the flat, ulcerative form of ruminal carcinomas in cattle is distinguished by a very marked development of the stroma. In the present series, there did not appear to be any relationship between the amount or density of connective tissue and the site, size or histological grade of the lesion.

Necrosis was a feature of the majority of lesions in this series; superficial necrosis with bacterial colonisation was to be expected in large lesions subjected to repeated trauma by the normal action of the alimentary tract. Central necrosis of large lesions was common and was presumably due to failure of blood supply. Necrosis of keratinised foci was a frequent feature and in many areas it was impossible to distinguish production of parakeratotic keratin from coagulative necrosis of partly differentiated cells presumably because keratinisation is a form of cell death.

The leucocytic infiltrations associated with malignant growths have been discussed by Willis (1967d). Their role or otherwise in the host's response to the presence of a tumour forms a large part of present day cancer research and will not be reviewed here. Suffice it to say along with Ashley (1978b) and in contrast to Willis (1967d) that infiltrates of lymphocytes and plasma cells may be taken as an indication of a cellular immune response to the tumour and, to agree with Oota and Sobin (1977) that cellular reaction and inflammatory infiltration may be related to tumour behaviour. However, the excessive mononuclear cellular infiltrates associated with some oropharyngeal lesions were probably a reflection of the proximity of pre-existent lymphoid nodules in the oropharynx.

Multiple tumours or satellite lesions adjacent to large growths were found in many of the 100 cases. The multifocal origin of squamous cell carcinoma has been described many times and Ashley (1978) states that squamous cell carcinomas can grow by incorporating adjacent lesions. The association with multiple papillomas seems to be restricted to the cattle tumours but is described by both Brazilian and Kenyan investigators (Plowright, 1955; Dobereiner et al., 1967; Tokarnia et al., 1969; Plowright et al., 1971) and by Trotter (1911). The significance of these lesions in relation to the possible aetiology of squamous cell carcinoma of the bovine UAT has been discussed elsewhere (Jarrett et al., 1978, 1980) and reviewed by Jarrett (1980, 1981). It remains an interesting and intriguing question.

SECTION B: TUMOURS OF THE URINARY BLADDER

Introduction and Review of the literature

Results

- Macroscopic appearances of tumours
- Microscopic appearances of tumours

Discussion

SECTION B TUMOURS OF THE URINARY BLADDER

Introduction and Review of the Literature

Tumours of the bovine urinary bladder have been recorded in most parts of the world. In the majority of such areas, the presence of urinary bladder tumours is associated with a condition termed bovine chronic enzootic haematuria (CEH). This condition is, as its name suggests, a chronic disease of cattle that is prevalent in certain regions and is characterised by the clinical sign of blood in the urine. CEH is also known as cystic haematuria or haematuria vesicalis and has occasionally been loosely called 'red-water'. It is of world-wide distribution but is usually restricted to fairly well-defined areas (Pamukcu, 1963). Affected areas have been variously described as new and uncultivated (Kalkus, 1913), poor and neglected (Hadwen, 1917) or mountainous (Rosca, 1969) whereas highly developed agricultural countries no longer have the disease (Rosenberger, 1971). Recent reports have emerged from India (Nandi, 1982), China (Zeiguan et al., 1981), Australia (McKenzie, 1978b) and Japan (Yoshikawa & Oyamada, 1975). It is generally agreed that it is upland farms that are usually involved but a few contradictory reports do exist (Datta, 1952). Affected cattle may be of any breed and almost any age although young animals do not usually develop the disease (Bull et al., 1932, Pamukcu et al., 1976). Either sex may be affected but most cases occur in middle-aged or older cows (Rosenberger, 1971; Pamukcu et al., 1976).

The diagnosis of CEH is essentially a clinical one and the associated pathological findings are variable (Bull et al., 1932; Plummer, 1944). In the majority of cases, lesions are confined to the urinary bladder (Pamukcu, 1955; Plummer, 1944, Bankier, 1943) but the nature of those lesions has been disputed. Pamukcu (1957) noted that some authors maintained that the changes were primarily inflammatory in nature whereas others considered them to be essentially neoplastic. Early lesions of CEH have been described as small patches of congestion of the mucosa showing punctate

haemorrhagic foci and mild inflammation (Pamukcu, 1955) but it is now recognised that tumours of the urinary bladder are a prominent feature of the disease at least in advanced cases (Munday, 1966; Rosenberger, 1971). Pamukcu (1962), reviewing his earlier results (Pamukcu 1955, 1957), stated that tumours of the bladder were the actual cause of the vesical bleeding in 92% of cases. Other authors (Pachauri et al., 1981) reported that the occurrence of tumours of the urinary bladder has been recorded invariably in all clinical cases of CEH.

Plummer (1944) examined the histopathology of enzootic haematuria and described a range of vascular lesions including two forms of angioma one designated a typical haemangioma and the other recorded as a 'wild' type of lesion with distinctly malignant features. Earlier, Kalkus (1913) recorded both vascular lesions and papillomatous new growth but papillomas were excluded from the category of red-water lesions by his statement "there is no doubt but what the two conditions developed independantly and had nothing in common, their development at one and the same time being simply a coincidence". Other authors, however, have described a wider variety of tumours including not only haemangioma and haemangiosarcoma but also transitional cell papilloma and carcinoma, adenocarcinoma and squamous cell carcinoma. (Zeiguan et al., 1981; McKenzie, 1978b; Pamukcu et al., 1976; Smith and Beatson, 1970; Dzuvic, 1969a). By 1931, Kalkus too had conceded that complications could be due to new growths such as papillomas or carcinomas. Other tumours that have been associated with CEH include small fibromas (Mckenzie, 1978b). The occurrence of bovine vesical fibromatosis was described as a distinct entity by Yoshikawa & Oyamada (1971) but included in their series were a number of animals with classic haematuria. In an abbatoir survey of bovine urinary bladder pathology, conducted in an area where clinical cases of enzootic haematuria were known to occur (McKenzie, 1978a), examination of 2832 bladders revealed 68 cases of neoplasia and 104 cases of chronic cystitis. The majority of tumours found were non-epithelial in origin with 59 cases of fibroma and 4 cases of haemangioma. Only one carcinoma was

identified. Up to 18 fibromas were found in individual bladders but they occurred singly in 31 cases and most were between 1.0 and 5.0mm in size.

The largest reported series of tumours of the bovine urinary bladder is that of Pamukcu et al, (1976) and this includes cases reported earlier by Pamukcu (1957, 1955). The series comprises 60 cows and 7 water buffalo, with a history of haematuria, examined at necropsy; together with 72 bovine urinary bladders with tumours, collected from abbatoirs in the enzootic area. Tumours of epithelial origin, alone or in combination with mesenchymal tumours, constituted 90% of bladder tumours in the 139 naturally occurring cases. Mixed epithelial and stromal tumours were encountered in 75/139 cases (54%), whereas non-epithelial tumours alone were found in 13/139 cases (9%). Over two thirds (69%) of the 124 epithelial lesions recorded were classified as carcinomas (85 cases). Haemangiomas or haemangio-endotheliomas were recorded in 78/139 cases of bladder tumour. Only 4 leiomyosarcomas, 2 fibrosarcomas and 3 fibroma cases were recorded in this series. The authors maintain that the tumours vary between geographic regions and that a greater variety and more malignant tumours have been reported in Yugoslavia and Turkey.

Various reports of CEH emphasise the simultaneous occurrence of different tumour types or "mixed tumours" within the same urinary bladder (Zeiguan et al., 1981; Pachauri et al., 1981) thus confirming the assertion made by Pamukcu (1962) that urinary bladder tumours are not a homogeneous entity but a complex group. Pamukcu (1962) also noted that, in a few instances, glandular cystitis was associated with the bladder tumours and he compared the lesions to those regarded as precancerous in other species. A number of writers have described pleomorphic and proliferative lesions of CEH ranging from hyperplasia to frank neoplasia (Pachauri et al., 1981; Dzuvic, 1969; Munday 1966). The non-tumorous lesions described include epithelial atrophy or hyperplasia, capillary ectasia and proliferation and stromal oedema or fibroplasia (Dzuvic, 1969; Munday, 1966; Rosenberger, 1971).

Cystitis has generally been regarded as a secondary complication in long-standing cases of CEH (Mugera and Nderito, 1968; Munday, 1966) but lymphoid accumulations have been noted even in early cases (Plummer, 1944; Pamukcu, 1957; Smith & Beatson, 1970) and recent authors have recorded the presence of chronic cystitis in many or all of their cases (Smith & Beatson, 1970; McKenzie, 1978b). The nature of the changes has prompted various workers, Cleland (1911), Bull et al, (1932) and later Pamukcu (1963), to suggest that although they are neoplastic in character, the lesions of CEH may be due to chronic irritation of the bladder mucosa.

Outwith the areas where CEH is recognised, tumours of the bovine urinary bladder are uncommon although isolated reports, of adenoma in particular, do appear (Brobst & Olson, 1963). Feldman (1932) commented on the comparative rarety of neoplasms affecting the urinary bladder in cattle and the compilation of Smith & Jones (1966) included only 8 tumours of the bovine urinary bladder in a total of 8159 neoplasms of domesticated animals.

The situation in Britain is unclear. The survey of Anderson et al., (1969) did not reveal any cases of neoplasia of the bovine urinary bladder, although that may be readily explained since the urinary bladder is not opened at slaughter and the majority of tumours are not easily detected in the closed viscus. Cotchin's series (1960) of 293 bovine tumours did not include any bladder lesions but his comments imply that haematuria vesicalis was well known. The absence of reports of bovine CEH in the recent veterinary literature in Britain has led at least one author to suggest that it does not occur in the United Kingdom (Widdop, 1967). Previous reports have stated that chronic haematuria of cattle is not uncommon in certain areas of Wales and that it has been observed in the North of Scotland and Cornwall (Craig, 1930). The condition has been recognised in the Fell district (Burnett, 1937), in the Forest of Dean (Hall Masheter, 1933) and apparently also in Shropshire (Downham, 1938 quoted by Datta, 1952). More recently, studies of cattle submitted to GUVS for examination have indicated that there is a high incidence of bovine haematuria in

the West of Scotland. In this area, CEH appears to be closely associated with the occurrence of UAT tumours.

Plowright et al., (1971) in their report of squamous cell carcinoma in cattle found no obvious link between UAT carcinoma and chronic haematuria although bovine CEH does occur in certain areas of Kenya (Mugera and Nerito, 1969). However, in Brazil, an association between squamous cell carcinoma of the UAT and CEH in cattle was a feature of the reports of several workers. Dobereiner et al., (1967) compiled a table based on the results of Curial (1964) listing 9 cases of urinary bladder tumours in cattle; 7 animals had adenoma or haemangioma, 7 had carcinoma (epidermoid, adenocarcinoma or transitional cell carcinoma) and 3 had papilloma, either alone or in various combinations. Six of those 9 animals had cancerous or precancerous lesions of the pharynx. Tokarnia et al., (1969) listed 44 cases of UAT carcinoma which included the 21 cases reported by Dobereiner et al., (1967). In that series of 44 cases there were 6 animals with haemangioma of the urinary bladder, 4 with capillary proliferation, 1 with adenocarcinoma and 1 which was listed as having adenocarcinoma and carcinoma. The urinary bladder was free of lesions in 9 cases but in 23 animals the bladder was not available for examination. Neto et al., (1975) carried out histopathological examination of tumours in 19 animals; two had haematuria alone and 6 had haematuria and upper alimentary tumours. The principal lesions found in the urinary bladder of those 8 cases were papilloma in 4 and carcinoma in 5.

Preliminary studies of urinary bladders from cattle in the West of Scotland suggested a situation broadly similar to that recorded in Brazil. The results of the subsequent detailed investigations form part of this thesis.

Results

Tumours of the urinary bladder were found in 21% of 100 cattle with squamous cell carcinoma of the UAT. The lesions comprised papillary outgrowths of the epithelium and subepithelial haemangiomatous and fibromatous nodules. All tumours were less than 2.0cms diameter and the majority were 0.5cms diameter or less. No secondary growths were detected. In only 2 animals were the bladder lesions responsible for the major clinical signs although haematuria was recorded in five cases (Grimshaw, personal communication).

A further 39 cattle without UAT carcinoma were found to have tumours of the urinary bladder. Two cases were received as pathological specimens, 31 were destroyed immediately before full post-mortem examination, 3 animals died and in 3 the manner of death was not recorded. In the majority of these animals (30/39), the bladder lesions were associated with the major clinical signs. Two other animals obtained as cull cows from known "cancer farms" had a history of haematuria (Grimshaw, personal communication). In a number of cases (5/39) tumours were found in old animals with no history of urinary tract abnormality and in two animals adenocarcinoma of the intestine was the main lesion. In about 40% of 39 animals with urinary bladder neoplasia the tumours were over 2.0cms diameter and in some cases the whole bladder appeared to be diffusely involved. Tumour cells were detected in the regional lymph nodes of four such cases. The majority of animals with smaller lesions had multiple tumours scattered over the bladder mucosa.

Combination of the two sets of cattle described above provided a series of 60 cases of urinary bladder neoplasia. Thirty-five per cent of those animals also had squamous cell carcinoma of the UAT. Many cases had co-existent UAT papillomas (43/60) and intestinal lesions (32/60). As noted in Section A, the occurrence and significance of bovine UAT papillomas have been discussed by Jarrett (1981; Jarrett et al., 1978). Lesions of the

bovine intestinal tract will also be described elsewhere. Table 7 lists the various neoplasms found outwith the urinary bladder in 60 cattle. In addition, hepatic telangiectasis was recorded in 22 cases (37%) but no discrete haemangiomas were seen in the liver.

The age distribution of 60 cattle with bladder tumours is shown in Figure 37. Three cases were designated aged, i.e. over 8 years, and in 6 cases the age was unknown. The recorded ages ranged from 5 to 17 years with a peak at 12 years and 87% (47/54) were known to be 8 years or older. The age distribution of 39 animals with bladder tumours alone is compared with that of 21 animals with UAT plus bladder tumours in Figure 38. Although the peak incidence was at 12 years in each case, only 5% (1/21) of animals with tumours at both sites was less than 8 years old whereas 15% (6/39) of animals with urinary bladder tumours alone were aged between 5 and 7 years.

A wide variety of breeds was represented and the distribution is shown in Table 8. The majority were beef-type crosses of Aberdeen-Angus or Shorthorn parentage and in 3 animals the breed of both sire and dam was recorded so that the total number of breeds exceeds the number of animals. No male animals were included in this series.

The urinary bladder neoplasms found in 60 cattle were of three types; epithelial, vascular and fibrous; and in most cases the tumours were multiple. More that two tumours were present in 62% of the cattle and in some individuals there were over twelve lesions. However, tumours of two different types were only found in 13 animals (22%) and there was no case with tumours of all three types. Single, discrete tumours were recorded in 23% of affected animals but in 5 other cases the diffuse, infiltrative nature of the growth precluded such distinction. Vascular tumours were present in 63% (38/60) of the cases and epithelial tumours in 45% (27/60). Fibrous tumours were present in 8 cases (13%) and were multiple in all but one case. The co-existence and types of tumours present are indicated in Tables 9 and 10. No predilection

site was noted for any of the bladder tumours; vascular lesions in particular were often scattered over the entire muscosa. One tumour of the bladder neck and urethra was included in the series. Secondary deposits were found in the regional lymph nodes of only 4 cases, one with a vascular lesion and three with epithelial growths. No distant metastases were recorded.

Macroscopic appearances of tumours

The vascular tumours which were found in the urinary bladder of 38 animals ranged in size from pin-point foci to irregular masses of 6.0 x 12.0cms diameter (Figs. 39,40,41,44). However, the vast majority of lesions were less than 0.5cms diameter and appeared as raised, red or purplish-red, hemispherical nodules which usually had a smooth glistening surface. A proportion of the slightly larger lesions were pedunculated and one tumour appeared as a dark red polypoid mass 2.5cm in diameter. In some cases the surface of the lesions was irregular with evidence of erosion and recent haemorrhage. Indeed, in many animals the bladder was distended with brown or bloody urine which frequently contained large clots of blood. However, the number or size of lesions was not related to the presence or absence of frank blood. The consistency of most lesions was soft and slightly fluctuating. Occasionally tumours felt firmer and more fleshy; such lesions were often mottled red and creamy-white and were occasionally necrotic. In many bladders with vascular tumours the adjacent mucosa was slightly thickened or oedematous and contained scattered petechial haemorrhages.

Epithelial tumours varied from small protrusions with a maximum diameter of 2mm to widely infiltrating lesions which were associated with thickening of the whole bladder wall and adhesion to other tissues in the area (Figs. 41,42). In one case the bladder had perforated. Most tumours took the form of small papillary outgrowths, polyps, or nodules with an irregular surface. Occasionally, lobulated cystic masses were present (Fig. 43). The colour was similar to that of the adjacent mucosa

or slightly more pink than normal. Larger lesions were often necrotic and formed fungating, cauliflower-like outgrowths or irregular plaques. Floccules of necrotic debris were sometimes found in the urine in such cases. The consistency of most lesions was firm although a few were friable and some could be designated scirrhous. Ulceration of the bladder mucosa and associated haemorrhage was sometimes evident, particularly in those cases with widespread tumour involvement. In many cases the epithelium adjacent to tumours was thickened and roughened.

Fibrous tumours were all less than 0.5cms diameter and they appeared as smooth, raised nodules that were paler than the adjacent mucosa (Fig. 44). These lesions were subepithelial in position and there were rarely any changes in the adjacent mucosa.

The size distribution of the various tumours is shown in Table 11.

Microscopic Appearances of Tumours

Vascular lesions were characterised microscopically by collections of blood-filled spaces in the subepithelial connective tissue (lamina propria) (Fig. 61.). Early lesions apparently comprised proliferation and ectasia of either the subepithelial capillary plexus or other capillaries lying deeper within the lamina propria (Figs. 65,66). Cases with either proliferation or ectasia of capillaries but without formation of a distinct collection of blood-filled spaces were excluded from this series.

The diameter of the blood spaces ranged from about 20µm to 400µm or more and lesions were designated capillary haemangiomas or cavernous haemangiomas depending on whether the smaller or larger spaces predominated (Figs. 45,46). Capillary and cavernous lesions occurred with about equal frequency but many animals had examples of both types and some lesions were noted to have large capillary or small cavernous spaces. In many cases the vascular channels formed a loose network of fine, oedematous connective tissue but in

other cases they were separated by bands of more dense fibrous tissue. Occasionally, there was very little connective tissue evident so that the walls of the spaces were apparently formed by a double row of lining cells.

The cells which lined the blood-filled spaces appeared to be of endothelial type (Fig. 48); they were generally elongated cells with faintly basophilic, scanty cytoplasm and small, dark nuclei. In many cavernous haemangiomas the lining cells appeared flattened. However, some capillary lesions were more proliferative with a lining of plump cells which had ample basophilic cytoplasm and ovoid nuclei with a stippled chromatin pattern. Such lesions sometimes extended to the muscle layer of the bladder wall and occasionally appeared to penetrate between muscle bundles.

In some cases there was papillary proliferation of cells into the vascular spaces and it appeared that progression beyond this stage resulted in the formation of bands or whorls of spindle shaped cells such as occur in various sarcomas. Indeed, the degree of cellular and nuclear pleomorphism in four such lesions together with hypercellularity, a high mitotic rate and variable numbers of abnormal mitotic figures prompted the designation haemangiosarcoma (Figs. 47,49). In one case, a thickened area of about 10 x 12cm within the bladder wall was considered to be an haemangiosarcoma and secondary deposits were found in the regional lymph nodes. The primary lesion was characterised by bands and whorls of spindle-shaped or polygonal cells with large, pleomorphic nuclei containing prominent nucleoli. Some blood-filled spaces, lined by tumour cells, were evident between more solid, cellular areas. Features of the secondary deposit in the lymph node included fibrosis, haemorrhage and necrosis. There were some solid, cellular areas but there were also numerous circlets of two or three cells suggestive of angiogenesis.

Local haemorrhage was a common feature of many haemangiomas (Figs, 39,46), particularly those with thin walls or erosion of the overlying epithelium. Erythrocytes were often present in both

adjacent connective tissue and overlying epithelium, apparently within as well as between the epithelial cells. Scattered macrophages laden with haemosiderin were frequently present in the lamina propria. In some cases there was thrombosis of vascular spaces or organisation of exudates and associated fibroplasia.

In every bladder with vascular tumours a degree of cystitis was also present. This ranged from a mild reaction comprising scattered mononuclear cells or small foci of lymphoid cells to severe inflammation with numerous polymorphs, lymphoid and plasma cells. In some cases lymphoid follicles were present in the lamina propria and occasionally they were well-organised with obvious germinal centres. The number of polymorphs present was greatest where there was erosion and infection of the surface of lesions. The mucosa adjacent to vascular tumours was frequently oedematous but in some animals there was severe oedema with lymphatic ectasia or thickening and fibroplasia of the lamina propria so that the term polypoid cystitis was applicable.

Various changes were seen in the epithelium overlying, or adjacent to, the haemangiomas. When present, the overlying epithelium was often attenuated but in approximately one third of cases there was erosion or ulceration. Hyperplasia was a feature of the overlying epithelium in only a few cases but the adjacent epithelium was hyperplastic in at least 50% of cases. The number of cell layers was increased and there appeared to be downgrowths of epithelial buds with subsequent formation of either Brunn's nests, i.e. collections of transitional epithelial cells in subepithelial tissues with or without any obvious connection to surface epithelium, or cysts lined by cuboidal epithelium. There was occasional metaplasia of surface or cyst epithelium to a colonic type characterised by columnar cells with a proportion of mucus-secreting cells. Cystic cystitis or glandular cystitis were justified terms for the more severe of these cases (Figs. 57,58).

In a number of bladders with epithelial hyperplasia there was also dysplasia suggestive of carcinoma (Fig. 65). The

epithelium showed cellular atypia with nuclear and cellular pleomorphism, hyperchromasia and loss of polarity. In three of such cases the changes were particularly marked and in some areas there appeared to be early infiltration of the subepithelial connective tissue so that a diagnosis of transitional cell carcinoma was made (Fig. 62). Two other animals showed similar epithelial changes intimately associated with haemangiomas. It appeared that malignant epithelial cells were infiltrating the vascular channels of the subepithelial capillary plexus and the subjacent haemangioma, lining the spaces or forming small nests or whorls of cells (Fig. 64). In one further example, a proliferative haemangioma contained similar groups and nests of cells, apparently within vascular channels, but the degree of atypia was such that the cells could not be identified. The overall impression was of an haemangiosarcoma but on close inspection, the cell nests resembled foci of carcinoma. Sections taken from deeper within the block failed to show any connection to surface epithelium but the appearance was more and more suggestive of carcinoma. It finally proved impossible to classify this lesion by light microscopy.

Epithelial tumours were mostly (13/27 cases) papillary outgrowths of transitional cell epithelium. The simplest lesions comprised delicate, branching villi made up of a fine, fibrovascular core covered with transitional epithelium (Fig. 50). In more florid lesions further branching, folding and fusion of villi produced a more tubular pattern with groups of transitional cells with a small central cavity surrounded by the fibrovascular connective tissue (Fig. 51). The epithelium resembled more or less closely the normal transitional epithelium of the urinary bladder and was made up of between 4 and 10 or more layers of cells. The cells were columnar or broadly fusiform in shape with their long axes generally perpendicular to the supporting stroma. The cytoplasm was finely vacuolated, granular or uniformly eosinophilic and the nuclei were ovoid or spindle-shaped with a stippled chromatin pattern. In some areas the nuclei, particularly of superficial cells were small, dark and apparently pyknotic.

A few tumours showed variations from this basic pattern with loss of cellular polarity leading to the formation of whorls and nests of transitional cells within the layers of epithelium. Such cells were often more distinctly spindle-shaped and showed evidence of nuclear crowding often accompanied by pleomorphism.

The base of many lesions was formed by a narrow pedicle but more solid tumours often had a broader base. Scatterred lymphoid cells were frequently seen at the base of the tumours and also in the connective tissue cores which were occasionally oedematous.

Using the strict criteria of Pamukcu (1974) all such outgrowths were classified as transitional cell carcinomas although the macroscopic appearance was that of a papilloma. In 11 animals the lesions examined were designated papillary, non-infiltrative carcinoma Grade I (Fig. 51). However, in two other cases the degree of cellular atypia, with loss of polarity and formation of whorls of spindle-shaped cells, indicated Grade II carcinoma (Fig. 52). It should be noted that in many of these cases more than one lesion was present and that each individual tumour was not always available for histological examination.

Other epithelial tumours found in a further 14 animals are detailed in Table 12. Two cases with epithelial hyperplasia and dysplasia in the absence of more advanced carcinoma are included because the changes were considered severe enough to warrant classification as early infiltrative carcinoma. In both these animals multiple haemangiomas were present as well as moderate cystitis and epithelial hyperplasia with formation of Brunn's nests. In addition, there were occasional foci with nests of transitional cells infiltrating the lamina propria and showing pronounced cellular pleomorphism and loss of polarity together with nuclear pleomorphism and hyperchromasia (see Figs. 62,65,66). The overall appearance of the epithelium in these cases would fall into Mostofi's category of "unstable mucosa" (1973).

In six animals, widely infiltrating transitional cell carcinomas were present. These were all classified as Grade III lesions although the appearance often varied from one area to another. In general, the bulk of these growths comprised trabeculae or nests of spindle-shaped cells with large, hyperchromatic nuclei (Fig. 53). Frequently, the nests of transitional cells contained a central cavity so that parts of a tumour had a glandular pattern. Occasionally, true glandular metaplasia was evident with formation of acini lined by columnar cells interspersed with goblet cells. In other instances, there were foci of squamous metaplasia with collections of prickle cells and some keratinisation.

In all these infiltrative growths the aggressive nature of the tumour was indicated by the cellular pleomorphism and high mitotic rate and by the marked nuclear pleomorphism with occasional binucleate cells or uninucleate giant cells. Some lesions were associated with a marked scirrhous reaction so that islands of neoplastic cells were embedded in a dense, collagenous stroma. In other cases, tumour cells infiltrated between muscle bundles and lined connective tissue spaces so that the lesion resembled haemangiosarcoma (Fig. 56). However, in all these cases despite occasional areas of anaplasia, the transitional cell origin of the tumour was obvious. Secondary tumour deposits were only detected in one case and these were apparently confined to the renal lymph node.

One poorly-differentiated, infiltrative growth is included in the series. It was presumed to be of transitional cell type although the cells could not be classified by light microscopy and the point of origin was not detected. The lesion comprised small groups of 4 or 5 cells scattered through the bladder wall and associated with a marked scirrhous reaction. Occasional cells had vacuolated cytoplasm and in some areas there appeared to be an acinar pattern but mucous stains proved negative. Although this tumour extended through the bladder wall to the serosa, no secondary deposits were detected.

Two animals in this series had large polypoid masses in their urinary bladders. These masses ranged from 5.0 to 20.0cms in diameter and had short but distinct stalks. Microscopy showed these to be glandular lesions with large numbers of dilated acini and mucous lakes embedded in a loose connective tissue stroma. Acini were lined by columnar cells or goblet cells and there was some variation in differentiation and mucus content which reflected the differentiation pattern of normal colonic mucosa. In some acini the lining epithelium was flattened by retained secretion. Special stains revealed the presence of neutral mucins and sialomucins including sulphomucins. Mitotic figures were infrequent and the general appearance suggested a diagnosis of adenoma. However, in one animal, secondary deposits of a poorly differentiated adenocarcinoma with only occasional foci of PAS-positive material were identified in local lymph nodes. In both cases, occasional areas of nuclear crowding, hyperchromasia or pseudostratification in irregular acini suggested that the tumours be classified as adenocarcinomas, albeit well-differentiated (Fig. 54).

The final type of epithelial lesion detected in this series was a squamous cell carcinoma found in one animal. This tumour formed an infiltrative growth with nests of squamous cells in a scirrhous stroma (Fig. 55). There was some central keratinisation of cell nests often merging with areas of necrosis. According to the criteria detailed in Section A of this Chapter the lesion was designated Grade III. Secondary tumour deposits were evident in abdominal lymph nodes, some of which were virtually replaced by highly scirrhous squamous carcinoma.

A degree of inflammatory or immunological cellular reaction was evident in the bladder mucosa of all animals with epithelial tumours. This ranged from occasional scattered lymphoid cells in cases with Grade I lesions to massive and extensive inflammatory reactions associated with ulceration and necrosis of infiltrative lesions. In the majority of cases there was some evidence of chronic cystitis with foci of lymphoid cells which occasionally

formed distinct follicles. Lesions with superficial erosion or ulceration were associated with an infiltrate of polymorphs. In some animals lesions were severe enough to constitute follicular cystitis or polypoid cystitis with oedema and thickening of the lamina propria. It is interesting to record that both cases of adenocarcinoma were associated with a marked cystitis characterised by large numbers of plasma cells and lymphoid cells and scattered polymorphs. This reaction was particularly heavy in the lamina propria of the tumour masses (Fig. 54).

More than half (18/27) of the urinary bladders in this series showed areas of epithelial proliferation in addition to the epithelial tumours described. In some animals this was limited to focal hypercellularity whereas in others there was marked proliferation with formation of intraepithelial whorls and subepithelial Brunn's nests or cysts. In one case, foci of carcinoma in situ were noted adjacent to an infiltrating spindle-cell carcinoma. Occasional cases showed glandular metaplasia (Fig. 58). Although impossible to quantify, the degree of hyperplasia and dysplasia seemed to be more severe in epithelium adjacent to infiltrative carcinoma or in those bladders with moderate or severe cystitis.

Fibrous lesions proved to be well-circumscribed tumours consisting of well-differentiated fibroblasts or fibrocytes and loose bundles of fine collagen fibrils (Figs. 59,60). In some specimens the loose texture of the growth imparted a myxoid appearance but stains for mucin were consistently negative. Connective tissue stains confirmed both the presence of collagen fibrils and the absence of smooth muscle fibres except in association with small blood vessels.

These tumours were confined to the lamina propria and were distinguished from the proliferative lesions of polypoid cystitis by the paucity of inflammatory cells within the growths. In addition, fibromas were associated with some compression of adjacent tissue so that in many cases concentric layers of

condensed collagen at the periphery of the lesion formed an indistinct capsule (Fig. 59).

The constituent fibrocytes or fibroblasts were small, spindle-shaped cells with elongated or ovoid nuclei which had a fine, stippled, chromatin pattern (Fig. 60). Mitotic figures were rare. Isolated polymorphs, lymphoid and other mononuclear cells were identified in some specimens.

The fibromas tended to form nodules which protruded above the mucosa and in the majority of lesions the overlying transitional epithelium was attenuated. Occasionally there was some erosion of this epithelium. However, in almost all cases there was some proliferation of adjacent epithelium. This was generally limited to slight hyperplasia and in some animals it was more marked with formation of Brunn's nests or cysts and focal metaplasia. There was mild or moderate cystitis in all cases but the tumours themselves contained few inflammatory cells. Scattered polymorphs and mononuclear cells were present in the lamina propria and focal collections of lymphoid cells were occasionally found adjacent to tumours. In a few cases, dilated lymphatics or capillaries were noted at the edges of the fibromas and in one animal the fibrous lesions were intimately associated with small haemangiomas (Fig. 63).

TABLE 7: Additional neoplasms found in 60 animals with urinary bladder tumours

Type of tumour	Number of cases	Percentage
Upper alimentary tract		
squamous cell carcinoma	21	35
UAT papillomas	43	72
ruminal lipomas	1	
Lower alimentary tract		
intestinal carcinoma	5	8
adenomatous intestinal lesions	31	52
Urinary system		
renal cortical adenoma	1	
Genital tract		
uterine haemangioma	1	
Endocrine system		
pituitary adenoma	1	
phaeochromocytoma of adrenal	1	
adrenal cortical adenoma	1	
thyroid tumour	2	
Liver and Gall bladder		
cystic duct adenoma	1	
Other		
melanoma	1	

TABLE 8: Breed distribution in 60 cases of urinary bladder tumour

Aberdeen Angus	13
Ayrshire	10
Blue Grey	4
Galloway	7
Hereford	5
Highland	6
Friesian	2
Shorthorn	10
Charolais	1
Red Poll	1
Jersey	1
Luing	1
Unknown	2
TOTAL	63

TABLE 9: Types of urinary bladder tumours in 60 cattle

Type	Number of Cases
Vascular	38
Epithelial	27
Fibrous	8
TOTAL	73

TABLE 10: Inter-relationships of types of urinary bladder tumour in 60 cattle

TYPE	Number of Cases	Percentage
Vascular alone	26	43
Epithelial alone	16	27
Fibrous alone	5	8
Vascular and epithelial	10	17
Vascular and fibrous	2	3
Epithelial and fibrous	1	2
Vascular, epithelial and fibrous	0	0
TOTAL	60	100

TABLE 11: Size distribution of urinary bladder tumours in 60 cattle

Maximum diameter	Number of Cases			
	Vascular	Epithelial	Fibrous	TOTAL
0-1cm	23	12	8	43
1-6cm	13	5	0	18
Over 6cm	2	10	0	12
TOTAL	38	27	8	73

TABLE 12: Types of epithelial tumours in 27 urinary bladders

Type of lesion	No.of Cases	Percentage
Transitional cell carcinoma		
Papillary non-infiltrative Grade I	11	41
Papillary non-infiltrative Grade II	2	7
Infiltrative Grade III	6	22
Dysplasia/early infiltrative carcinoma	2	7
Infiltrative carcinoma in haemangioma	2	7
Poorly differentiated carcinoma	1	4
Adenocarcinoma	2	7
Squamous cell carcinoma	1	4
TOTAL	27	100

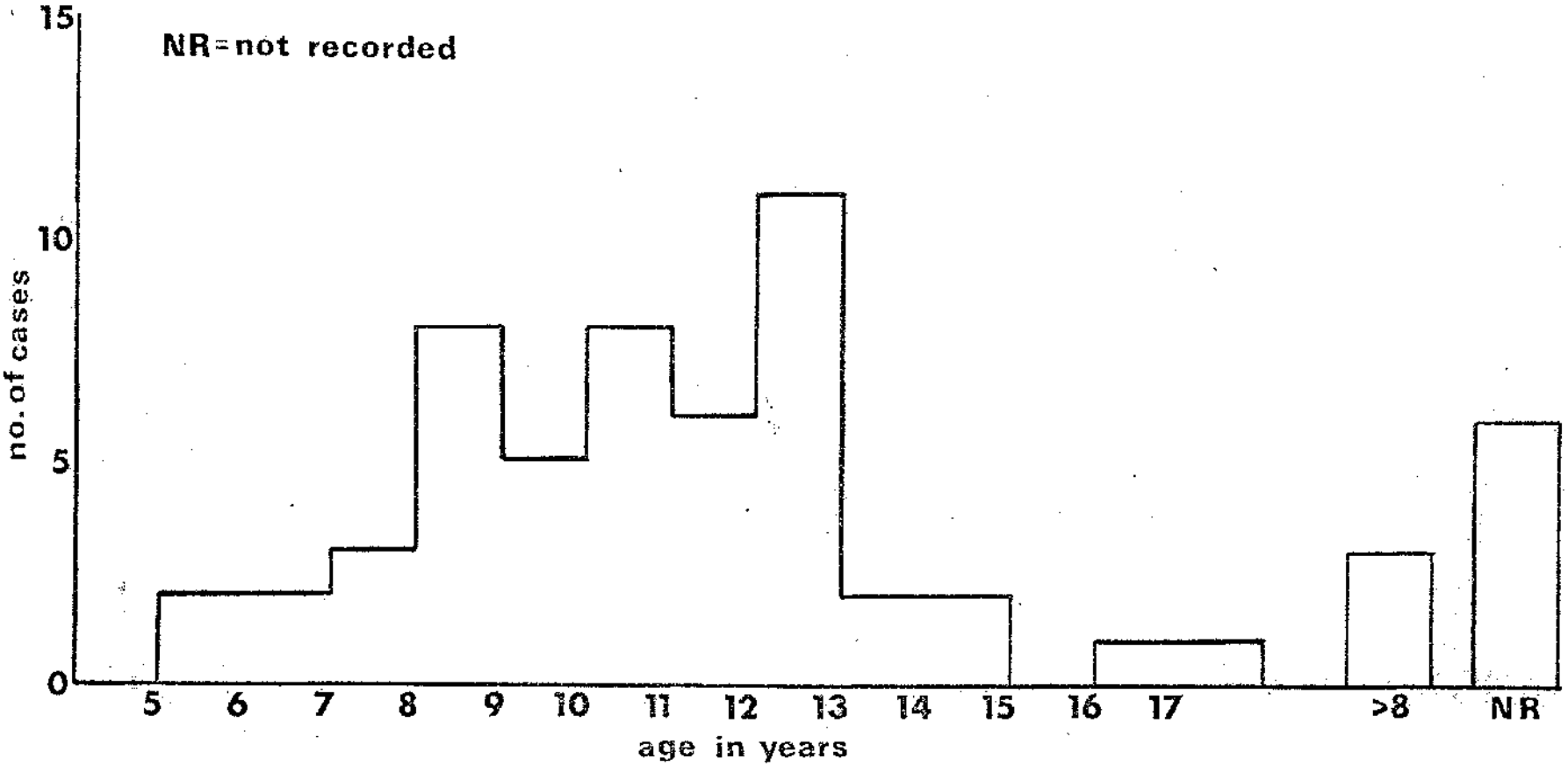


FIG. 37: Age distribution of 60 cattle with urinary bladder tumours.

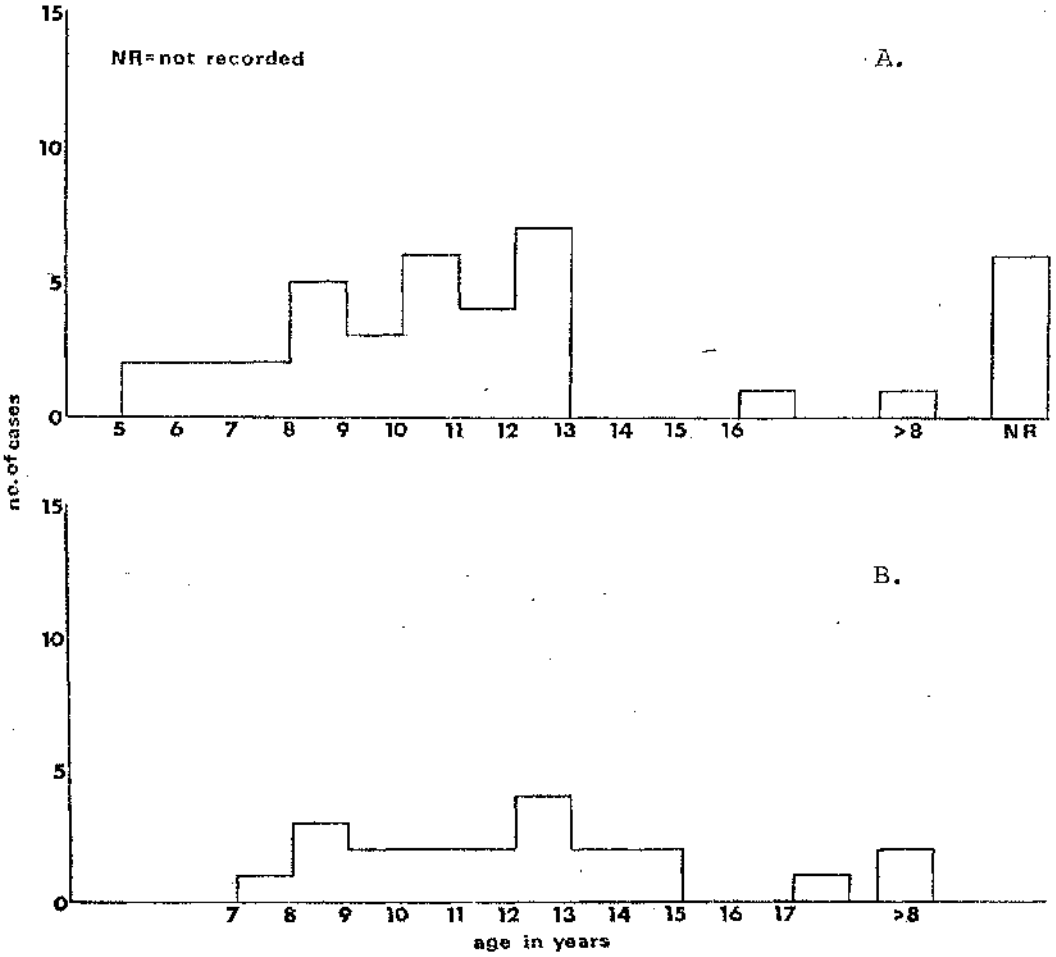


FIG. 38: Ages of 60 animals with bladder tumours.

A. 39 cases with bladder tumours alone.

B. 21 cases with UAT plus bladder tumours.

SECTION B: TUMOURS OF THE URINARY BLADDER

FIGURES 39-44: Macroscopic appearances of tumours.

FIG 39: Multiple haemangiomas of the urinary bladder. Raised vascular lesions of various sizes (small arrows) are scattered over the bladder mucosa. Areas of subepithelial haemorrhage are also present (large arrow).

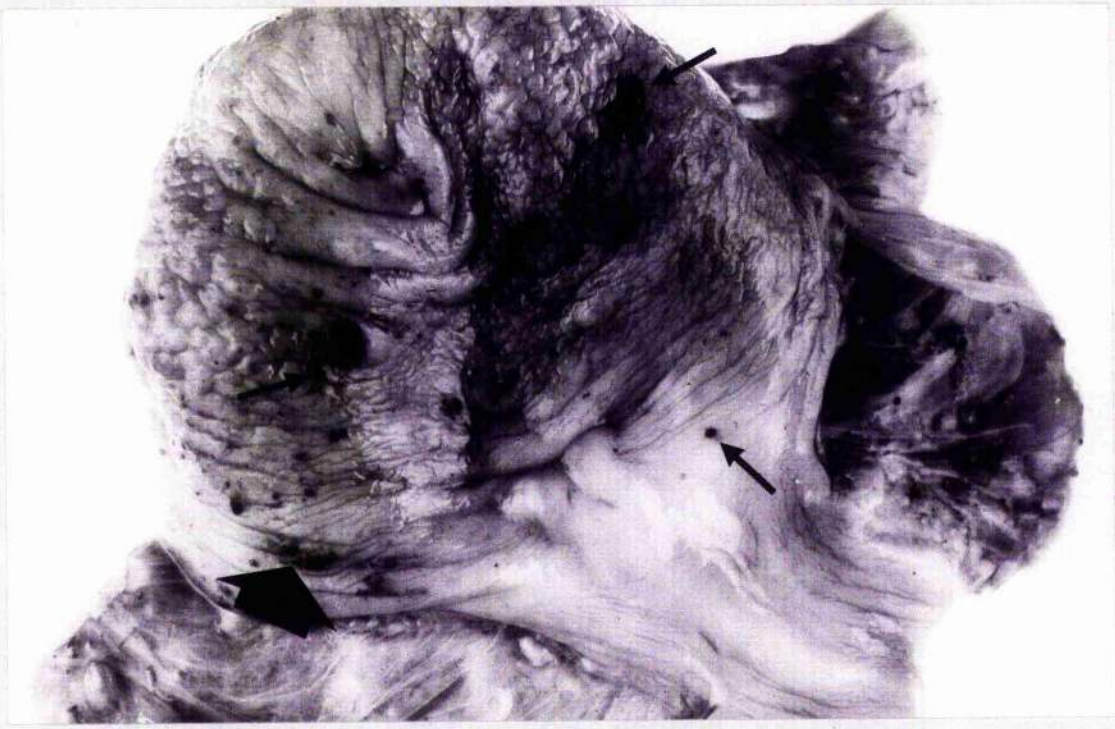


FIG. 40: Haemangioma of the urinary bladder. A nodular, haemorrhagic plaque bulges from the bladder mucosa.

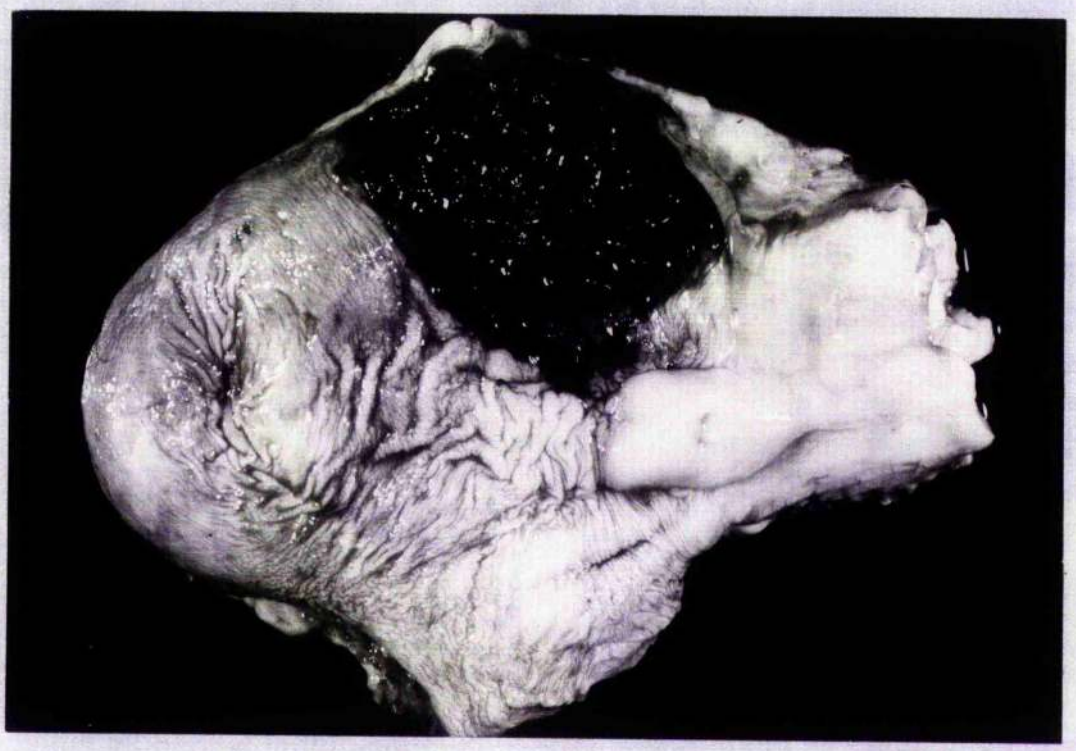


FIG. 41: Transitional cell carcinoma of the urinary bladder. A papillary carcinoma forms a broad-based polyp (large arrow). Small haemangiomas are also present (small arrows) and the intervening mucosa is hyperplastic and oedematous.

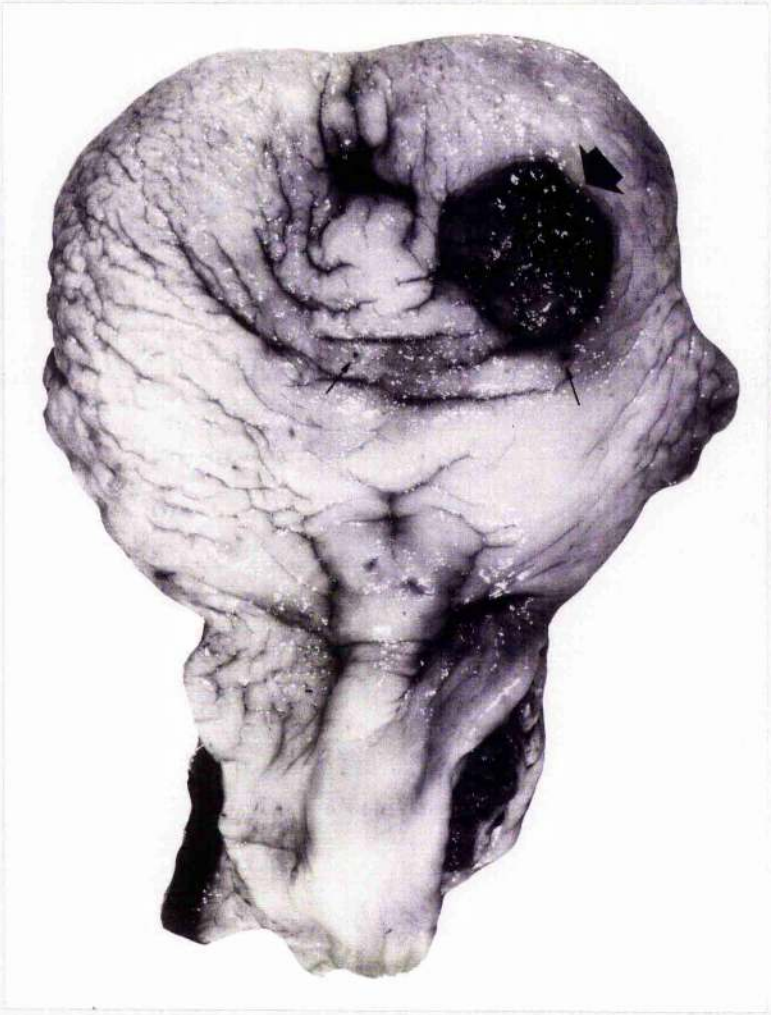


FIG. 42: Transitional cell carcinoma of the urinary bladder. Vertical section of the bladder wall reveals an irregular tumour mass with central necrosis (N). The base of the tumour is irregular and there is infiltration of the muscle layers (arrow).

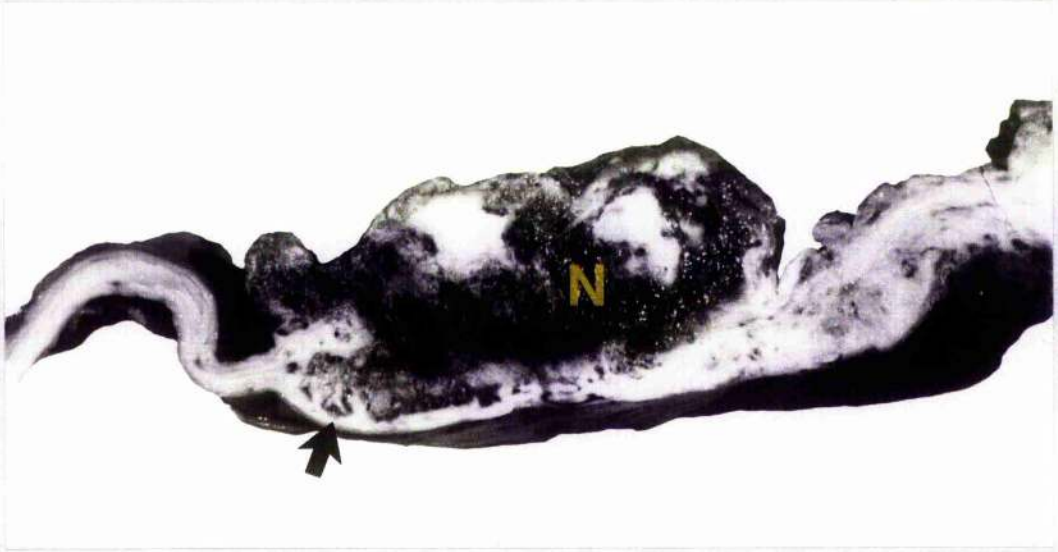

N

FIG. 43: Adenocarcinoma of urinary bladder. A large polypoid mass (asterisk) protrudes from the mucosa of the everted bladder. Similar, smaller masses are also present (arrows).

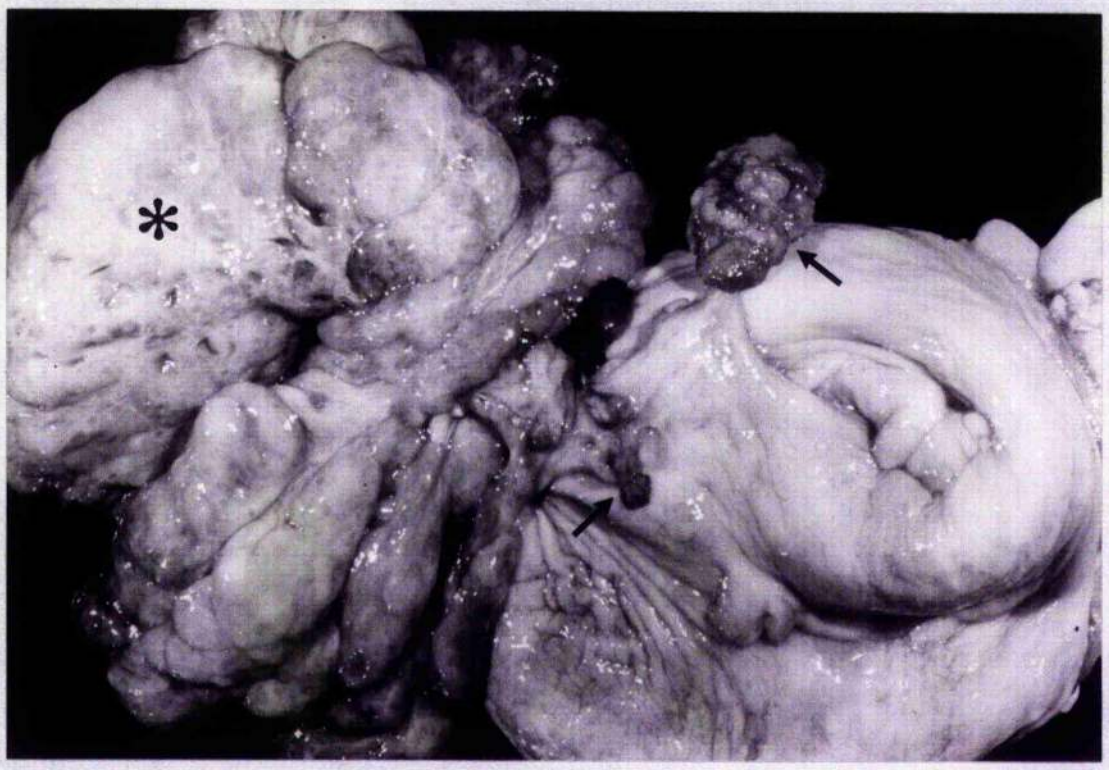
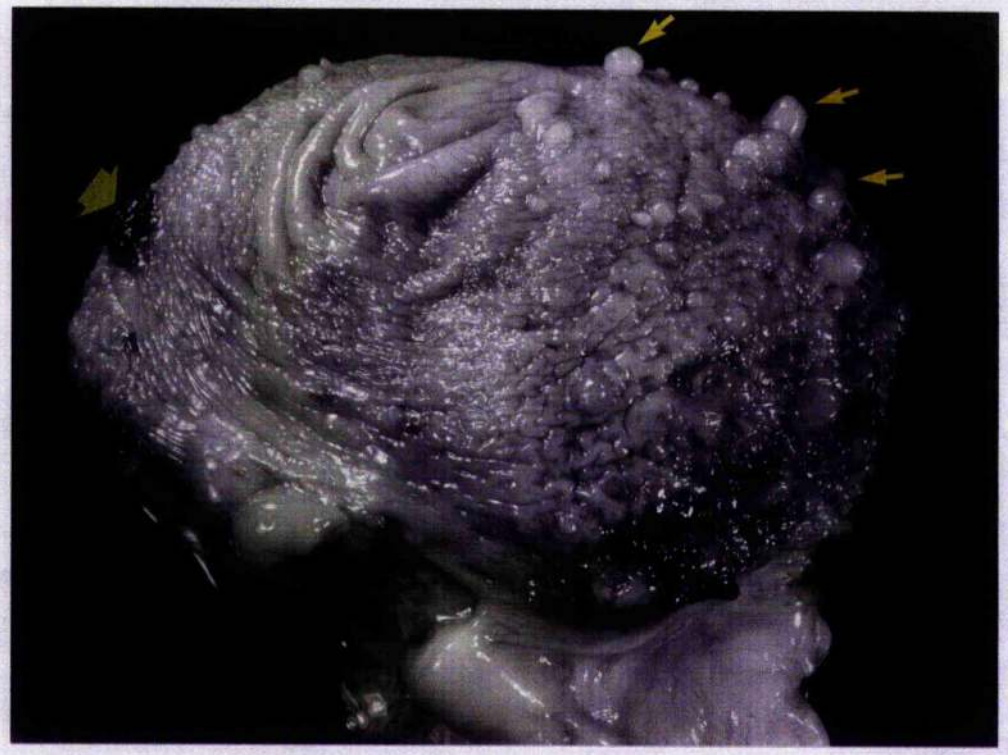


FIG. 44: Multiple bladder tumours. Sub-epithelial fibromas appear as smooth-surfaced nodules projecting from the mucosa (small arrows). Vascular lesions are seen as dark, irregular swellings (large arrow).



SECTION B: TUMOURS OF THE URINARY BLADDER

FIGURES 45-66: Microscopic appearances of tumours.

FIG. 45: Cavernous haemangioma (H&E x 35). A vascular polyp protrudes into the lumen of the bladder. It comprises blood-filled cavernous spaces (s) in a moderately abundant fibrous stroma (CT) and is covered by attenuated transitional epithelium.

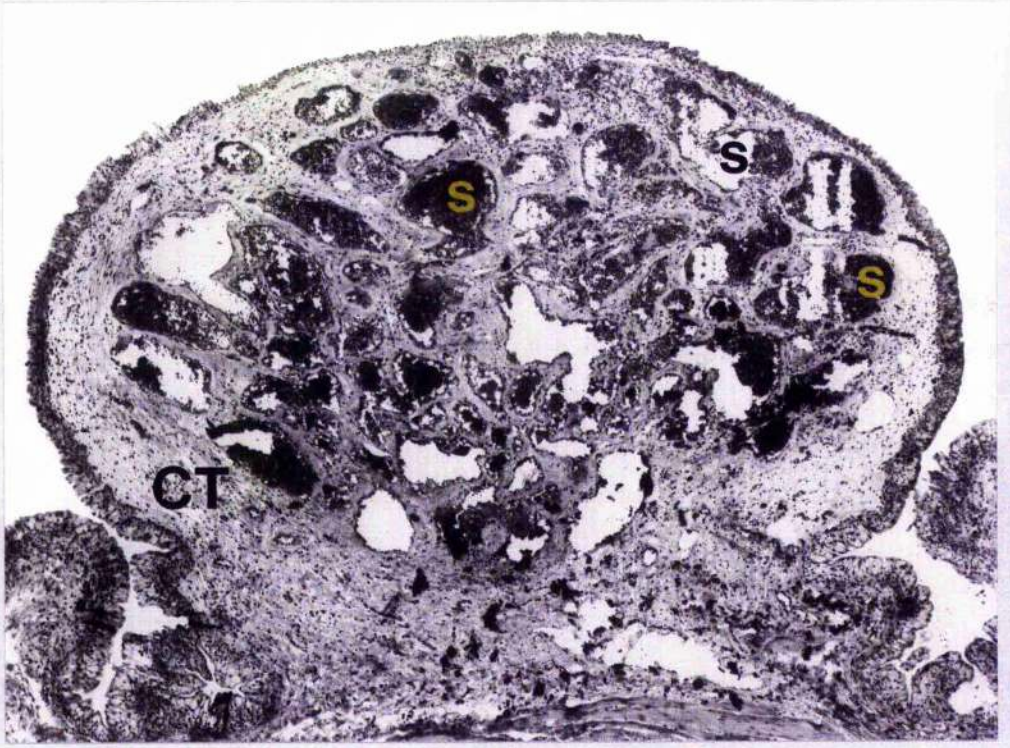

S
S
S
CT

FIG. 46: Capillary haemangioma (H&E x 35). An ill-defined haemangioma (H) consists of irregular capillary spaces in the subepithelial connective tissue (CT). There is superficial thrombosis, ulceration and haemorrhage (asterisk).

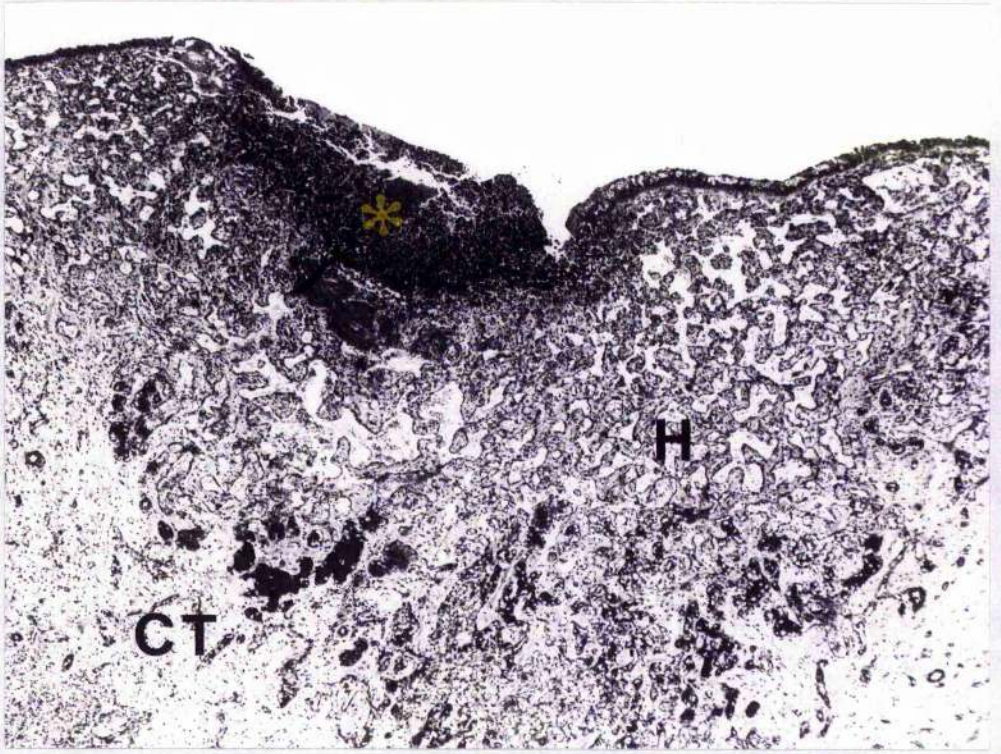

H
CT

FIG. 47: Haemangiosarcoma (H&E x 110). Irregular vascular spaces (s) lined by plump hyperchromatic cells (small arrows) lie immediately beneath attenuated transitional epithelium. Deeper layers of the growth comprise clumps and whorls of tumour cells (large arrows).

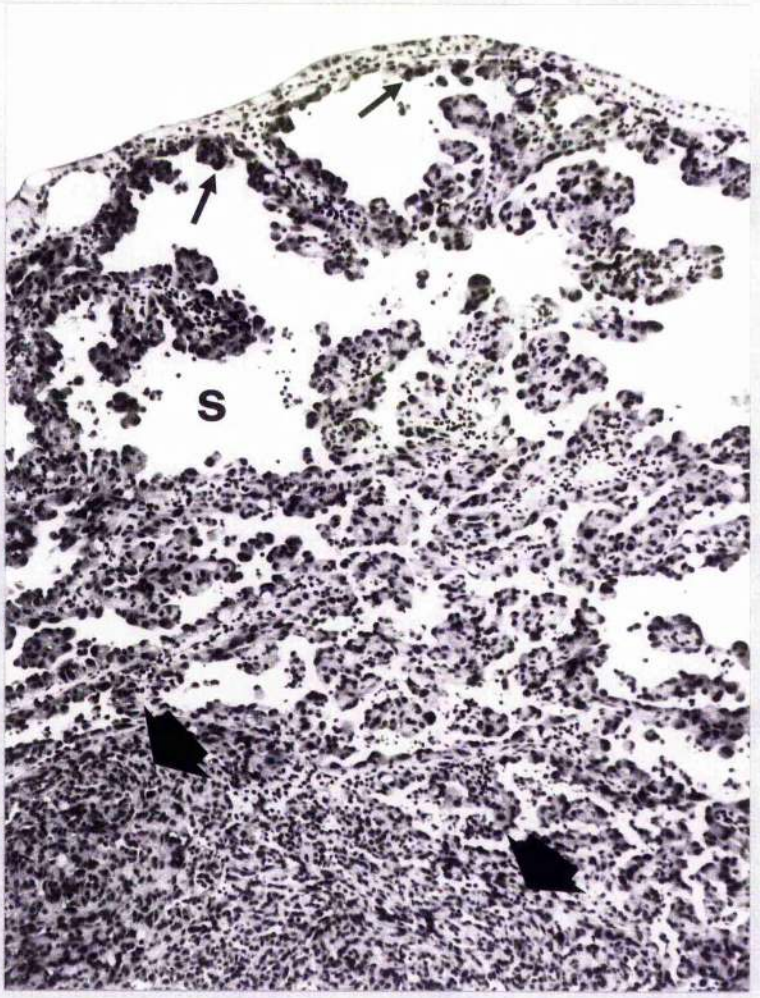

S

FIG. 48: Endothelial cells in haemangioma (H&E x 400). Capillary and cavernous vascular spaces are lined by flat endothelial cells with ovoid or elongated nuclei and attenuated cytoplasm (arrows).

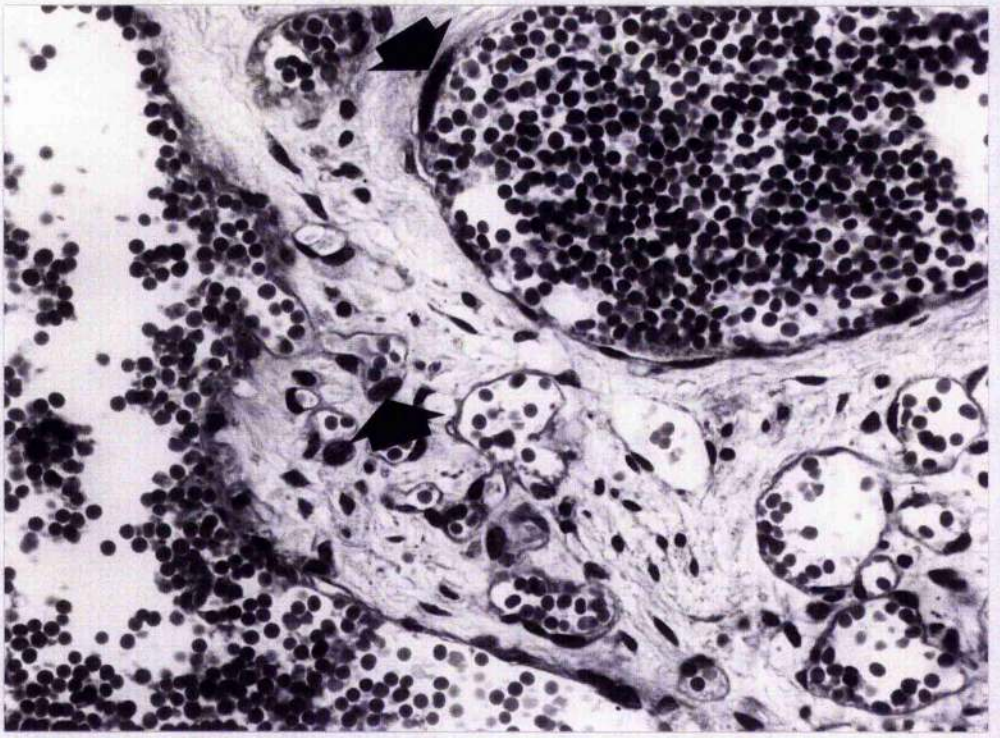


FIG. 49: Endothelial cells in haemangiosarcoma (H&E x 400). Irregular vascular spaces are lined by abnormal endothelial cells (arrows). The tumour cells have hyperchromatic cytoplasm and large nuclei with coarsly clumped chromatin.

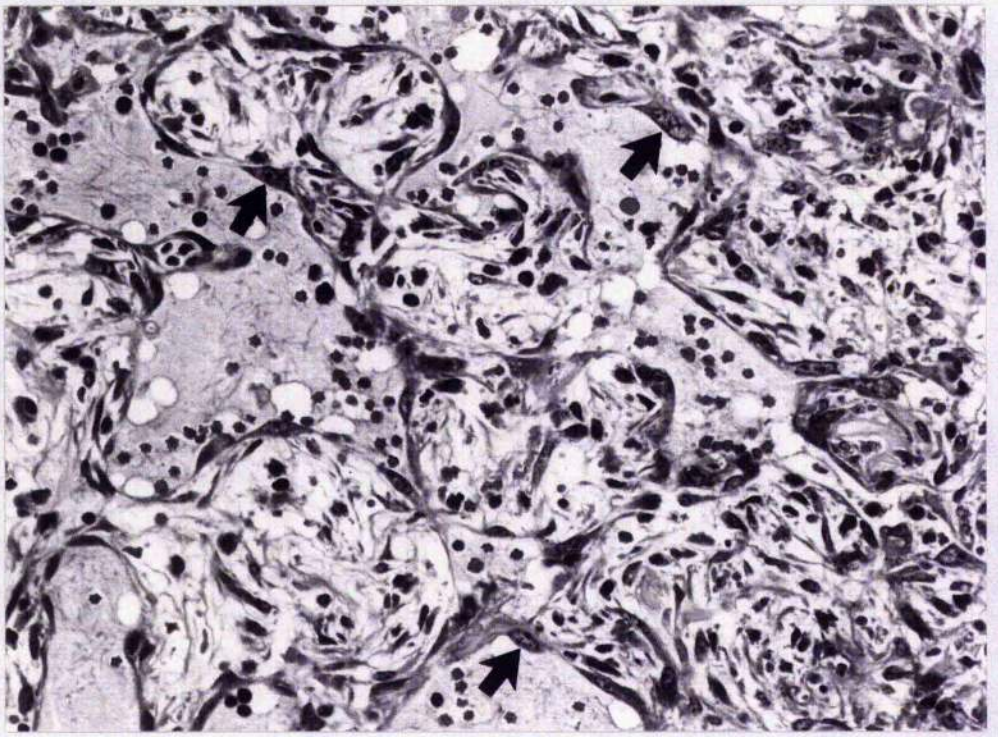


FIG. 50: Papillary transitional cell carcinoma (H&E x 15). Delicate, branching fingers of transitional epithelium extend into the lumen of the bladder supported by a fine fibrovascular stroma.

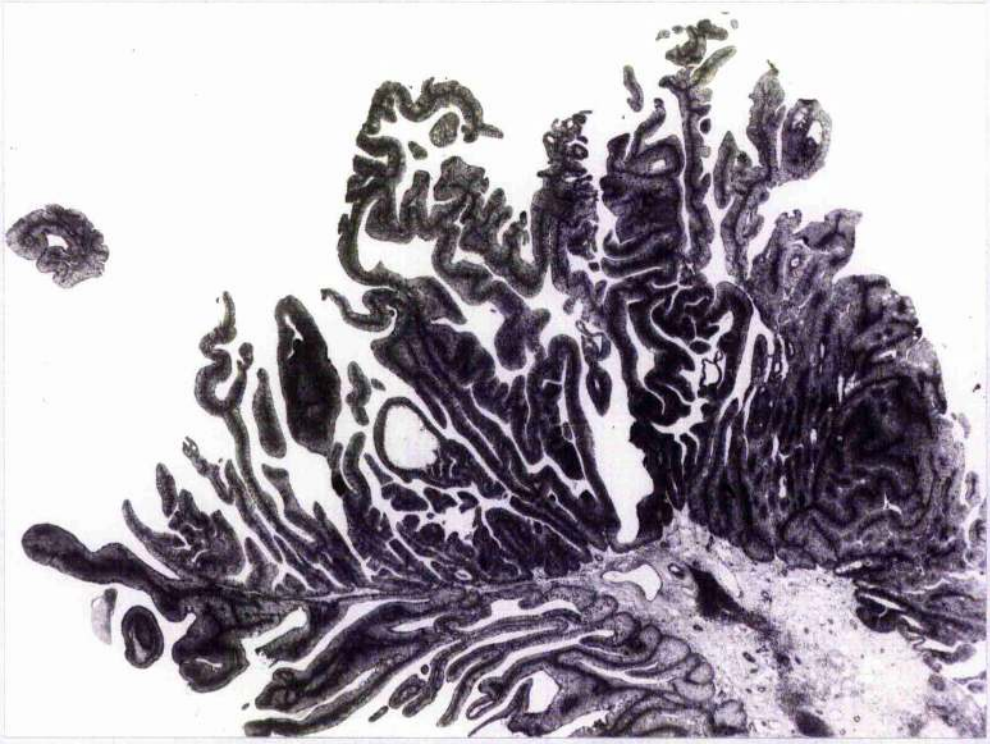
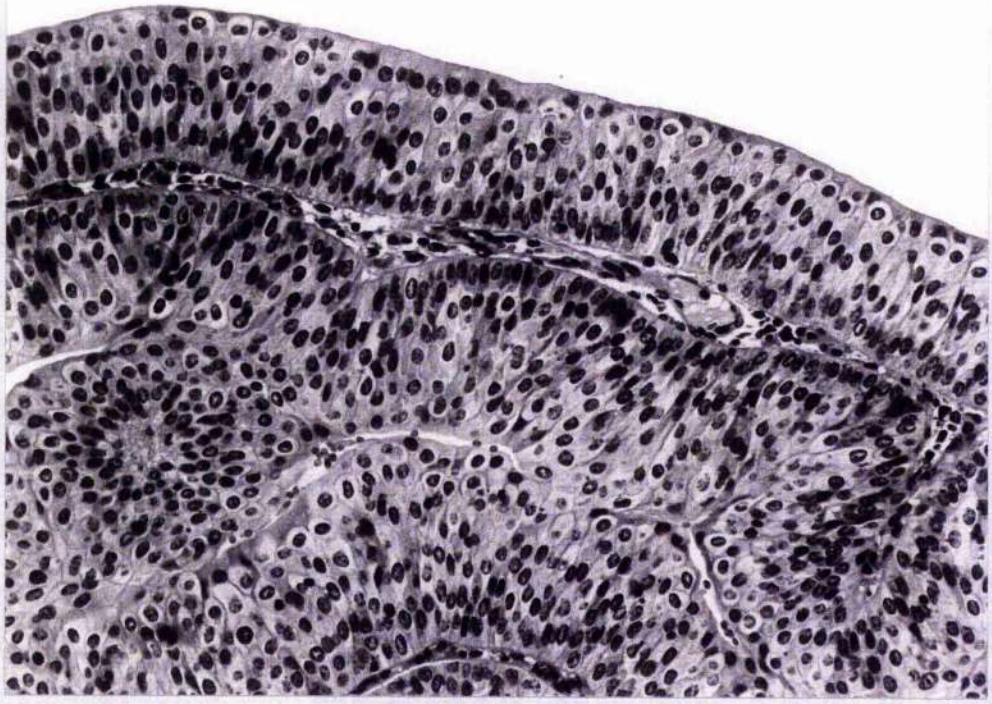


FIG. 51: Transitional cell carcinoma, Grade I (H&E x 250). Layers of transitional cells cover delicate strands of fibrovascular connective tissue. Branching and fusion of the villi imparts a solid appearance. The transitional cells are fairly uniform in appearance and arranged at right angles to the supporting stroma.

FIG. 52: Transitional cell carcinoma, Grade II (H&E x 250). Layers of transitional cells cover delicate strands of fibrovascular tissue. Branching and fusion of the villi imparts a solid appearance. There is loss of polarity, cellular crowding and some nuclear and cellular pleomorphism.



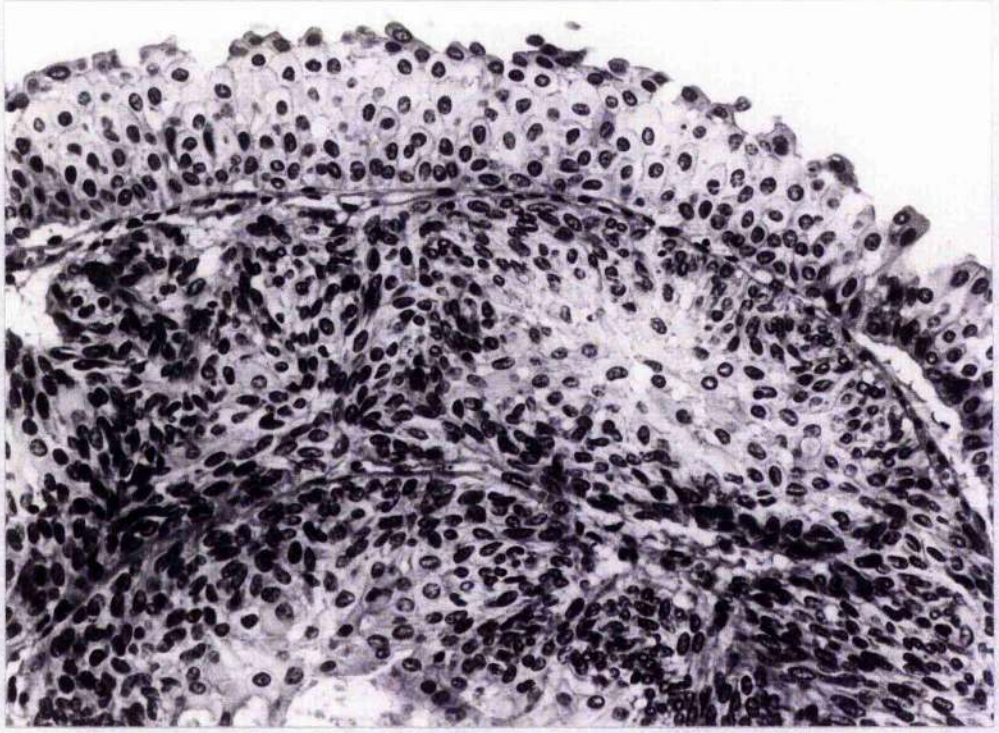


FIG. 53: Infiltrating transitional cell carcinoma (Grade III) (H&E x 250). Pleomorphic epithelial cells infiltrate between surviving strands of muscle (M).

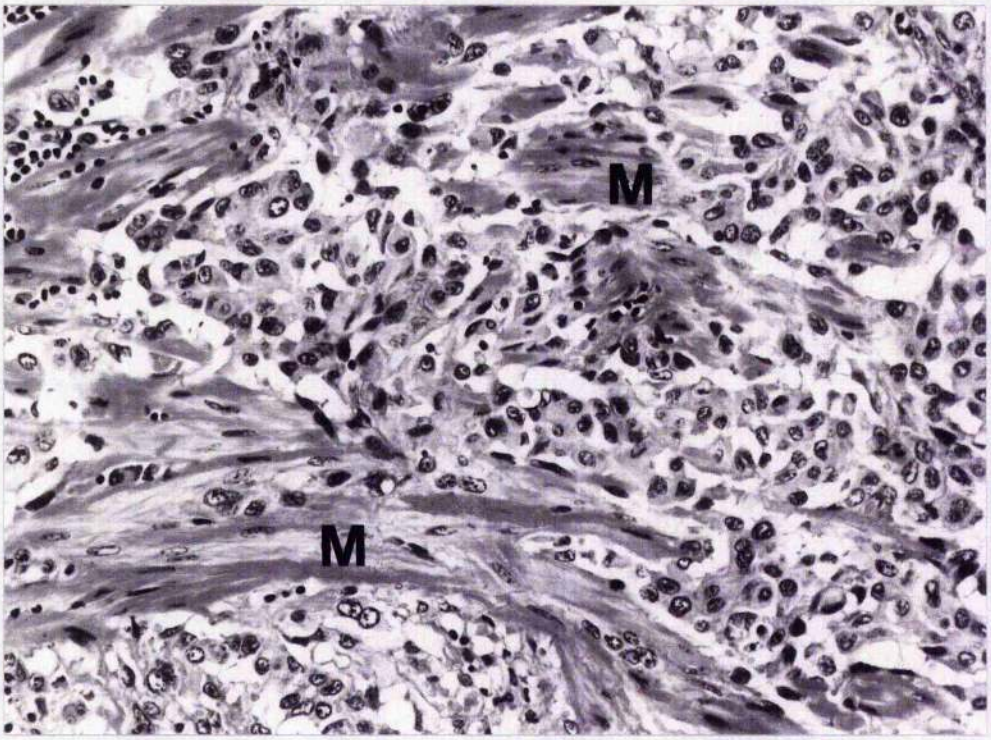

M
M

FIG. 54: Mucus-producing adenocarcinoma of the urinary bladder (H&E x 250). Irregular acini (A) of columnar and goblet cells are surrounded by a connective tissue stroma containing large numbers of lymphoid and plasma cells. The tumour cells show nuclear crowding.

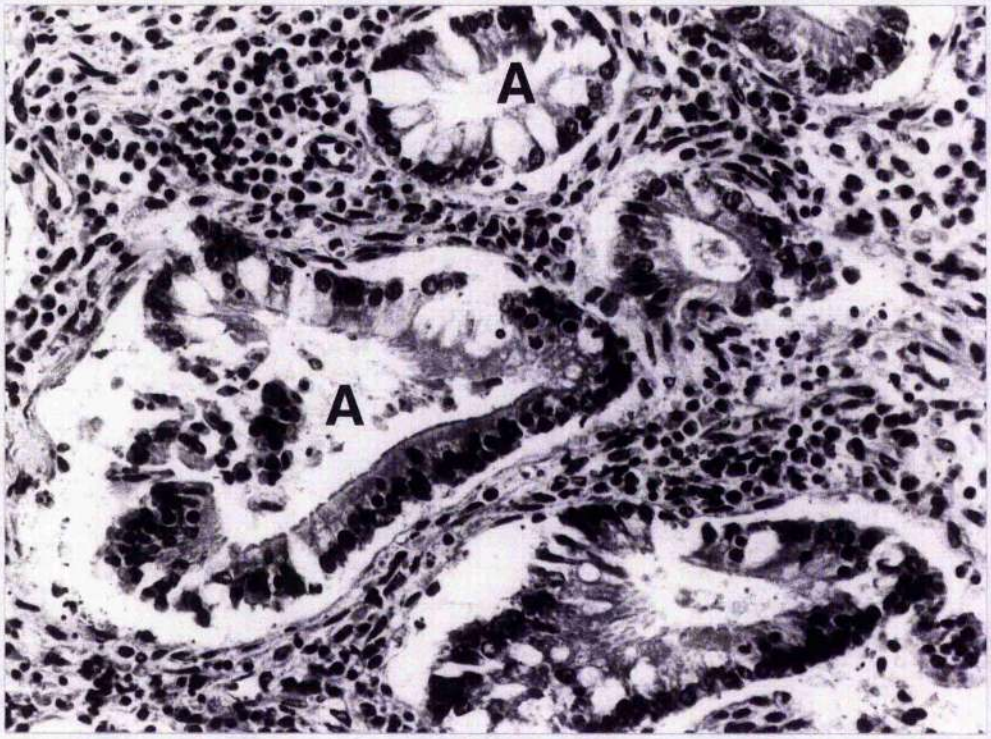

A
A

FIG. 55: Squamous cell carcinoma of the urinary bladder (H&E x 250). Irregular islands (large arrows) and nests (small arrows) of pleomorphic squamous cells are surrounded by fibrous connective tissue. There is central cavitation and necrosis (asterisks) of some tumour islands but no real evidence of keratinisation in this area.

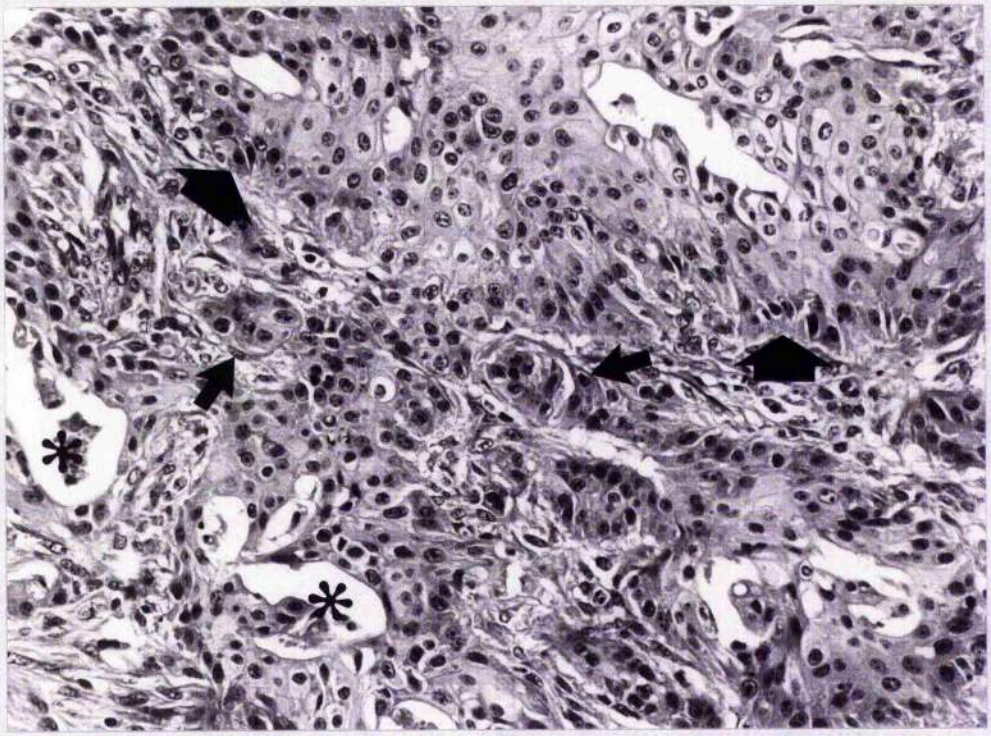
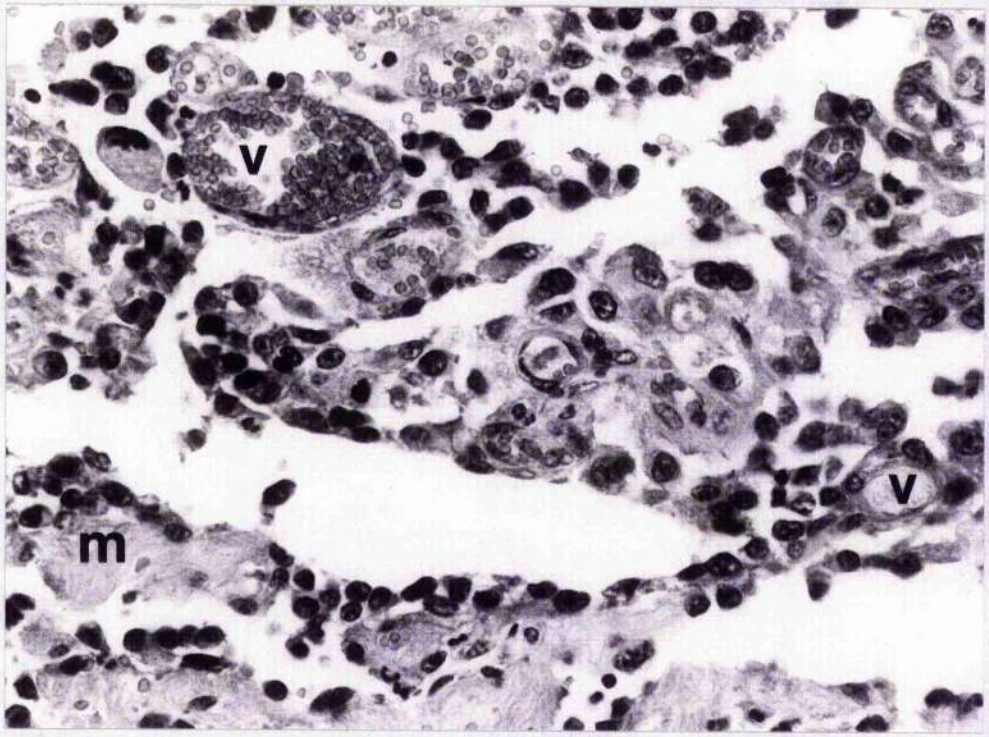


FIG. 56: Poorly-differentiated bladder tumours (H&E x 420).

a) Vascular carcinoma resembling haemangiosarcoma. Hyperchromatic tumour cells clothe small blood vessels (v) and line irregular spaces between surviving muscle bundles (m). The appearance mimicks that of endothelial lined spaces in haemangiosarcoma.

b) Haemagiosarcoma resembling adenocarcinoma. Hyperchromatic tumour cells line irregular vascular spaces (s) which contain detached tumour cells but only a few red blood cells (arrows). Attenuated transitional epithelium (e) covers the surface of the growth. The appearance mimicks that of irregular acini in an adenocarcinoma.


v
v
m

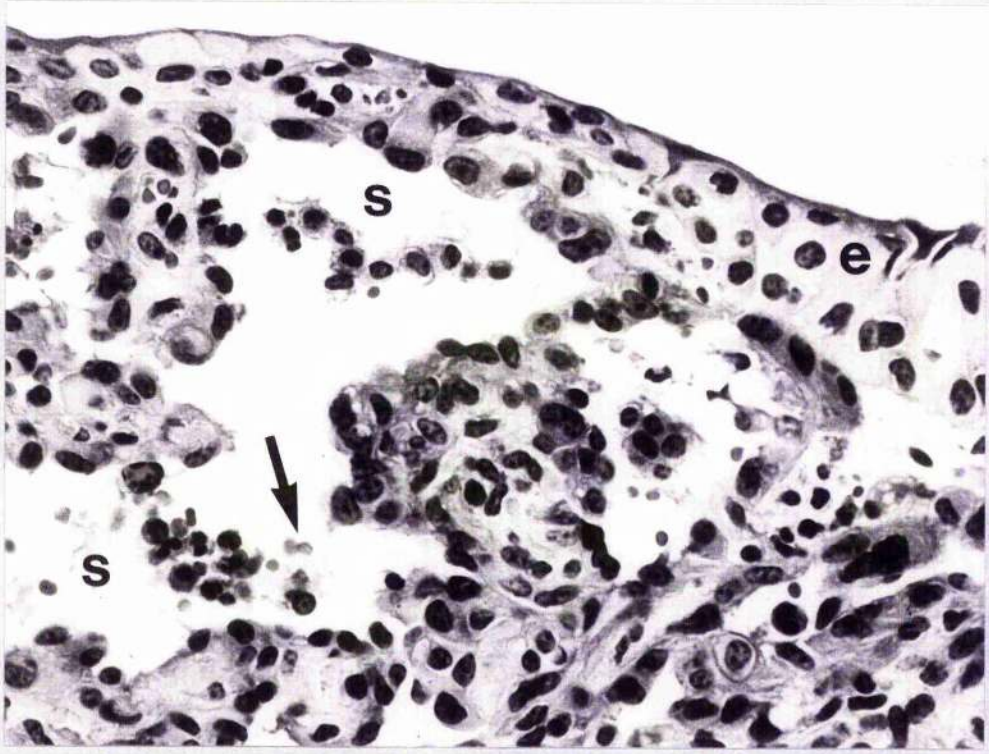
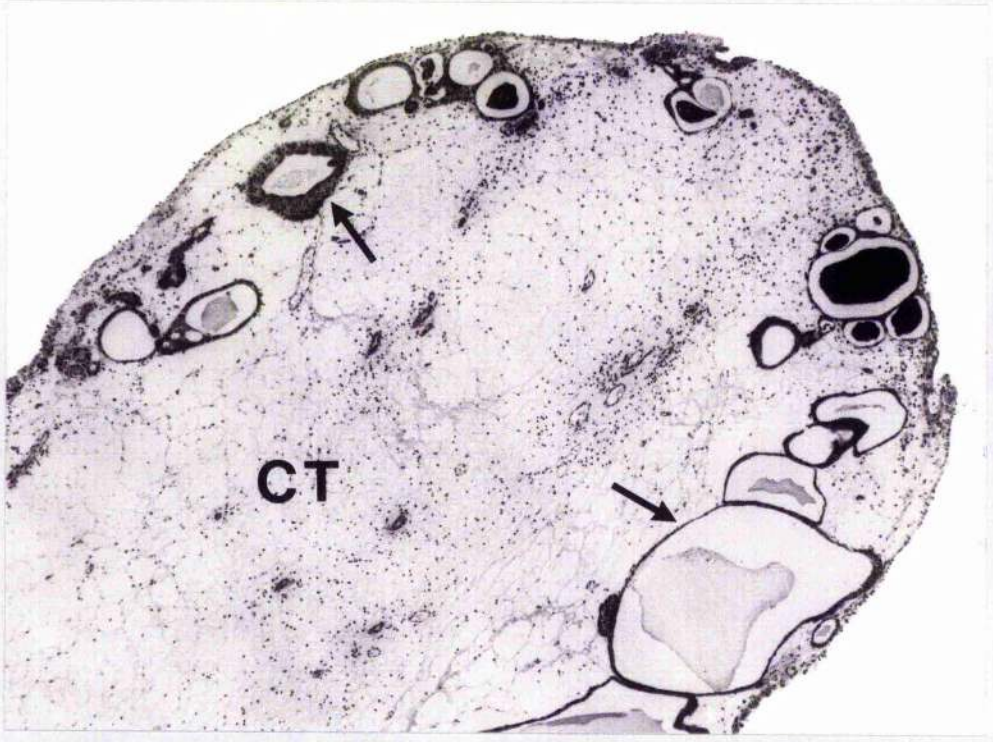

s
e
s

FIG. 57: Cystic cystitis (H&E x 35). Cystic spaces (arrows) lined by one or more layers of transitional epithelium lie below the surface of a mucosal polyp. The bulk of the polyp comprises loose fibroblastic connective tissue (CT) containing scattered mononuclear cells.

FIG. 58: Glandular cystitis (H&E x 35). Well-differentiated mucous glands (arrows) extend into the subepithelial connective tissue. The mucosal surface is thrown into irregular folds and the lamina propria is hypercellular.


CT

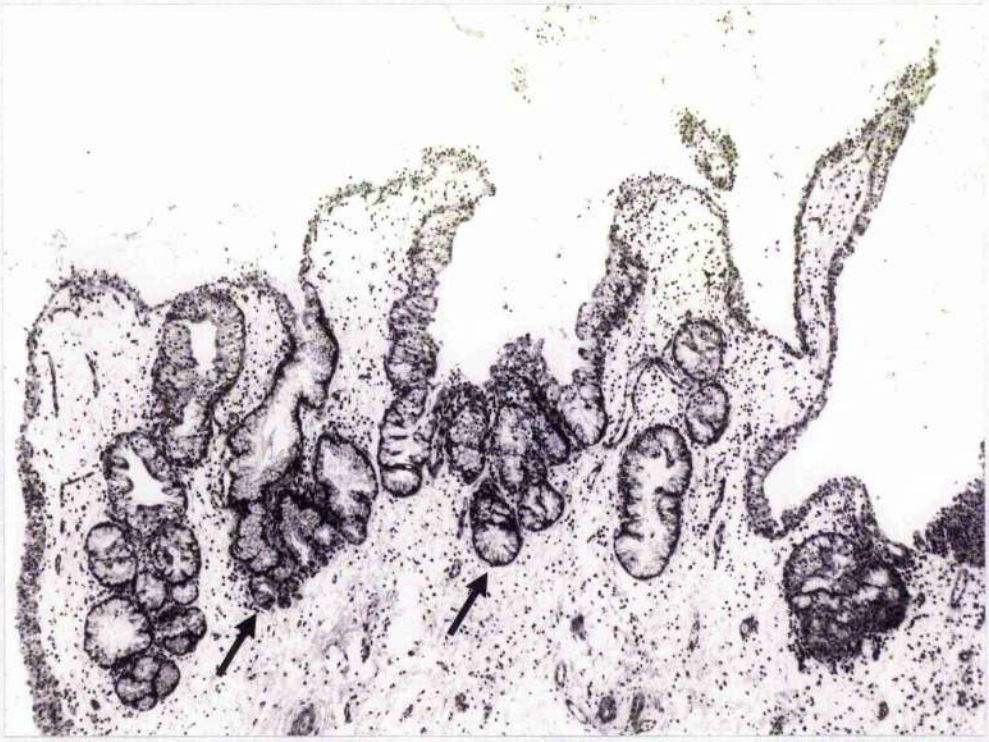


FIG. 59: Fibroma of the urinary bladder (H&E x 25). A well-defined, subepithelial nodule protrudes into the bladder lumen. An indistinct capsule is formed around the tumour by compressed connective tissue.

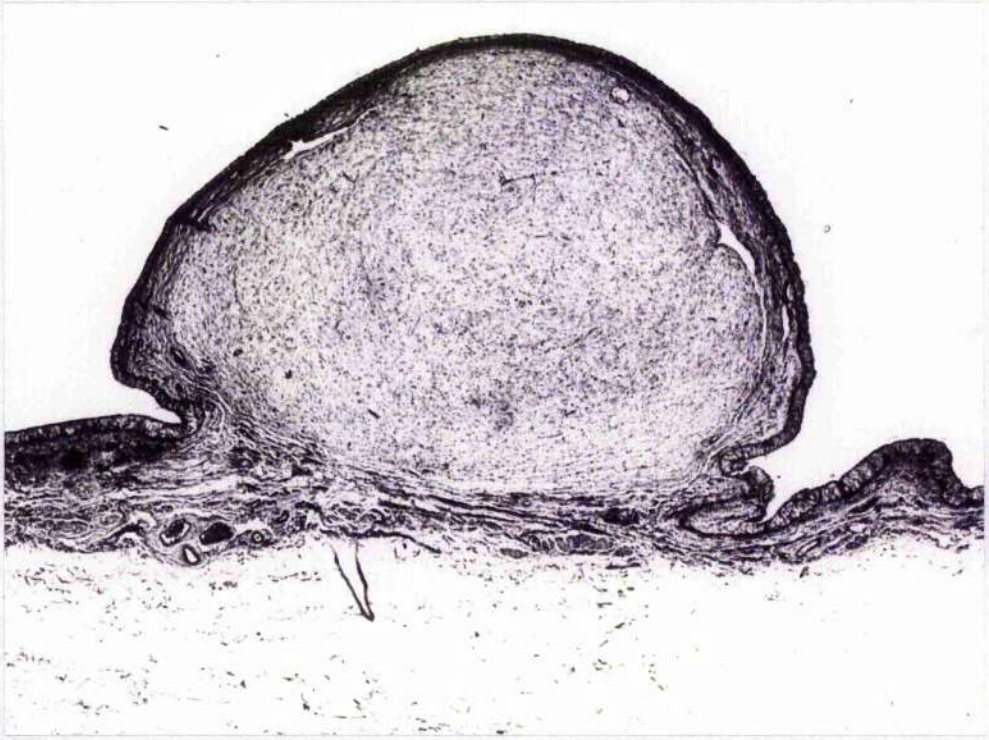


FIG. 60: Fibroma of the urinary bladder (H&E x 400). The tumour comprises loose bundles of fine collagen fibres (f) and fibroblasts (arrows). The cells are arranged haphazardly but there is no marked pleomorphism.

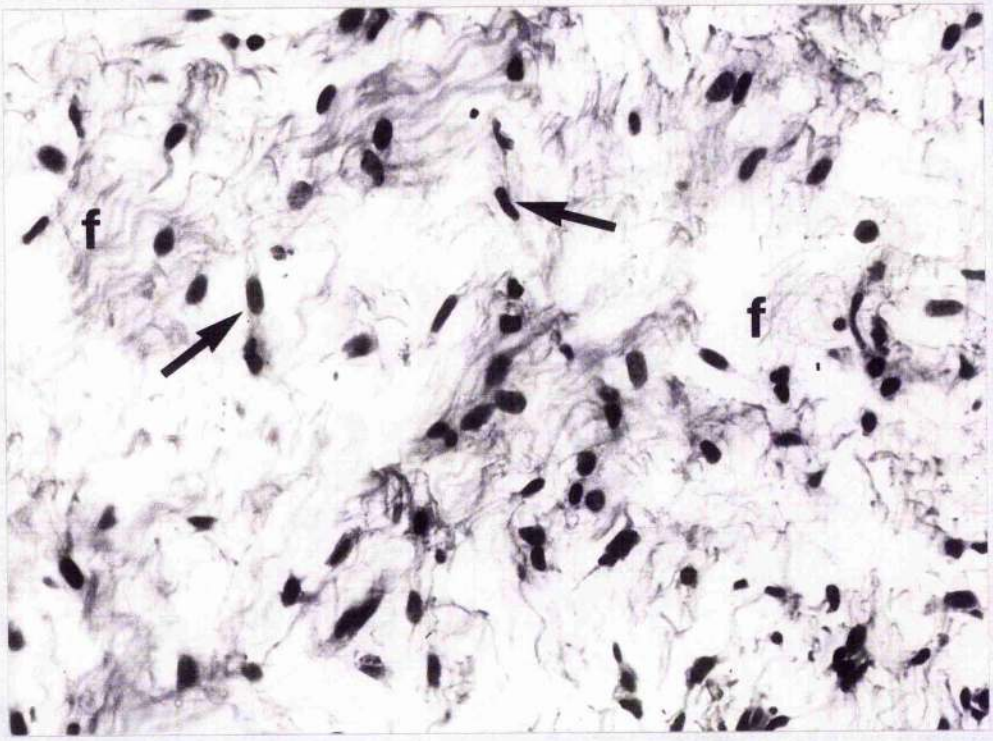

f
f

FIG. 61: Simple haemangioma (H&E x 5). An irregular vascular polyp comprises cavernous spaces overlying a broad connective tissue base.

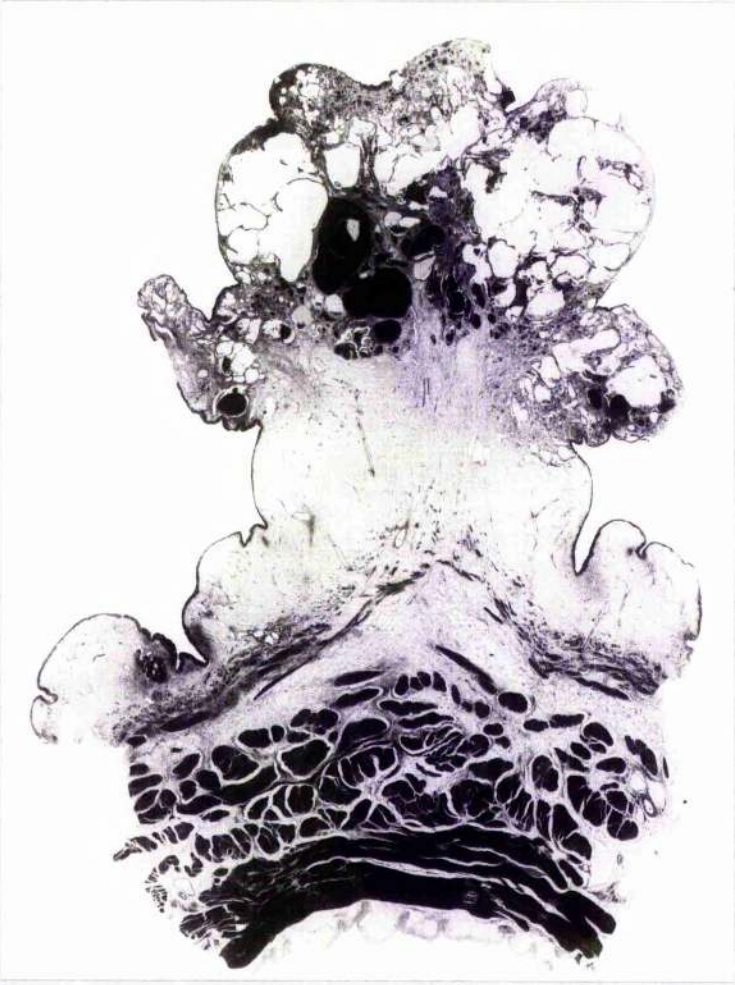


FIG. 62: Haemangiomatous polyp (H&E x 15). Foci of haemorrhage (small asterisk) are associated with haemangiomatous areas (H) comprising collections of blood-filled spaces in a dense connective tissue stroma (CT). The overlying epithelium is hyperplastic and there is a central area of ulceration and haemorrhage (large asterisk). A focus of early infiltrating carcinoma is also present (arrow).

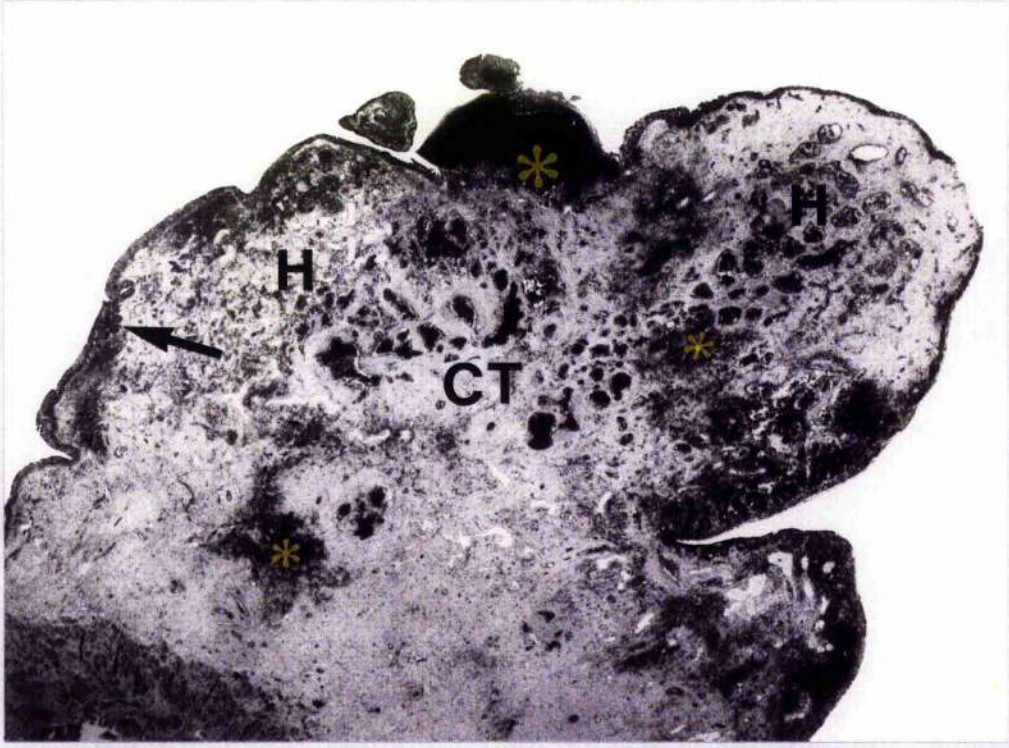

H
H
CT

FIG. 63: Co-existent fibroma and capillary haemangioma (H&E x 50). A loose whorl of fibrous tissue (F) lies adjacent to, and is partly overlain by, a capillary haemangioma (H). Above the fibroma there is an area of subepithelial haemorrhage (asterisk).

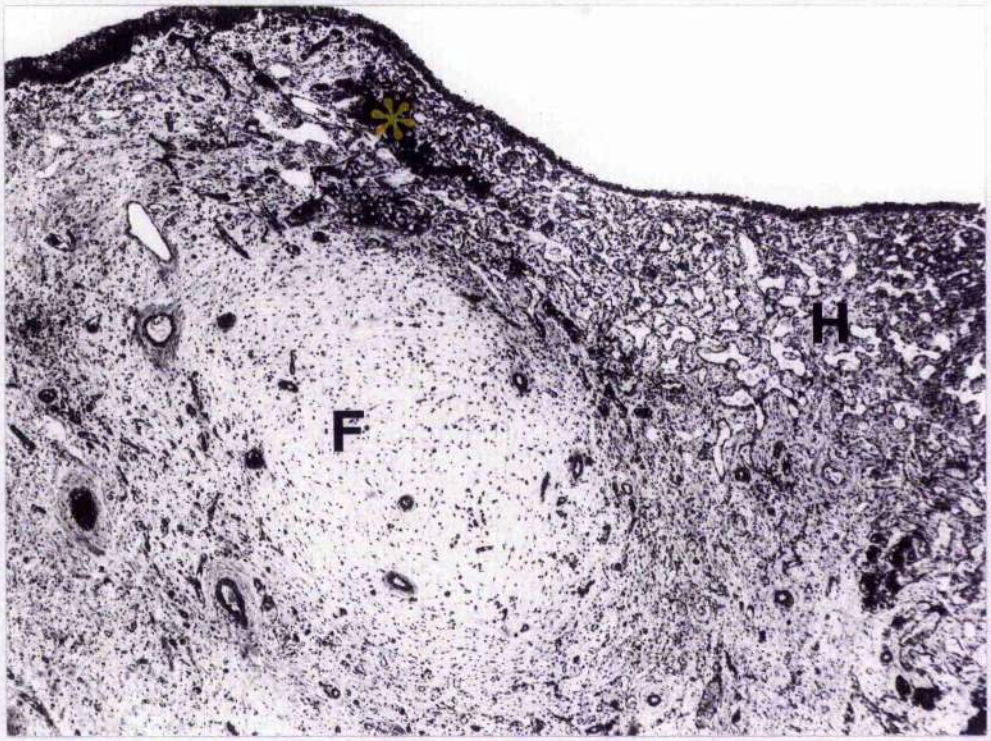

H
F

FIG. 64: Co-existent carcinoma and haemangioma (H&E x 110). Dilated vascular spaces mark the junction between areas of carcinoma (Ca) and haemangioma (H).

FIG. 65: Proliferative lesions of the urinary bladder (H&E x 250).

a) Capillaries. There is proliferation and ectasia of capillaries in the lamina propria (thin arrow) and subepithelial plexus (thick arrow). Scattered inflammatory cells are present in the connective tissue (CT). The overlying epithelium shows some loss of polarity.

b) Epithelium. There is epithelial hyperplasia and dysplasia with nodules projecting into the underlying connective tissue (arrows). The cells show nuclear and cellular pleomorphism with loss of polarity and some whorling. Scattered inflammatory cells are present in the lamina propria.

FIG. 66: Early tumour formation in the urinary bladder (H&E x 250).

a) Haemangioma. A small collection of dilated capillaries (arrows) lies below hyperplastic epithelium (E) and compresses the surrounding connective tissue (CT). There is little evidence of haemorrhage.

b) Carcinoma. Irregular islands of pleomorphic epithelial cells (arrow) infiltrate the subepithelial connective tissue forming a small focus of carcinoma. There is haemorrhage into the surrounding tissues (asterisk).

Discussion

A series of 60 animals with tumours of the urinary bladder was compiled. There were clinical signs of urinary tract abnormality in 35 cases and there was a history of haematuria in a further 2 cases (Grimshaw, personal communication). These findings indicate that bovine CEH still exist in Britain, at least in the West of Scotland. It has been noted by other workers that the urinary bladder lesions associated with CEH are diverse and that in early cases they may not be frankly neoplastic (Munday, 1966; Pamukcu, 1955). The series described here was limited to cases with distinct tumours of the urinary bladder.

Co-existent UAT carcinoma was found in 21 (35%) of those animals with urinary bladder tumours. The situation in the West of Scotland in therefore broadly similar to that described in Brazil by Tokarnia et al., (1969); a geographical area with a high incidence of bovine CEH also has a high incidence of UAT carcinoma in cattle. Some individual animals within that area may be affected concurrently with both disorders. The situation is somewhat different from that recorded in Turkey and other regions where bovine haematuria is enzootic (Pamukcu et al., 1976); bladder tumours have been studied in detail in such areas but UAT carcinoma in cattle has not been recognised.

A wide variety of breeds was represented in this series but no male animals were included. The sex distribution merely reflects the type of animal kept by the farms concerned and does not contradict the findings of other workers that any breed and either sex may be affected by CEH (Pamukcu et al., 1976; Rosenberger, 1971; Bull et al., 1932).

Most of the affected cattle were between 8 and 12 years of age with the peak incidence at 12 years (Fig. 37) thus confirming that most cases of CEH occur in middle-aged or older cows (Rosenberger, 1971; Pamukcu et al., 1976). The age distribution

resembles that for cases of UAT carcinoma (Fig. 1) except that more animals with bladder lesions were less than 8 years of age while fewer were over 12 years. Comparison of animals with bladder tumours alone and those with UAT plus bladder tumours (Fig. 38) also showed that animals with UAT carcinoma formed a slightly older group than those with bladder tumours alone. Similar results were recorded in Brazil with haematuria being observed in animals aged 2 years or more but UAT carcinoma only being observed in animals aged 5 years or more (Dobereiner et al., 1967). Those authors attributed the occurrence of tumours at both sites to the oncogenic action of bracken and suggested that the age at which an animal starts eating fern, the duration of feeding and the amount consumed may determine the appearance of acute bracken poisoning, or CEH, or UAT carcinoma. Results quoted by Grimshaw and Evans (1980) indicated that animals with bladder tumours alone probably had less exposure to bracken fern than animals with UAT carcinoma. Such differences in access to bracken fern and age incidence of the various tumours provide some explanation for the lack of reports of UAT carcinoma from other countries where CEH and bladder tumours are well recognised.

In addition to UAT carcinoma, associated findings in 60 animals with urinary bladder tumours included UAT papillomas, intestinal adenomas and carcinomas and hepatic telangiectasis. As noted earlier (Section B. Results), the significance of the alimentary tract lesions has been or will be discussed elsewhere. Hepatic telangiectasis was recorded in 22 cases (37%) and similar changes have been noted in association with bladder tumours by other authors (McKenzie, 1978b; Mugera and Nderito, 1968). In this present study, however, no hepatic tumours were found and the lesions were indistinguishable from those described by Ponomarkov and Mackey (1974) as being common in adult dairy cows. They were considered not to be significant.

Tumours of the urinary bladder ranged from discrete lesions of less than 1.0cm diameter to irregular, nodular masses of over 10.0cms diameter or widely infiltrating growths which produced

diffuse thickening of the bladder wall. In the majority of animals (62%) two or more tumours were present and a number of individuals (13/60) had more than one type of tumour present. Tumours were classified as either vascular, epithelial or fibrous and most cases (63%) had vascular lesions. Epithelial lesions were present in only 27 cases (45%). This contrasts with the findings of Pamukcu et al., (1976) who recorded epithelial growths in more than 90% of their cases. They contend that a greater variety and more malignant tumours are seen in Turkey and Yugoslavia and our results do not refute that. Lesions described by Brazilian workers vary from one report to another. Tokarnia et al., (1969) recorded haemangioma or capillary proliferation in 10 out of 12 animals with bladder lesions whereas Neto et al., (1975) recorded papillomas and carcinomas of the bladder in 8 animals with haematuria. There was no obvious predilection site for the tumours in 60 bladders and vascular tumours in particular were frequently scattered throughout the mucosa. Secondary tumour deposits were found in the regional lymph nodes of only 4 cases (7%) and there were no distant metastases. This is in accordance with the described behaviour of urinary bladder tumours in cattle and in other species, including man (Pamukcu, 1974; Mostofi et al., 1973).

The microscopic appearances of urinary bladder lesions in 60 cattle were similar to those described by other authors (Pamukcu, 1962; Dzuvic, 1969a; Smith & Beatson, 1970; McKenzie, 1978b). Vascular tumours were seen as subepithelial collections of capillary and cavernous blood-filled spaces. Many were lined by flattened endothelial cells, others appeared more proliferative and some infiltrated the deeper layers of the bladder wall. Epithelial tumours formed delicate papillary outgrowths or appeared as nodular or infiltrative downgrowths. Fibrous tumours appeared as discrete subepithelial collections of fine collagen fibrils and associated fibroblasts.

Vascular lesions may be considered to be characteristic of CEH (Plummer, 1944; Rosenberger, 1971) and certainly haemorrhage was regularly associated with the presence of haemangiomas in this

series. However, haematuria was not confined to animals with vascular lesions and also occurred in cases with epithelial lesions. Moreover, since red cells were present at all levels of intact epithelium, apparently within as well as between the epithelial cells, it appears that erosion of the epithelium is not a prerequisite for haematuria. Similar findings have been noted in cases of CEH by other authors (McKenzie 1978b; Plummer, 1944). Haemorrhage through the epithelium and erythrophagocytosis by transitional epithelial cells has been demonstrated in the urinary bladder of experimental rats treated with a variety of cytotoxic chemicals (Wakefield and Hicks, 1974). Haematuria might therefore precede any morphological changes in the earliest cases of CEH.

Five of 38 vascular growths were considered to be histologically malignant whereas in all 27 cases the epithelial lesions were classified as carcinomas. The classification was based on the strict criteria of Pamukcu (1974) and includes lesions which were macroscopically indistinguishable from transitional cell papillomas. The prognostic implications of such fine distinctions in animals are still unknown but a standard classification system aids description and at least provides a uniform base from which to compare reports from various sources. Following Pamukcu (1974) the malignant vascular growths were designated haemangiosarcoma, terminology which reflects their spindle-cell or polymorphic character and their aggressive nature. Other authors favour malignant haemangioendothelioma as a label, emphasising that the neoplastic cells are of endothelial origin. Some insist that malignant haemangioendotheliomas are neoplasms sui generis and do not arise by transformation of a pre-existing haemangioma (Weiss, 1974). In a later publication, Pamukcu et al., (1976) classify vascular lesions as either haemangiomas (benign) or haemangioendotheliomas (malignant). A number of cases in the present series showed infiltrative vascular growth merging imperceptibly with areas of typical (benign) haemangioma suggesting that transformation does indeed occur. Whatever terminology is employed this author feels it should be emphasised that a spectrum of lesions is seen in CEH, ranging from classical haemangiomas to

aggressive cellular growths with many individual lesions being of intermediate appearance. The designation haemangioendothelioma (unqualified) probably reflects this most accurately.

Tumours of transitional epithelial cells were classified as papillary non-infiltrating in 13 cases and as infiltrating in 8 cases. No cases of papillary infiltrating tumours were recorded although Pamukcu (1974) states that such tumours account for nearly 55% of carcinomas in cattle. The deficiency in this series may be due to lack of experience in classifying these lesions or to inadequate sampling since not every tumour was examined in every bladder. Other epithelial tumours found in 60 animals included two mucous adenocarcinomas and one keratinising squamous cell carcinoma. The incidence of these tumours is similar to that recorded by Pamukcu (1974). Areas of mucous and squamous metaplasia were also observed in other carcinomas.

One spindle-cell tumour was classified as a poorly-differentiated carcinoma, largely on the basis of the uniformity of the cell population and the pattern of infiltration. However, the author is aware of the many pitfalls involved in tumour classification and of the subjective nature of the art. In this instance, considerable difficulty was encountered in distinguishing infiltrating carcinomas from malignant vascular lesions since one often mimicked the other, particularly when located between bundles of dying muscle (Fig. 56). Experienced pathologists have similar difficulties and even Ashley (1978c) admits that any haemorrhagic carcinoma showing sinusoidal blood spaces is difficult to distinguish from an angiosarcoma. Such difficulties can ultimately only be resolved by techniques more sensitive than light microscopy. Electron microscopy can play a useful role in tumour identification (McLay & Toner, 1981) and the use of antibodies to intermediate filament proteins has great potential (Ramaekers et al., 1982; Osborn & Weber, 1983).

Fibrous tumours of the type described here have not been regularly associated with bovine CEH although isolated reports of

their occurrence do exist (Pamukcu et al., 1976; Pachauri et al., 1981). Occasional cases of fibrosarcoma and leiomyosarcoma have been reported (Pamukcu et al., 1976) but none was found in the present series. Yoshikawa and Oyamada (1971) described vesical fibromatosis as a distinct entity although a number of their cases also had chronic haematuria or vesical papillomas. Those authors noted the simultaneous occurrence of the two conditions and commented on the histological similarities between the fibromas and the marked stromal changes seen in some cases of CEH. The lesions they described appear to have been identical to the fibrous tumours found in 13% of cattle (8/60) in this study. In Australia, McKenzie (1978b) found fibromas in 2 out of 19 cattle with CEH and in a high proportion of urinary bladders from an abbatoir survey (McKenzie 1978a). It may be that these fibromas are indeed a separate entity and not aetiologically related to the vascular and epithelial lesions of CEH. They may well have been overlooked in the past because of their small size and insignificant appearance. A much larger series of cases would need to be examined to confirm or refute this suggestion.

A striking feature of the majority of cases in this study was the variety and multiplicity of lesions in each bladder. In at least two cases, the inter-relationship of vascular and epithelial lesions was such that infiltrating transitional cells extended into the channels and spaces of the subepithelial capillary plexus and subjacent haemangioma. Even where only one tumour type was present there was frequently more than one individual tumour present. In addition, adjacent tissues usually showed evidence of epithelial hyperplasia, metaplasia or dysplasia and/or capillary proliferation and ectasia. Such changes were particularly noted in animals with epithelial tumours but were also found adjacent to haemangiomas and fibromas. A degree of cystitis was present in all animals with bladder tumours, even those where there was no ulceration or necrosis; there was infiltration by various inflammatory cells, often with oedema of the lamina propria, and in some cases there was also stromal fibroplasia. The combination of lesions was such that the appearance was often suggestive of chronic cystitis with

consequent hyperplasia and dysplasia and subsequent progression to neoplasia of epithelial or mesenchymal elements or both. Proliferation of epithelium, capillaries and fibrous stroma as described here in association with bladder tumours has also been recorded in early cases of CEH with so-called microhaematuria (Varenika, 1958). It is extremely difficult, if not impossible, to distinguish between reactive and preneoplastic hyperplasia except under retrospective experimental conditions (Hicks & Chowaniec, 1978). Nevertheless, it seems highly probable that the proliferative cystitis seen in CEH is a preneoplastic condition rather than a secondary event. A similar sequence of haemorrhagic cystitis succeeded by bladder neoplasia has been put forward by other workers to explain their findings in bracken-fed sheep (McCrea & Head, 1981).

The pattern of urinary bladder tumours in cattle, as in man and other animals, is consistent with the action of a urine-borne carcinogen (Munday, 1966; Hicks, 1977) but the nature of the specific agent (or agents) remains unknown. Nevertheless, the role of bracken fern in the aetiology of bovine bladder tumours seems well established with a large body of both circumstantial and experimental evidence supporting the hypothesis. The association between the occurrence of CEH and the distribution of bracken has long been known and it is recognised that bladder tumours are rare outwith the bracken-infested enzootic areas (Pamukcu et al., 1976). The present study provides further evidence for the role of bracken fern since all 60 animals with urinary bladder tumours had access to the fern for some, if not all, of their lives (Grimshaw, personal communication).

The possible role of agents other than bracken fern in the aetiology of these tumours has never been excluded and constant reference is made to the bovine papillomaviruses (Yoshikawa and Oyamada, 1971 and 1975; Pamukcu et al., 1976; McKenzie, 1978b). Papilloma virus-like activity has been demonstrated in suspensions of bovine urinary bladder tumours (Olson et al., 1965) but the virus was never visualised and has been described as a passenger

virus (Pamukcu et al., 1976; Olson et al., 1965). The possible role of papillomavirus in the aetiology of fibromas of the urinary bladder is particularly interesting since those lesions resemble closely the fibromatous components of the viral fibropapillomas described in the skin, alimentary tract and teat (Jarrett et al., 1984a and 1980; Campo et al., 1981).

Experimental inoculation of the urinary bladder with suspensions of cutaneous warts has induced fibromatous tumours and epithelial polyps (Olson et al., 1959; Brobst & Olson, 1965; Dobereiner et al., 1966). The tumours produced have since been described as self limiting (Pamukcu et al., 1976). Also, bracken fern intake under experimental conditions did not affect the development of the lesions (Dobereiner et al., 1966).

The association between the various bovine papillomaviruses and tumours of the bovine urinary bladder, including their putative aetiological role, is currently under investigation by Jarrett and his co-workers. Additional evidence for the role of bracken fern in the aetiology of urinary bladder tumours in cattle is provided in Chapter II of this thesis.

CHAPTER II

BRACKEN FERN, CARCINOGEN AND TOXIN

CHAPTER II: BRACKEN FERN, CARCINOGEN AND TOXIN

INTRODUCTION AND REVIEW OF THE LITERATURE

BRACKEN FEEDING EXPERIMENT

INTRODUCTION AND REVIEW OF THE LITERATURE

Bracken fern (Pteridium aquilinum (L.) Kuhn) is widely distributed throughout the world and is now recognised as having both a potent toxic and a carcinogenic effect. The literature has been reviewed by a number of authors, notably I.A. Evans (1976a, 1972, 1968), Farnsworth et al. (1976) and Braid (1959). More recently, in 1982, the Royal Society of Edinburgh held a symposium entitled "Bracken in Scotland" in which various aspects of the bracken problem were reconsidered in the light of current knowledge (Proceedings, 1982). In addition, Grimshaw and Evans (1980) have published a review of the inter-relationship between bovine neoplasia and bracken fern and they included, without permission, many of the results which constitute the first part of this thesis.

The fact that bracken might be toxic to livestock was recognised independantly by Freeman, Penberthy and Storrar in 1893 and by Muller in 1897 (quoted by Braid, 1959). Since that time, investigations of naturally-occurring disorders, biochemical studies and bracken feeding experiments have confirmed that bracken contains a thiaminase which induces the classic neurological symptoms of thiamine deficiency in monogastric animals and a bone marrow toxin which induces neutropaenia and thrombocytopaenia with consequent septicaemia, haemorrhage and death in a variety of species (see W.C. Evans, 1976; I.A. Evans, 1972).

Cattle appear to be particularly susceptible to the bone marrow toxin and in clinically affected animals, acute bracken poisoning is usually fatal. The potency of the bracken may be variable but the effect is believed to be cumulative (W.C. Evans, 1964). It has been estimated that a dose of 10g dried bracken per kilogram body weight for 35 days is sufficient to produce a fatal outcome in cattle whereas sheep are at least twice as resistant (I.A. Evans, 1972). The earliest sign of bone marrow failure is leukopaenia, more particularly neutropaenia (I.A. Evans et al., 1972a; Naftalin & Cushnie, 1954b), while the platelet count is said to be the most useful prognostic indicator (I.A. Evans et al., 1958). The characteristic post-mortem features of acute bracken

poisoning in cattle were adequately described by Penberthy (1893), Storrar (1893) and Stockman (1917); there are numerous petechial and ecchymotic haemorrhages of all tissues, there may be haemorrhagic ulcers of the alimentary tract, the intestines frequently contain frank blood and the faeces are black and tarry. There may also be bacterial infarcts of kidney, liver or lung (Naftalin & Cushnie, 1954a).

The oncogenic properties of bracken fern have been demonstrated in a variety of species under experimental conditions and tumours have been induced in a variety of organs. Recent studies have concentrated on various extracts of bracken in an attempt to identify the offending compounds (see I.A. Evans et al., 1982).

A rat experiment, in which dried-bracken was fed for 64 days, provided the first demonstration of bracken's potent carcinogenic activity (Evans & Mason, 1965). Multiple adenocarcinomas were induced in the ileum of all experimental animals and none was found in the control animals. That result has since been confirmed by other workers with induction of intestinal adenomas, adenocarcinomas and sarcomas (Hirono et al., 1973). By continuous feeding of bracken for 29 weeks or more, co-existing tumours of the intestine and urinary bladder were induced in rats (Pamukcu & Price, 1969). The urinary bladder tumours included papillomas, transitional cell carcinomas and squamous cell carcinomas. Other workers reported that after bracken was included in the rats' diet for 20 weeks, tumours were found in the subcutis, kidney and palate as well as in the intestine and urinary bladder (Yoshikawa et al., 1981). As noted by Pamukcu et al. (1976) there are various factors which influence the pattern, location and incidence of neoplastic lesions induced in rats by bracken fern. These include not only the duration of ingestion but also the age at initial exposure (I.A. Evans, 1968). A variety of supplements may alter the incidence of tumours at various sites; for example,

thiamine increases the incidence of bladder tumours (Pamukcu et al., 1970) as does bicarbonate, but the latter decreases the incidence of intestinal lesions (Pamukcu et al., 1977b). Recently, it has been shown that the induction of upper alimentary tract tumours in rats by other carcinogens may be enhanced by feeding bracken fern (Hirono et al., 1982).

In early experiments, the feeding of bracken fern to mice induced pulmonary tumours or leukaemia but no intestinal or urinary bladder tumours (Widdop, 1967; Pamukcu et al., 1972). In contrast, various bracken extracts implanted as pellets into the urinary bladder of mice resulted in a range of hyperplastic and neoplastic lesions (Wang et al., 1973; Pamukcu et al., 1970). Subsequently, urinary bladder tumours were produced in bracken-fed mice by implanting glass beads into the urinary bladder (Miyakawa and Yoshida, 1975). Those results illustrated that different species vary in their subseptibility to the bracken carcinogen. A difference in response between strains of animals was shown by Hirono et al., (1975) when feeding bracken fern for four months induced intestinal tumours in one of two strains of mice. No urinary bladder tumours were produced in that experiment. It was later shown by Pamukcu et al., (1977a) that simultaneous urinary bladder and intestinal neoplasms could be induced in mice by feeding bracken fern for their lifetimes.

Other species which have been shown to be susceptible to the oncogenic action of bracken fern or its extracts include guinea pigs, Japanese quail and possibly hamsters (Maeda, 1975; I.A. Evans, 1968; Widdop, 1967). Tumours were found in the urinary bladder of guinea pigs and the intestines of quail and hamsters.

Long-term feeding of dried bracken to sheep induced urinary bladder carcinomas in 7 out of 8 animals within five years; the eighth individual died of acute bracken poisoning (McCrea and Head, 1981). One of those seven animals also had fibrosarcoma of the maxilla and mandible.

The link between bracken fern and cattle cancer has always been closely associated with investigations of bovine CEH. The oncogenic potential of bracken fern was first indicated by the work of Rosenberger and Heeschen (1960) who succeeded in producing the proliferative vascular lesions of enzootic haematuria in experimental cattle by successive feeding of fresh bracken and bracken hay. Previously, the coincidence of the distribution of bracken fern and the occurrence of bovine CEH had been noted by numerous authors (see Datta, 1952; Bull et al., 1932). Only rarely have other plants been incriminated in the aetiology of CEH and, even then, bracken fern has not been entirely excluded (Nandi, 1982; McKenzie, 1978b). Pamukcu et al., (1976) emphasized that bladder tumours are rare in cattle outside the enzootic haematuria areas and relatively infrequent in the absence of bracken fern. The report by Mugera et al. (1969) of their findings in Zebu cattle in Kenya is perhaps a notable exception although other workers have observed bracken fern even in that particular area (Jarrett, personal communication).

Since the initial work of Rosenberger and Heeschen (1960) a number of workers have successfully induced lesions of the urinary bladder by including fresh or dry bracken fern in the diets of experimental cattle (Sofrenovic et al., 1965; Rosenberger, 1965; Pamukcu et al., 1967; Dzuvic, 1969a,b). In almost all those cases the lesions were described as being practically indistinguishable from those of naturally occurring CEH (see Chapter I Section B). More recently, Pamukcu et al., (1976) compiled a series of 20 experimental cattle with bladder lesions by including cases which had been reported previously (Pamukcu et al., 1967; Price & Pamukcu, 1968). Thirty cows weighing 100-150kg were fed from 300 to 600gm dried bracken or 400 to 1000gm fresh bracken daily. Twenty of those animals developed bladder tumours within 5.3 years (between 276 and 1920 days). Lesions were macroscopic in 13 cases but only microscopic in 7 cases. The gross appearances were said to be similar to, and the histological features indistinguishable from, those of naturally occurring urinary bladder tumours. Epithelial lesions were described in 18 cases (90%) either alone or

in combination with mesenchymal lesions and 10 of those were classified as carcinomas. Mesenchymal lesions were found in 13 cases (65%) and the majority of those (10/13) were haemangioma or haemangioendothelioma, usually found in combination with epithelial lesions (9/10). All animals developed bracken poisoning with multiple haemorrhages, including submucosal haemorrhages in the urinary tract.

Bracken fern, although originally a woodland plant, is now prevalent in many different environments, being tolerant of a wide range of climatic conditions. If its numerous varieties are included then bracken fern is found on every continent, except perhaps South America and the Polar Regions (Mitchell, 1973).

However, it has been suggested that this highly successful plant achieves its greatest vigour, extent and density of growth in Britain (Page, 1982). In Scotland, the Agricultural Census returns for 1957 indicated that over 187,000 hectares of land were infested and that 25% of that was in Argyllshire with a further 12.9% of Scottish bracken being found in Inverness-shire and Ross and Sutherland (MacLeod, 1982; Hendry, 1958). The association between bracken fern distribution and the occurrence of both urinary bladder tumours and UAT tumours in cattle in Scotland was first noted by Jarrett (1973). A similar association had previously been recorded by workers in Brazil (Dobereiner et al., 1967; Tokarnia et al., 1969) and this was subsequently confirmed by others (Neto et al., 1975). In contrast, bracken fern had been excluded from the possible causes of UAT tumours in Kenya (Plowright et al., 1971) although it was later conceded that bracken fern was eaten by some of the cattle in that particular valley (Linsell, unpublished results).

The occurrence of UAT tumours and urinary bladder tumours in cattle in the West of Scotland has been described in Chapter One of this thesis. Investigations into the origins of these cattle revealed that the majority of cases (over 80%) came from the bracken infested areas of Argyll or Inverness although those areas

accounted for only 21.3% of all bovine admissions to GUVS (Grimshaw & Evans, 1980).

In addition, in all cases where the origins of the individual animals could be traced, there had been exposure to bracken for most, if not all, of their lives (Grimshaw, personal communication). However, there was some suggestion that animals with bladder tumours alone had less exposure to bracken since farms from which they originated had only light infestation or the animals had been denied access to bracken after about 3 years of age (Grimshaw & Evans, 1980).

In view of the large body of circumstantial evidence linking bracken fern with bovine neoplasia, a number of bracken feeding experiments were established. The first of these experiments in cattle will be described.

BRACKEN FEEDING EXPERIMENT

Introduction

Materials and Methods

Results

Discussion

BRACKEN FEEDING EXPERIMENT

Introduction

This was originally designed as a pilot experiment employing both infection with bovine papilloma virus and feeding of dried bracken fern in an attempt to induce tumours of the UAT. Four calves were given intraruminal inoculations of bovine papilloma virus and were fed intermittently with cubed or powdered mixes incorporating a percentage of dried bracken fern. Feeding was initiated at low dose levels in an attempt to induce tumours but avoid the toxic effects of the plant. The experimental diet was withdrawn whenever neutrophil counts fell to unacceptably low levels.

The experiment was conceived as a long term project and continued in this way for about four years. Then, in view of the minimal haematological changes observed to this point, the feeding regime was reviewed. It was decided to increase the levels of bracken consumption and this was most easily achieved by replacing dried fern with fresh, green bracken. It was considered this would also eliminate any processing faults which might have reduced the potency of the dried bracken.

Fresh green bracken was fed as and when available over three seasons. One animal was destroyed before this, at the end of year 4, due to unfortunate but unrelated circumstances. A second animal died while being fed fresh bracken and the experiment was terminated when the remaining two animals were killed almost 7½ years after the project began.

Bracken Feeding Experiment

Materials and Methods

Animals

Papilloma Virus

Bracken Fern

Feeding Regime

Haematology

Urine Analysis

Papilloma Detection

Necropsy Procedures

Materials and Methods

Animals

Four Ayrshire calves, two male (28,99) and two female (27,98), all aged approximately 3 months, were purchased from the Medicine Department of GUVS. The day they were first received and bled for haematology was designated Day 0. The animals were weighed at the beginning of the experiment and at intervals thereafter so that consumption of bracken fern could be calculated on a basis of body weight.

The male animals were castrated at approximately 9 months of age on day 182. All four animals were dehorned at 15 months of age on day 383 of the experiment.

Other unrelated but significant events which occurred during the experimental period included an episode of pneumonia which affected all four animals during the third month of the experiment. This was treated successfully by intramuscular injections of tetracycline (Terramycin, Pfizer). In addition, during the second year of the experiment, Stirk 28 escaped on day 553, gorged himself with available feed and suffered metabolic acidosis (overeating disease). This rapidly responded to treatment by starvation and rest.

Papilloma Virus

Skin warts were collected from a bovine animal at slaughter. The warts were chopped and stored in 50:50 phosphate buffered saline (PBS) glycerol at +4°C. Fifteen months later, 3g of wart tissue was homogenised with PBS, on ice, in a Silverson homogeniser, to produce a 10% W/V suspension. This was clarified by centrifugation at 2500 rpm for 10 mins at +5°C in an MSE Mistral 4L centrifuge. The supernatant was aliquoted into bijoux bottles and stored at -70°C as a crude papilloma virus preparation. These

particular warts were chosen because extraction and electron microscopy of portions of these tissues had demonstrated the presence of large numbers of virus particles.

Three animals were infected on day 34 of the experiment and one (98) was infected on day 35. Routine anaesthetic and surgical procedures were adopted. Rumenotomy was performed under general anaesthesia via a paracostal incision and the cut edge of the rumen was temporarily stitched to the skin. Some ruminal contents were removed but it was still not possible to gain clear access to the cardia. Injections were therefore made by means of a 2ml syringe and small bore needle held in one hand and the required sites were located by touch through the fluid ruminal contents. Five sites at, or adjacent to, the cardia were used to inject a total of 2ml of crude virus preparation in each calf i.e. approximately 0.4ml per site. As far as possible the inoculations were made into the subepithelial tissues. A suspension of penicillin and streptomycin was instilled into the peritoneal cavity of each animal before surgery was completed and further prophylactic doses of the same antibiotics were given intramuscularly for 3 successive days.

Bracken Fern: a) Dried Bracken

Year One: Bracken fern was cut by sickle or scythe and collected during the summer months when the fronds were fully developed but still green. This material was dried by laying it on shelves of chicken wire in a room with several large fans and electric fires which produced on ambient temperature of approximately 120°F. About half a ton of bracken was dried in three batches over a period of 9 days. Twenty kilogrammes of the dried material were then incorporated into 200kg of cubed cattle feed according to the following formula:

10 parts Dried Bracken
44 parts Barley
20 parts Maize
5 parts Soya
5 parts Groundnut
5 parts Cottonseed
5 parts Fishmeal
3 parts Molassine meal
3 parts Minerals/Vitamins

The product had a dried bracken content of 10% and a crude protein content of roughly 16%.

In addition, 28kg dried bracken was subjected to chemical extraction similar to the methods of Leach et al (1971) and Evans et al (1958). The bracken was treated with acetone, then extracted with hot ethyl alcohol. The volume of the extract was reduced by rotary evaporation then it was washed and eluted with methanol. Ethyl acetate was then added; the resulting precipitate was discarded but the volume of supernatant was reduced to form a thick oil that was stored at +4°C. Several months later all the solvent in this product was evaporated off under reduced pressure. The residue was disolved in hot tap water and made up to a final volume of 900cc which was again stored at +4°C.

Years Two to Four: Quantities of green bracken were collected and dried as above except that a commercial electric fan heater was used to produce the required temperature of 120°F. The dried bracken was then put through a commercial hammer mill and molasses was added at the rate of 1cwt molasses to approximately 6cwt bracken in an attempt to reduce dust formation. This bracken-molasses mix was then milled with other ingredients to produce a cattle feed with a total crude protein level of about 13%. The following formula was used:

2 parts Bracken-molasses
14 parts Barley
1 part Soya
1 part Groundnut
1 part Cottonseed
1 part Minerals/vitamins/Molasses

This feed was presented in powdered form and had a dried bracken content of approximately 9.2%.

Bracken Fern: b) Fresh Bracken

Year Five and thereafter: During the fifth year of this experiment fresh, green bracken was fed to surviving animals. This bracken was initially picked by hand to obtain young, uncurled fronds but later was cut by means of a hand-held, petrol-driven, rotary scythe. The green bracken was packed into polythene bags and fed to the animals either on the day it was cut or on the following day.

This system was maintained over several seasons and bracken was generally suitable for cutting from late May or early June. Standing fronds of comparatively green material were still available in November or December and bracken harvesting was only abandoned in the face of frost damage or heavy snowfall.

Feeding Regime

The experimental animals were kept in loose boxes or in cow-standings with neck chains. Hay and water were available at all times on an ad libitum (ad lib) basis. In addition, when not receiving experimental diet, each animal was given 2-3lbs (1.0-1.5kg) of a commercial, standard cattle concentrate twice daily. Since the fatal dose of dried bracken was said to be approximately 10g per kg body weight daily for a period of 35 days (I.A. Evans, 1972). Feeding levels in this experiment were adjusted so that the daily intake of dried bracken was about 2g per kg body weight or less. An exception was the one week when concentrated extract was administered.

Year One The cubed feed containing 10% dried bracken was introduced to the cattle on day 15. Several pounds of feed were given to each animal twice daily and this replaced the cattle concentrate that was normally given. By day 22 each animal was eating approximately 8lb (3.5kg) of the 10% bracken compound per day. After about one month of bracken feeding, two animals (98 and 99) had low neutrophil counts and were taken off the experimental diet on day 47. The other two animals continued eating the bracken feed for a further week until all the remaining diet was consumed by day 54.

The alcohol extract of bracken was given orally as a drench for seven days. On days 96 to 102 inclusive, each animal was given 30cc of extract which was further diluted with water to facilitate administration.

Year Two: Powdered bracken mix was first offered to the four cattle towards the end of the first year of the experiment on day 341. By day 343 each animal was consuming about 10lb (4.5kg) of the 9.2% bracken mix each day. Feeding at this level continued for over 3 months (100 days) to day 443. At this time neutrophil counts were again below the normal range and the bracken mix was withdrawn and replaced by normal maintenance diet.

Bracken feeding was resumed about 10 weeks later on day 651. Approximately 10lb (4.5kg) of the powder was eaten daily by each animal for more than 2 months until day 695. At that time an attempt was made to increase the average daily intake to 20lb (9kg) of bracken feed. However, the animals were unable to cope with this quantity of concentrate and daily intake gradually declined until after a week or so it was again about 10lb (4.5kg).

Year Three: Feeding with the powdered mixture continued at this level of 10lb (4.5kg) per animal per day for a further 15 months. The diet finally ran out after day 1155 (month 38) by which time a total of 10 tons of 9.2% bracken mix had been consumed by the four cattle over a period of 20 months.

Year Four: Normal maintenance diet was resumed after day 1155 and continued until month 46 when the four animals were put out to grass in a field free of bracken-fern. They were brought in 2 months later and the normal maintenance diet of hay and cattle cake was again provided. One animal (28) was destroyed during this period (day 1471).

It was at this stage in the experiment that the feeding regime was reviewed and bracken consumption increased by supplying fresh green bracken as a supplement to the maintenance diet. At the start of each season the cattle were often reluctant to eat the bracken and at such times the amount of hay and commercial cake offerred was either reduced or witheld until the bracken fern had been consumed. Various methods were tried to encourage bracken consumption e.g. chopping the fronds and mixing them with the hay ration or spraying the fronds with a mixture of molasses and water. Finally, however, the bracken fern was fed as it had been cut and only the toughest, dry stalks were left uneaten.

Whenever bracken fern was not being given, the cattle were fed the standard maintenance diet of hay ad lib and 2-3kg of commercial cattle cake per day.

Year 5-6: Fresh green bracken was fed to the three surviving animals from day 1673 (month 55) onwards. The regime was somewhat intermittent with bracken sometimes unavailable for one or two days at weekends and for up to 2 weeks during summer holiday periods. However, feeding continued at daily levels of approximately 3kg bracken per animal for almost 7 months until day 1881 (month 62).

Year 6-7: Bracken feeding was resumed on day 2049 (month 68) and again continued at daily levels of approximately 3kg per animal for over 6 months until day 2239 (month 74).

Year 7-8: Bracken feeding was restarted on day 2421 (month 80). The ration was increased to daily levels of approximately 6kg of green fronds per animal. One animal (98) died three weeks later on day 2442. The remaining two animals continued to receive fresh fern as a supplement to their diet until bracken feeding was finally stopped on day 2585 (month 86). Normal maintenance diet was then fed until the experiment was terminated, almost 90 months after it had begun, on day 2695.

Table 13 and Figure 67 summarize and illustrate the scheme of bracken feeding during the 7½ years of this experiment.

TABLE 13: Summary of bracken feeding

Year	Days of bracken feeding	Duration Days	Months (approx)
One	15-47/54*	35d	1m
One	96-102	7d	1wk
Two	3341-443	100d	3m
Two-Four	651-1155	504d	17m
Four	normal maintenance diet then out at grass		
Five-six	1673-1881	208d	7m
Six-seven	2049-2239	190d	6m
Seven-eight	2421-2585	164d	5½m
	TOTAL	1208d	40m

*see page 139.

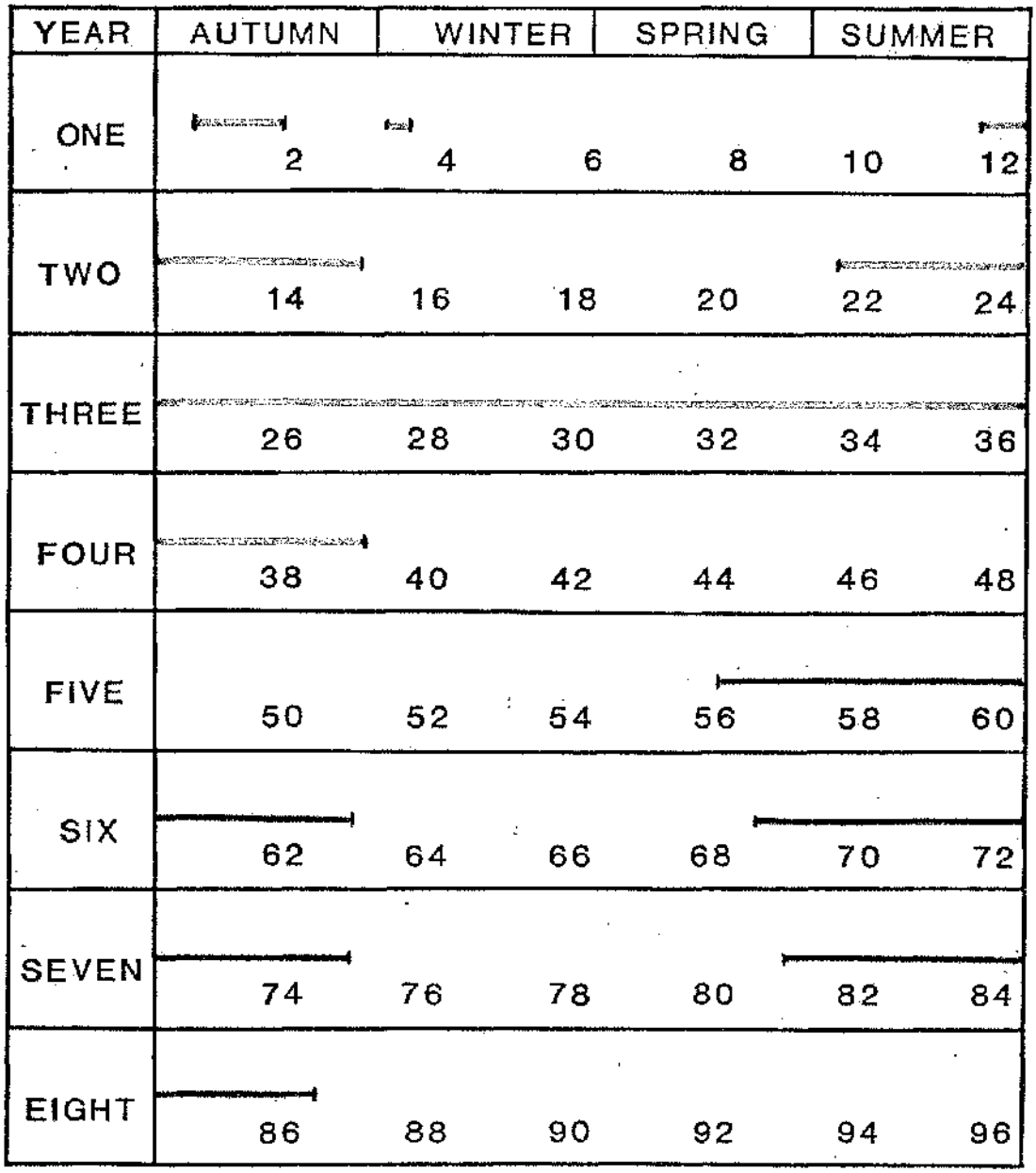


FIG. 67 : PLAN OF BRACKEN FEEDING OVER 90 MONTHS

Dried bracken

Fresh bracken

Haematology

Blood samples were taken for haematological examination because bone marrow failure is the main component of acute bracken poisoning. In particular, the absolute neutrophil counts were recorded since a precipitous fall in the neutrophil count is said to be the first indication of the bone marrow damage (I.A. Evans, 1972). Bracken feeding was interrupted on occasions when the neutrophil counts were considered to be unacceptably low.

Animals were bled by jugular venepuncture and samples were collected into bottles containing 1mg ethylenediaminetetra-acetic acid (EDTA) per ml of blood as anticoagulant. Initially animals were bled every four days or twice weekly but this was later reduced to once weekly. When the animals were not receiving experimental diet blood samples were taken once per month.

Standard haematological techniques were employed.

Packed Cell Volume This was estimated by means of capillary tube samples spun in a Hawksley microhaematocrit centrifuge (Gelman-Hawksley Ltd).

Total Red Cell and White Cell Counts These were measured with an electronic particle counter (Model B, Coulter Electronics Ltd.) after dilution of blood with PBS according to the manufacturers instructions.

Haemoglobin Determination This was done by the cyanmethaemoglobin method using Drabkin's cyanide-ferricyanide solution as described by Dacie and Lewis (1970).

Platelet Count This was done by visual means. Blood was diluted with 1% ammonium oxalate and the platelets were counted in an improved Neubauer counting chamber.

Differential Leucocyte Counts Blood films were prepared and stained with Leishman's stain and a minimum of 200 cells were counted.

Following the purchase of a new electronic particle counter and associated equipment (System ZF6, Coulter Electronics Ltd.) at the end of year 5 of the experiment, total red cell and white cell

counts, haematocrit and haemoglobin levels were all determined as per the manufacturer's instructions. In addition, as from year 6 of the experiment, platelet counts were measured electronically following adaptation of the Model B counter.

Urine Analysis

In view of the fact that other workers had recorded the induction of bladder lesions in bracken feeding experiments (see Rosenberger, 1971), the urines of all animals were examined occasionally for macroscopic evidence of haematuria during the early years of this experiment. Following the introduction of green bracken in year 5, samples were taken more frequently and tested for microhaematuria either by means of commercial dipsticks (Haemastix, Ames Company, Division of Miles Laboratories Ltd.) or by microscopy of urine sediment or cytospin preparations. Urine with a high cell content was counted as for haematology and occasional samples were submitted for routine bacteriological examination.

Although it was extremely difficult and time-consuming to obtain samples, occasionally from all animals and at all times from some individuals, catheterisation was deliberately avoided to prevent traumatic damage to urinary bladder mucosa.

Papilloma Detection

The animals were examined periodically for the presence of skin papillomas. In addition, following the death of Stirk 28 in the fourth year of the experiment, oesophagoscopy was performed on the three surviving animals. This was done once only, under general anaesthesia, using a fibre-optic colonoscope (Olympus CF Type LB2, light source Olympus CLE-3).

Necropsy Procedures

The techniques employed for destruction and post-mortem examination were identical to those detailed in Chapter 1.

Bracken Feeding Experiment

Results

Summaries: Heifer 27
Stirk 28
Heifer 98
Stirk 99

Bracken Consumption

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Results

Summary of Results

Heifer 27 This animal was destroyed on day 2695 when the experiment was terminated after approximately 7½ years. Post-mortem examination revealed a thickened urinary bladder with multiple haemagiomas and areas of infiltrating transitional cell carcinoma. There was associated chronic cystitis and much of the epithelium showed hyperplasia and dysplasia. In addition, two papillomas were present on the side of the tongue and one papilloma was found in the oesophagus. Skin papillomas had never been seen in this animal and oesophagoscopy during the fourth year of the experiment had proved negative.

Haematuria was first recorded during the sixth year of the experiment, approximately 8 months after the introduction of fresh green bracken to the diet. At first it was intermittent but ultimately all urine samples tested were found to contain blood.

The haematological picture was largely unremarkable. A low neutrophil count, $0.54 \times 10^9/l$, which occurred during the second year of the experiment, prompted the withdrawal of bracken (similar results were recorded in heifer 98). However, this result occurred after 3 months of feeding with dried bracken and similar results were subsequently recorded on occasions when bracken was not being fed. Red blood cell counts were maintained at normal levels throughout the experiment. Despite the haematuria there was no obvious decline in the values although results were rather erratic during year 6. The minor variations noted in platelet and lymphocyte counts could not be correlated with periods of bracken feeding.

Stirk 28 This animal was destroyed during the fourth year of the experiment (day 1471). Post mortem examination revealed early pleurisy and abomasal ulcers. Numerous oesophageal papillomas were present and two small fibromas were found in the

rumen. This animal was never known to be haematuric.

A neutrophil count of less than $1.0x10^9/l$ was recorded on only one occasion, approximately 7 weeks after re-introduction of bracken feeding. This neutrophil count of $0.24x10^9/l$ on day 702 coincided with a low platelet count of $51.0x10^9/l$ and may have been a manifestation of mild bracken poisoning. There were no significant changes in lymphocyte or red cell numbers.

Heifer 98 This animal died during the 7th year of the experiment on day 2442 following a period of persistent haematuria and declining red blood cell counts. Post-mortem examination revealed a ruptured urinary bladder with haemorrhage into the pelvis and posterior abdomen. Infiltrating haemangiosarcoma had caused thickening of the bladder wall around the insertion of the ureters and there was associated hydronephrosis. Multiple haemangiomas were also present in the urinary bladder and there was severe cystitis. An additional finding was hepatic telangiectasis.

Haematuria had first been recorded during the sixth year of the experiment, approximately 13 months after the introduction of fresh green bracken. Moderate haematuria continued until death.

The main haematological changes in this animal were associated with the persistent haematuria and approaching death. The degree of haematuria was indicated by the declining red cell count throughout the last year of this animal's life. The precipitous fall in red cell numbers and the accompanying rise in neutrophil numbers recorded immediately prior to death were presumably due to haemorrhage and shock following the rupture of the urinary bladder.

Low neutrophil numbers, similar to those recorded in heifer 27, prompted cessation of bracken feeding during the second year of the experiment. However, in subsequent years, similar low counts were noted outwith the periods of bracken feeding.

Platelet numbers in this animal, as in the others, were frequently below the normal range. Lymphocyte numbers, however, were frequently above the normal range particularly during the first five years.

Skin papillomas were noted to be present when intraruminal inoculation was performed on this animal at the age of 3 months. However, no papillomas were detected by oesophagoscopy at four years and none was seen at necropsy either on the skin or in the alimentary tract.

Stirk 99 This animal was destroyed on day 2660, over 7 years after the start of the experiment. Necropsy revealed a diffusely thickened urinary bladder with proliferative lesions of epithelium and connective tissue which included formation of both small carcinomas and small haemangioma-like foci.

Haematuria was first recorded with only scanty amounts of blood present, 19 months after the introduction of fresh green bracken. Macroscopic haematuria was not evident until 10 months later.

The neutrophil counts in this individual were consistently lower than those of the other animals in this experiment although they were generally within the normal range. However, counts less than $0.55 \times 10^9/l$ were occasionally recorded during periods of bracken feeding. Platelet counts, although slightly lower than usual at those times were not markedly reduced.

Lymphocyte numbers were also generally lower than those in the other animals throughout this experiment. Red cell counts were normal apart from a slight decline during the last year of the experiment.

No papillomas were detected by oesophagoscopy at 4 years and none was found at necropsy.

Bracken consumption

The bodyweights of individual animals are shown in Table 14 together with an indication of the periods of bracken feeding.

The estimated daily intake of bracken fern is detailed in Tables 15 & 16. Although most of the dried feed was readily consumed there was some spillage, particularly of the powdered mix. Fresh bracken was not eaten so avidly and wastage was extremely difficult to quantify. Accordingly the quantities consumed by individual animals were not measured accurately and the figures quoted probably overestimate the actual intake.

Table 15 shows the maximum daily intake of bracken for any of the experimental animals during a given period. This was calculated using the lowest body weight recorded during each period. It appears that during year 1 and years 2-6 the highest dosage was received by Heifer 98. During year 2 Stirk 99 received the highest dosage while in years 6-8 the highest dosage of fresh bracken was received by Heifer 27. It can be seen that for much of the experimental period the intake of dried bracken was 2g/kg body weight or less. The variable intake quoted for years 2-4 reflects the unsuccessful attempt to increase this. Higher dosage rates were achieved by the introduction of fresh green bracken to the diet.

Table 16 shows the average daily intake for individual animals during the various periods of bracken feeding. This was estimated using the average body weight calculated from the results available for each period.

TABLE 14: Body weights of cattle in kilograms

Solid Bars [indicate Periods of Bracken Feeding

Day	Animal 27	28	98	99
6	181.8	190.9	136.4	127.3
13	195.5	206.8	143.2	143.2
[20	188.6	197.7	140.9	140.9
[27	186.4	204.5	140.9	145.5
[34	181.8	190.9	131.8	140.9
[41	181.8	197.7	140.9	138.6
[48	184.1	200.0	145.5	147.7
56	190.9	211.4	154.5	150.0
63	193.2	222.7	154.5	156.8
70	200.0	225.0	156.8	161.4
76	195.5	222.7	159.1	165.9
83	193.2	213.6	154.5	163.6
90	202.3	234.1	163.6	179.5
[97	204.5	236.4	170.5	186.4
104	209.1	240.9	172.7	190.9
111	211.4	247.7	175.0	188.6
118	220.5	247.7	177.3	195.5
125	227.3	259.1	188.6	211.4
132	222.7	256.8	186.4	204.5
139	227.3	263.6	190.9	213.6
146	215.9	263/6	190.9	211.4
153	250.0	306.8	225.0	236.4
173	245.5	300.0	211.4	240.9
273	284.1	377.3	261.4	290.9
338	311.4	397.7	286.4	318.2
[363	322.7	406.8	318.2	279.5
[412	322.7	400.0	290.9	325.0
[433	327.3	418.2	302.3	336.4

TABLE 14: Body weights of cattle in kilograms (Cont'd).

Day	Animal 27	28	98	99
[441	331.8	409.1	311.4	338.6
447	340.9	411.4	295.5	336.4
455	318.2	415.9	306.8	345.5
468	338.6	434.1	320.5	343.2
496	354.5	447.7	338.6	359.1
511	361.4	454.5	352.3	384.1
524	359.1	440.9	334.1	356.8
531	368.2	452.3	347.7	381.8
539	370.5	459.1	350.0	379.5
560	377.3	447.7	354.5	393.2
642	404.5	486.4	386.4	436.4
[659	413.6	500.0	388.6	438.6
[695	427.3	522.7	400.0	454.5
[762	454.5	550.0	427.3	486.4
[874	500.0	600.0	490.9	536.4
		Dest. Day 1472		
1559	531.8		468.2	550.0
1661	590.9		513.6	604.5
[2183	581.8		604.5	668.2
[2217	572.7		613.6	654.5
[2239	577.3		613.6	663.6
2332	590.9		604.5	681.8
2388	586.4		595.5	686.4
[2428	559.1		572.7	681.8

TABLE 15: Estimated daily intake of bracken fern

Year	Feeding Period	kg bracken per animal	maximum intake g/kg body weight
1	1m	0.4 dried	3.0 (98)
	1wk	1.0 dried (extract)	5.9 (98)
2	3m	0.5 dried	1.8 (99)
2-4	17m	0.4-0.8 dried	2.1 (98)
5-6	7m	approx 3.0 fresh	5.8 (98)
6-7	6m	approx 3.0 fresh	5.2 (27)
7-8	5½m	approx 6.0 fresh	10.7 (27)

TABLE 16: Estimated average daily intake of bracken fern g/kg body weight

Year		Animal 27	28	98	99
1	dried	2.2	2.0	2.9	2.8
	dried (extract)	4.9	4.2	5.9	5.4
2	dried	1.5	1.2	1.6	1.6
2-4	dried	1.3	1.1	1.4	1.3
5-6	fresh	5.1		5.8	5.0
6-7	fresh	5.2		4.9	4.5
7-8	fresh	10.7		10.5	8.8

Urine Tests

The results of urine tests for haematuria are shown in Table 17. Haematuria was first detected in Heifer 27 on day 1898. This was over five years after the start of the experiment but eight months after the introduction of fresh green bracken to the diet. Five months later Heifer 98 was haematuric but Stirk 99 did not show haematuria until a further six months had passed i.e. 19 months after green bracken had first been fed. In all animals the haematuria was initially slight and intermittent but it gradually increased in quantity and frequency until Heifer 98 in particular showed persistent haematuria, clearly visible to the naked eye with occasional clots of blood present.

Examination of the few samples of urine suitable for bacteriology failed to reveal any pathogenic microorganisms.

TABLE 17: Urine tests for haematuria

DAY	ANIMAL 27	98	99
1696	ND	-	-
1828	ND	ND	-
1832	ND	-	ND
1898	++	ND	ND
1903	ND	ND	-
1920	++	ND	-
1932	+	ND	ND
1946	-	ND	-
2056	-	ND	-
2057	ND	+	ND
2073	ND	++	ND
2135	ND	ND	-
2137	-	++	ND
2143	ND	++	ND
2220	+++	+++	-
2239	++	++	+
2324	+	++	+
2387	+	++	+
2427	++	++	+
2466	+	Died Day 2442	+
2514	ND		+++
2518	++		ND
2655	+		+++

\+ scanty amount of blood = microhaematuria

++ moderate amounts of blood

+++ copious amounts of blood = obvious macroscopic haematuria

ND test not done

Papillomas

Skin papillomas were never recorded on animals 27, 28 and 99. Heifer 98 had skin papillomas at the age of four months and these were present at the time of experimental virus inoculation. These lesions resolved approximately two months later and no further skin papillomas were recorded.

Oesophageal papillomas (19) were found at necropsy in Stirk 28 four years after inoculation of the rumen with skin papilloma virus. Two small fibromas were found in the rumen at or near the presumed site of virus inoculation adjacent to the cardia.

Six months later, oesophagoscopy of the three surviving animals failed to reveal the presence of any oral or oesophageal papillomas. The rumen was not examined.

Two oral papillomas and one oesophageal papilloma were found in Heifer 27 at necropsy, over seven years after the experimental virus inoculation. Alimentary papillomas were not found in Heifer 98 nor Stirk 99.

Haematology

The haematological parameters of four animals recorded during the course of this experiment are illustrated in Figures 68-94. Each illustration covers 350 days and so roughly corresponds to one experimental year. Each graph includes an indication of the range within which normal values might be expected to fall. These estimates are largely based on the results obtained by Straub and others (Bovine Haematology, 1981) during their investigations prior to establishing a leukosis key. The figures used are somewhat arbitary but are broadly similar to the normal values and ranges quoted in standard textbooks such as Comparative Clinical Haematology (1977) and Veterinary Haematology (1975).

The range of values accepted as normal for the purpose of this thesis are indicated in Table 18.

The salient features of results obtained for each animal will be summarised here.

Heifer 27 Neutrophil counts were generally within the normal range with only occasional low values. A count of less than $0.6x10^9/l$ at day 441 was thought to be associated with bracken feeding but similar values of less than $1.0x10^9/l$ were subsequently recorded on a few occasions both during and outwith periods of bracken feeding.

Raised neutrophil counts were noted on a number of occasions but these were usually related to clinical events such as surgical intervention in Year 1 and sepsis following dehorning in Year 2.

Lymphocyte counts showed more variation than neutrophil counts and in some periods were distinctly erratic. Nevertheless, values were never markedly outwith the accepted normal range.

Red blood cell counts were maintained within the normal range throughout the experiment. Results were somewhat erratic during year 6 and this coincided with the recorded onset of haematuria.

Platelet values were consistently low when compared to the accepted normal range of $200\text{-}600 \times 10^9/l$. However, results were rarely lower than $100 \times 10^9.l$ and peak values were around $400 \times 10^9/l$. Variations from this overall pattern were seen with higher counts in year 5 and occasional counts as low as $80 \times 10^9/l$ but there was no clear correlation between these variations and the periods of bracken feeding.

Stirk 28 Neutrophil counts were largely within the accepted normal range throughout the experiment. Occasional peaks were related to clinical events such as surgery and pneumonia in Year 1 and acidosis in Year 2.

However, at day 702 a precipitous fall in the neutrophil count occurred with a recorded value of $0.2 \times 10^9/l$. Bracken feeding was continued and values rose to remain within the normal range apart from a rise immediately prior to death.

(In comparison with the results in animals 27 and 98 low neutrophil counts were not recorded at day 441).

Lymphocyte counts were somewhat erratic but generally within the normal range apart from a decline immediately prior to death.

Red blood cell counts were well within the normal range throughout the experimental period.

Platelet counts initially showed a wide variation but later were mostly within the range $100\text{-}400 \times 10^9/l$. An exception was the low value of $51 \times 10^9/l$ recorded on day 702 in association with the low neutrophil count.

Heifer 98 Neutrophil counts were occasionally below the accepted lower normal limits and in the early years of the experiment this precipitated withdrawal of bracken feeding (days 47 and 441 cf. heifer 27). However, in later years, occasional low values were recorded outwith the periods of bracken feeding. Raised neutrophil counts were occasionally recorded, some could be related to clinical events such as experimental surgery (day 37) whereas others could not (e.g. day 202). There was a dramatic rise in the neutrophil count immediately prior to death.

Lymphocyte counts showed more variation than neutrophil counts with values frequently above the upper limit of the normal range. This pattern was maintained until the sixth year of the experiment when values fell within the normal range. Occasional peaks (e.g. day 1856) and troughs (e.g. day 791) were recorded but their significance, if any, was unclear.

Red blood cell counts remained steadily within the accepted normal range until the sixth year of the experiment. There was then a gradual decline in values until a precipitous fall occurred just before death.

Platelet counts initially showed wide variation but subsequently were below the accepted normal range with most results between 100 and $400 \times 10^9/l$. There were isolated exceptions to this pattern but no changes obviously associated either with bracken feeding or with the declining red cell count.

Stirk 99 Neutrophil counts were generally at the lower end of the accepted normal range throughout the period of the experiment. Exceptions were the peaks related to clinical events such as rumenotomy and castration in year 1 and dehorning in year 2. In addition, counts of less than $0.55 \times 10^9/l$ were recorded during periods of bracken feeding in the third, sixth and last years of the experiment.

Lymphocyte counts were initially erratic and, unlike the other animals, many values were below the expected normal range. In later years, counts were mostly within the normal range although frequently in the lower half. A small trough with a minimum value of $1.62 \times 10^9/l$ was recorded during the last year of the experiment in association with the neutropaenia noted above. In year 6 results were extremely erratic with many counts above the normal range and this could not be related to any obvious clinical or environmental changes.

Red blood cell counts were maintained within the normal range with only a slight decline in values during the last year of the experiment.

Platelet counts were initially erratic with values up to $800 \times 10^9/l$. In later years, counts were generally between 100 and $400 \times 10^9/l$ i.e. many results were below the accepted normal range. Counts were not markedly reduced during the recorded periods of neutropaenia.

TABLE 18: Haematological values accepted as normal

Neutrophils:

all age groups 0.6-4.6 $x10^9/l$

Lymphocytes:

age (years)	range ($10^9/l$)
0-1	4.4-8.4
1-2	4.1-8.1
2-3	2.9-6.9
3-4	2.3-6.3
4-5	1.7-5.7
5-6	1.4-5.4
6-7	1.4-5.4
8	1.0-3.0

Red Blood Cells:

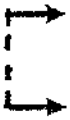
age (years)	range ($10^{12}/l$)
0.1	5.9-9.9
1-2	5.1-9.1
2-3	4.4-8.4
3-4	4.2-8.2
4-5	4.1-8.1
5-6	3.9-7.9
6-7	3.8-7.8
7-8	3.8-7.8
8	3.7-7.7

Platelets:

all age groups 200-600 $x10^9/l$ (Gentry et al., 1975).

FIGURES 68-94: Haematology Results.

Solid bars indicate the accepted normal ranges for neutrophils and platelets.



Interrupted bars indicate the accepted normal ranges for lymphocytes and red blood cells.

———— Bracken feeding:

a) Dried. Graphs One to Four. Days 0 - 1400
b) Fresh. Graphs Five to Eight. Days 1450 - 2700

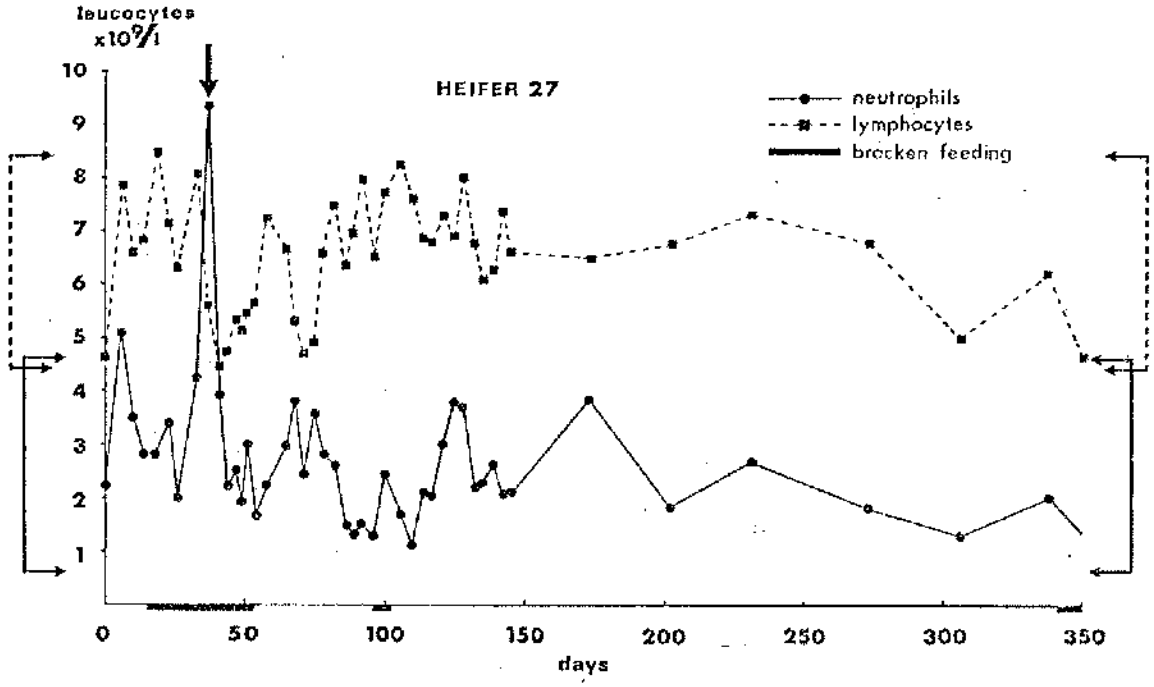
FIG. 68: Haematology results. Heifer 27, Graph One.

Lymphocytes Results appear rather erratic but are mostly within the normal range.

Neutrophils Results are variable but minimum values are never below $1.00 \times 10^9/l$. The peak recorded at day 37 (↓) follows the experimental surgery performed on day 34.

Red Blood Cells Although somewhat erratic, these results are predominantly in the lower half of the normal range.

Platelets Initially there was marked variation in the results although they fall mostly within the accepted normal range. The significance, if any, of the apparent trough at days 65-75 is unclear. (Compare later results for this animal).


leucocytes
x10⁹/l
HEIFER 27
neutrophils
lymphocytes
broken feeding
10
9
8
7
6
5
4
3
2
1
0
50
100
150
200
250
300
350
days

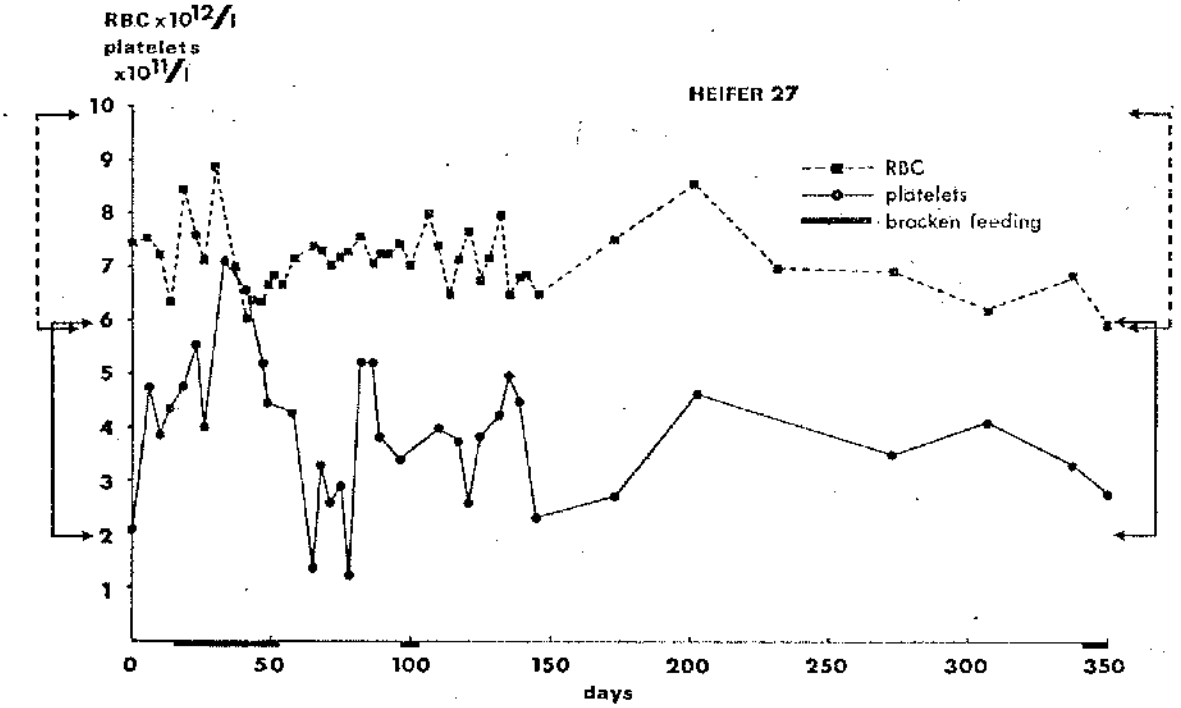

RBC x10¹²/l
platelets
x10¹¹/l
HEIFER 27
RBC
platelets
broken feeding
10
9
8
7
6
5
4
3
2
1
0
50
100
150
200
250
300
350
days

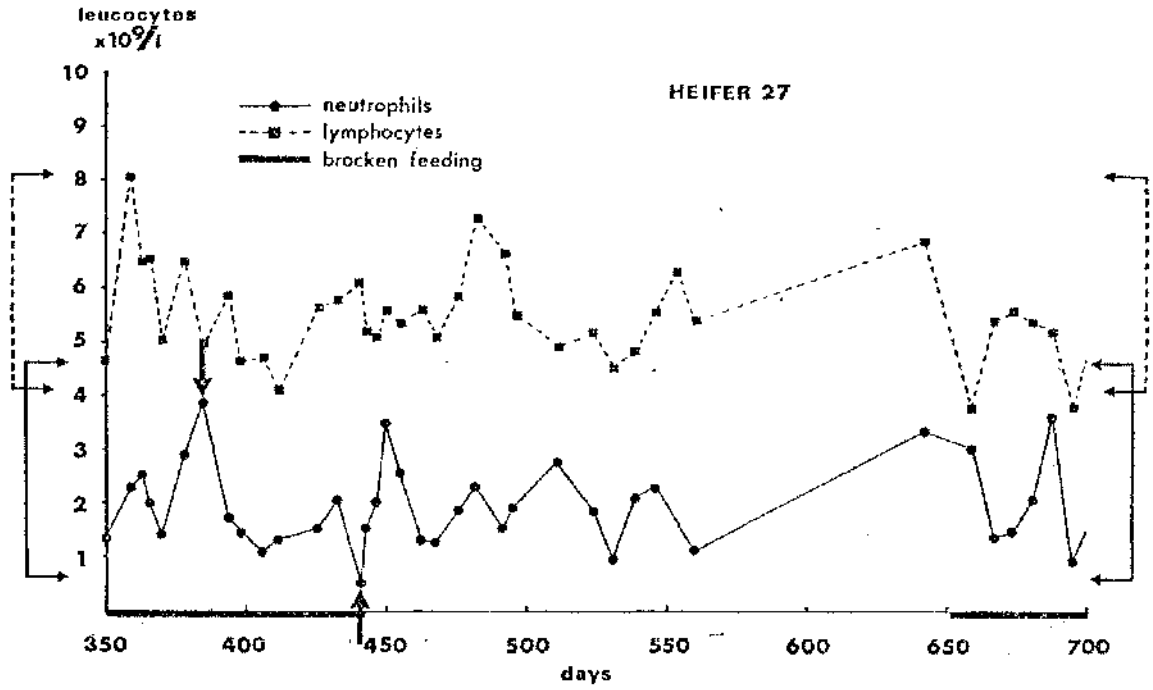
FIG. 69: Haematology results. Heifer 27,Graph Two.

Lymphocytes Results appear to be somewhat lower during periods of bracken feeding but are never markedly outwith the normal range.

Neutrophils Bracken feeding was stopped at day 443 (↓) because of the low neutrophil count (also shown by Heifer 98) Results at other times are within the normal range although there is slight peak at day 385 (↓) following dehorning on day 383.

Red Blood Cells Within normal range.

Platelets Results are consistently rather low when compared to the accepted normal range. Troughs below normal are seen both during and outwith the periods of bracken feeding.


leucocytes
x10⁹/l
HEIFER 27
neutrophils
lymphocytes
brocken feeding
10
9
8
7
6
5
4
3
2
1
350
400
450
500
550
600
650
700
days

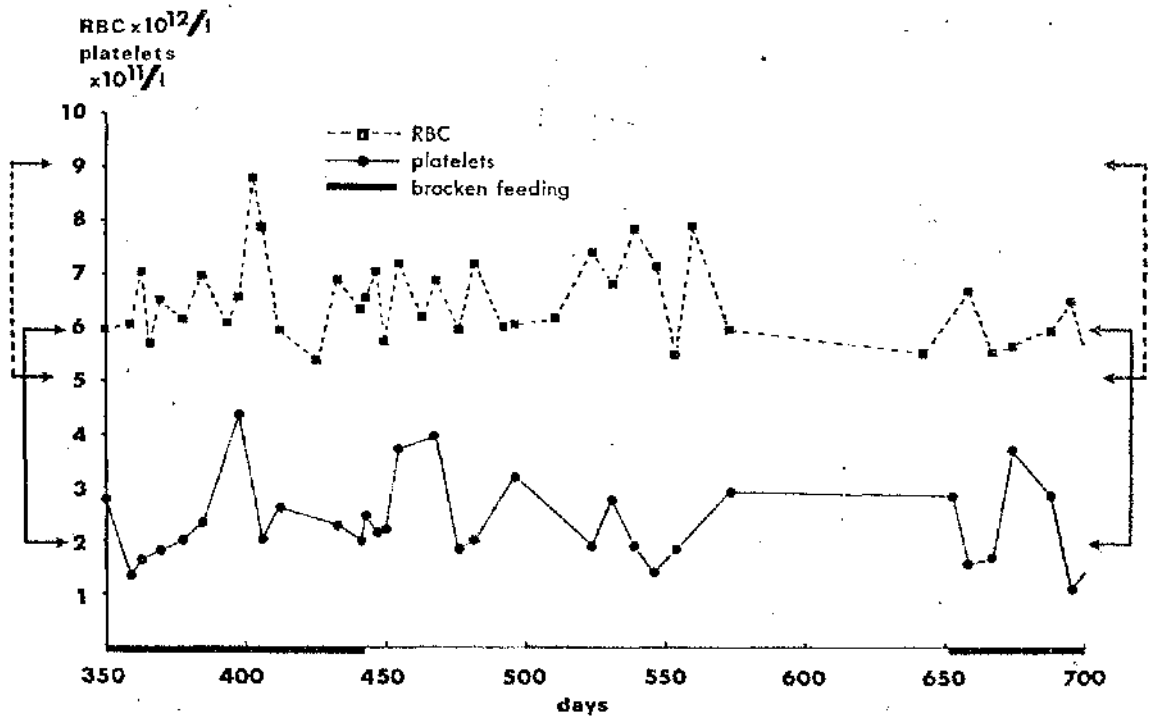

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
brocken feeding
10
9
8
7
6
5
4
3
2
1
350
400
450
500
550
600
650
700
days

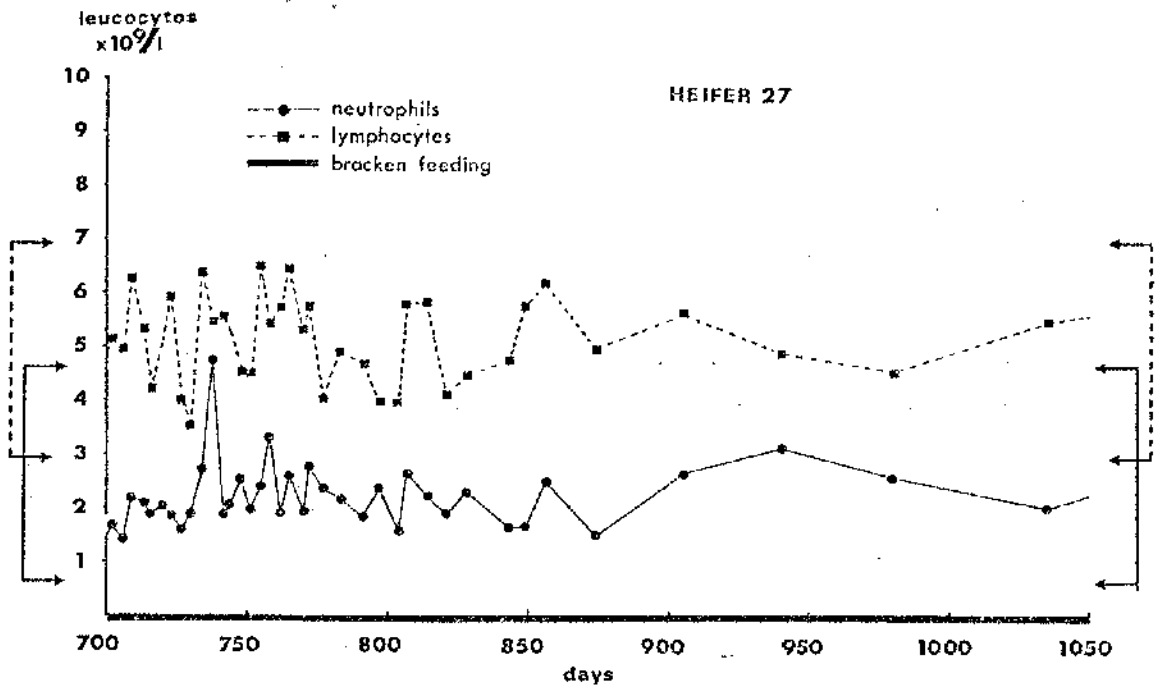
FIG. 70: Haematology results. Heifer 27,Graph Three.

Lymphocytes Values are well within the normal range although there is more variation than with neutrophil counts.

Neutrophils Values are well within the normal range throughout this period of bracken feeding. Occasional small peaks were not related to any obvious clinical or environmental change.

Red Blood Cells Values are steadily within the accepted normal range.

Platelets Counts are frequently below the accepted normal range with values of between 100 and $400 \times 10^9/l$.


leucocytes
x10⁹/l
HEIFER 27
neutrophils
lymphocytes
bracken feeding
10
9
8
7
6
5
4
3
2
1
700
750
800
850
900
950
1000
1050
days

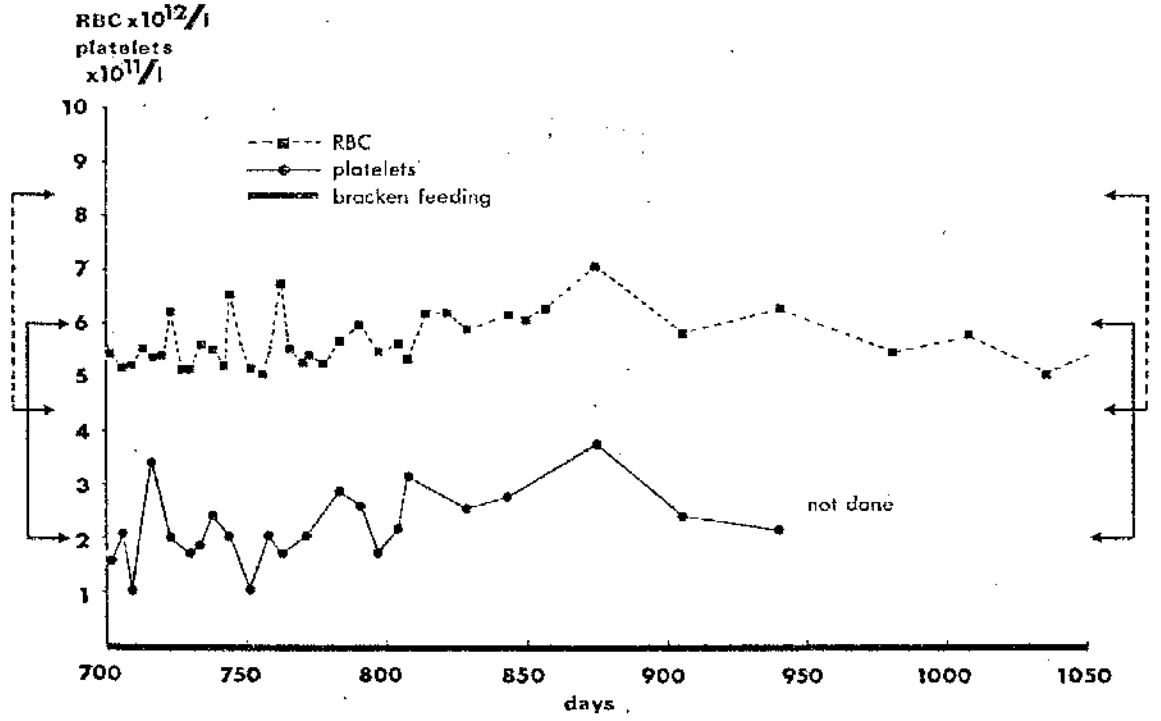

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
not done
10
9
8
7
6
5
4
3
2
1
700
750
800
850
900
950
1000
1050
days

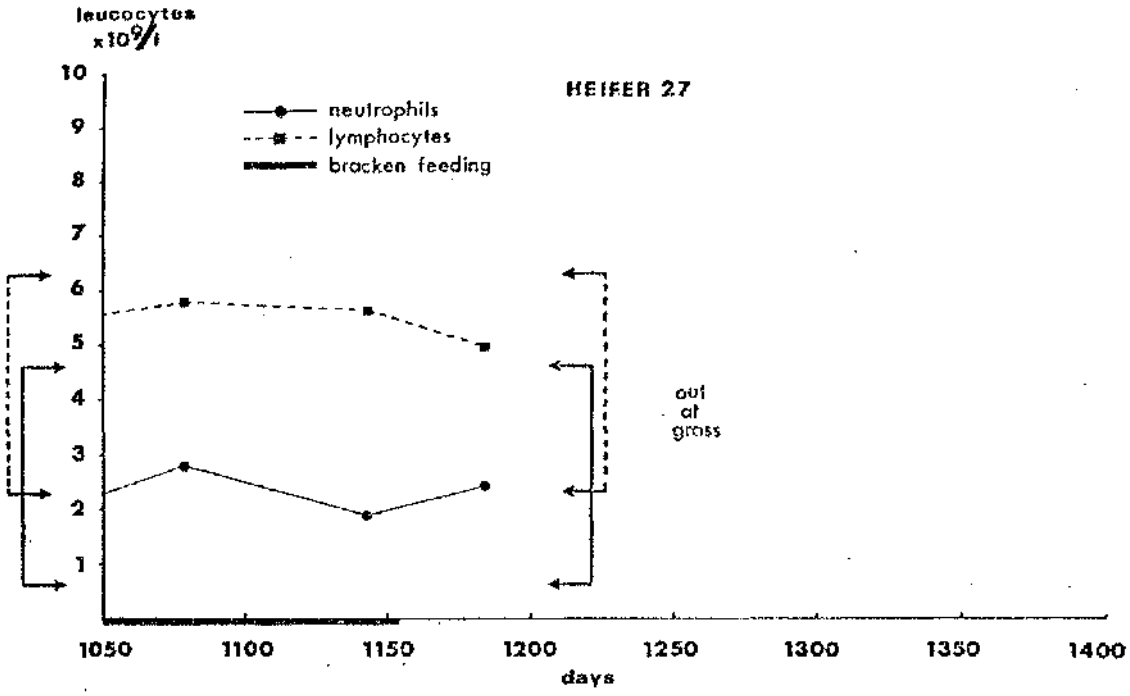
FIG. 71: Haematology results. Heifer 27,Graph Four.

Lymphocytes Results are within the normal range.

Neutrophils Results are within the normal range.

Red Blood Cells Results are within the normal range.

Platelets Not done.


leucocytes
x10⁹/l
HEIFER 27
neutrophils
lymphocytes
bracken feeding
out
of
grass
10
9
8
7
6
5
4
3
2
1
1050
1100
1150
1200
1250
1300
1350
1400
days

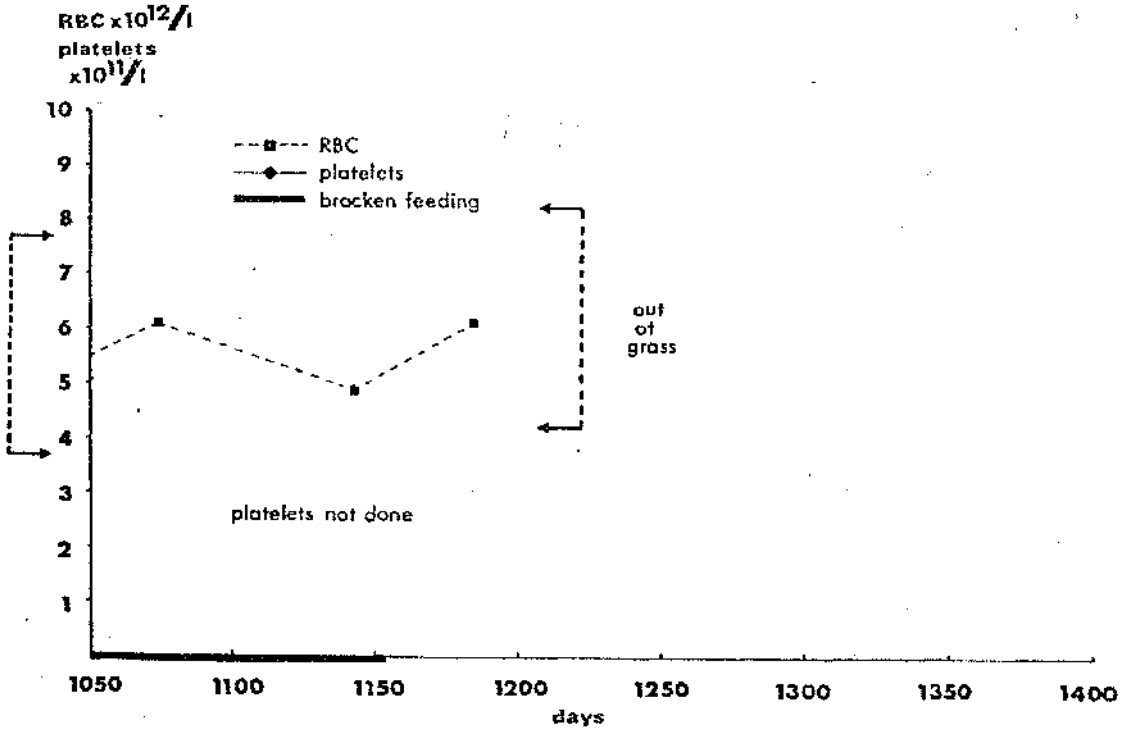

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
out
of
grass
platelets not done
10
9
8
7
6
5
4
3
2
1
1050
1100
1150
1200
1250
1300
1350
1400
days

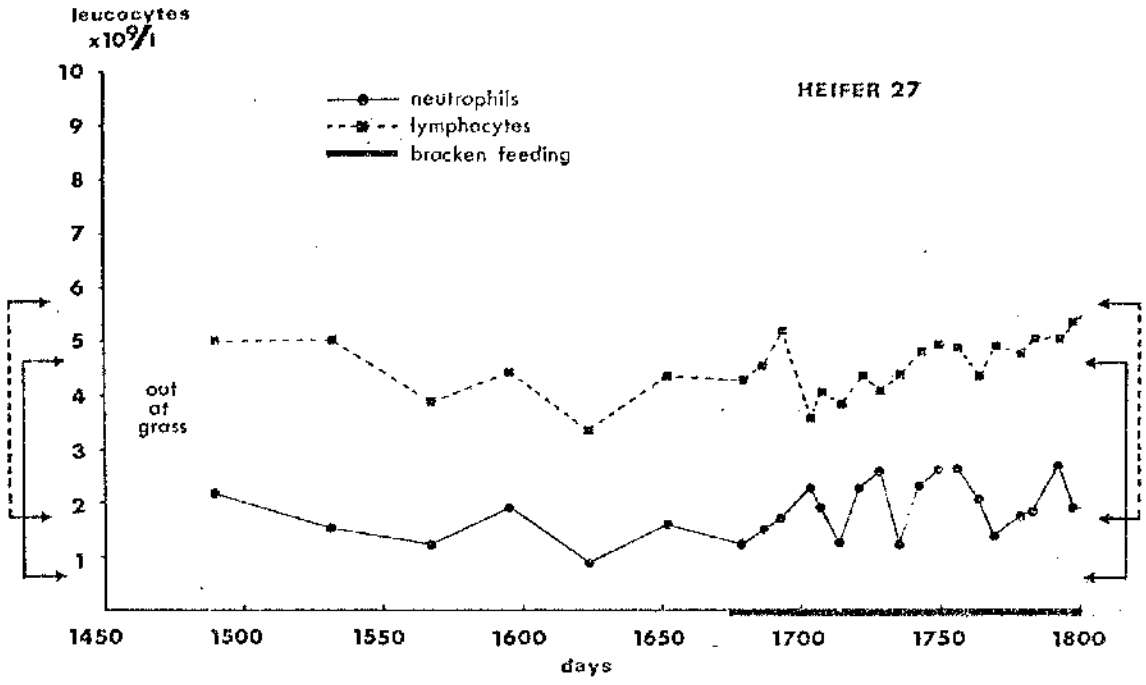
FIG. 72: Haematology results.Heifer 27,Graph Five.

Lymphocytes Counts remain within the normal range throughout this period.

Neutrophils Values remain steadily within the lower half of the normal range despite reintroduction of bracken feeding.

Red Blood Cells Counts are within the normal range although lower than in previous years.

Platelets Values are within the accepted normal range (i.e. somewhat higher than in previous years) apart from a few isolated counts when bracken feeding is reintroduced.


leucocytes
x10⁹/l
HEIFER 27
neutrophils
lymphocytes
bracken feeding
out
at
grass
10
9
8
7
6
5
4
3
2
1
1450
1500
1550
1600
1650
1700
1750
1800
days

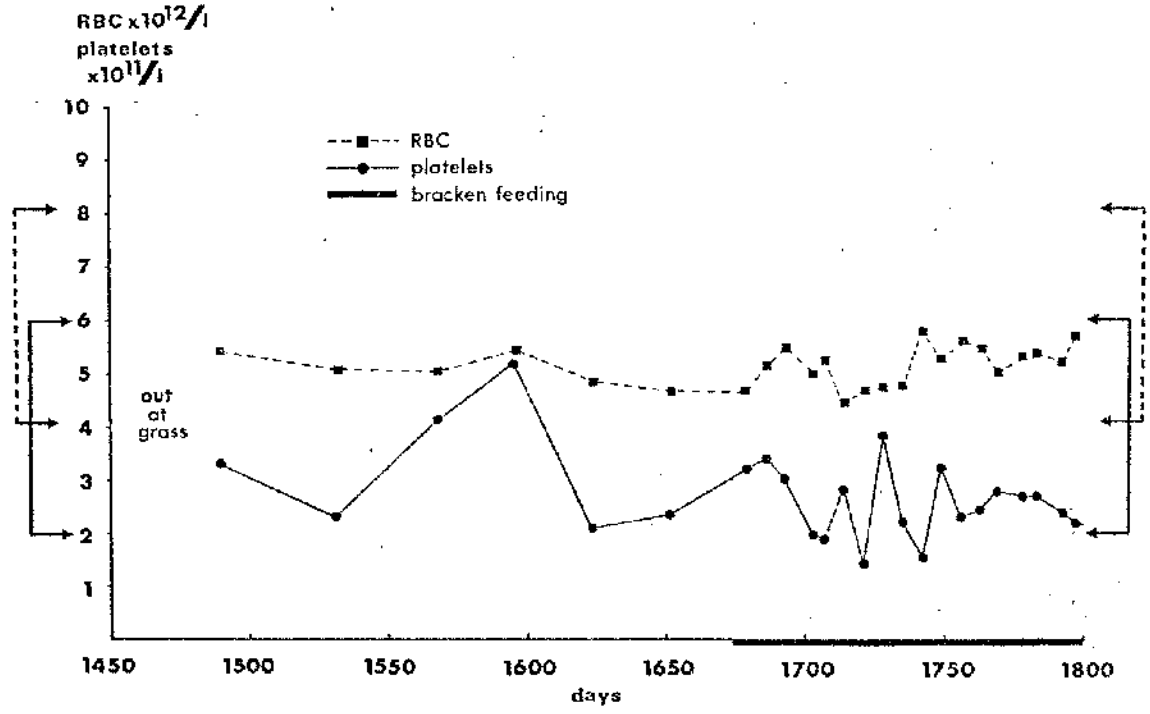

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
out
at
grass
10
9
8
7
6
5
4
3
2
1
1450
1500
1550
1600
1650
1700
1750
1800
days

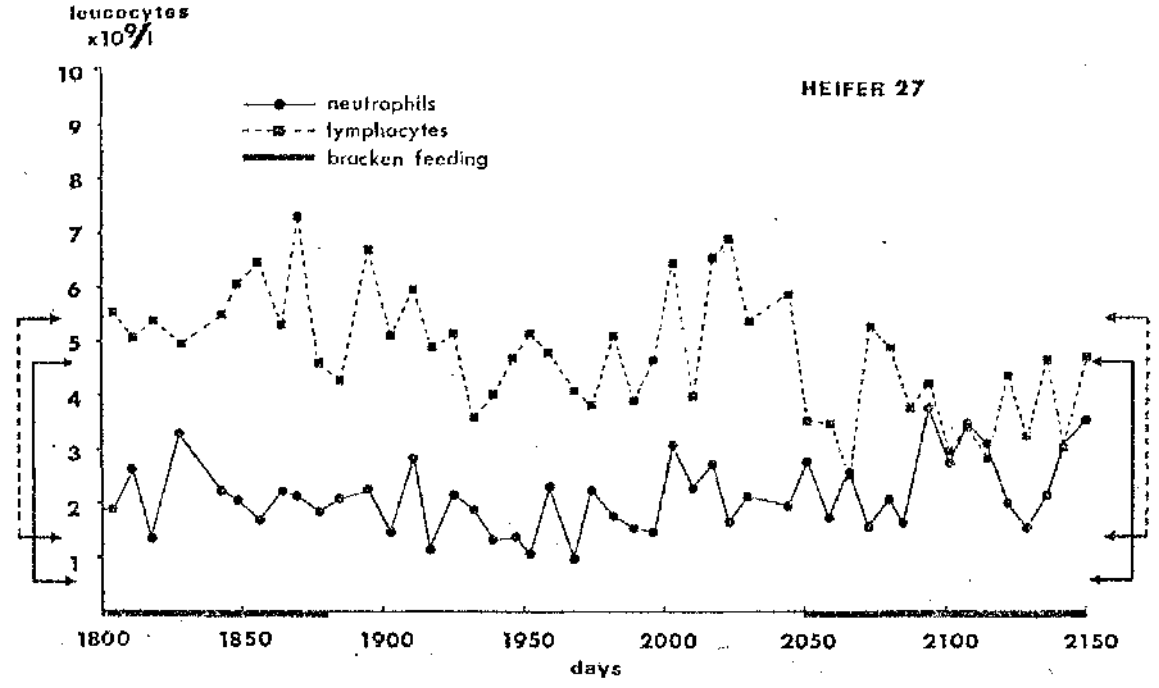
FIG. 73: Haematology results. Heifer 27,Graph Six.

Lymphocytes Values appear to decline during this period, particularly when bracken feeding is reintroduced. However, numerous individual counts are above normal limits throughout the period.

Neutrophils Values are within the accepted normal range with no large peaks or troughs.

Red Blood Cells Counts are largely within the normal range although results are somewhat more erratic than in previous years. (Haematuria was present at this stage).

Platelets Values oscillate around the lower limits of normal with a minimum count of $80x10^9/l$ (†) more than 3 months after bracken feeding was reintroduced.


leucocytes
x10⁹/l
HEIFER 27
neutrophils
lymphocytes
bracken feeding
10
9
8
7
6
5
4
3
2
1
1800
1850
1900
1950
2000
2050
2100
2150
days

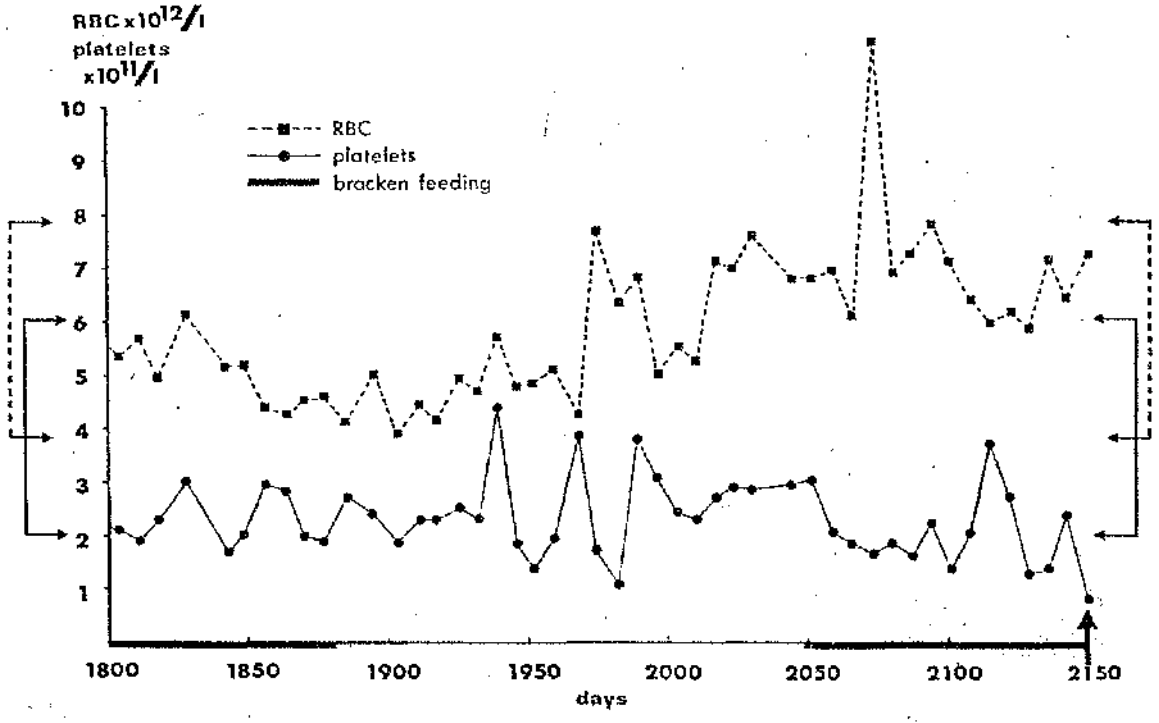

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
10
9
8
7
6
5
4
3
2
1
1800
1850
1900
1950
2000
2050
2100
2150
days

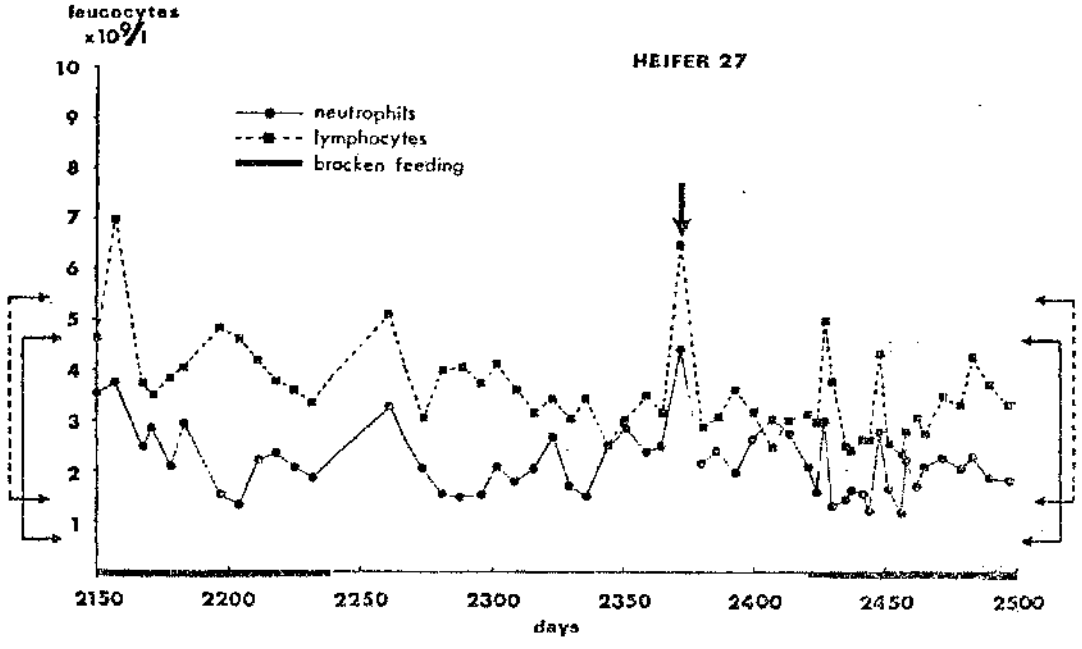
FIG. '74: Haematology results. Heifer 27, Graph Seven.

Lymphocytes There appears to be a slight but steady decline in values throughout this period with only occasional counts above normal limits.

Neutrophils Counts are within the normal range except a small peak is evident at day 2372 (↓). No associated clinical or environmental change was recorded.

Red Blood Cells Values are within the upper half of the accepted normal range.

Platelets Values oscillate around the lower limits of normal with most counts between 100 and $400 \times 10^9/l$.


leucocytes
x10⁹/l
HEIFER 27
neutrophils
lymphocytes
bracken feeding
10
9
8
7
6
5
4
3
2
1
2150
2200
2250
2300
2350
2400
2450
2500
days

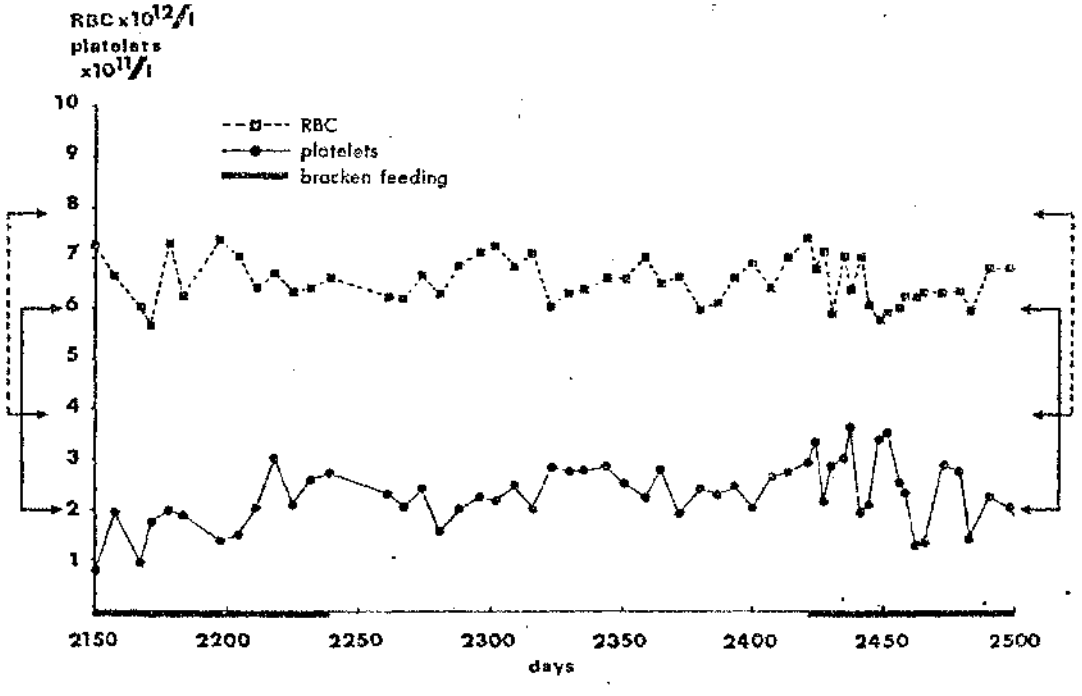

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
10
9
8
7
6
5
4
3
2
1
2150
2200
2250
2300
2350
2400
2450
2500
days

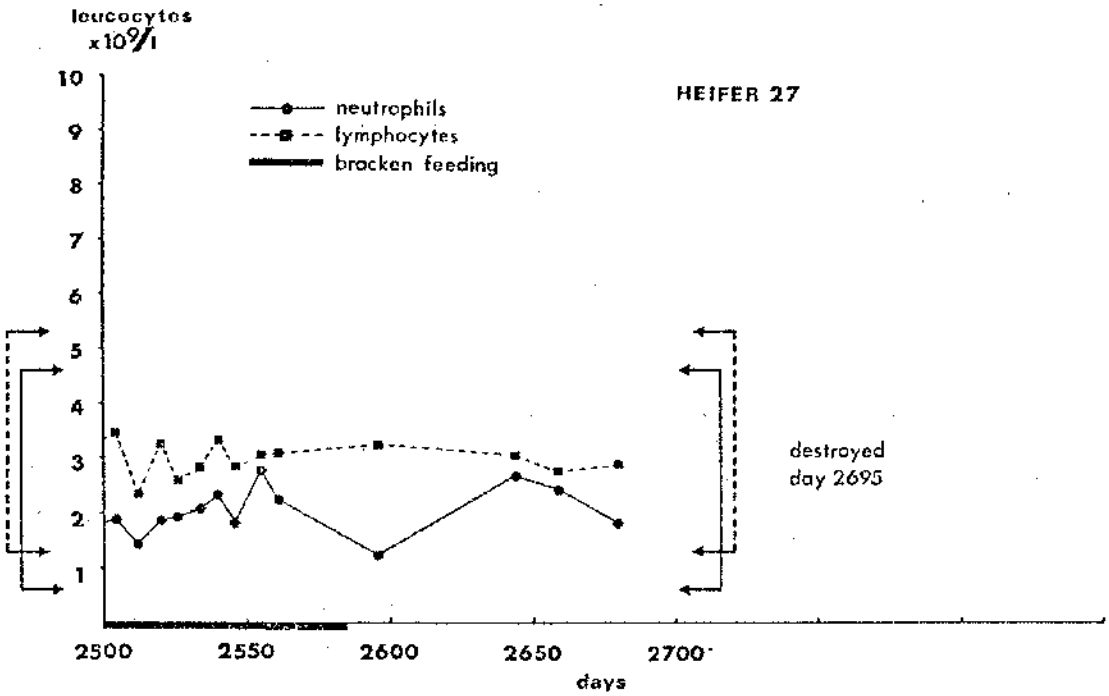
FIG.,75: Haematology results. Heifer 27,Graph Eight.

Lymphocytes Values are consistently within the normal range.

Neutrophils Values are consistently within the lower half of the normal range.

Red Blood Cells Normal values are maintained.

Platelets Values remain within the range 100-400x10^9/l.


leucocytes
x10⁹/l
HEIFER 27
neutrophils
lymphocytes
bracken feeding
destroyed
day 2695
10
9
8
7
6
5
4
3
2
1
2500
2550
2600
2650
2700
days

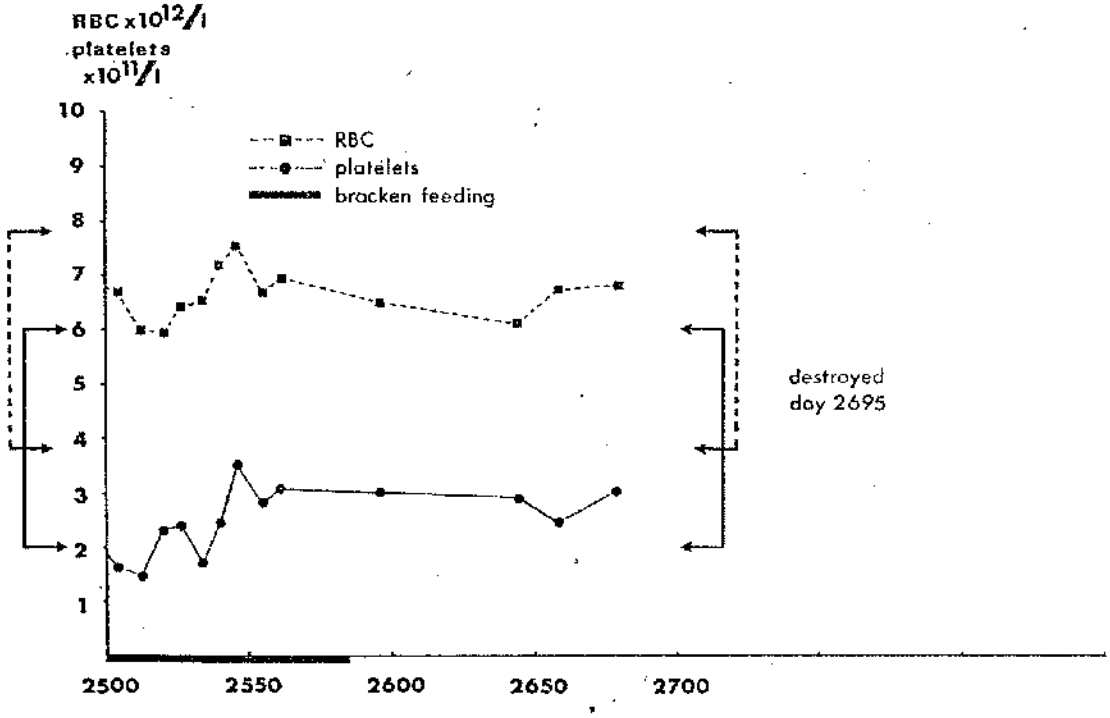

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
destroyed
day 2695
10
9
8
7
6
5
4
3
2
1
2500
2550
2600
2650
2700

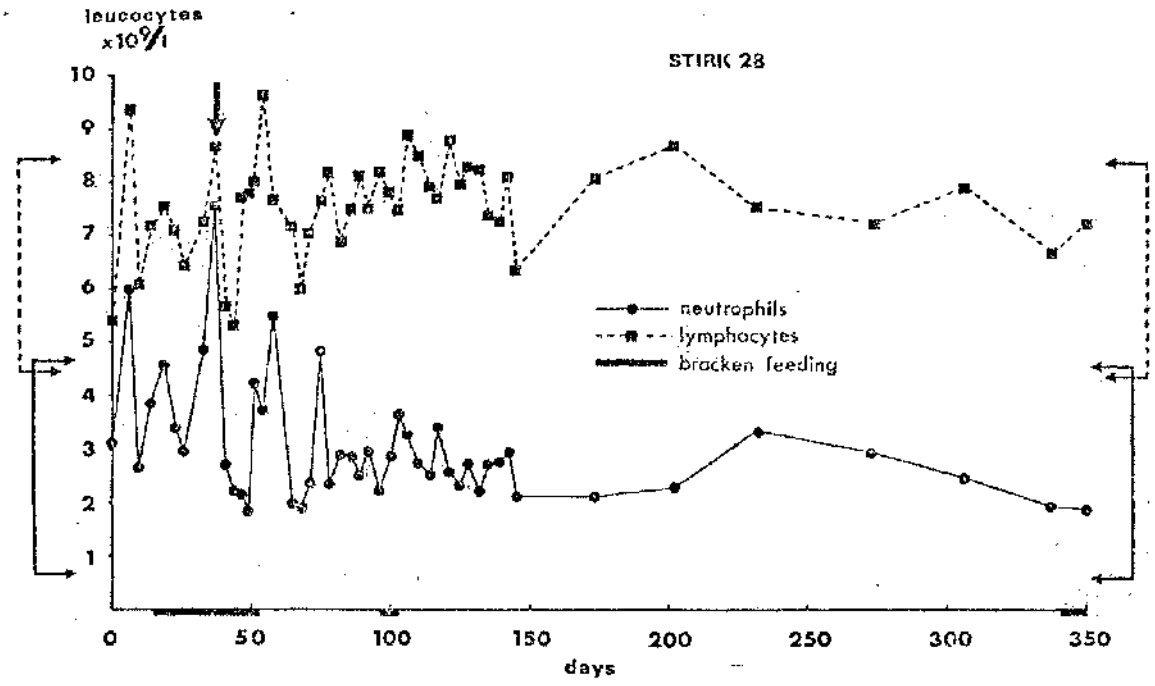
FIG. '76: Haematology results. Stirk 28,Graph One.

Lymphocytes The results were initially erratic with values at the upper limits of the normal range.

Neutrophils Values are raised during the initial days of the experiment with the peak at day 37 (↓) following experimental surgery on day 34. Elevated values occurring between days 55-80 are related to the recorded episode of pneumonia.

Red Blood Cells Values are well within the accepted normal range.

Platelets There is wide variation in results with various peaks above the accepted normal range.


leucocytes
x10⁹/l
STIRK 28
neutrophils
lymphocytes
bracken feeding
days

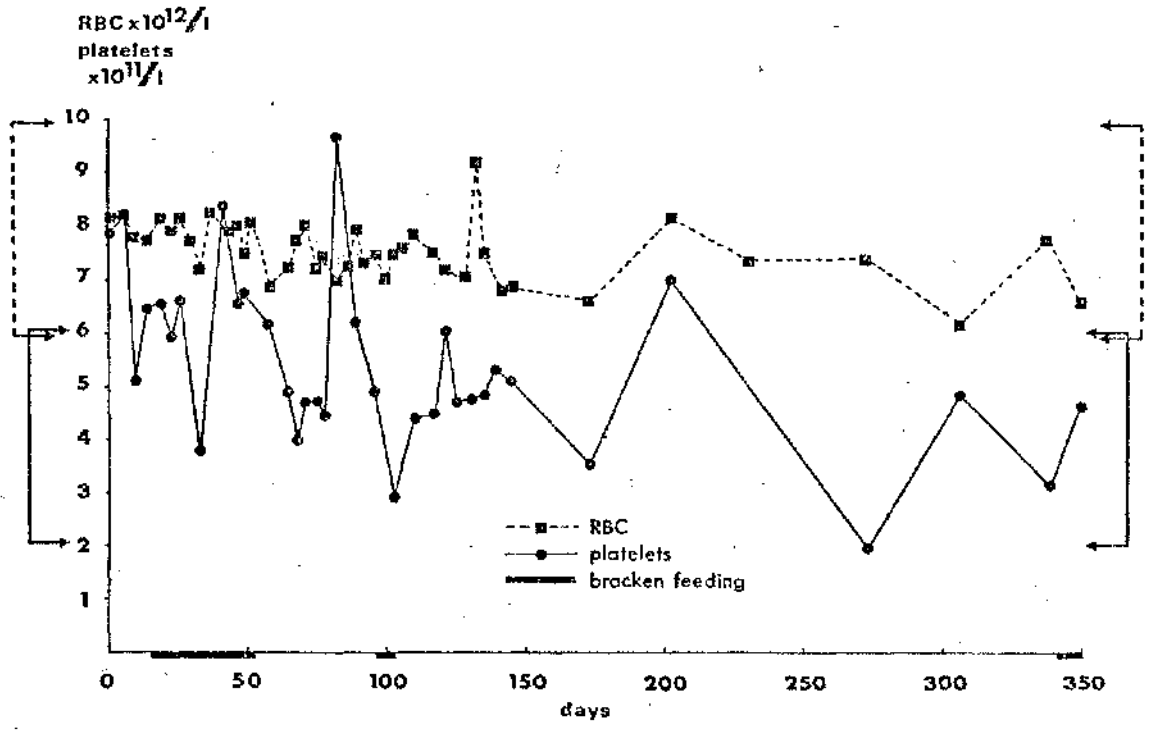

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
days

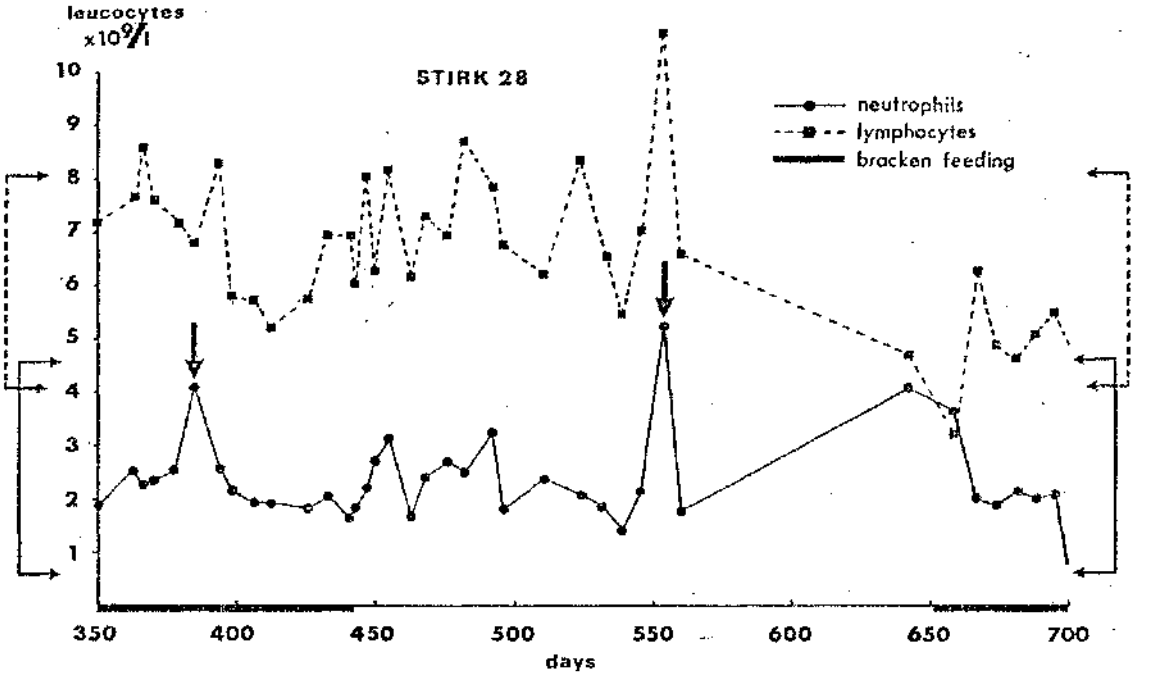
FIG. 77: Haematology results. Stirk 28, Graph Two.

Lymphocytes Results are rather erratic with various peaks and troughs most of which were not related to any obvious clinical events or environmental changes.

Neutrophils Values are within the normal range apart from an isolated peak at day 554 (↓). This follows the animal's escape on day 553 when he gorged himself with available feed and suffered metabolic acidosis (overeating disease). A small peak at day 385 (↓) follows dehorning on day 383.

Red Blood Cells Values are within the accepted normal range.

Platelets Results are somewhat erratic but tend to be at or below the lower end of the normal range throughout this period. (Compare results at day 443 with the low counts recorded for animals 27 and 98.)


leucocytes
x10⁹/l
STIRK 28
neutrophils
lymphocytes
bracken feeding
10
9
8
7
6
5
4
3
2
1
350
400
450
500
550
600
650
700
days

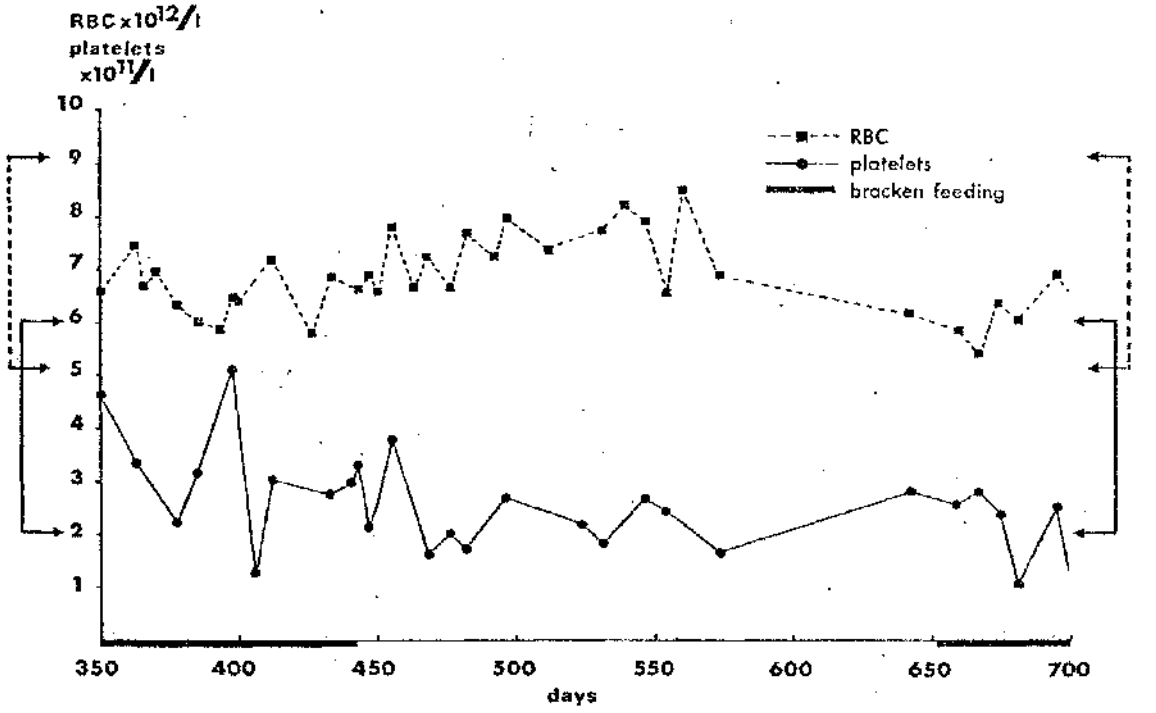

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
10
9
8
7
6
5
4
3
2
1
350
400
450
500
550
600
650
700
days

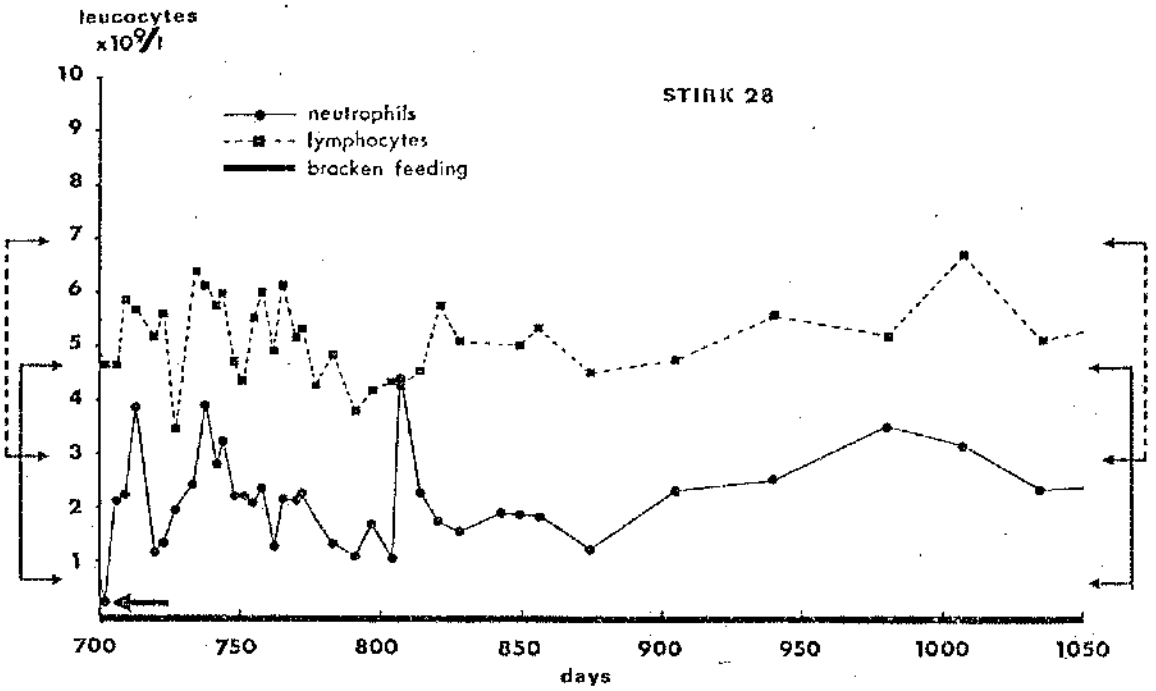
FIG.,78: Haematology results. Stirk 28,Graph Three.

Lymphocytes Values are well within the normal range throughout this period of bracken feeding.

Neutrophils There is a precipitous fall in the neutrophil count at day 702 (←). Bracken feeding was continued and values recover to remain within the normal range.

Red Blood Cells Counts are steadily within the normal range throughout this period.

Platelets The minimum value of $50x10^9/l$ at day 702 (↑) coincides with the fall in the neutrophil count. Values recover to over $100x10^9/l$ but are frequently below the accepted normal range.


leucocytes
x10⁹/l
STIRK 28
neutrophils
lymphocytes
bracken feeding
10
9
8
7
6
5
4
3
2
1
700
750
800
850
900
950
1000
1050
days

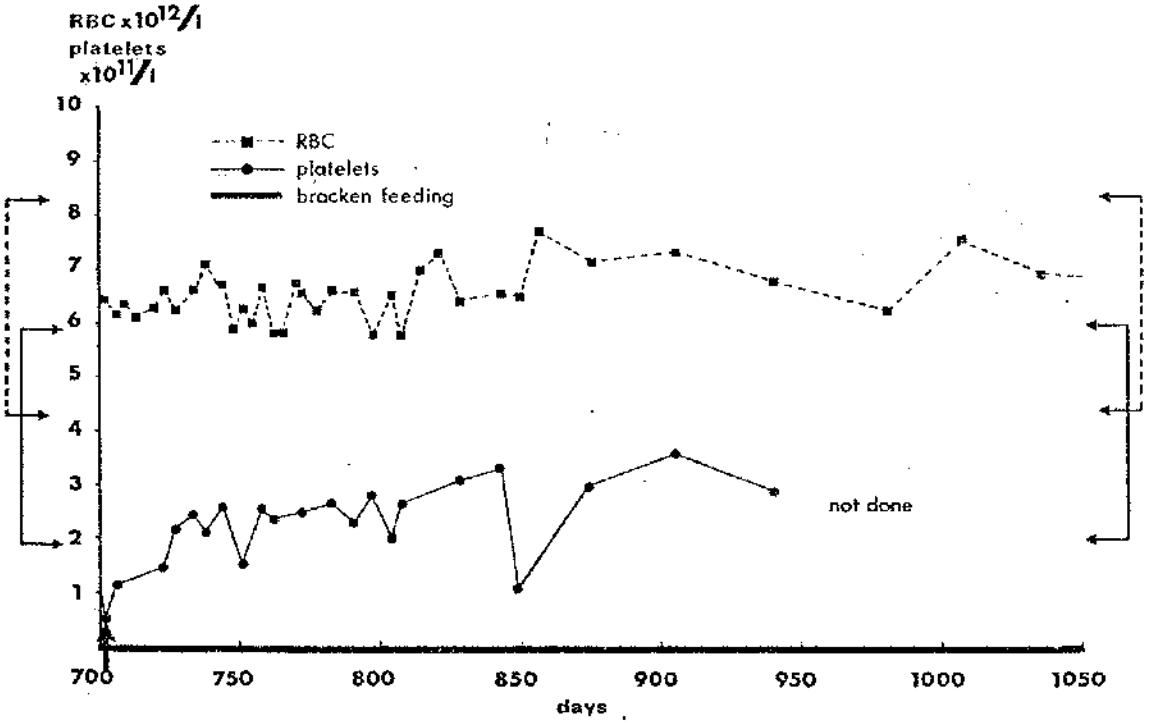

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
not done
10
9
8
7
6
5
4
3
2
1
700
750
800
850
900
950
1000
1050
days

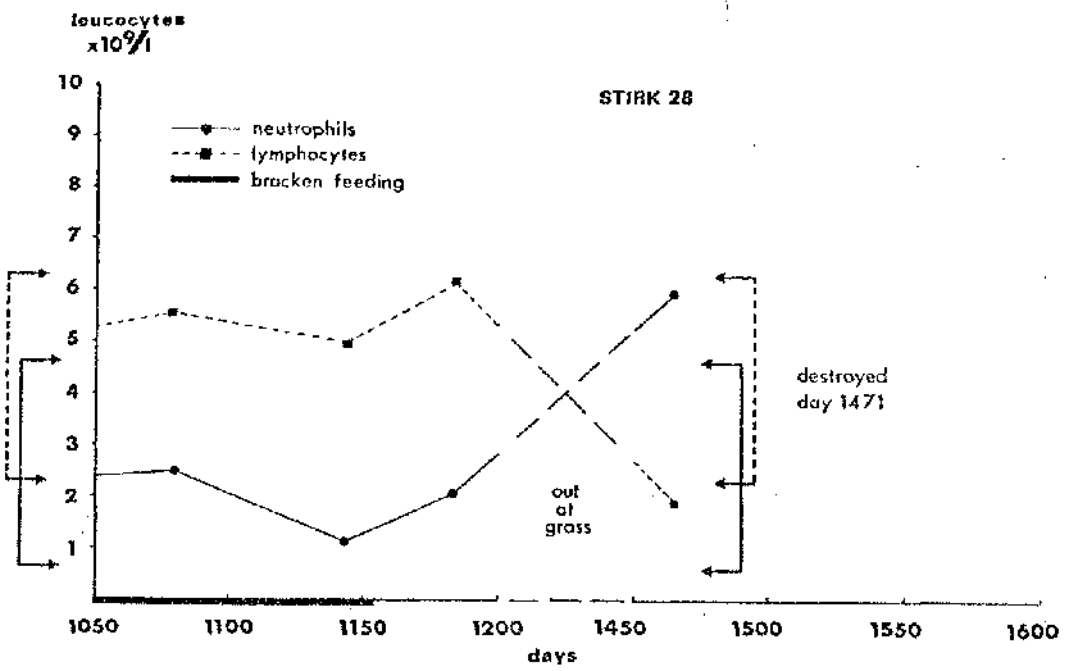
FIG. 79: Haematology results. Stirk 28,Graph Four.

Lymphocytes Values are apparently within the normal range apart from the terminal low count.

Neutrophils Values are apparently within the normal range apart from the terminal rise.

Red Blood Cells Values are within the normal range throughout this period.

Platelets Although initially not done, terminal counts were within the normal range.


leucocytes
x10⁹/l
STIRK 28
neutrophils
lymphocytes
bracken feeding
destroyed
day 1471
out
of
grass
10
9
8
7
6
5
4
3
2
1
1050
1100
1150
1200
1450
1500
1550
1600
days

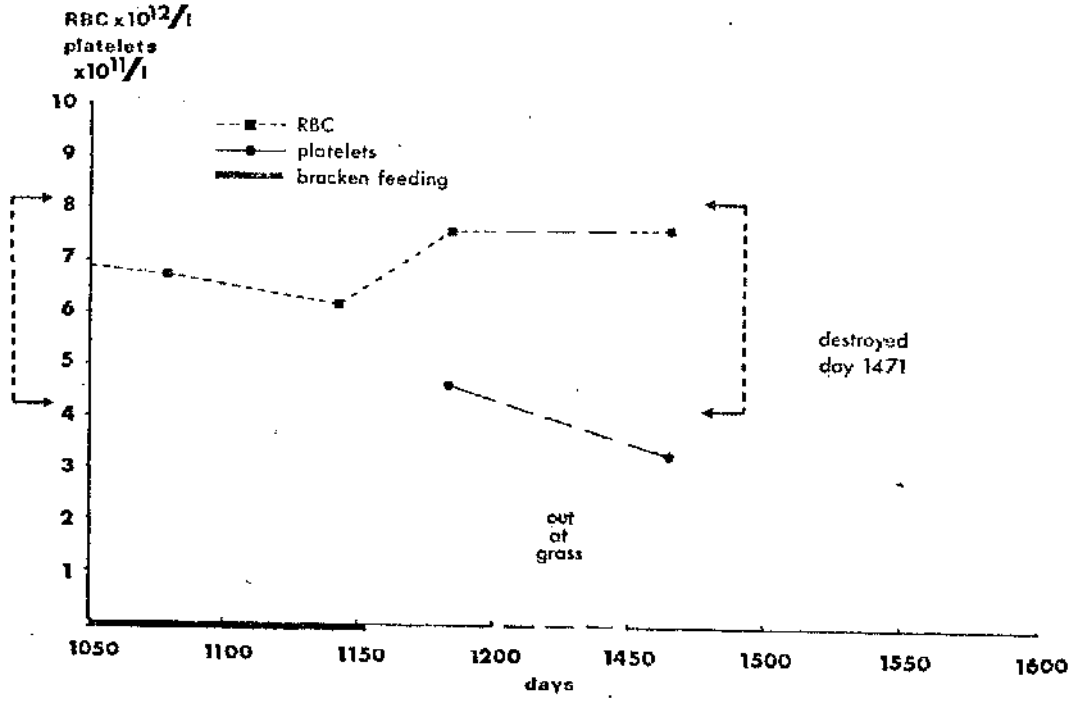

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
destroyed
day 1471
out
of
grass
10
9
8
7
6
5
4
3
2
1
1050
1100
1150
1200
1450
1500
1550
1600
days

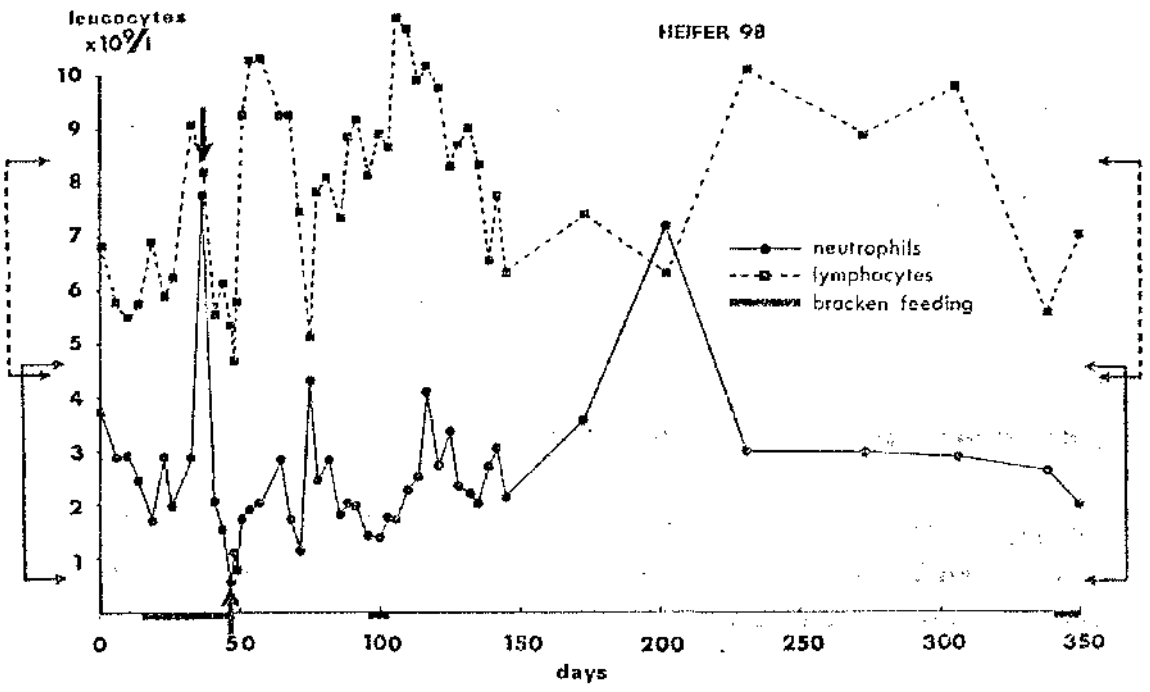
FIG. 80: Haematology results. Heifer 98,Graph One.

Lymphocytes Results are erratic with various peaks above the expected normal range which were not obviously related to any clinical or environmental changes.

Neutrophils The recorded peak at day 37 (↓) follows experimental surgery on day 35. The trough at day 47 (↑) precipitated withdrawal of bracken feed. A raised count was also recorded on day 202 (compare Stirk 99).

Red Blood Cells Values are well within the accepted normal range.

Platelets There is wide variation in results with many counts above the accepted normal range although few are above $800 \times 10^9/l$. Occasional troughs of less than $200 \times 10^9/l$ are not associated with periods of bracken feeding.


leucocytes
x10⁹/l
HEIFER 98
neutrophils
lymphocytes
bracken feeding
days

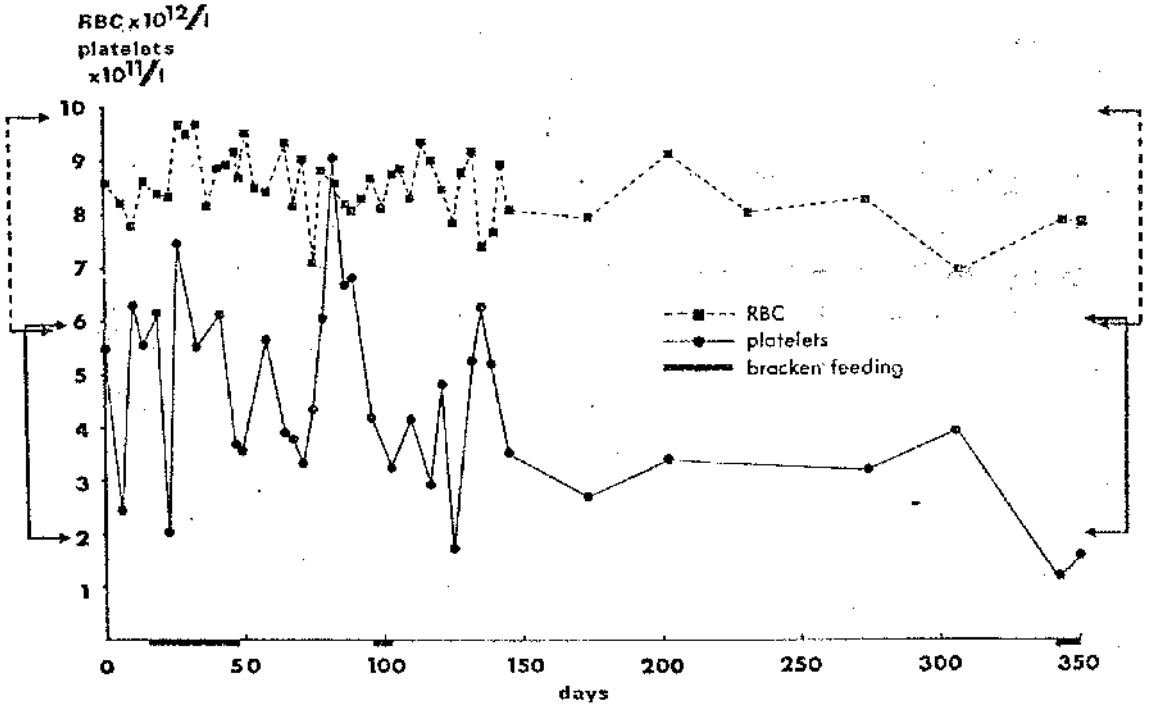

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
days

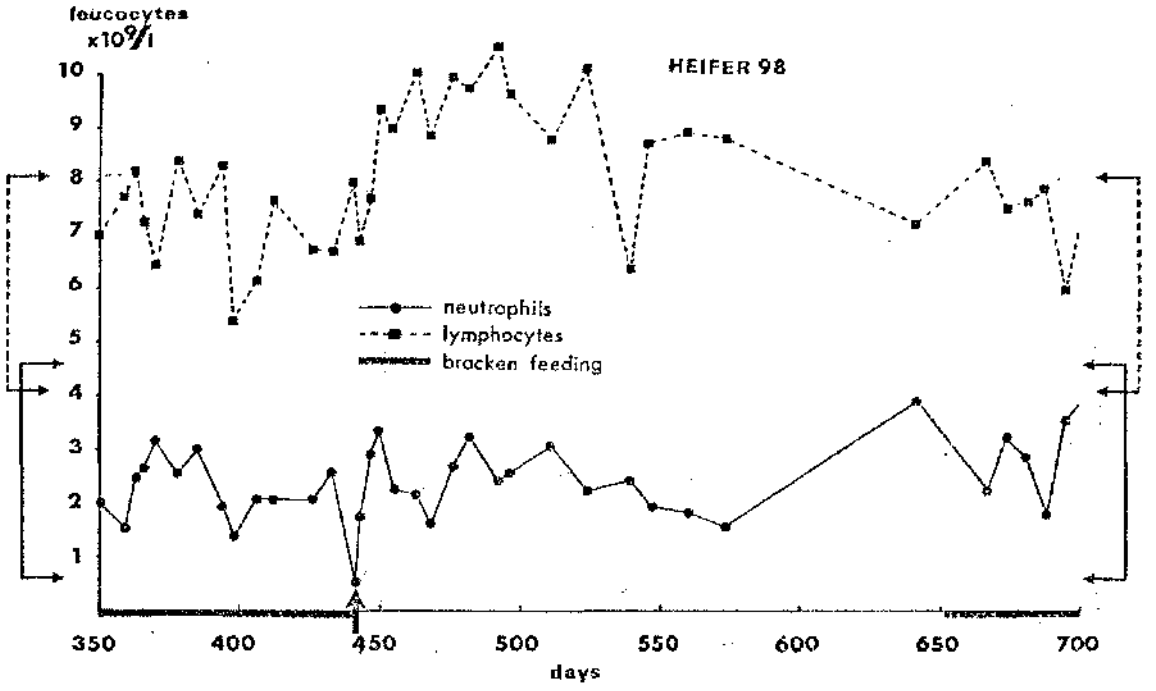
FIG. 81: Haematology results. Heifer 98,Graph Two.

Lymphocytes Results are erratic with counts frequently above the accepted normal range particularly when bracken was not being fed.

Neutrophils Bracken feeding was stopped at day 443 in view of the low neutrophil count (also shown by Heifer 27). There appears to be rapid recovery and thereafter counts are maintained within the normal range.

Red Blood Cells Values are largely within the normal range apart from isolated peaks slightly above this.

Platelets Values are consistently at the lower end of the normal range with numerous counts of less than $200x10^9/l$ particularly during periods of bracken feeding.


leucocytes
x10⁹/l
HEIFER 98
neutrophils
lymphocytes
bracken feeding
days

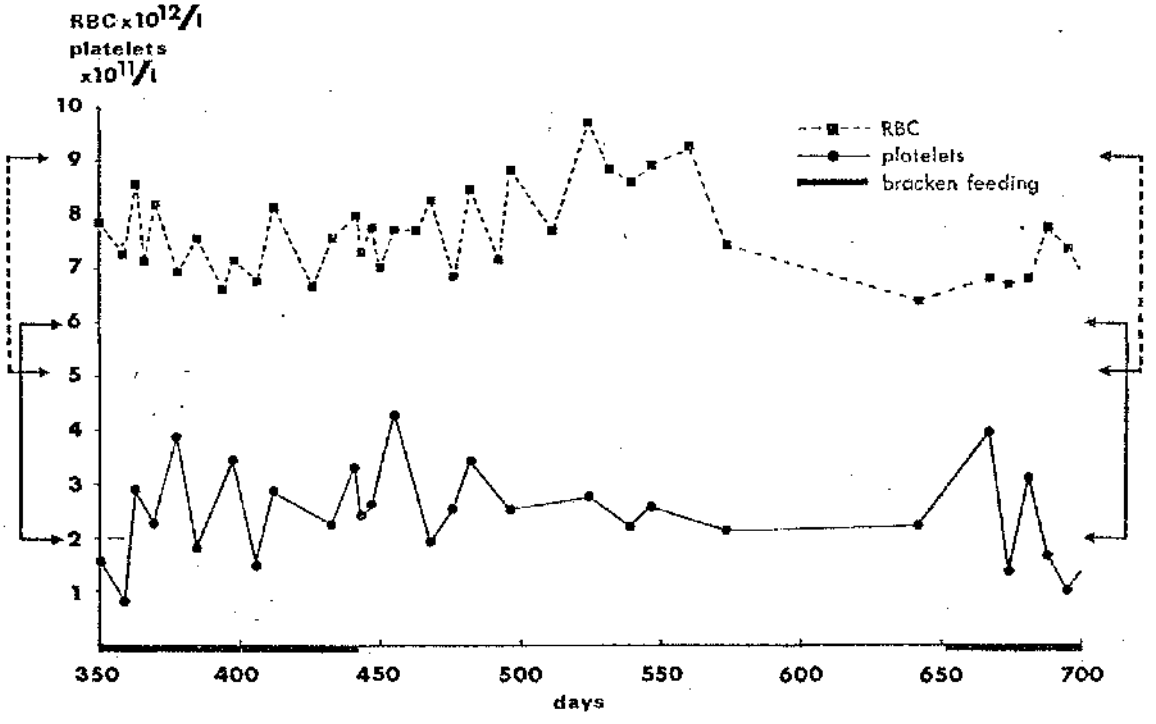

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
days

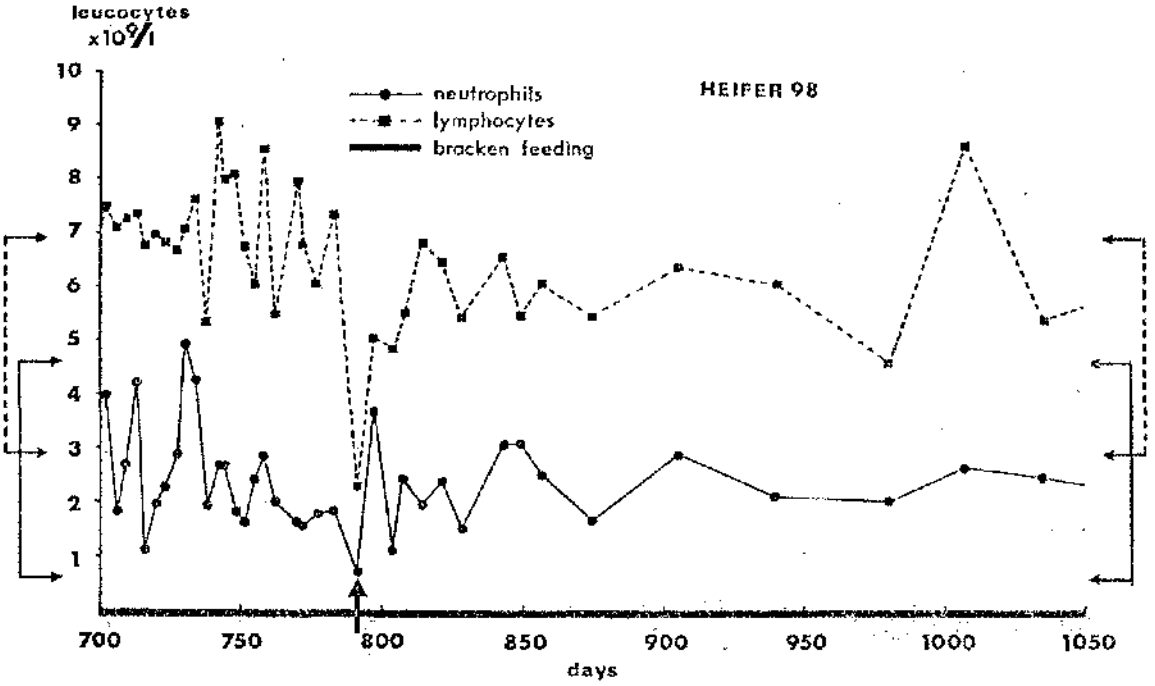
FIG. 82: Haematology results. Heifer 98, Graph Three.

Lymphocytes Results are rather erratic with a number of values above the accepted normal range and most counts in the upper half of the range. One isolated result on day 791 (†) appears as a precipitous drop but there is a rapid recovery to normal values.

Neutrophils Counts are largely within the accepted normal range although there is a slight trough at day 791 (†) which coincides with the lymphopaenia.

Red Blood Cells Values remain steadily within the normal range throughout this period of bracken feeding.

Platelets Values are frequently below the accepted normal range with the majority of counts between 100 and $400 \times 10^9/l$.


leucocytes
x10⁹/l
HEIFER 98
neutrophils
lymphocytes
bracken feeding
10
9
8
7
6
5
4
3
2
1
700
750
800
850
900
950
1000
1050
days

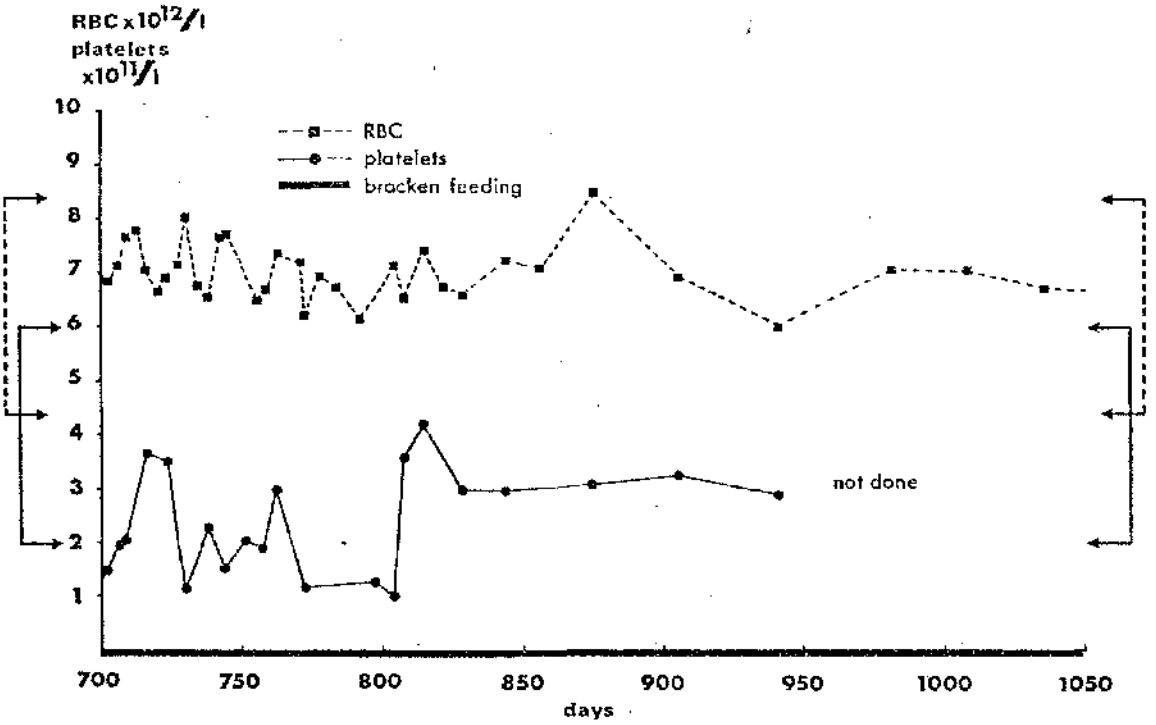

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
not done
10
9
8
7
6
5
4
3
2
1
700
750
800
850
900
950
1000
1050
days

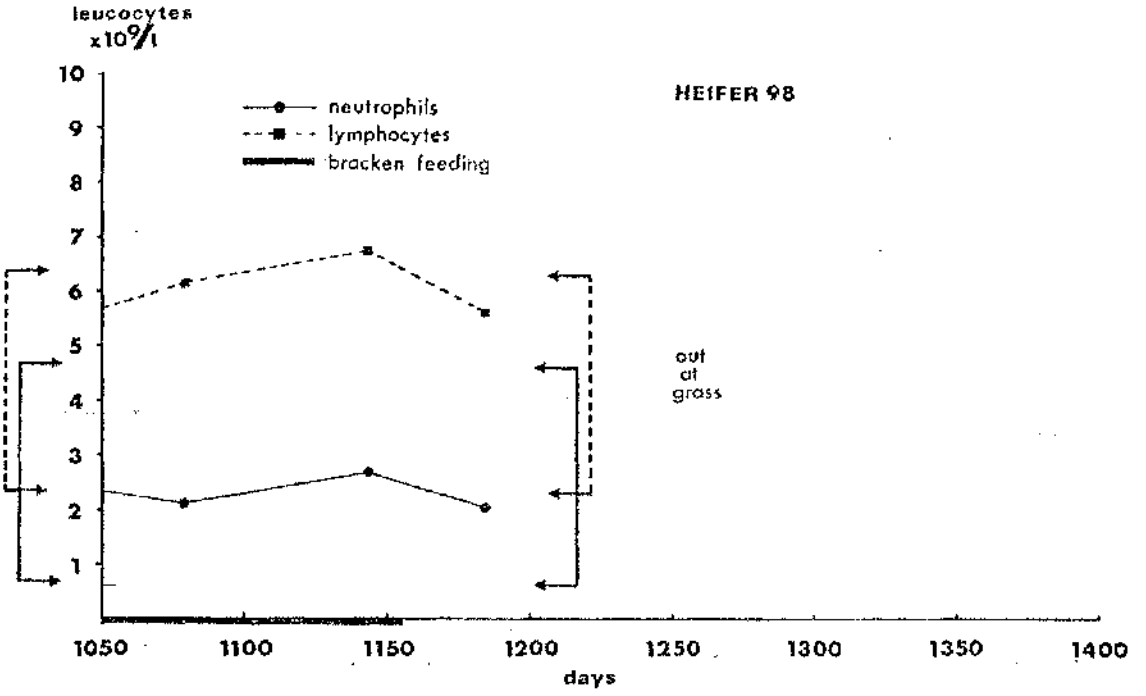
FIG. 83: Haematology results. Heifer 98,Graph Four.

Lymphocytes Values oscillate around the upper limits of the accepted normal range.

Neutrophils Results are within the normal range.

Red Blood Cells Results are within the normal range.

Platelets Not done.


leucocytes
x10⁹/l
HEIFER 98
neutrophils
lymphocytes
bracken feeding
out
at
grass
10
9
8
7
6
5
4
3
2
1
1050
1100
1150
1200
1250
1300
1350
1400
days

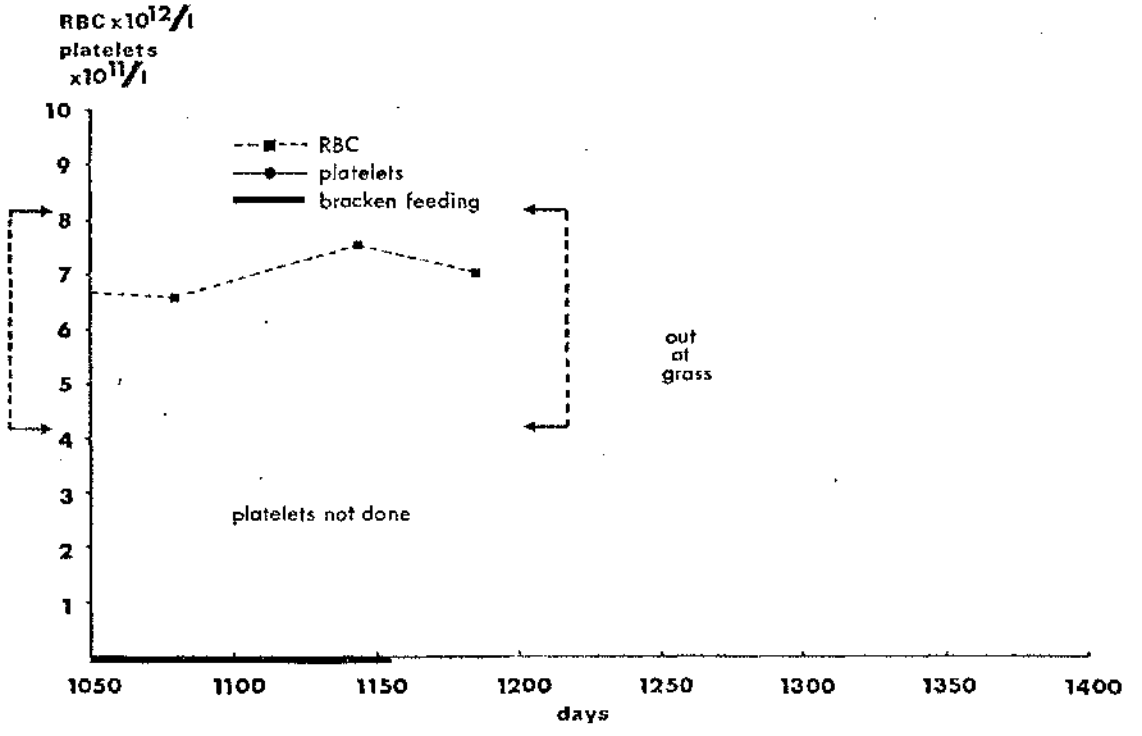

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
out
at
grass
platelets not done
10
9
8
7
6
5
4
3
2
1
1050
1100
1150
1200
1250
1300
1350
1400
days

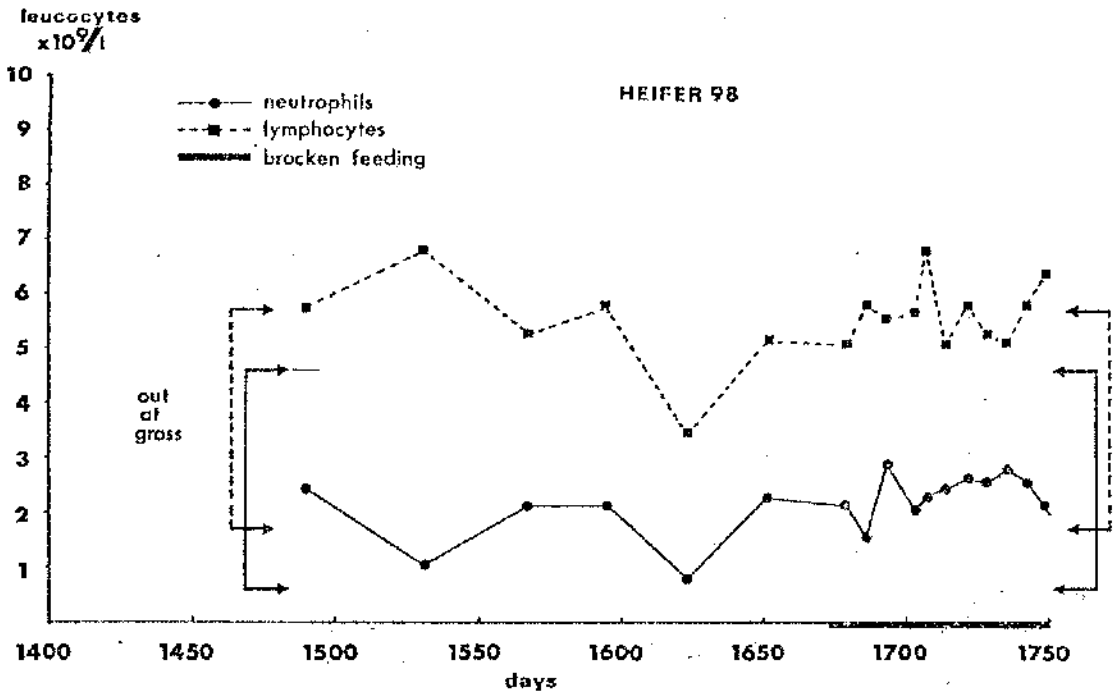
FIG. 84: Haematology results. Heifer 98,Graph Five.

Lymphocytes Results appear to be rather erratic with a number of counts above the normal range.

Neutrophils Values are largely within the lower half of the normal range.

Red Blood Cells Values are largely within the upper half of the normal range.

Platelets There appears to be a gradual decline in counts during this period although values are generally above $200x10^9/l$ (i.e. within normal range).


leucocytes
x10⁹/l
HEIFER 98
neutrophils
lymphocytes
bracken feeding
out
at
grass
10
9
8
7
6
5
4
3
2
1
1400
1450
1500
1550
1600
1650
1700
1750
days

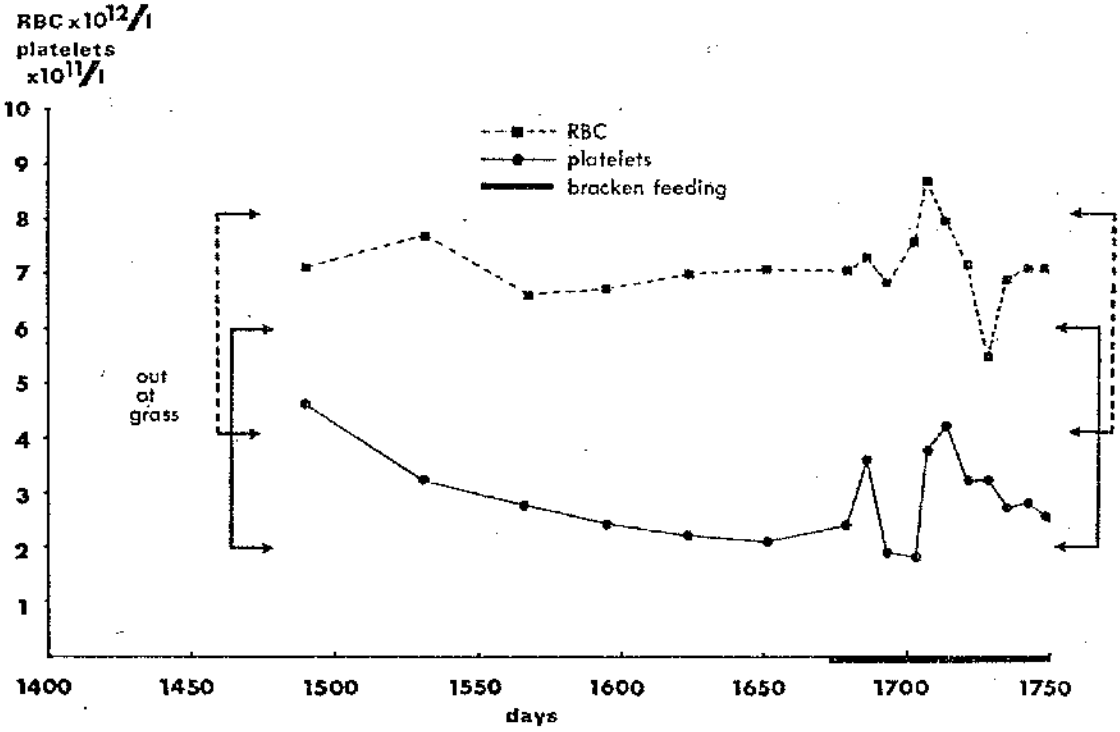

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
out
at
grass
10
9
8
7
6
5
4
3
2
1
1400
1450
1500
1550
1600
1650
1700
1750
days

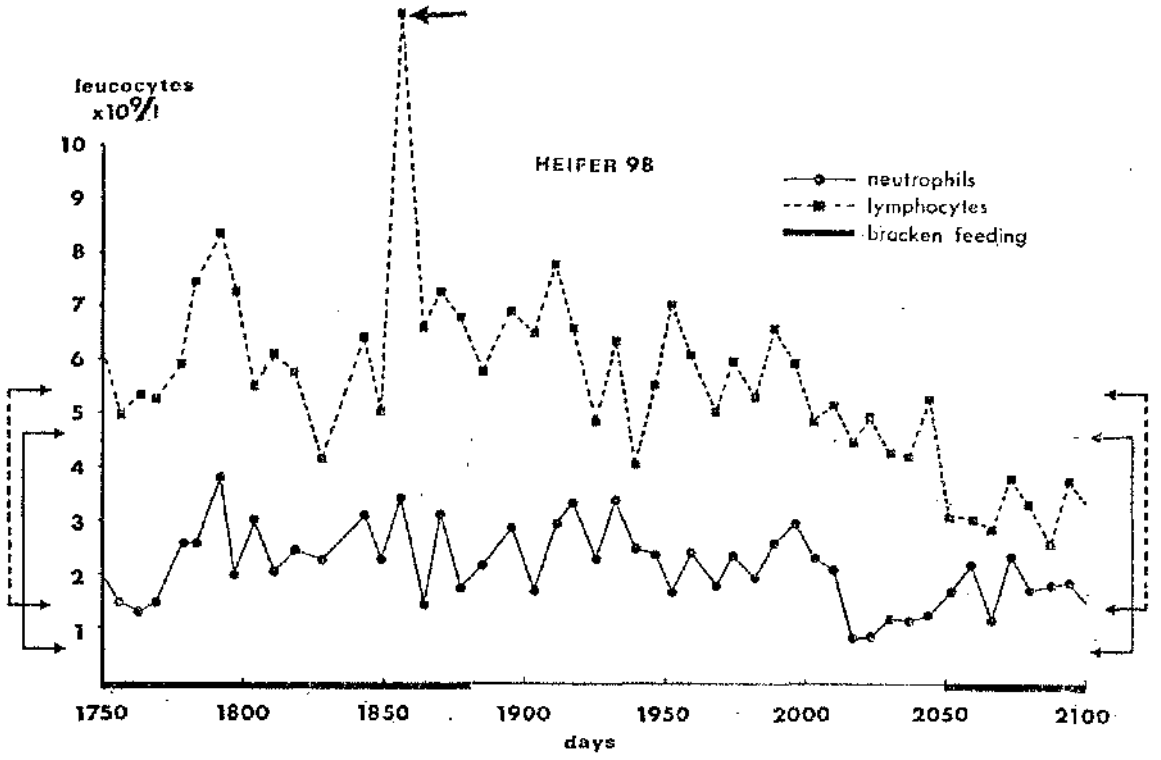
FIG. 85: Haematology results. Heifer 98,Graph Six.

Lymphocytes There is a decline from values above normal to within the normal range. The peak at day 1856 (←) could not be related to any obvious clinical or environmental change.

Neutrophils Values are within the normal range with the lowest counts occurring outwith the periods of bracken feeding.

Red Blood Cells Values are largely within the normal range although there is a slight decline towards the end of this period. Haematuria was recorded at this time.

Platelets Counts are almost all within the range $100\text{-}400 \times 10^9/l$ with no obvious change associated with bracken feeding or the declining red cell count.


leucocytes
x10⁹/l
HEIFER 98
neutrophils
lymphocytes
bracken feeding
10
9
8
7
6
5
4
3
2
1
1750
1800
1850
1900
1950
2000
2050
2100
days

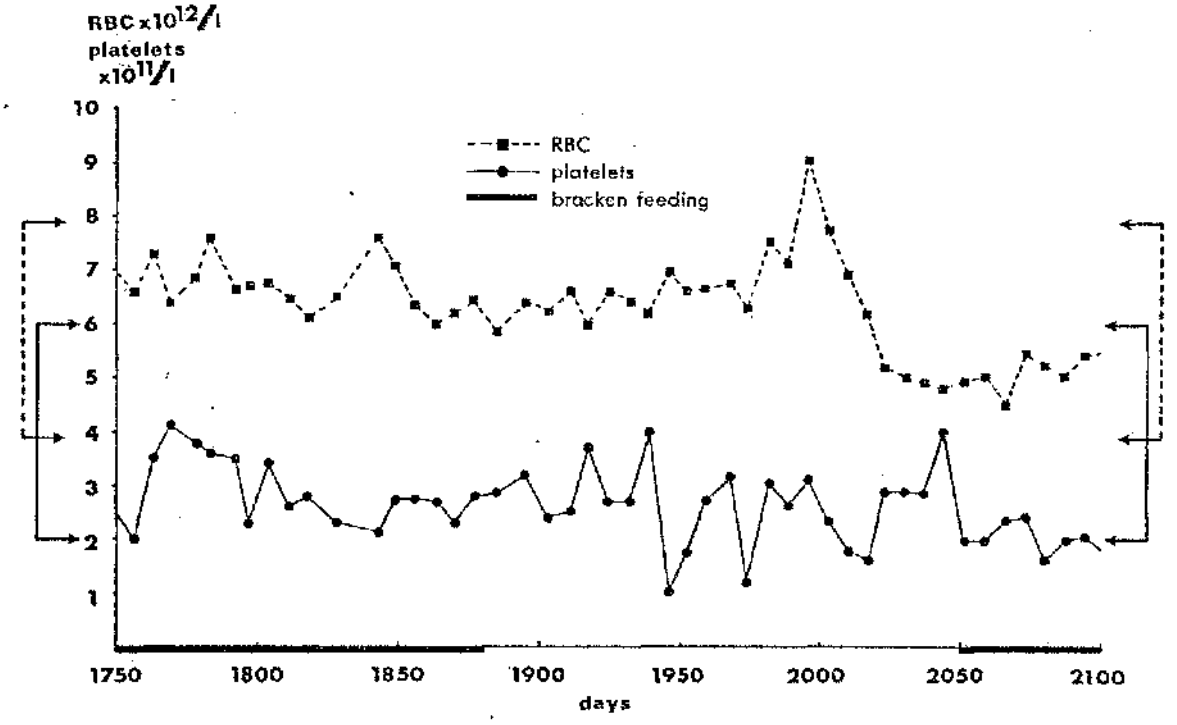

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
10
9
8
7
6
5
4
3
2
1
1750
1800
1850
1900
1950
2000
2050
2100
days

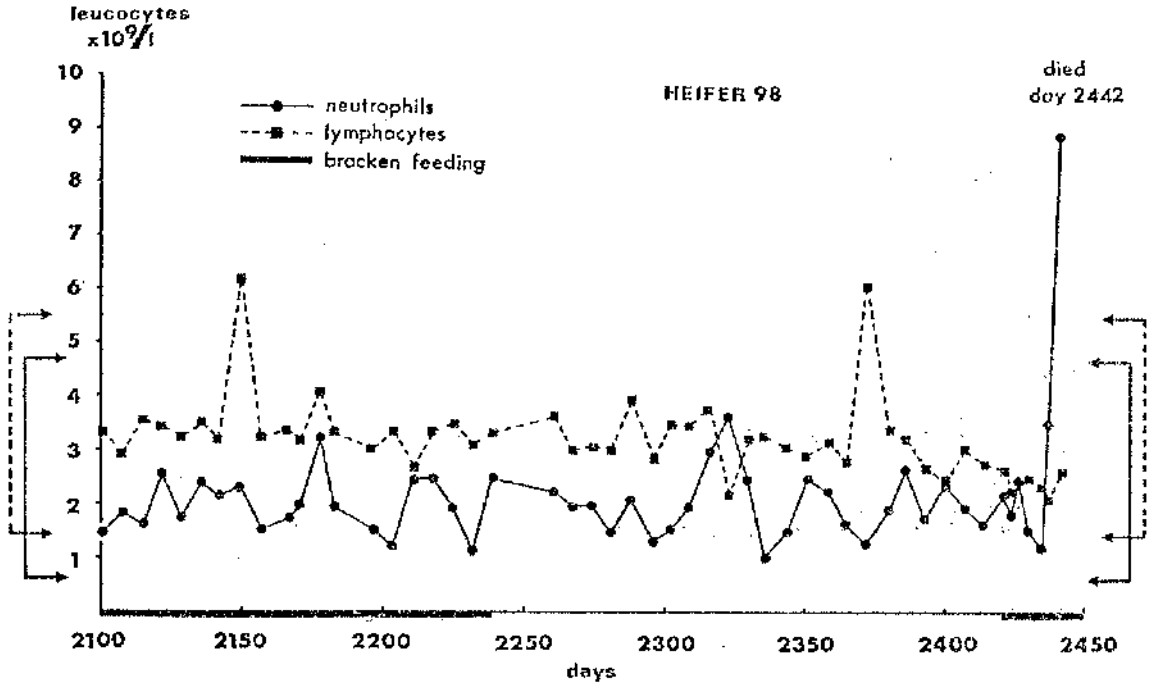
FIG. 86: Haematology results. Heifer 98,Graph Seven.

Lymphocytes Counts are largely within the normal range although isolated peaks occur at days 2150 and 2373.

Neutrophils Counts are within the accepted normal limits apart from a dramatic rise immediately prior to death.

Red Blood Cells Counts show a gradual decline with a precipitous fall immediately prior to death.

Platelets Counts remain around the lower normal values with isolated results of less than $150x10^9/l$.


leucocytes
x10⁹/l
HEIFER 98
died
day 2442
neutrophils
lymphocytes
bracken feeding
10
9
8
7
6
5
4
3
2
1
2100
2150
2200
2250
2300
2350
2400
2450
days

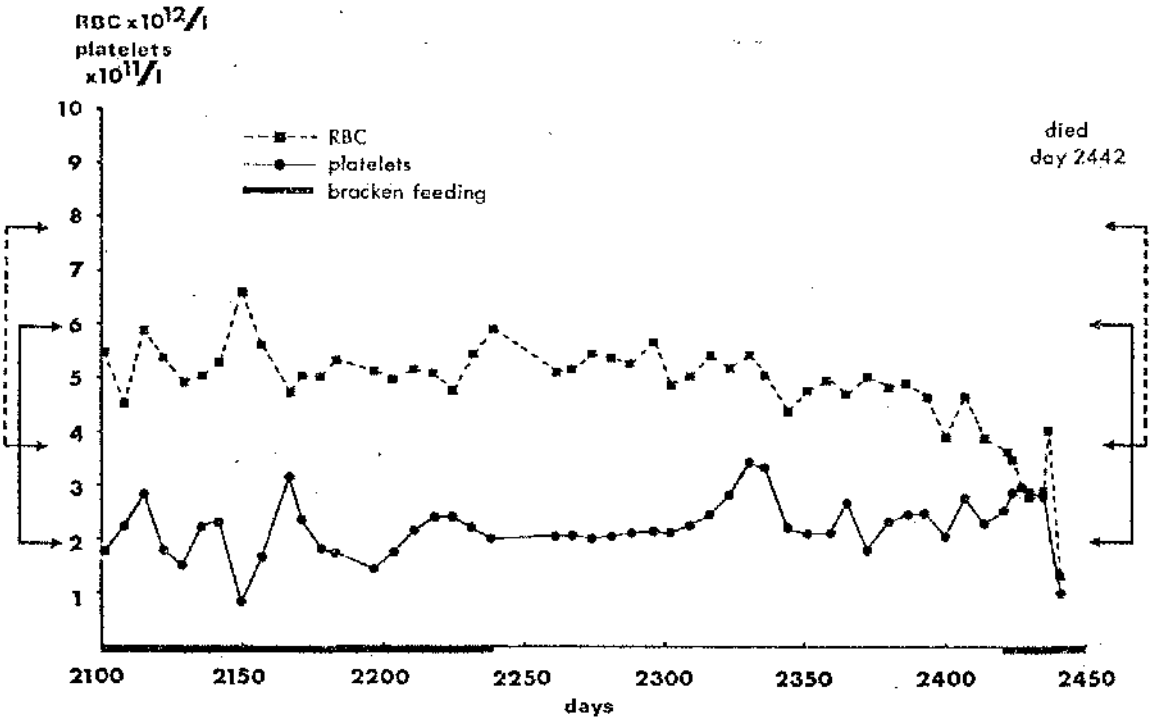

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
died
day 2442
10
9
8
7
6
5
4
3
2
1
2100
2150
2200
2250
2300
2350
2400
2450
days

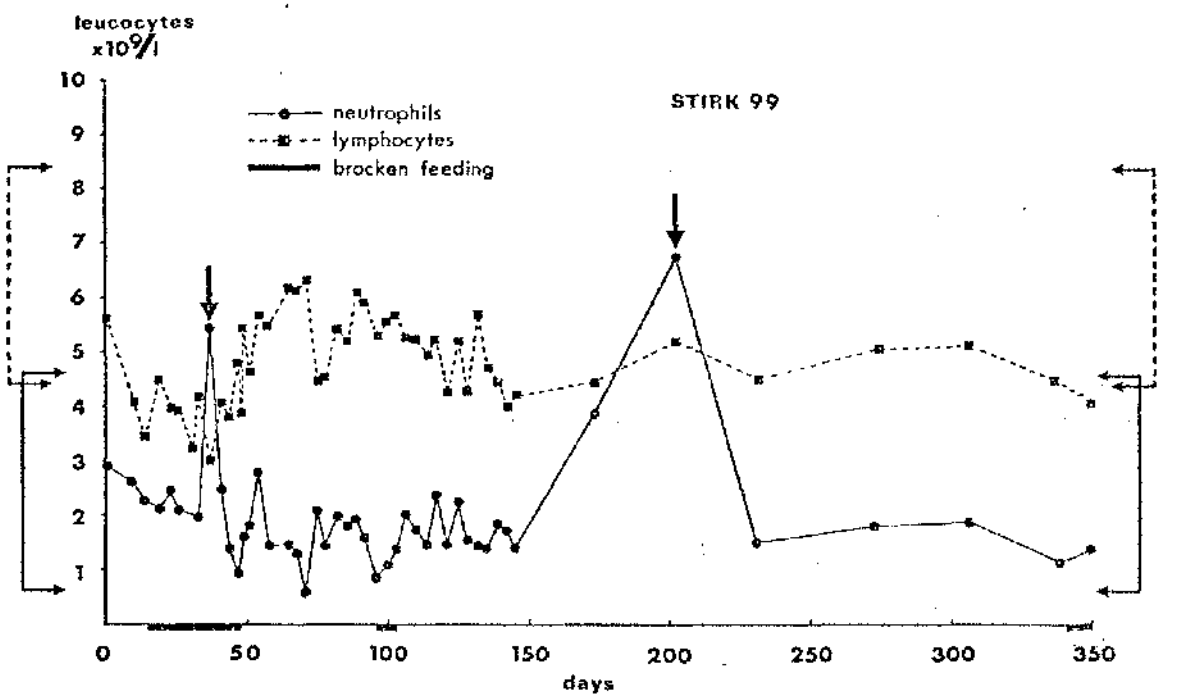
FIG. 87: Haematology results. Stirk 99,Graph One.

Lymphocytes The initial results are erratic with numerous values below the normal range (compare other individuals).

Neutrophils The raised result at day 37 (↓) follows the experimental surgery performed on day 34. The peak at day 202 (↓) follows castration on day 182 (but compare Heifer 98). The low result recorded on day 70 appears not to be related to bracken feeding.

Red Blood Cells Values are well within the normal range.

Platelets Results are erratic, particularly at first. There are numerous counts above the accepted normal range but few above $800x10^9/l$.


leucocytes
x10⁹/l
STIRK 99
neutrophils
lymphocytes
brocken feeding
10
9
8
7
6
5
4
3
2
1
0
50
100
150
200
250
300
350
days

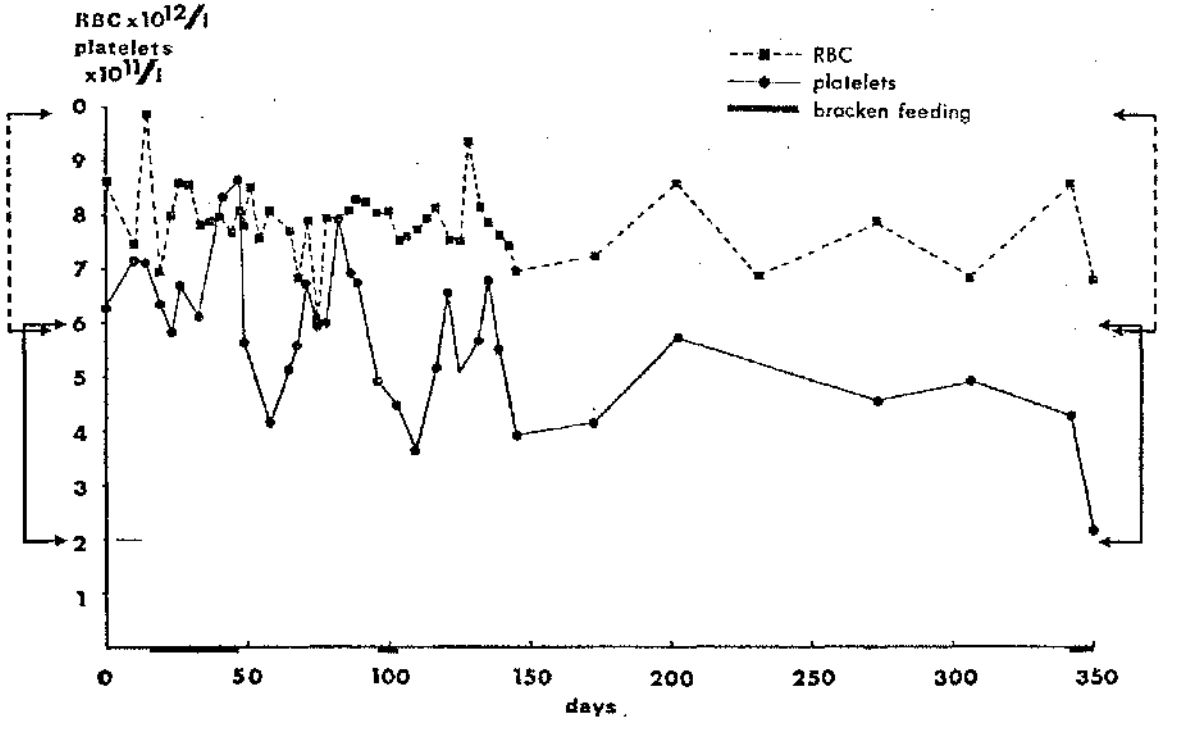

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
brocken feeding
0
9
8
7
6
5
4
3
2
1
0
50
100
150
200
250
300
350
days

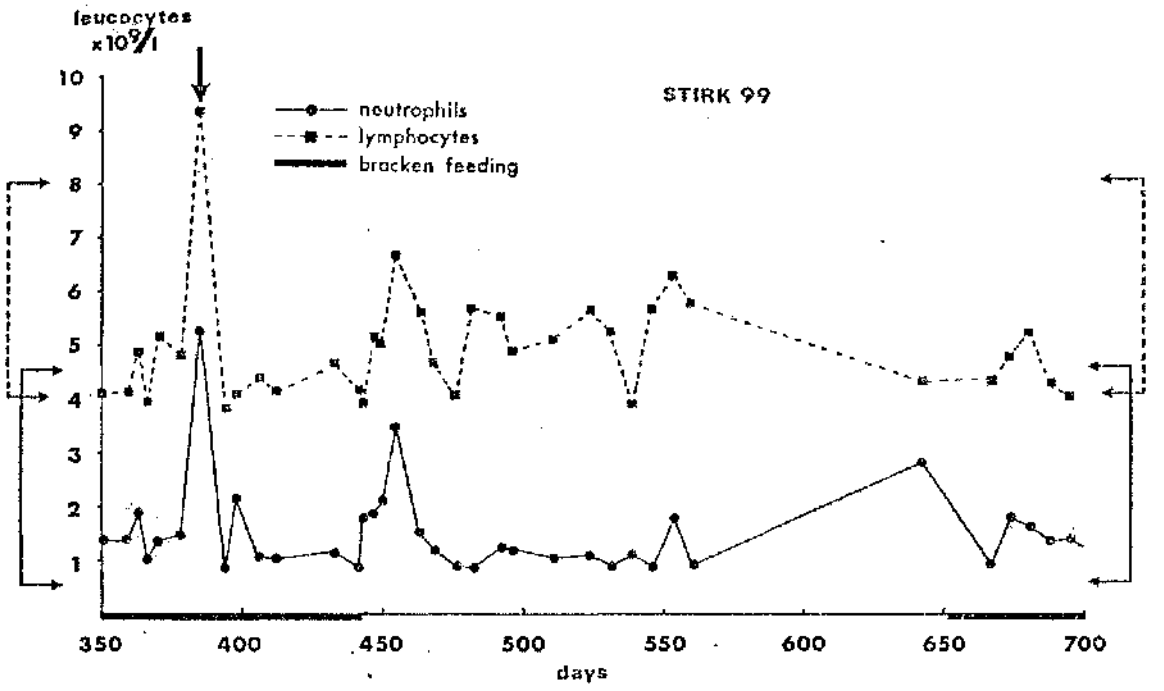
FIG. '88: Haematology results. Stirk 99,Graph Two.

Lymphocytes Most values are at the lower end of the normal range. The peak at day 385 (↓) follows dehorning on day 383.

Neutrophils Most results are at the lower end of the normal range both during and outwith the periods of bracken feeding. Neutrophilia coincides with the lymphocytosis on day 385 (↓).

Red Blood Cells Values are within the accepted normal range.

Platelets Results are mostly in the lower half of the normal range with several counts of less than $200 \times 10^9/l$ both during and outwith periods of bracken feeding.


leucocytes
x10⁹/l
STIRK 99
neutrophils
lymphocytes
bracken feeding
10
9
8
7
6
5
4
3
2
1
350
400
450
500
550
600
650
700
days

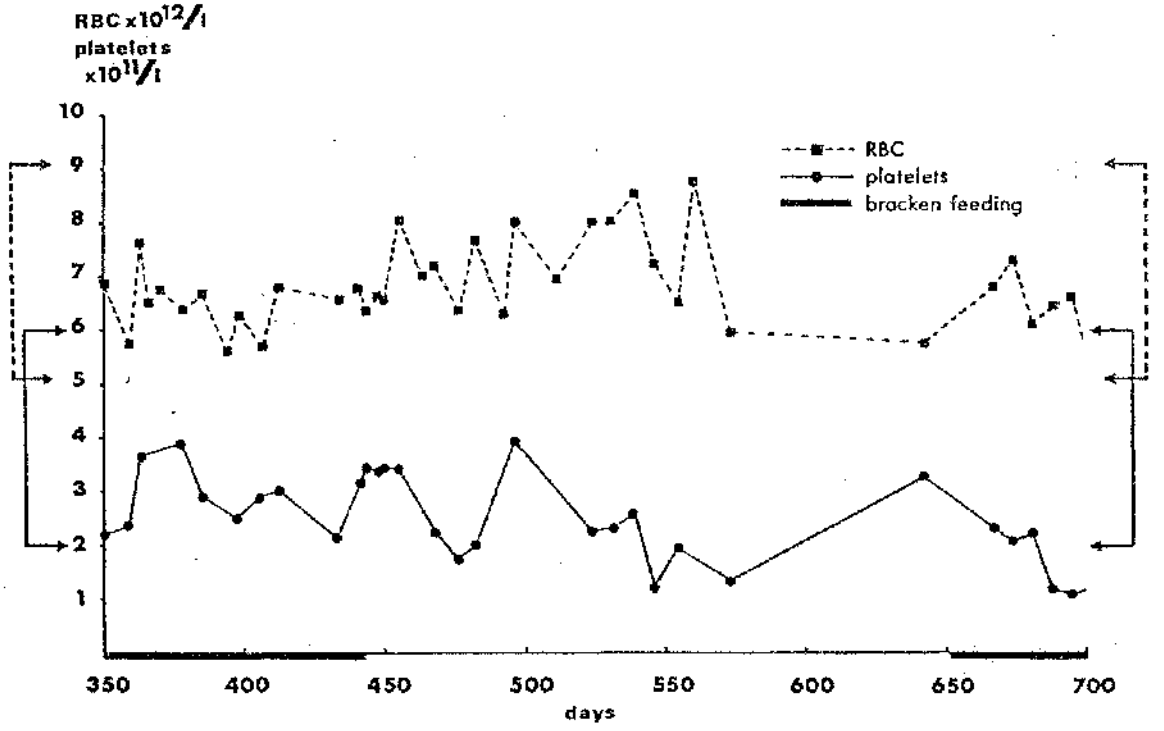

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
10
9
8
7
6
5
4
3
2
1
350
400
450
500
550
600
650
700
days

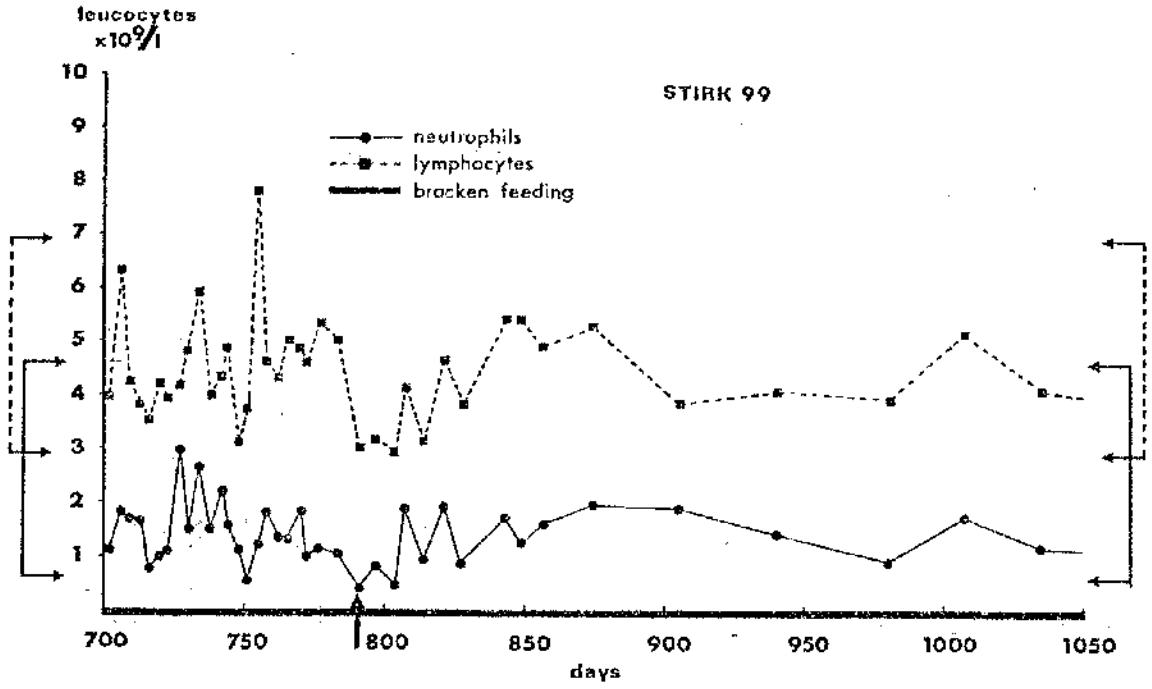
FIG. 89: Haematology results. Stirk 99, Graph Three.

Lymphocytes Values are generally within the normal range although many counts are in the lower half of the range. An exception is the peak at day 755 which was not related to any obvious clinical or environmental change.

Neutrophils Values are consistently at or below the lower end of the accepted normal range with occasional results as low as $0.43 \times 10^9/l$ (†) during this period of bracken feeding.

Red Blood Cells Values are steadily within the normal range.

Platelets Counts are frequently below the normal range with values falling approximately between 100 and $400 \times 10^9/l$.


leucocytes
x10⁹/l
STIRK 99
neutrophils
lymphocytes
bracken feeding
10
9
8
7
6
5
4
3
2
1
700
750
800
850
900
950
1000
1050
days

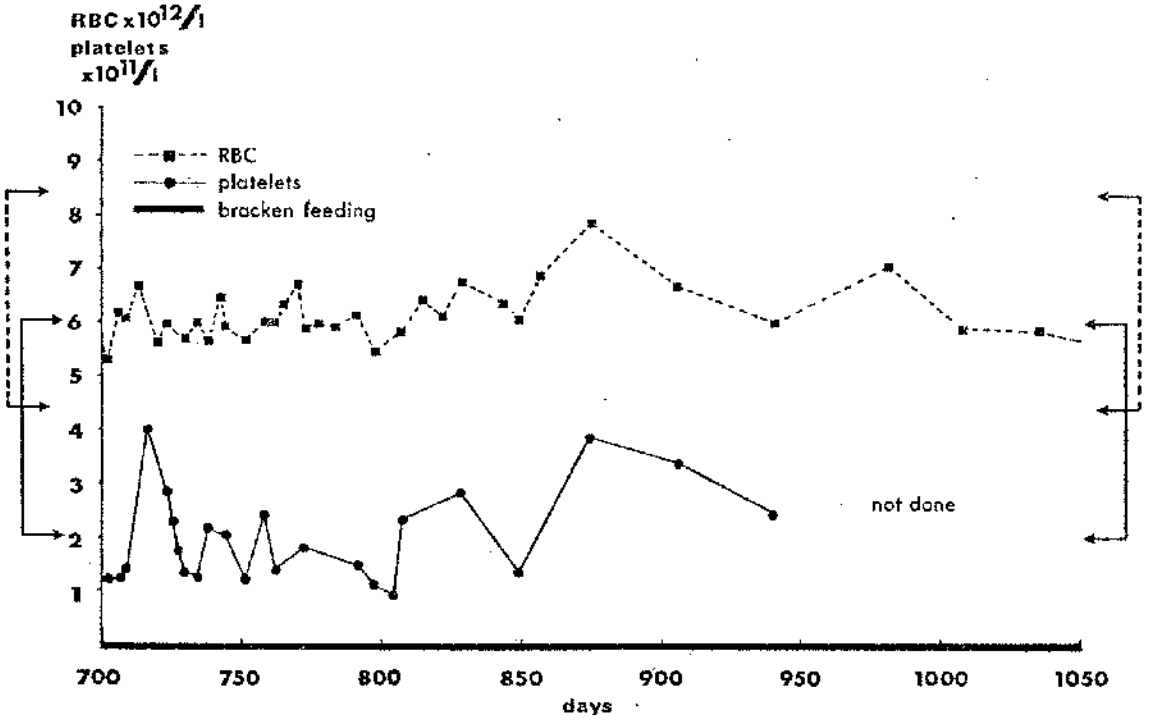

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
not done
10
9
8
7
6
5
4
3
2
1
700
750
800
850
900
950
1000
1050
days

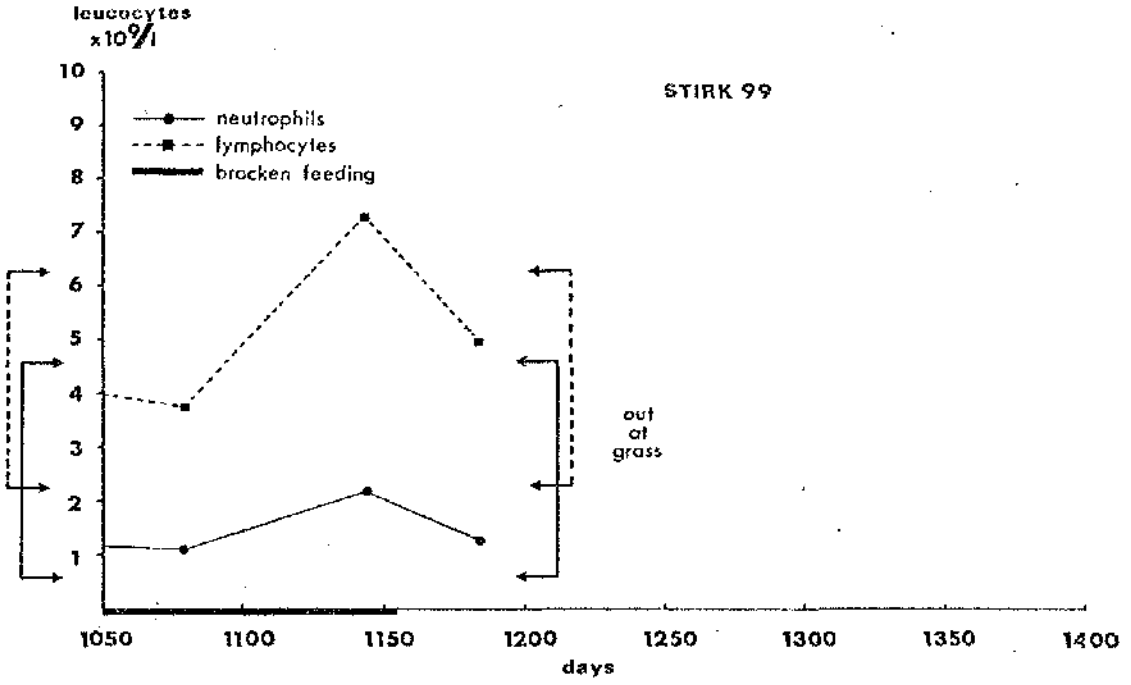
FIG. 90: Haematology results. Stirk 99,Graph Four.

Lymphocytes Results appear to be somewhat erratic with one count above the normal range.

Neutrophils Counts are at the lower end of the normal range.

Red Blood Cells Counts are within the normal range.

Platelets Not done.


leucocytes
x10⁹/l
STIRK 99
neutrophils
lymphocytes
bracken feeding
out
at
grass
10
9
8
7
6
5
4
3
2
1
1050
1100
1150
1200
1250
1300
1350
1400
days

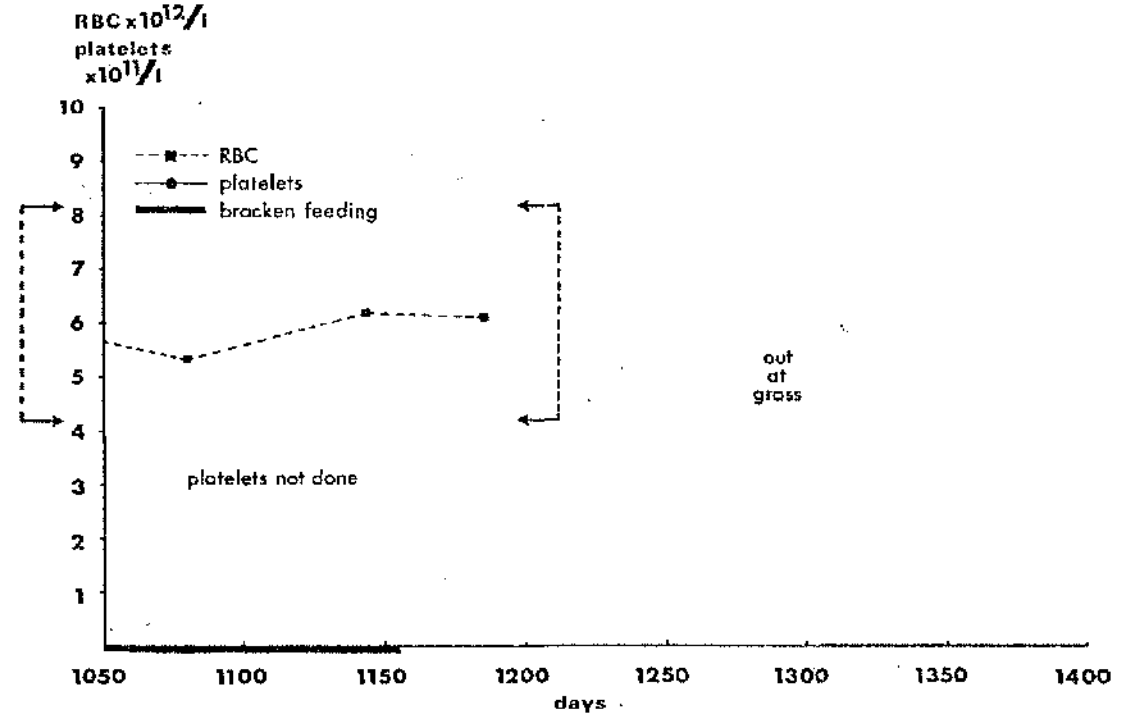

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
out
at
grass
platelets not done
10
9
8
7
6
5
4
3
2
1
1050
1100
1150
1200
1250
1300
1350
1400
days

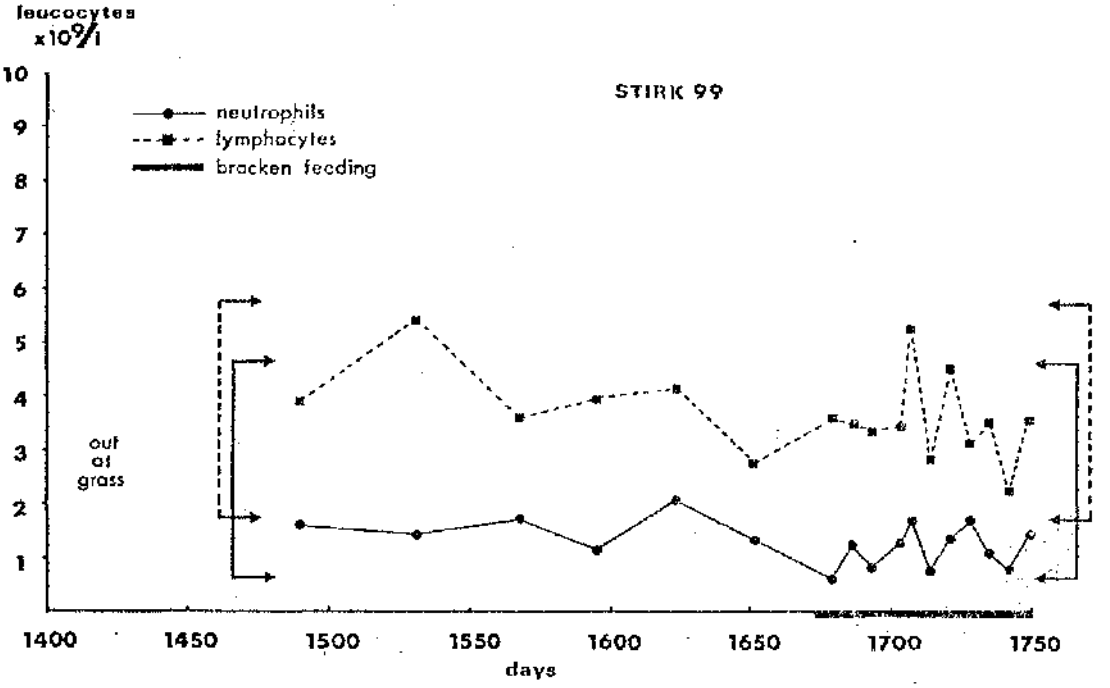
FIG. 91: Haematology results. Stirk 99, Graph Five.

Lymphocytes There is wide variation between individual results but all counts are within the normal range.

Neutrophils Counts are consistently at the lower end of the accepted normal range.

Red Blood Cells Values remain within the normal range.

Platelets Results are rather erratic but minimum values are never less than $150 \times 10^9/l$.


leucocytes
x10⁹/l
STIRK 99
neutrophils
lymphocytes
bracken feeding
out
at
grass
10
9
8
7
6
5
4
3
2
1
1400
1450
1500
1550
1600
1650
1700
1750
days

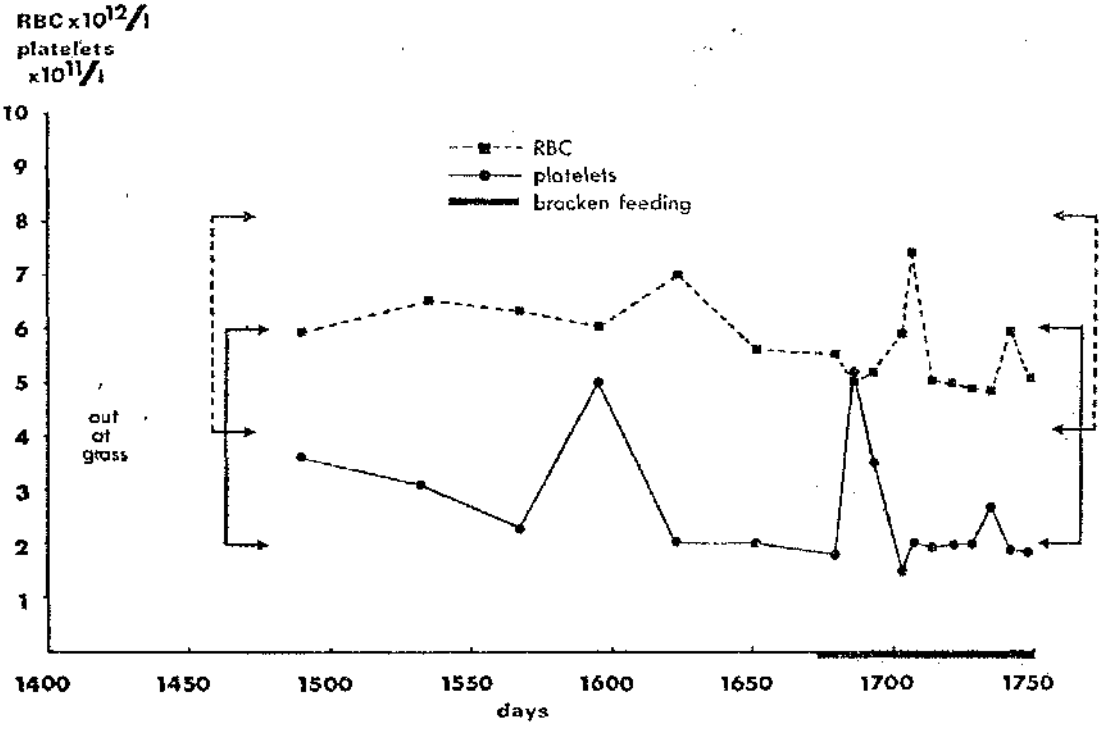

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
out
at
grass
10
9
8
7
6
5
4
3
2
1
1400
1450
1500
1550
1600
1650
1700
1750
days

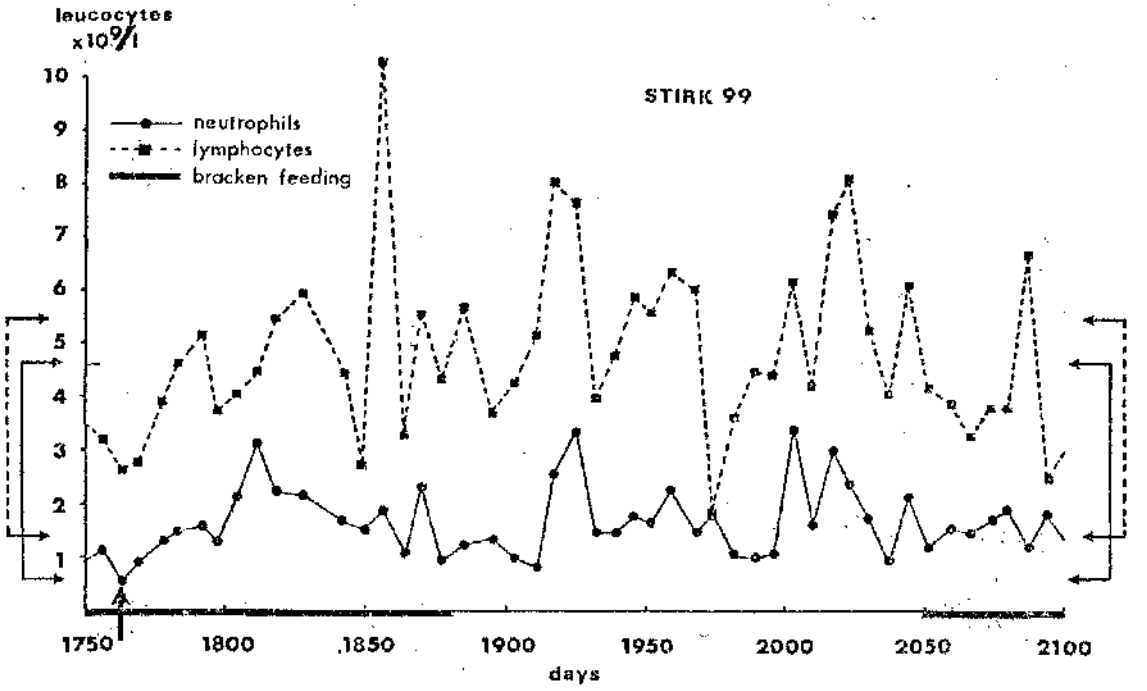
FIG. 92: Haematology results. Stirk 99,Graph Six.

Lymphocytes Results are extremely erratic with numerous counts above the accepted normal range.

Neutrophils Counts are generally within the lower half of the normal range. A minimum value of 0.54 was recorded on day 1763 (†) during the period of bracken feeding.

Red Blood Cells Values are mostly within the normal range with only a few counts slightly above this.

Platelets Values are consistently at or below the lower limit of the normal range (†) but only occasional counts are below $100x10^9/l$ and those occurred outwith the period of bracken feeding.


leucocytes
x10⁹/l
STIRK 99
neutrophils
lymphocytes
bracken feeding
10
9
8
7
6
5
4
3
2
1
1750
1800
1850
1900
1950
2000
2050
2100
days

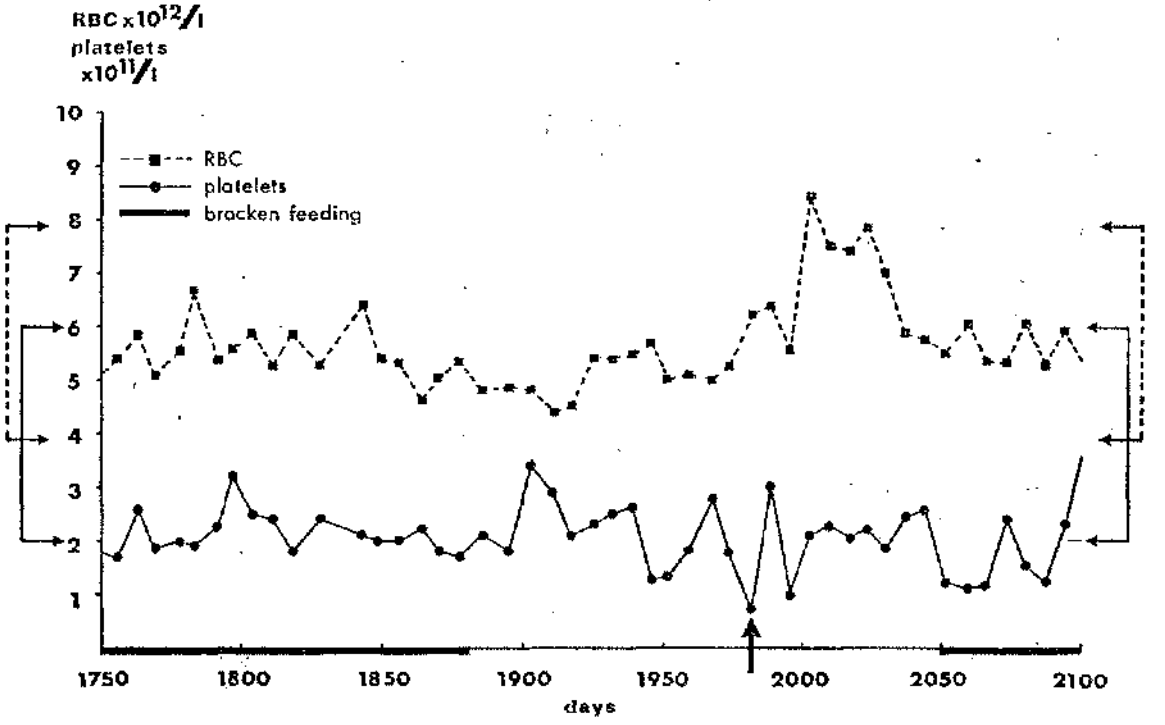

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
10
9
8
7
6
5
4
3
2
1
1750
1800
1850
1900
1950
2000
2050
2100
days

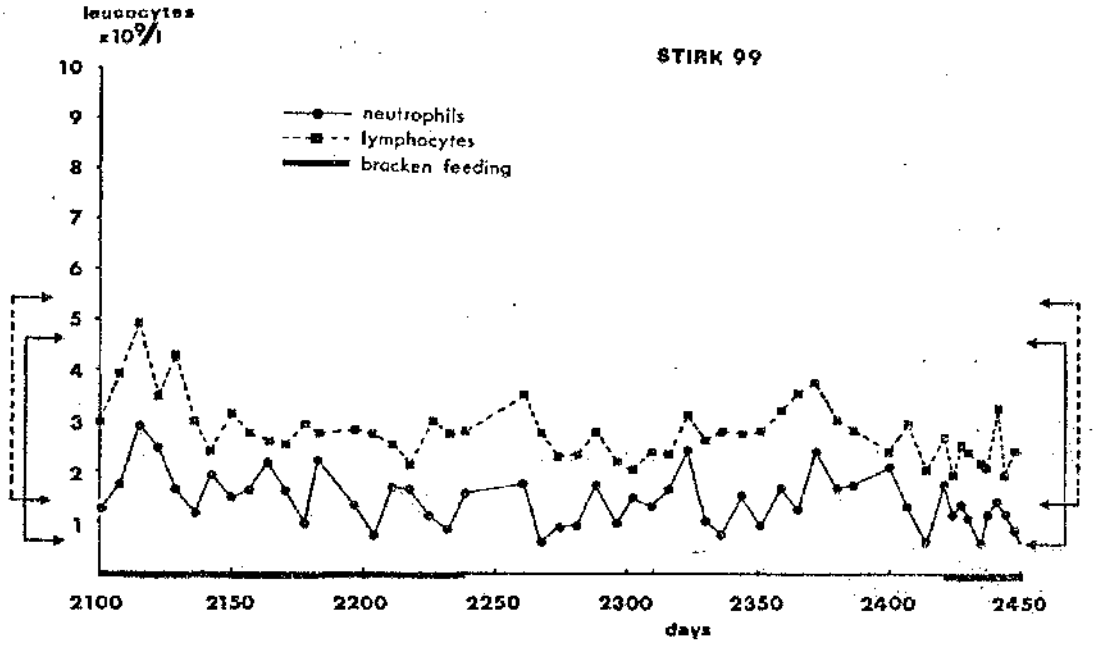
FIG. 93: Haematology results. Stirk 99,Graph Seven.

Lymphocytes Counts show an apparent decline during this period but remain within the normal range. Results are much less erratic than in the previous year.

Neutrophils Counts are consistently at the lower end of the normal range with no obvious alteration associated with bracken feeding.

Red Blood Cells Counts remain well within the accepted normal range.

Platelets Values are almost all between 100 and $400 \times 10^9/l$ i.e. many counts are below the accepted normal range.


leucocytes
x10⁹/l
STIRK 99
neutrophils
lymphocytes
bracken feeding
10
9
8
7
6
5
4
3
2
1
2100
2150
2200
2250
2300
2350
2400
2450
days

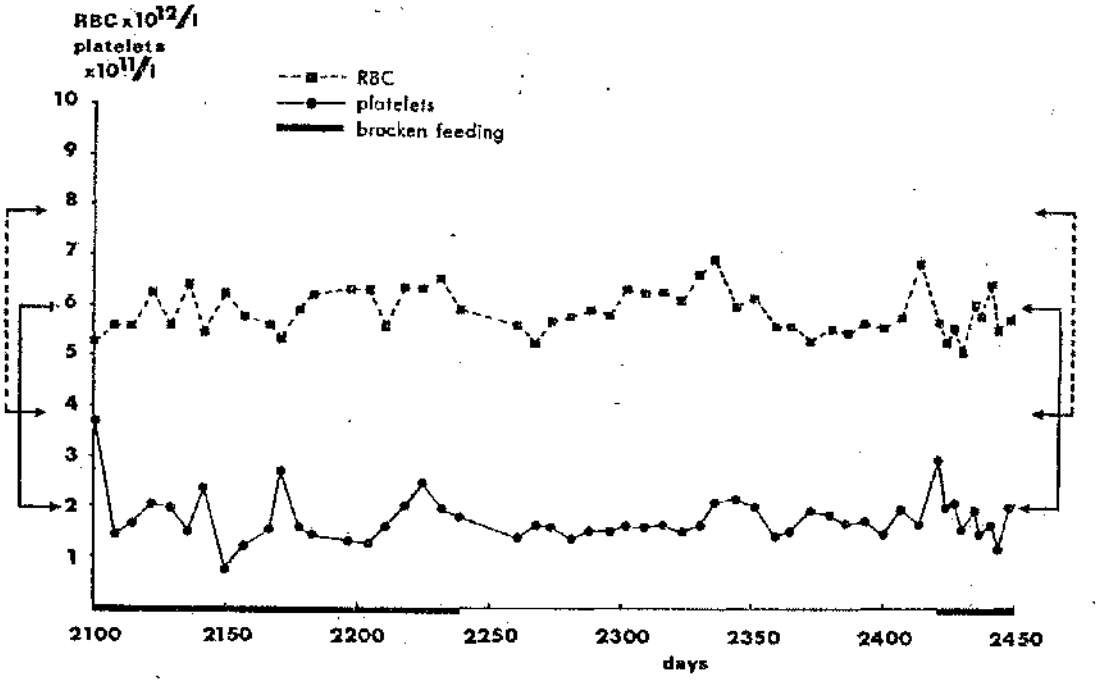

RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
10
9
8
7
6
5
4
3
2
1
2100
2150
2200
2250
2300
2350
2400
2450
days

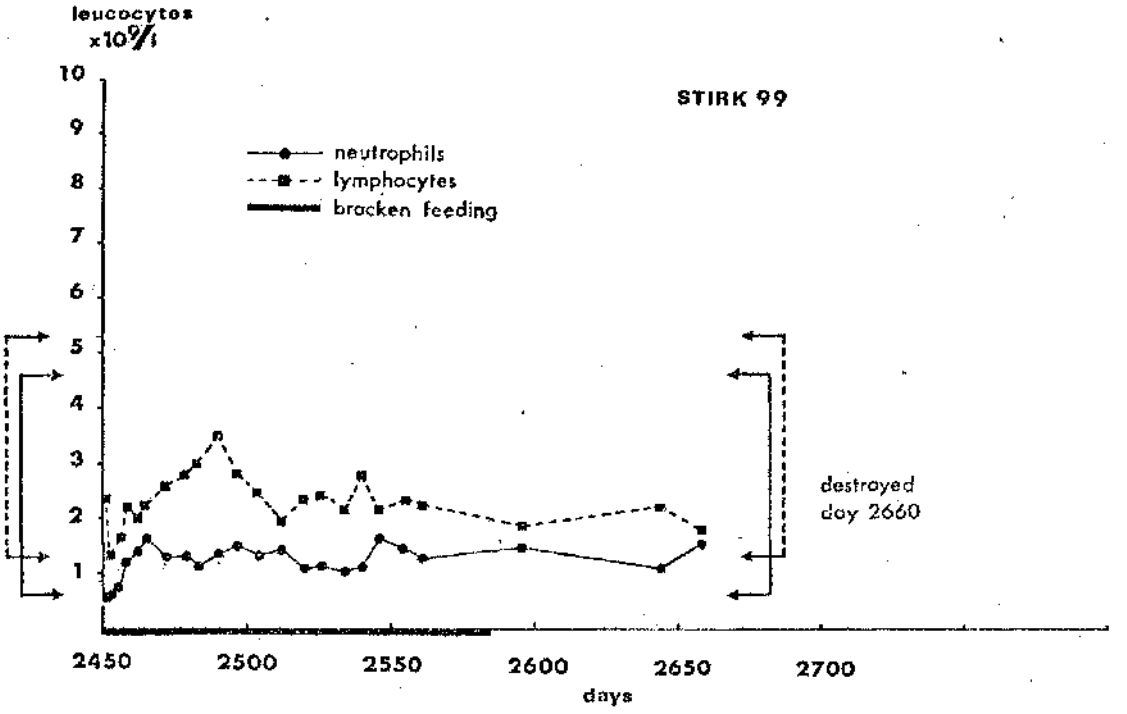
FIG. 94: Haematology results. Stirk 99,Graph Eight.

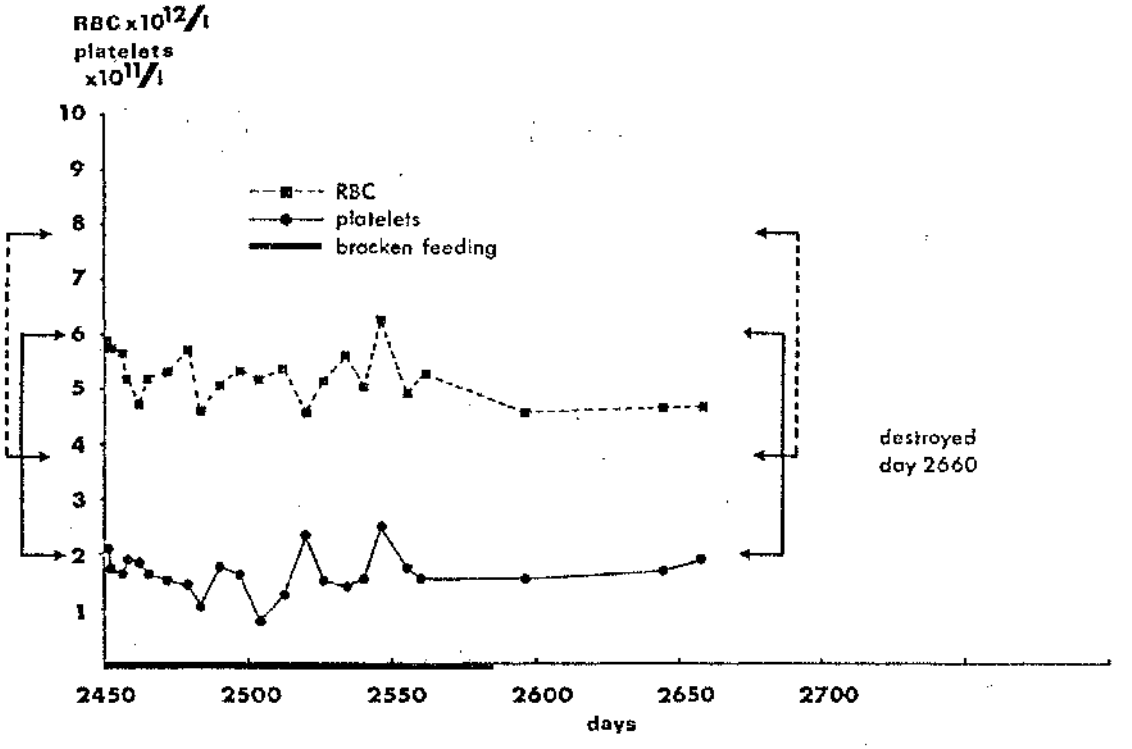
Lymphocytes Counts are at the lower end of the normal range with a minimum value of $1.62 \times 10^9/l$ on day 2456.

Neutrophils Counts are at the lower end of the normal range with a minimum value of $0.54 \times 10^9/l$ on day 2452.

Red Blood Cells Counts are within the lower half of the normal range.

Platelets The majority of counts are below the accepted minimum of $200 \times 10^9/l$ but above $100 \times 10^9/l$.


leucocytes
x10⁹/l
STIRK 99
neutrophils
lymphocytes
bracken feeding
destroyed
day 2660
days


RBC x10¹²/l
platelets
x10¹¹/l
RBC
platelets
bracken feeding
destroyed
day 2660
days

Necropsy Report

Heifer 27 This animal was destroyed on day 2695, 7½ years after the start of the experiment. There was a history of intermittent haematuria for more than 2 years before death.

At necropsy the main findings were confined to the urinary bladder. A tumour mass of 10x7cms was present in the left lateral wall of the bladder. This was a raised lobulated mass infiltrating the bladder wall so that it was 2.5cms thick. The lesion appeared to be a carcinoma but also contained haemangiomatous foci. Throughout the bladder the mucosa was thickened and thrown into 0.5 to 1.0cm folds with both subepithelial oedematous tissue and thickened epithelium. Protruding from this, 24 distinct carcinomatous plaques were identified, each approximately 2x1cm in size with injected or haemorrhagic surfaces.

Additional findings in this case were two papillomas on the side of the tongue and one papilloma in the oesophagus.

Microscopical examination of various sections of urinary bladder confirmed the presence of transitional cell carcinoma together with a number of other changes. All sections showed degrees of epithelial hyperplasia and dysplasia which in some areas warranted the description of carcinoma in situ. There was whorling of the hyperplastic epithelium with formation of Brunn's nests and epithelial cysts. Some foci showed variable loss of polarity, hyperchromasia and nuclear pleomorphism with occasional uninucleate giant cells. Well-developed areas of infiltrative carcinoma were classified as Grade II in view of the degrees of cellular and nuclear pleomorphism present. On some sections there was epithelial metaplasia with intercellular bridges evident and in a few areas there was thickening or condensation of the basement membrane below the dysplastic epithelium.

Subepithelial alterations included a thickened, vascular lamina propria with dilated lymphatics and some subepithelial haemorrhage. There were foci of capillary ectasia and endothelial proliferation which occasionally formed distinct haemangiomas of large capillary or small cavernous type.

There was condensation of the connective tissue around some of the larger vessels and occasional whorls of loose connective tissue were seen such as are found in fibromas. Polymorphs, lymphocytes and plasma cells were scattered through all sections with occasional collections of lymphoid cells present so that there appeared to be a chronic proliferative cystitis interspersed with areas of carcinoma and haemangioma.

Summary

Transitional cell carcinoma of urinary bladder
Haemangiomas of urinary bladder
Chronic cystitis
Papillomas of tongue and oesophagus

Necropsy Report

Stirk 28 This animal was destroyed during the fourth year of the experiment. He had been brought in from grass for supplementary feeding but unfortunately slipped and fell while tethered in the byre. Thereafter he grew gradually weaker and was unable to stand. He was destroyed one week later on day 1471.

Post-mortem examination revealed fibrinous pleurisy over the right lung and focal pulmonary collapse which was more severe on the left. There was a slight excess of pericardial fluid which contained a fibrin clot. The hindquarters showed superifical traumatic damage and wasting of the large muscles.

Examination of the gastrointestinal tract revealed severe ulceration of abomasum and omasum. Numerous small lesions were scattered throughout the mucosae including the edges of the folds. One large ulcer of 2.0cm diameter was present in the pyloric region. Microscopy confirmed the presence of non-specific ulceration with surface colonisation by bacteria.

In addition, nineteen papillomas were present in the oesophagus and two small fibromas were found in the rumen. The oesophageal papillomas were of filiform type and most were located in the cranial third of the oesophagus. Ten were between 4 and 10mm in diameter but nine were only 1 or 2mm in size. Microscopy confirmed the presence of papillomas comprising fronds of hyperkeratotic epithelium. Occasional intranuclear inclusion bodes were seen (Fig. 95). These were homogeneous, but rather indistinct, basophilic structures which nevertheless suggested that the papillomas were of viral origin.

The ruminal lesions were represented by a smooth lesion of 6mm diameter, located on the upper surface of the anterior pillar. This structure was slightly raised above the surface, had a broad base and an almost square outline and was not pigmented. The second lesion was a raised, sessile nodule of 2mm diameter located

on the right wall of the dorsal sac, slightly above the larger growth. It had a smooth surface and again was not pigmented. Microscopy revealed that these were both well-defined subepithelial nodules composed of bands and whorls of fibroblasts with fine collagen fibrils. The cells showed some nuclear pleomorphism with occasional large, hyperchromatic nuclei but the mitotic rate was low. The overlying epithelium showed fewer rete pegs than normal. Occasional plasma cells and polymorphs were evident around and within the lesions.

There were no other notable findings in this case.

Summary

Oesophageal papillomas
Ruminal fibromas
Abomasal and omasal ulceration
Mild pleurisy
Focal pulmonary collapse

Necropsy Report

Heifer 98 This animal died on day 2442 over 7 years after the start of the experiment. Haematuria had by then been present for more than 14 months and latterly had become both continuous and severe. On the day before death haematology revealed a low red cell count and raised neutrophil count consistent with haemorrhagic shock.

At necropsy the carcase was pale with blanched mucosae. The ventral wall of the urinary bladder had ruptured and clots of blood were present in the posterior abdomen and pelvic cavity. The bladder wall was less than 1.0cm thick and the normal mucosal corrugation was absent suggesting that the viscus had been distended for some time. It had a maximum diameter of approximately 15.0cm and contained almost 600ml of blood and urine. The entire mucosa was mottled with subepithelial haemorrhages but discrete lesions were confined to the posterior third of the bladder. One distinct haemangioma of 1.0cm diameter was present and there were also a few irregular, raised, haemorrhagic areas. In the region of the trigone, the bladder wall was firm and thickened to a depth of 1.5cm with apparent cicatrix formation and both ureters were thickened at their insertion into the bladder wall.

The right kidney contained focal, wedge-shaped areas of haemorrhage and necrosis but the left kidney was pale and slightly enlarged. Section of the left kidney revealed hydronephrosis with only a thin rim of compressed renal tissue surviving in some lobules. The left ureter was thickened and dilated throughout its length.

Other findings in this case included hepatic telangiectasis and nodular fat necrosis of posterior mesenteric and perirectal fat. Autolytic changes were pronounced throughout the intestinal tract.

Microscopical examination of sections of urinary bladder revealed the presence of a malignant tumour extending from subepithelial tissue to the serosal surface and infiltrating around the ureters. The malignant cells were seen individually, in small circlets, or separating and coating the muscle bundles of the bladder wall. The cells were characteristically spindle shaped, triangular or polygonal with elongated or polygonal nuclei but there was marked pleomorphism. The nuclei tended to have a stippled chromatin pattern and multiple small nucleoli. There was a moderately high mitotic rate with abnormal mitotic figures and some multinucleate tumour giant cells. The tumour was considered to be an haemangiosarcoma and in some areas, poorly-differentiated tumour merged with distinct haemangioma or collections of blood filled spaces lined by malignant cells. The surface epithelium showed some hyperplasia and dysplasia with formation of Brunn's nests but there was no clear evidence of epithelial neoplasia.

The serosal surface was haemorrhagic with granulation tissue adjacent to the site of rupture. Focal accumulations of mononuclear cells were present on all sections and in some areas there was mucosal oedema and thrombosis of submucosal vessels.

Microscopical examination of sections from other organs confirmed the gross findings. No secondary deposits of tumour were identified.

Summary

Haemangiosarcoma of urinary bladder
Haemangiomas of urinary bladder
Cystitis
Renal infarcts
Hydronephrosis
Hepatic telangiectasis

Necropsy Report

Stirk 99 This animal was killed on day 2660, 7½ years after the start of the experiment. Haematuria had been present for 14 months but had only been macroscopically evident for 4 months.

At necropsy, major changes were confined to the urinary bladder. The whole mucosa was thickened and nodular with what appeared to be a mixture of epithelial and haemangiomatous foci.

Microscopy revealed hyperplasia and dysplasia of transitional cells with epithelial whorls, large Brunn's nests and occasional small cysts. There were also occasional papillary projections comprising columns of 8 or more cells. One section showed a papillary lesion, which, in view of the increased cellularity, nuclear crowding, loss of polarity and occasional hyperchromasia, was considered to be a Grade I, non-infiltrating carcinoma.

The underlying connective tissue was thickened and vascular with condensation of connective tissue fibres around a few of the larger vessels. There was capillary proliferation and ectasia with some associated subepithelial haemorrhage and there also appeared to be some lymphatic ectasia. However, no distinct haemangioma was identified on the sections examined.

Evidence of cystitis was provided by the scattered mononuclear cells and occasional focus of lymphoid cells.

Summary

Transitional cell carcinoma of urinary bladder
Cystitis with capillary proliferation and ectasia

CHAPTER II: BRACKEN FERN, CARCINOGEN AND TOXIN.

FIGURES 95-107: Lesions found at necropsy in bracken-fed cattle.

FIGURES 95-97: UAT tumours in bracken-fed cattle.

FIG. 95: Oesophageal papilloma.

a) Low power view (H&E x 20). Elongated fronds of stratified squamous epithelium supported by delicate connective tissue cores form filiform projections from the oesophageal epithelium.

b) High power view (H&E x 1600). Intranuclear inclusions (arrows) are evident in the deeper layers of cornified cells.

FIG. 96: Ruminal fibroma (H&E x 35). Hyperkeratotic epithelium with loss of rete pegs overlies a circumscribed fibrous nodule (arrows) which has compressed the subepithelial connective tissue.

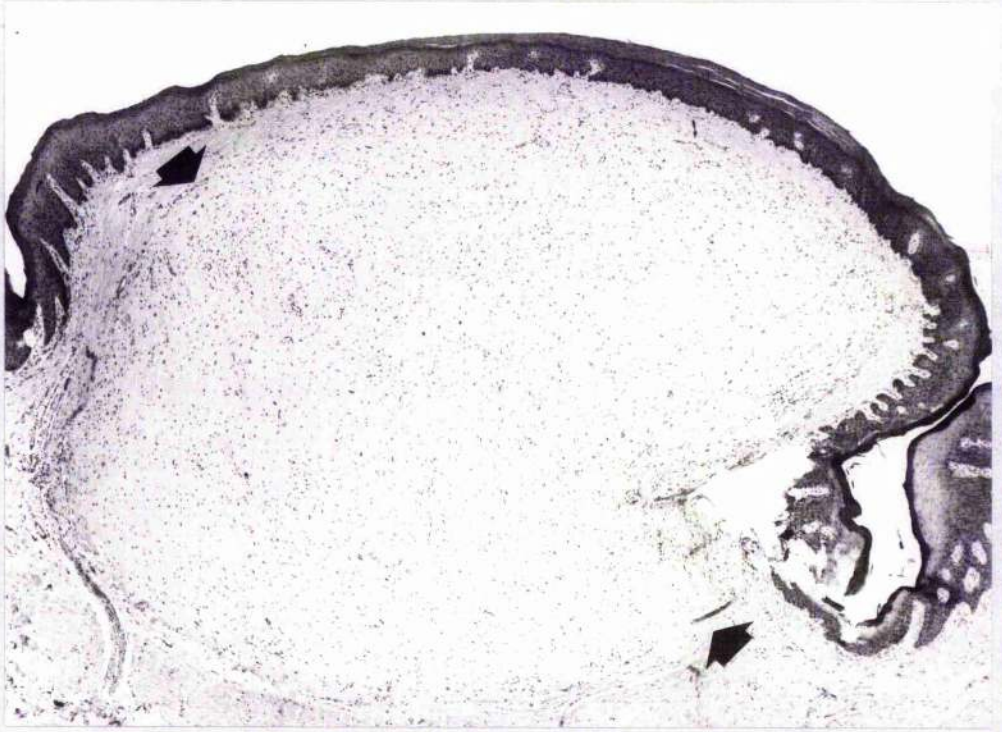
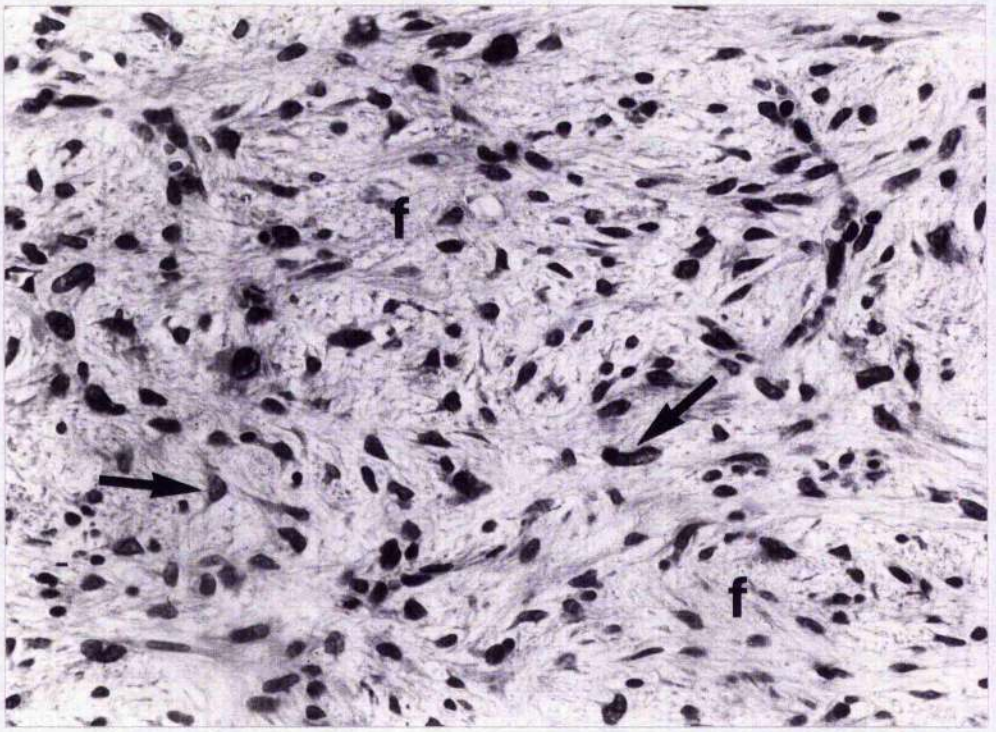
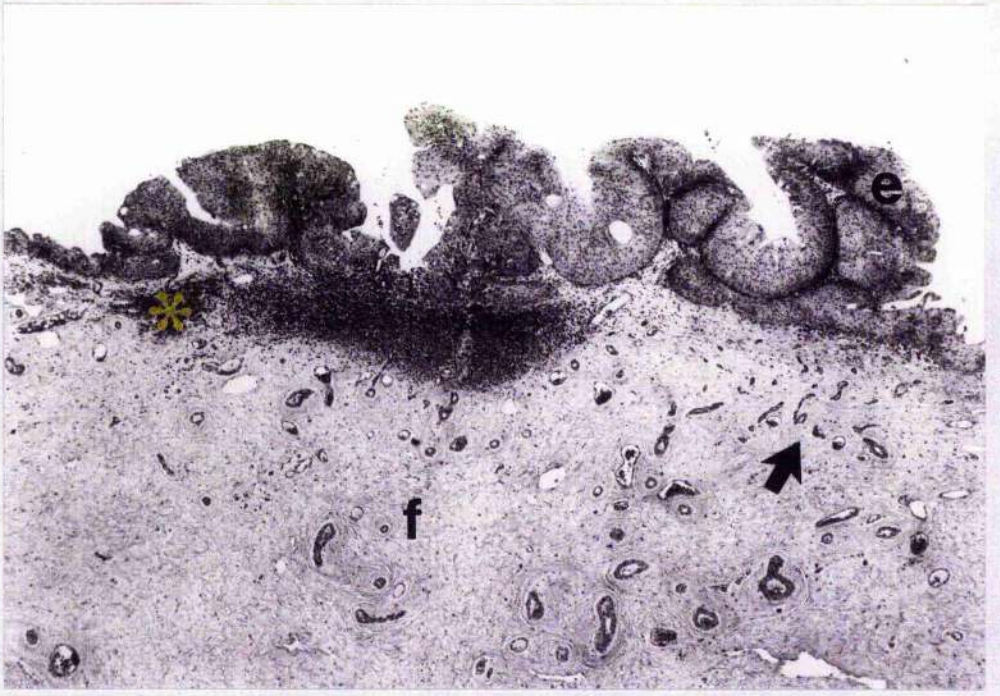


FIG. 97: Ruminal fibroma (H&E x 400). The tumour comprises haphazardly arranged groups of fine collagen fibres (f) and fibroblasts (arrows) showing some nuclear pleomorphism.


f
f

FIGURES 98-107: Urinary bladder lesions in bracken-fed cattle.

FIG. 98: Epithelial hyperplasia and dysplasia (H&E x 35). Adjacent areas of the bladder wall show a variety of changes including papillary and nodular hyperplasia of transitional epithelium (e) which also shows loss of polarity within nodules. Stromal changes include fibroplasia (f), capillary and lymphatic ectasia (thick arrow) and capillary proliferation (thin arrow). There are focal accumulations of lymphoid cells which appear as dark patches interspersed with areas of subepithelial haemorrhage (asterisks).


e
f

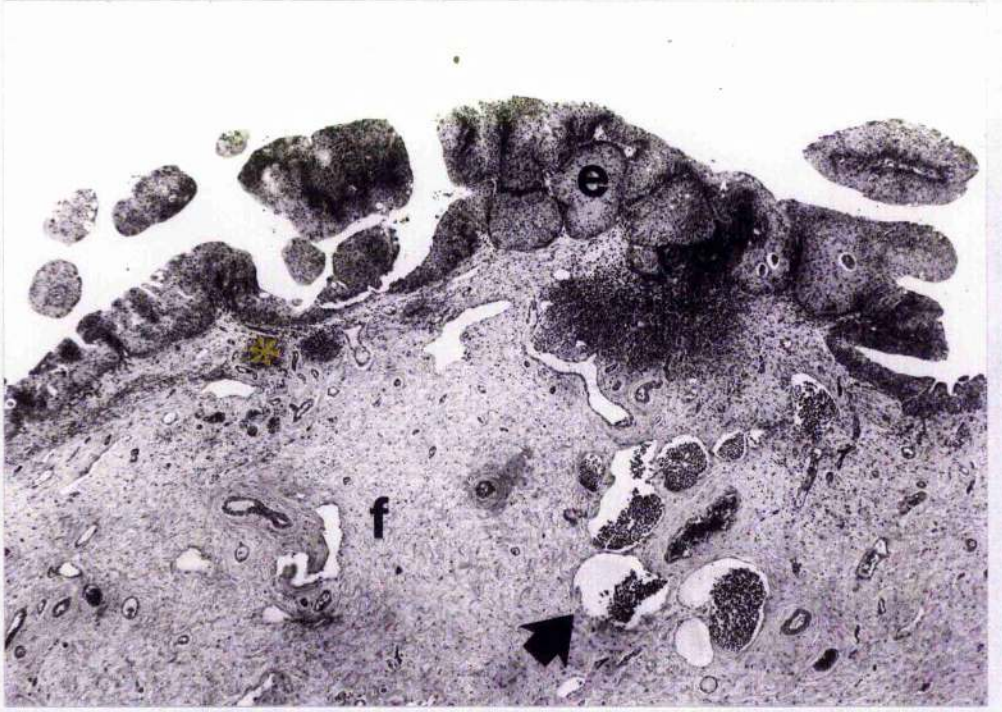

e
f

FIG. 99: Nodular hyperplasia of transitional cells (H&E x 250). Surface epithelium (e) and subepithelial nodules (n) of transitional cells show nuclear crowding and slight loss of polarity.

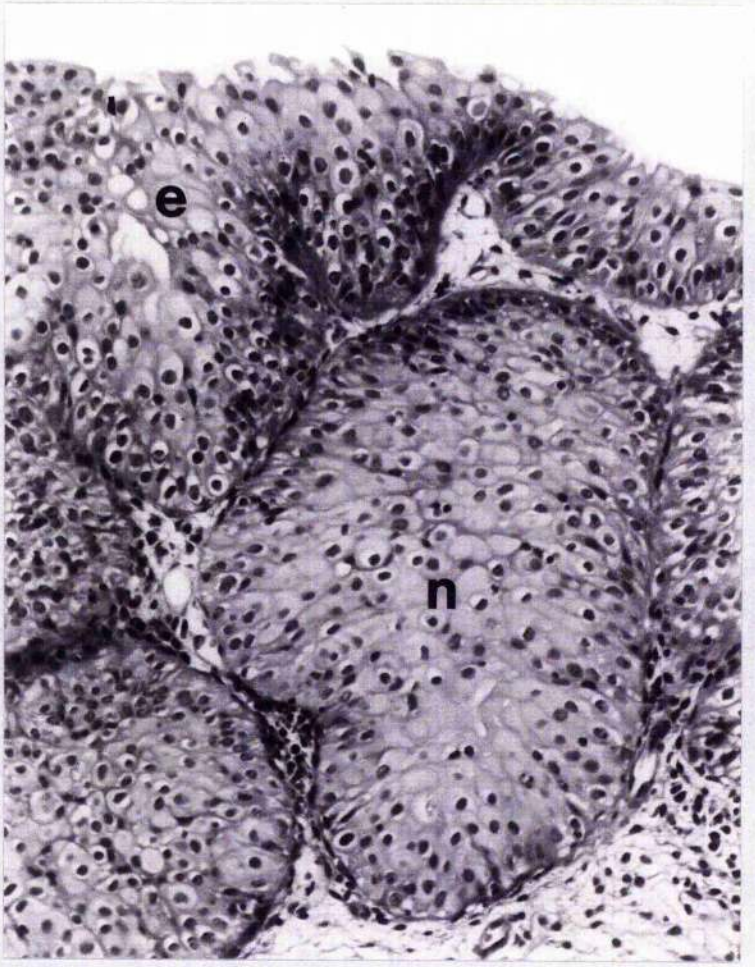

e
n

FIG. 100: Nodular hyperplasia with squamous metaplasia (H&E x 400). Nodular downgrowths of epithelium (e) overlie the margins of an infiltrating carcinoma (Ca). Prominent intercellular bridges can be seen (arrows).

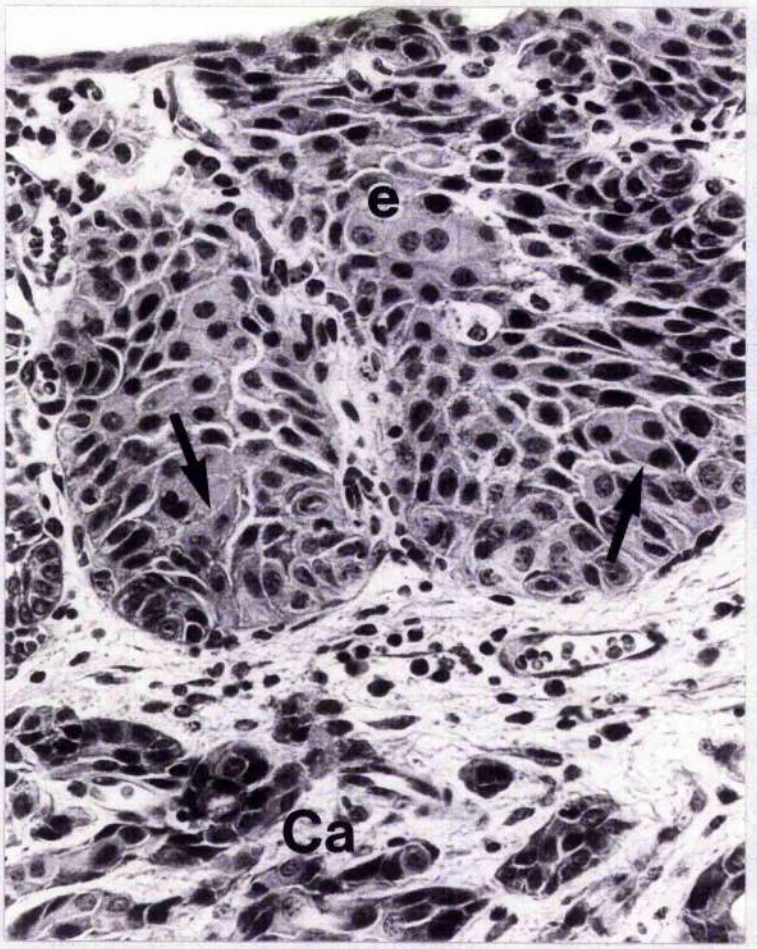

e
Ca

FIG. 101: Carcinoma of urinary bladder (H&E x 35). An ulcerated focus of infiltrating carcinoma (Ca) lies adjacent to an area of nodular hyperplasia and dysplasia (arrows).

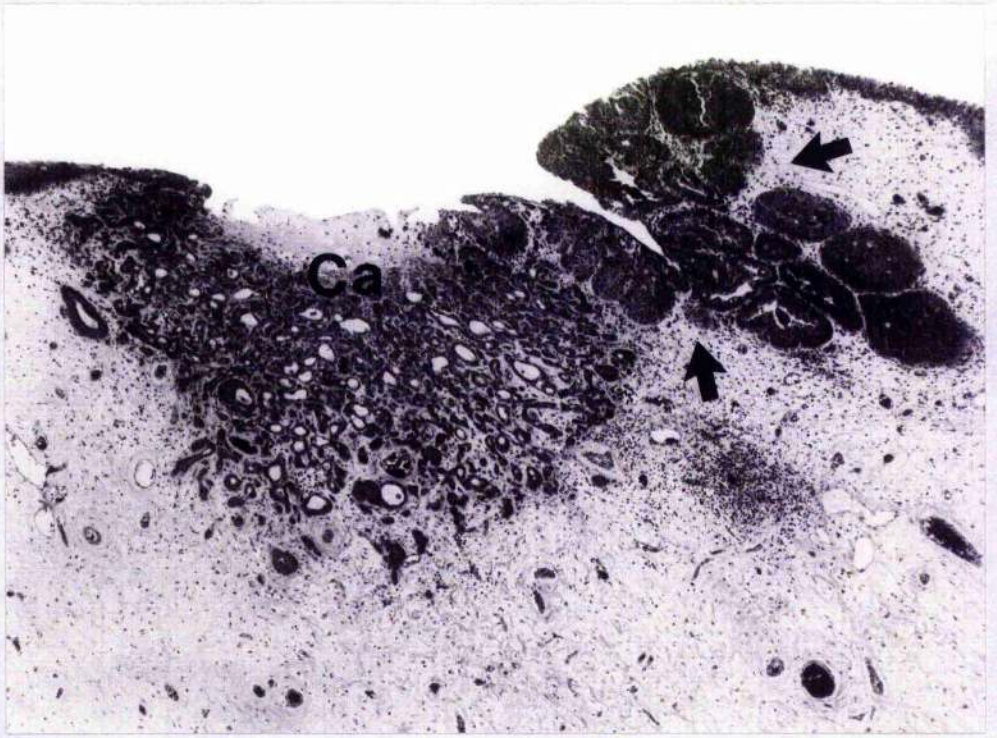

Ca

FIG. 102: Bladder carcinoma (H&E x 400. High Power view of Fig. 101). Infiltrating nests and acini of neoplastic epithelium (A) impart a glandular appearance although there is squamous metaplasia of the lining cells.

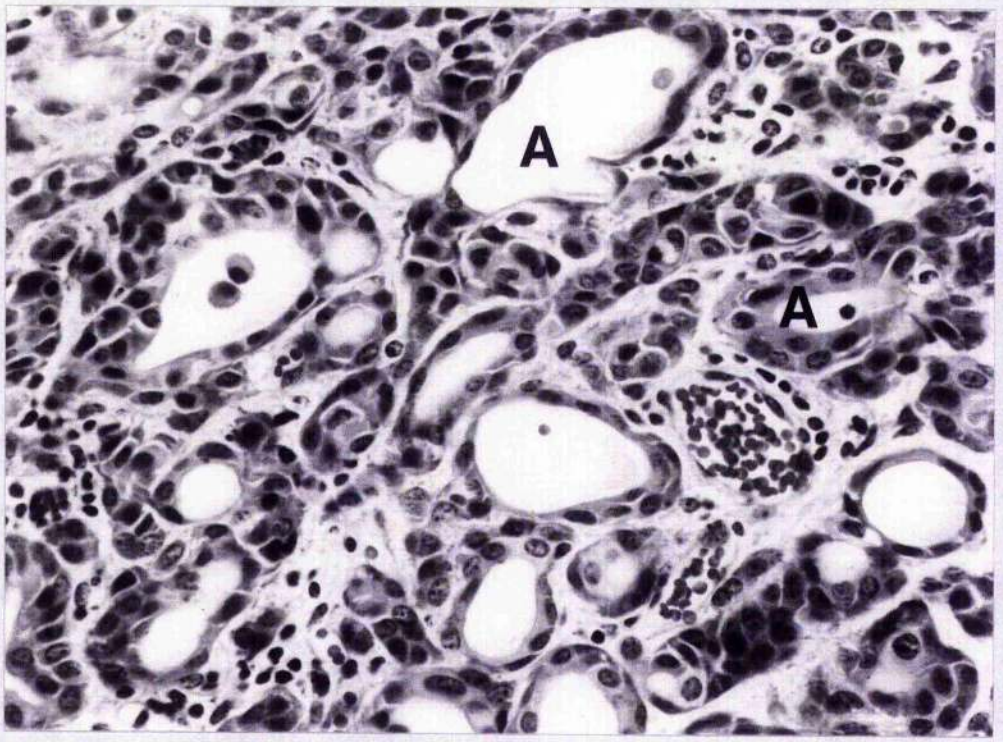

A
A

FIG. 103: Epithelial hyperplasia plus haemangioma (H&E x 35). Hyperplastic transitional epithelium (E) overlies an ill-defined haemangioma (H) with cavernous and capillary blood-filled spaces in a fibrous stroma.

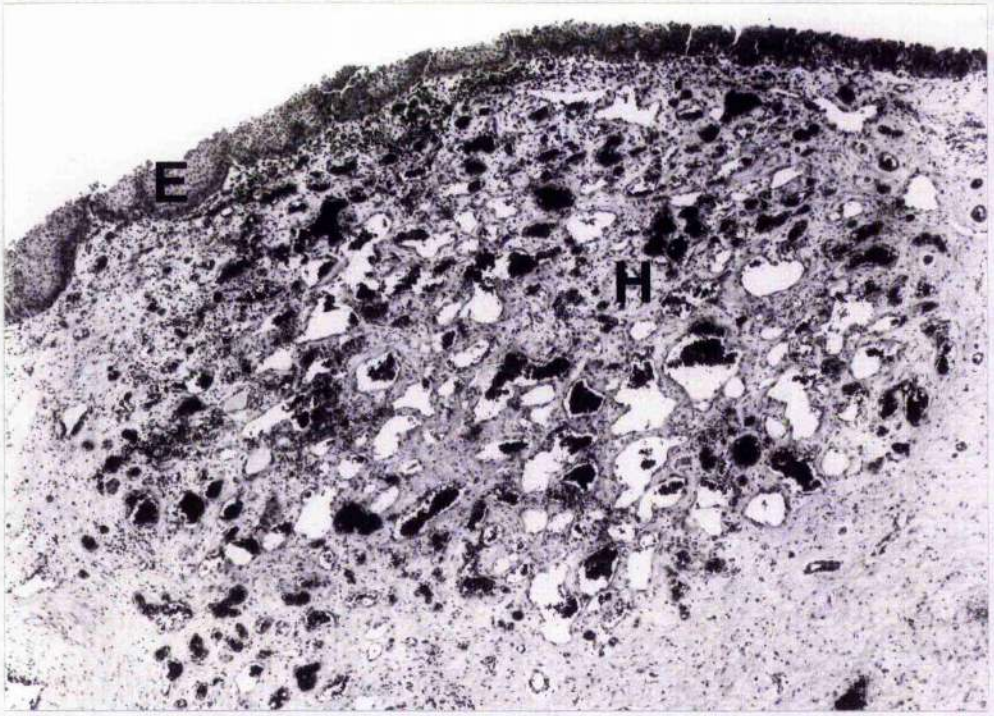

E
H

FIG. 104: Early vascular lesions (H&E x 110). Collections of dilated, congested capillaries (arrows) lie within fibroblastic connective tissue (CT). The overlying epithelium (E) is hyperplastic.

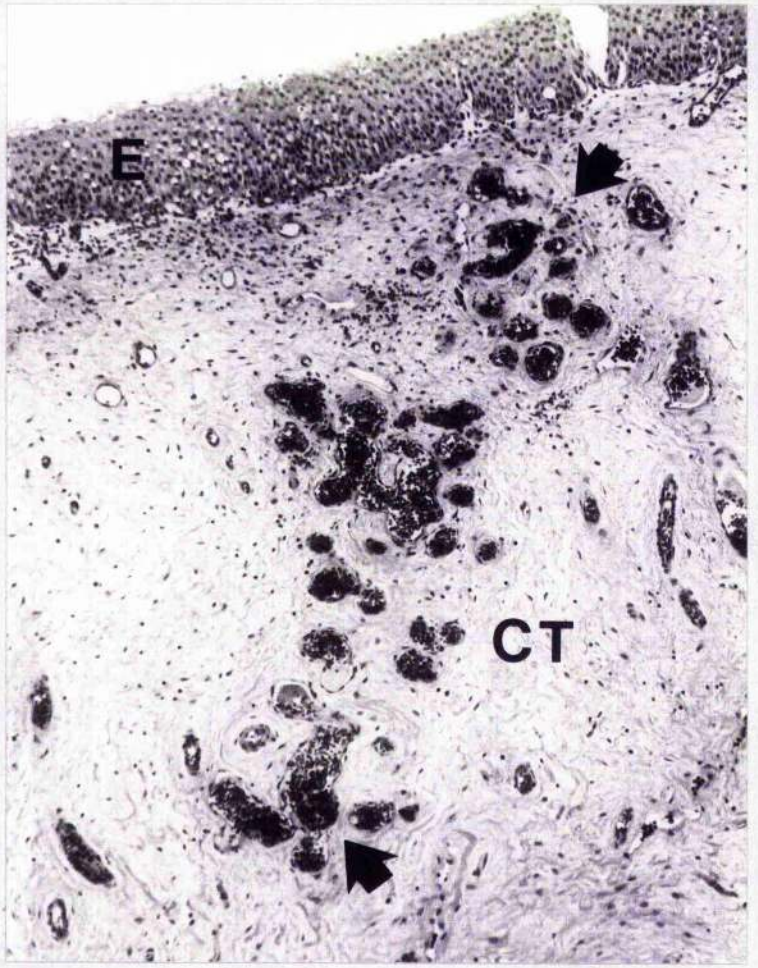

E
CT

FIG. 105: Haemangioma (H&E x 110). A circumscribed sub-epithelial collection of blood-filled capillary and cavernous spaces (s) is surrounded by fibrous connective tissue (CT).

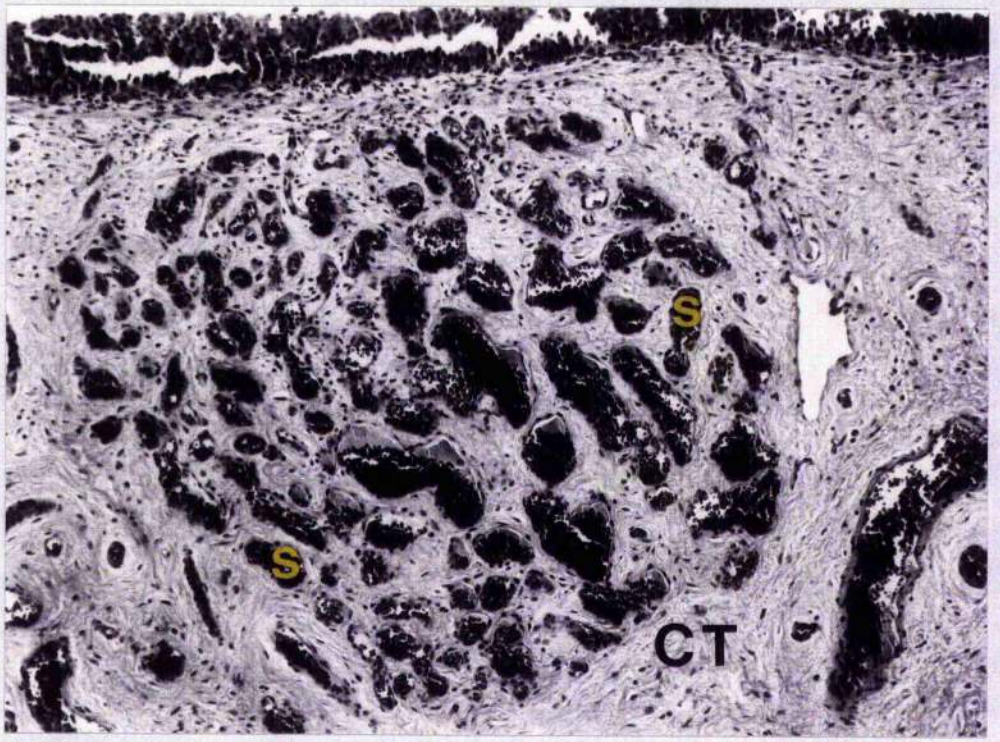
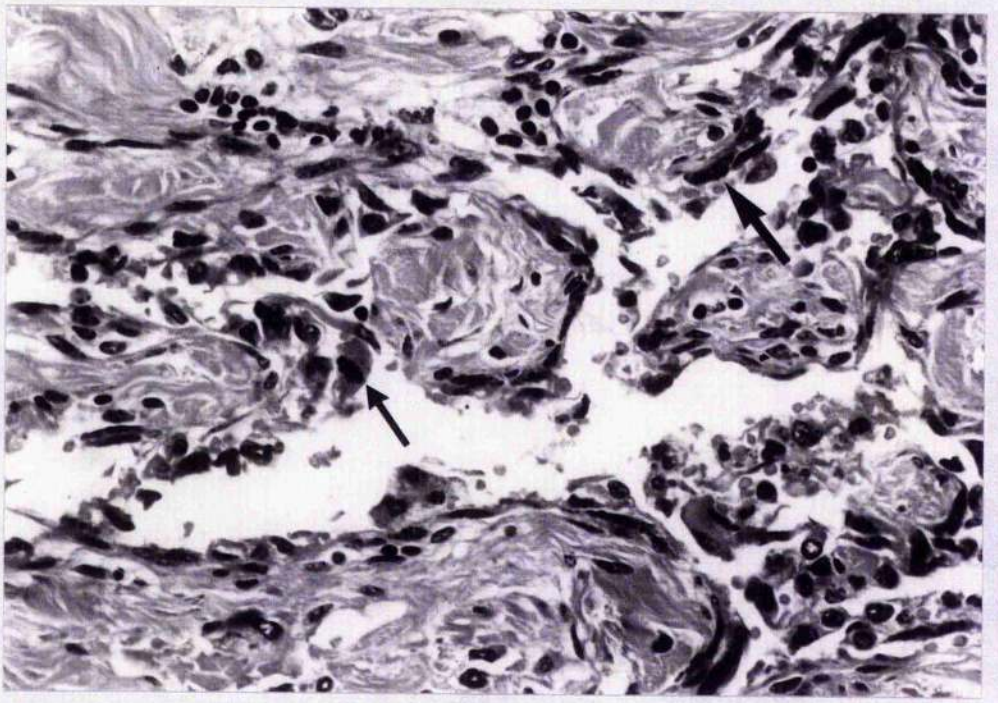

S
S
CT

FIG. 106: Haemangiosarcoma (H&E x 400). Pleomorphic tumour cells clothe surviving muscle and line irregular, vascular spaces. Tumour cells have elongated, hyperchromatic nuclei (large arrow) and occasional mitoses are evident (small arrow).



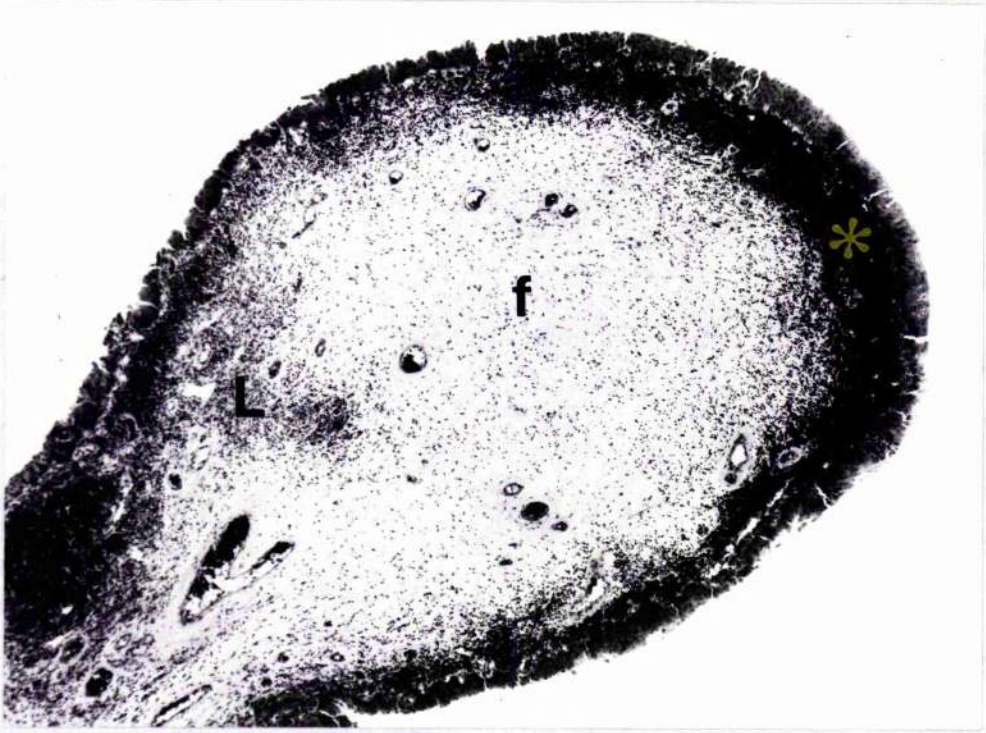

f
L
*

FIG. 107: Fibroblastic polyp (H&E x 35). The outgrowth comprises fibroblastic stroma (f) with capillary congestion and superficial haemorrhage (asterisk) overlain by hyperplastic transitional epithelium. Collections of lymphoid cells (L) are present in the stroma.

Discussion

This experiment was designed as a preliminary investigation of the possible roles of bracken fern and bovine papilloma virus in the aetiology of UAT cancer in cattle. Papilloma virus was inoculated into the rumen of each of four animals and bracken, either dried or fresh, was fed intermittently for almost eight years. Throughout this period there were no signs of acute bracken poisoning but each of three animals which survived more than five years developed haematuria and had urinary bladder tumours at necropsy. In addition, although UAT cancer was not found, oesophageal papillomas and rumenal fibromas were present in one animal which died after four years and lingual and oesophageal papillomas were found in one animal which survived over 7 years.

Since the project was started, major advances have been made in the study of papillomaviruses in general and bovine papilloma virus in particular. Jarrett and his co-workers have made large contributions to this work (see Campo et al., 1980; Jarrett et al., 1980) and the possible role of papillomavirus in cancers has been reviewed (Jarrett, 1981). It is now known that there are at least six bovine papilloma viruses, each with its own predilection sites (Jarrett et al., 1984b). The virus used in this experiment was derived from large "angleberry" type, skin fibropapillomas and as such was probably BPV-2 (Jarrett, personal communication). It would therefore be safe to assume that, although the ruminal fibromas found in one experimental animal may have been due to the inoculated virus, the oral and oesophageal epithelial papillomas recovered in two animals were probably due to natural infections with BPV-4. The significance of such lesions in relation to the aetiology of UAT cancer in cattle remains speculative.

During this experiment, bracken fern was fed intermittently for almost eight years yet the acute toxic effects of the plant were successfully avoided. There were no clinical signs of acute

bracken poisoning in the experimental animals and there were only minimal haematological changes.

The damage sustained by the bone marrow in acute bracken poisoning is said to be indicated by a precipitous drop in the number of circulating leucocytes and platelets (W.C. Evans et al., 1982, 1959) and to perhaps be shown most clearly by the neutrophil count which may approach zero (I.A. Evans et al., 1972a). In this bracken feeding experiment, neutrophil values in the four animals were rarely less than 1.0 $x10^9/l$ although occasional counts of around 0.5 x $10^9/l$ were recorded. Such counts prompted withdrawal of bracken feed in the early years of the experiment but in retrospect it was appreciated that there were no associated clinical abnormalities and similar counts occurred outwith the periods of bracken feeding. For example, the low leukocyte values recorded at day 443 were considered to have been a consequence of the bracken feeding. Certainly, at this time, the affected animals, Heifers 27 and 98, were apparently consuming more bracken per kg body weight than Stirk 28 which showed no evidence of leucopaenia. In contrast, on day 702, a precipitous fall in the neutrophil count was recorded for Stirk 28. (neutrophils 0.2 x $10^9/l$) and this was associated with a similar drop in the platelet count (51.0 x $10^9/l$). No such alterations were recorded in the other animals yet it appeared that Stirk 28 was still receiving the lowest dosage of fern. Although suggestive of acute bracken poisoning those were isolated results, there were no associated clinical abnormalities and the counts returned to normal levels despite continued bracken feeding.

Low platelet counts are the second main feature of bone marrow damage in acute bracken poisoning and I.A. Evans et al., (1958) consider them the most useful prognostic indicator. Counts of less than 100 x $10^9/l$ are said to indicate that a calf is likely to succumb to the effects of experimentally-induced bracken poisioning (W.C. Evans et al., 1982). This appears not to be the case in adult animals exposed to intermittent, low levels of bracken feeding. Platelet values of between 100 and 150 x $10^9/l$

were not uncommon in the four experimental animals and counts of less than 100 x 10^9/l were recorded occasionally. Those low values were not consistently associated with the period of bracken feeding neither was there any clinical evidence of an increased bleeding tendency in the individuals concerned. The normal range of platelet values accepted in this study, 200-600x10^9/l, is that of Gentry et al., (cited by Doxey, 1977) but many of the observed results were below that. Schalm 1972 (cited by Doxey, 1977) quotes the normal range as 200-800 x 10^9/l and this would encompass some higher results recorded for Stirks 28 and 99 in year one. However, in Veterinary Haematology (1975a) Schalm et al. note that platelet numbers have been variously reported to be between 100 and 800 x 10^9/l. Platelet counts are notoriously difficult to evaluate and it has been suggested that the wide "normal" ranges quoted in the literature may be due to variations in technique and that although better reproducibility is obtained by electronic counting such methods cannot overcome errors caused by platelet clumping or fragmentation (Dacie & Lewis, 1975). It is possible that some of the high results in this experiment were due to platelet fragments or other particles. Also, platelet clumping was recognised as a recurrent problem and may account for some of the very low results. Nevertheless, the majority of platelet counts in the four cattle fell within the range 100-400 x 10^9/l. It is not clear whether these rather low platelet values, consistently recorded throughout the experiment, were due to a constant technical error or if they indicate prolonged but mild bone marrow suppression. Alternatively, and perhaps more likely, they may simply reflect a natural pattern. Certainly, with counts of more than 50 x 10^9/l there is no increased bleeding tendency and this is in agreement with the findings of other workers who have examined a variety of species (Dale & Hurley, 1977; Mustard & Packham, 1977; see also Schalm et al., 1975b and Comparative Clinical Haematology, 1977).

It has been suggested that in certain species the bracken toxin induces successive bone marrow suppression and reparative overcompensation which leads to fluctuations in cell counts which

are twice as large as normal (I.A. Evans et al., 1972b). This might go some way towards explaining the wide variations in lymphocyte counts which were recorded in certain individuals at various times during this experiment. However, such variations were not maintained during the periods of bracken feeding and were also recorded at other times. Other authors have noted that variations in leucocyte counts occur in healthy cattle both during the day and from one day to another (Dalton, 1964). Blood samples in this experiment were not taken on the same day every week nor at the same time during the day. It seems more likely that the variations recorded in this experiment were due to natural irregularities rather than to intermittent bone marrow suppression although cyclic variations have been recorded in other bovine, bracken-feeding experiments (Jarrett, personal communication).

Red cell counts remained remarkably constant in all individuals throughout the experiment. Results were rather more erratic in Heifer 27 in Year 6 and this coincided with the recorded onset of haematuria. Similarly, in the latter years of the experiment, Stirk 99 showed a slight decline in red cell numbers and Heifer 98 showed a rather greater decline with a terminal precipitous fall. Those results merely reflect the degree of haematuria which occurred in those two individuals.

Thus, the results obtained, from one animal during four years and from three animals during almost eight years of sampling, suggest that this was largely an exercise in normal haematology. This is somewhat surprising since the amounts of bracken consumed (up to 6kg fresh bracken per day) were equivalent to or greater than those employed by other workers. Rosenberger (1970) and Pamukcu et al., (1976) fed up to 3kg of fresh bracken daily (approximately 6g per kg body weight) and observed bracken poisoning in most, if not all, of their animals.

The first clinical indication of the presence of lesions attributable to bracken in this experiment was the haematuria recorded in Heifer 27. This occurred over five years after the

start of the experiment but only eight months after fresh green bracken was introduced. In the remaining two animals haematuria was recorded 13 months and 19 months after the introduction of fresh bracken. Haematuria was not recorded in any of the experimental animals while dried fern was being fed. In a similar experiment, Rosenberger (1965) noted that haematuria was seen at the earliest 10½ months and at the latest 15 months after the start of bracken feeding and microhaematuria had been detected some 2 months earlier. Stamatovic et al., (1965) used somewhat lower dosage (up to 1kg green bracken per day) which did not induce bracken poisoning but, even with intermittent feeding they observed macrohaematuria within 32½ months.

In view of these results it would appear that the green bracken was the important factor in our experiment and that the dried bracken fern was either fed at too low a dosage or was inactivated for some reason. Other workers have succeeded in inducing bladder tumours within three years by feeding dried bracken at the rate of about 2g/kg body weight per day (Price and Pamukcu, 1968; Rosenberger, 1971). The estimated average daily intake of dried bracken fern in this experiment was frequently less than 2g/kg body weight and may simply have been too low. However, high dosage rat and mouse experiments, conducted in parallel to the bovine experiments also failed to demonstrate any marked oncogenic activity in the dried fern (McNeil, unpublished results). It was suggested that the active principle might have been destroyed during preparation of the various mixes but Japanese workers have shown that drying and mincing do not affect the carcinogenic activity of the fern (Mori et al., 1977). More recently, however, it was shown that prolonged storage of dried bracken powder did result in decreased carcinogenic activity (Kawai et al., 1981). The bracken diets used in this experiment were in storage for up to 18 months.

Once haematuria had developed, it increased and progressed most rapidly in Heifer 98. The duration of haematuria ranged from 13 months in Heifer 98 to 19 months in Stirk 99 and 25 months in

Heifer 27. It might be considered that such variations in response reflected a variation in bracken fern intake. Dose-related variations in the action of bracken fern have been recorded such as increased multiplicity and malignancy of tumours induced in rats receiving higher dosages (Hirono et al., 1982). However, although the average bracken fern intake per kilogram bodyweight in year 5-6 was higher for Heifer 98 than for the other two animals, that was not true during the following two years. Equally, the earlier onset of haematuria in Heifer 27 was not related to a high dose rate since, in relation to bodyweight, that animal consumed less than Heifer 98 during the first year of fresh bracken feeding.

The successful induction of haematuria in the absence of even minimal haematological changes attributable to bracken fern is difficult to explain, particularly when comparatively large quantities of fresh bracken were consumed in the later years of the experiment. It could be that prolonged intake of small amounts of bracken, especially of reduced potency, might induce a degree of tolerance to the bone marrow toxin.

It has been suggested that induction of bladder tumours requires only comparatively short exposure to the toxin (Grimshaw & Evans, 1980) or conversely, that development of urinary bladder cancer requires a high dose of bracken carcinogen (Pamukcu & Bryan 1979). Others have noted that urinary bladder tumours develop in rats when a relatively small amount of bracken carcinogen is given for a long period (Hirono et al., 1975). The experiment described here does not clarify the situation.

Nevertheless, the presence of urinary bladder tumours in three experimental cattle at necropsy confirmed the oncogenic potential of bracken fern. A variety of lesions was found in each bladder and the changes ranged from chronic inflammation to frank neoplasia and included capillary proliferation and ectasia; epithelial hyperplasia, metaplasia and dysplasia and formation of Brunn's nest or cysts. The tumours were haemangiomas and haemangiosarcoma as well as both non-infiltrating and infiltrating

transitional cell carcinoma. The lesions were indistinguishable from those associated with bovine CEH thus confirming the results of other workers, notably Pamukcu et al., (1976), which indicate that bracken fern is a major factor in the aetiology of CEH. The results also emphasise the neoplastic and aggressive nature of the lesions with one animal succumbing to rupture of the tumefied viscus.

The most striking feature in the experimental animals, as in the field cases, was the variety and multiplicity of lesions in each bladder. The impression is gained of widespread epithelial and subepithelial abnormalities with proliferative and dysplastic lesions of epithelial and endothelial cells progressing to frank neoplasia through a spectrum of morphological changes. This would be in accordance with current theories of urine-borne carcinogens and promoters inducing transformation of the entire urothelium and subsequent progression to overt malignant tumours by a series of stages (see Hicks, 1983).

The presence of carcinogens in the urine of bracken fed cattle has been demonstrated (Pamukcu et al., 1966; Georgiev et al., 1963). It is worth noting that endothelial tumours of the urinary bladder are uncommon in species other than cattle even after experimental induction by bracken feeding (e.g. sheep, McCrea and Head, (1981); rats, Pamukcu & Price (1969).

Studies on the histogenesis of urinary bladder tumours in bracken fed rats have concentrated on the epithelial lesions (Yoshikawa et al., 1981; Pamukcu et al., 1976). Other workers have recorded that chemical induction of tumours in the rat urinary bladder is associated with abnormal proliferation of subepithelial capillaries (Hicks and Chowaniec, 1978). They have further suggested that this proliferation may be the first morphological indicator of neoplastic change in the bladder and that the subsequent pattern of tumour development may be dependent on it. It is tempting to speculate that in bracken fed cattle a similar process continues several stages further and results in the

formation of vascular tumours. The pathogenesis of the broad spectrum of vascular and epithelial lesions found in the urinary bladder of bracken fed cattle has yet to be explored.

FINAL DISCUSSION AND CONCLUSIONS

This thesis records the occurrence of upper alimentary and urinary bladder tumours in 139 cattle in the West of Scotland. Twenty-one animals (15%) had lesions at both sites, 79 (57%) had UAT tumours alone and 39 (28%) had bladder tumours alone. In Chapter I the pathology of 100 cases of squamous cell carcinoma and 60 cases of urinary bladder neoplasia is described. Preliminary attempts to induce the tumours in experimental cattle are described in Chapter II.

Squamous cell carcinoma of the upper alimentary tract in cattle is uncommon except in two other localised geographical areas, one in Kenya and the other in Brazil (Plowright et al., 1971; Plowright, 1955; Tokarnia et al., 1969; Dobereiner et al., 1967). The predilection sites of UAT carcinoma proved to be the tongue, palate and oropharynx; the oesophagus; the cardia and reticular groove and the rumen. The majority of tumours in the Scottish cattle were found in the oesophagus or rumen. This parallels the situation in Brazil where oesophageal cases predominated and in Kenya where ruminal cases predominated. The incidence of oropharyngeal lesions varied, being absent from the Kenyan series but occurring in over a quarter of the Scottish cattle and in more than half of the Brazilian cases. The reasons for this discrepancy remain unclear.

The macroscopic and microscopic appearances of UAT carcinomas were similar in each area and were indistinguishable from those described in other sites and other species. As noted by Wahi et al., (1971) and emphasised by Kreyberg and Whimster (1978), squamous cell carcinomas may resemble any or all of the layers of normal stratified squamous epithelium.

Three main cell types constituted the squamous carcinomas in 100 Scottish cattle; basal-type cells, squamous cells and cornified cells. In any individual tumour, one cell type might predominate or the lesion might consist of a mixture of two types or all three types. Kreyberg and Whimster (1978) stress that differentiation should be the foundation of tumour typing whereas

cell size, pleomorphism, number of mitoses etc. are criteria for grading malignancy. The opportunity to compare the microscopic appearance of squamous cell carcinomas of the UAT in 100 cattle was unique so an attempt was made to categorise the tumours and so determine if there was any relationship between the histological grade and the incidence of secondary tumours. This was done largely as an aid to description but also in response to a plea in the veterinary literature (Head, 1976). It must be restated that the microscopic appearance of squamous cell carcinoma is extremely variable, even within a given tumour. Grading was based on the overall appearance of each tumour (the largest tumour in each animal) taking care to avoid the growing edges of the lesion or areas directly adjacent to ulcerative or inflammatory processes. A system was finally devised in which carcinomas were classified as Grade I, II, III or IV on the basis of cellular atypia and anaplasia. It was found that keratin, the product of differentiation in most stratified squamous epithelia, was present even in the more anaplastic lesions of Grades III and IV. Thus the presence of keratin is clearly an indicator of tumour type rather than tumour grade. It was also found that the incidence of secondary tumours was much higher in cases with Grade III or IV lesions than in those with Grade I or II. Whether such a grading system is of prognostic value in cattle or any other species remains to be tested.

Tumours of the urinary bladder in cattle are also uncommon except in association with the clinical syndrome of CEH. The incidence of bovine CEH closely follows the distribution of the bracken fern, Pteridium aquilinum, being largely absent from highly developed agricultural countries (Rosenberger, 1971). The urinary bladder lesions found in 60 Scottish cattle included epithelial, vascular and fibrous tumours as well as a variety of proliferative changes which were believed to be preneoplastic. The lesions were indistinguishable from those associated with bovine CEH (Pamukcu, 1962) and haematuria had in fact been recorded in more than half of our cases (Grimshaw, unpublished results). Vascular lesions predominated, being present in 63% of the 60 animals, but it was

noted that haematuria had not been recorded in each of those cases and that it had occurred in some individuals without vascular lesions. In view of the distribution of red cells in some bladders it was suggested that haematuria could occur through intact epithelium and that this might be important in early cases of CEH.

Bladder tumours were classified according to the criteria of Pamukcu (1974) and 5 vascular lesions were designated haemangiosarcoma. However, it appeared that there was a spectrum of lesions between haemangioma and haemangiosarcoma and that transformation of the former into the latter could indeed occur. It was suggested that haemangioendothelioma might be a more appropriate term for the range of vascular tumours encountered in CEH. Epithelial tumours were found in 45% of 60 cattle with urinary bladder neoplasia and this is in marked contrast to the findings of Pamukcu et al, (1976) who recorded epithelial growths in more than 90% of their 139 cases. Following Pamukcu (1974), the epithelial tumours in all 27 cases were classified as carcinomas although infiltration was seen in only 14 cases and secondary tumour deposits were found in only 3 cases. The prognostic value of such strict classification in cattle remains unknown. The fibrous tumours recorded in 8 cases occurred as small, subepithelial nodules. Such tumours have not been regularly associated with CEH but have been recorded by a few authors, notably McKenzie (1978a, 1978b) and Yoshikawa and Oyamada (1971). They may have been overlooked in the past because of their small size but a large number of additional cases must be examined to determine whether these lesions are part of the CEH complex or whether they constitute a distinct entity.

The coincidence of UAT carcinoma and urinary bladder neoplasia as described in cattle in the West of Scotland has previously been reported only in Brazil. In both these areas, individual animals may be affected concurrently with both conditions. It seems likely that such areas with a high incidence of neoplasia would provide some clue to the aetiology of the tumours concerned. Brazilian workers attributed both UAT and

bladder tumours to the oncogenic actions of bracken fern. In the West of Scotland too, it has been found that almost all cattle with UAT or bladder tumours had access to bracken for most, if not all, of their lives (Grimshaw & Evans, 1980; Grimshaw, unpublished results).

In view of the known oncogenic activity of bracken fern and the strong body of circumstantial evidence linking it with the occurrence of cattle cancer, a number of long-term feeding experiments were initiated. The first of these experiments in cattle is included in this thesis. Four animals, obtained as calves, were fed dried or fresh bracken intermittently for up to almost eight years. There were never any signs of acute bracken poisoning yet three animals which survived more than five years developed haematuria and had bladder tumours at necropsy. Carcinoma of the UAT was not found.

The urinary bladder lesions found in three animals were indistinguishable from those associated with field cases of CEH. The outstanding feature of those lesions in both natural and experimental cases was the range, variety and multiplicity of changes found even within a single bladder. The changes include proliferative and dysplastic lesions which, although they may be preneoplastic, are morphologically indistinguishable from the lesions of chronic inflammation and reactive hyperplasia. At the other extreme there is frank neoplasia which may be so aggressive as to cause widespread infiltration of the bladder wall and occasional rupture of the viscus with catastrophic consequences. The conclusion that proliferative lesions of epithelium, blood vessels and connective tisue progress into transitional carcinomas, haemangioendotheliomas and fibromas is highly attractive.

The results of our preliminary bracken feeding experiment confirm that the fern is a factor in the aetiology of bovine bladder tumours. In contrast, a number of contradictions exist concerning the possible role of bracken fern in the aetiology of UAT carcinoma. One of these is that, with the exception of Brazil

and now Scotland, squamous cell carcinoma of the UAT in cattle has not been reported from areas where bracken fern is abundant and the occurrence of bladder tumours is well documented. There are a number of possible explanations. Firstly, it may be that the cattle are destroyed before the latent period of the tumour has been passed; our results and those of the Brazilian workers show that cattle affected with squamous carcinoma form a somewhat older group than those with bladder tumours. The difference, however, is not marked and the age of peak incidence at 12 years is the same for both groups. Secondly, it may be that the tumours are present but are either not seen or not recognised; it is possible to confuse the macroscopic appearance of squamous cell carcinoma with granulomatous lesions such as occur in tuberculosis or actinobacillosis. Indeed, individual animals in the West of Scotland and Brazil had co-existent lesions of actinobacillosis and UAT carcinoma in close proximity. Also, early carcinomas could be misinterpreted as papillomas or as ulcers with secondary infection and necrosis. Thirdly, as suggested by the Brazilian authors (Dobereiner et al., 1967), the timing and volume of bracken consumption may influence the development of the various disorders.

The comment by Jarrett et al. (1978) that as yet there has been no experimental induction of bowel cancer in cattle remains valid. This failure to induce UAT squamous cell carcinoma in experimental cattle casts furthur doubt on the role of bracken fern in the aetiology of the condition. However, there are again a number of explanations for this failure. Firstly, in many bracken feeding experiments the animals were killed when well below the age of peak incidence of squamous cell carcinoma and so may also have been below the threshold age for the tumour. Secondly, the amount of bracken consumed by the experimental animals may simply have been too low. In the experiment described in this thesis, bracken feeding began in early life and continued for almost eight years but was intermittent. There was also some suggestion that the dried fern may have been inactivated, or at least reduced in potency, following prolonged storage. Some authors have suggested that induction of urinary bladder tumours requires only

comparatively short exposure to the toxin (Grimshaw & Evans, 1980) while others have stated that development of urinary bladder cancer requires a high dose of bracken carcinogen (Pamukcu & Bryan, 1979). Others have noted that, in rats, urinary bladder tumours develop when a relatively small amount of bracken carcinogen is given for a long period (Hirono et al., 1975). It is possible that in our experiment, too little bracken was employed even over a prolonged period. Alternatively, it could be that too much bracken was fed for too short a time. It has been reported that various active components of bracken appear to have greater activity at lower rather than higher doses (I.A. Evans et al., 1982). It may be that only very low doses of bracken are necessary for the induction of alimentary carcinoma and that there is a very long latent period, higher doses fed for shorter periods would then obscure the issue by inducing bladder tumours which destroy the animal before the age at which UAT carcinoma might occur. In mice, bracken extracts have occasionally been associated with the induction of squamous carcinoma of the fore-stomach (I.A. Evans et al., 1982). The mice had received single doses of the bracken extracts in acute toxicity tests (usually via the intraperitoneal route) and survivors were retained until they were between 1 and 2 years of age. Those results also suggested a low dose but a prolonged latent period.

One major point which needs to be considered is whether bracken fern is the only aetiological agent involved, particularly in the induction of UAT carcinoma but also in relation to bladder tumours in cattle. It has been stated and accepted that carcinogenesis is a multistep process, involving at the very least, initiation, promotion and progression of tumour growth (Hicks & Chowaniec, 1977). It is by no means clear whether bracken fern acts as a complete carcinogen, an initiator, a promoter or a late stage carcinogen. Chemical carcinogens can have different effects on different tissues and in different species. For example, some carcinogens may be effective initiators but poor promoters in one system while they are weak initiators but powerful late-stage carcinogens in a different system (Hicks, 1983). It is also now

accepted that most, if not all, cancers have a multifactorial causation (Anon, Lancet 1978). It has recently been shown by Hirono and his colleagues (1982) that feeding bracken fern to rats enhances the induction of UAT tumours by the chemical carcinogen N-propyl-N-nitrosourethan (PNU). The incidence of tumours in the pharynx, oesophagus and forestomach was increased and the multiplicity of oesophageal tumours was higher in bracken-fed animals. In addition, there were increased numbers of squamous carcinomas as compared to papillomas. All bracken-fed rats (with or without PNU) developed urinary bladder tumours. These results suggest that bracken fern may be a complete carcinogen for the rat urinary bladder whereas additional factors are necessary for the development of UAT carcinoma in that species. However, papillomas were found in rats given a 30% bracken diet without PNU and 1 rat also had squamous cell carcinoma of the pharynx. The authors therefore suggested that papilloma and squamous cell carcinoma of the UAT in cattle may be induced even by consecutive ingestion of bracken alone. They also noted that it was not clear whether bracken was acting as a syncarcinogen or as a promoter. In man, epidemiological studies have shown that daily intake of bracken can significantly enhance the risk of developing oesophageal cancer (Hirayama, 1979). It is tempting to speculate that, in cattle as in rats, bracken fern acts as a complete carcinogen for the urinary bladder but as a co-carcinogen or promoter for UAT carcinoma. However, recent results tend to support the idea of multifactorial aetiology even for the bladder tumours (see later).

The second main contender for an aetiological role in the induction of UAT and bladder cancer in cattle is a papillomavirus. Since the work described in this thesis was started, the understanding of the nature and distribution of the bovine papillomavirus has increased enormously. A papillomavirus was incriminated at the outset because of the high incidence of UAT papillomas in animals with UAT carcinoma and the consistency of the sites affected (Jarrett et al., 1978). It is now known that those alimentary papillomas are the result of infection with a specific papillomavirus, BPV-4, and furthermore, sequences of that viral

genome have been demonstrated in the cells of carcinomas (Jarrett, 1980; Campo et al, 1980). The possibility of virus as initiator and bracken fern as promotor, or even of bracken as initiator and virus as promotor of tumour growth, is immediately apparent. Studies indicated that a marked amplification in the number of papillomas and the multiplicity of affected sites occurred in animals in the high incidence area as compared to those in an abbatoir survey (Jarrett et al., 1980). The inference is that bracken fern could enhance papillomatosis prior to development of squamous carcinoma. More recently, there is evidence that sequences of BPV-2 virus genome are present in bovine urinary bladder tumours (Jarrett, personal communication). Other authors who investigated the role of papillomavirus in bovine bladder cancer ultimately dismissed it as a passenger virus (Pamukcu, 1976; Olson et al., 1965).

In our preliminary bracken feeding experiment, bovine papillomavirus was inoculated into the rumen of each experimental animal. However, as noted in Chapter II, this was probably BPV-2 since it was derived from typical skin warts or angleberries. As such it would not be expected to be involved in the induction of UAT carcinoma but, since bladder lesions were found in three experimental animals at necropsy, its role in the aetiology of bovine bladder tumours remains unclear. It should be noted that multiple alimentary papillomas, similar to those caused by BPV-4, were found in two experimental animals at necropsy yet no squamous cell carcinomas were observed. If we assume that the papillomavirus is an initiator and bracken fern a promoter of tumour growth there still appears to be a missing link in the aetiological chain attached to UAT carcinoma in cattle. This, as yet unidentified factor (or factors), would be present in the West of Scotland and Brazil. It would act, as all late stage carcinogens may, by accelerating tumour development (Hicks, 1983). It would therefore reduce the latent period necessary for tumour induction and increase the risk of squamous cell carcinoma appearing (and being recognised) during the animal's lifetime.

In conclusion, this thesis confirms and amplifies the work of others in describing the occurrence of urinary bladder tumours and squamous cell carcinomas of the UAT in cattle. It details the pathology of the various tumours found in cattle in a high incidence area and preliminary experimental work confirms that the bracken fern Pteridium aquilinium, plays a major role in the aetiology of the bladder tumours. It also serves to emphasise the many questions which remain unanswered. Although this thesis is finally complete, the investigations continue.

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