AETIOLOGICAL FACTORS IN BLADDER TUMOURS

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Bladder tumours have developed in recent years into a problem of considerable interest to many branches of the medical profession. The apparent increase in incidence must be analysed and explained by the statistician. The industrial risk is of vital importance to the industrial medical officer lest chemicals be used in industry which produce these tumours; the aetiology of the spontaneously occurring tumours presents a challenge to the biochemist. The facility for investigation by serial biopsies and clinical staging lends itself to intensive study of tumour behaviour by the pathologist. To the surgeon the methods of diagnosis available lend themselves to meticulous assessment prior to operative surgery while the radiotherapist can use isotopes for intracavitary irradiation (cobalt, gold, bromine, sodium, yttrium or arsenic), or for interstitial irradiation (tantalum, gold, chromic phosphate or cobalt), or for external tele therapy with cobalt or caesium bombs.

A knowledge of the behaviour and aetiology of bladder tumours is, therefore, of paramount

importance to these and to other branches of the profession.

Incidence

The incidence of bladder tumours is increasing in a peculiar manner in as much as the increase is occurring mainly in the younger male age groups, especially those living in towns. There are two major factors that must be considered before claiming that this is, in fact, a real increase; the effect of the increasing number of elderly people—an ageing population—and also the possibility of more accurate diagnosis due to the introduction of a new diagnostic aid or investigation.

Case (1956) has been able to show that in bladder tumours neither of these two factors is playing a significant part. By plotting the age at death of series of groups of people born in the first five years of each succeeding decade (each group termed a cohort) he has been able to show that each male cohort is dying at a slightly earlier age than the preceding cohort. Successive curves

appear to be moving to the left. The female cohorts, with a much lower incidence, are not changing. This effect of the shift to the left is seen, significantly, only in one other tumour—carcinoma of the lung.

The possibility of more accurate diagnosis must also be considered. If this was occurring there would be an angulation in each curve of cohort mortality, but as this factor would apply to each cohort at a fixed point in time the angulation would appear at different age positions for each curve.

There are many possible explanations for this increase. As lung is the only other organ showing a significant increase in tumour formation, apart from the leukaemias, it is tempting to attribute the increase to similar factors. However, there is very little evidence to prove an association between smoking and bladder tumours. Several reports have suggested that there are more heavy smokers in a group of bladder tumour patients than in a matched series of control cases (Denoix, 1956). Experimentally, too, extracts from tobacco have been applied in various ways to experimental animals but without conclusive proof of tumour formation.

The occupational history is of much greater significance and it is especially among the younger male age groups working in towns that an industrial factor might be expected to produce an increased incidence of tumours.

The idea that industrial bladder tumours were found only in men working in the aniline dye factories has been shown to be too limited a concept. Industrial tumours may be produced by a variety of chemical substances such as 2-naphthylamine, benzidine or 4-aminodiphenyl. These chemicals have been (and may still be in certain centres) used in the processing of rubber or plastic materials. Melick (1955) has reported an 'epidemic' in one factory due to the use of 4-aminodiphenyl as a 'plasticizer' where over 11 per cent. of the workmen in contact with this substance have already developed bladder tumours. This particular chemical, also known as xenylamine, was recognized as a possible carcinogen many years ago (Walpole, Williams and Roberts, 1954; Case, 1954). It can be obtained in this country, but so far it has not been possible to find out which, if any, British industries use or have used this compound.

It is the duty of the clinician to enquire into the occupational history of any patient with a bladder tumour since, if the patient can prove that he was ever exposed to an environmental risk from a scheduled substance such as 2-naphthylamine (or any of its trade names), then he or his

2-NAPHTHYLAMINE

BENZIDENE

4-AMINO DI PHENYL-
XENYLAMINE

2-AMINO-1-NAPHTHOL

3-HYDROXY ANTHRANILIC ACID.

3-HYDROXY KYNURENIN.

Chemical formula

widow will be entitled to a pension or compensation.

Experimental

The story of bladder carcinogenesis is at last beginning to take definite shape. The evidence, however, is best reviewed under several headings, not taken in chronological order.

Bonser *et al.* (1952) demonstrated, by implanting pellets of various chemicals into the bladders of mice, that these can give rise to tumours of similar types as occur naturally in man. 2-naphthylamine rarely gave rise to tumours when applied in the form of intravesical pellets, but the metabolic end-product of this substance, 2-amino-1-naphthol, was highly carcinogenic. When, however, this compound was conjugated with a sulphate radical and applied as the sulphuric ester it was again non-carcinogenic. Boyland and Watson (1956) repeated this series of experi-

Illustration showing comparisons between Bonser and Jull, and Boyland and Watson techniques.

ments and were able to show that several other substances, all of a similar chemical composition, basic aromatic and amine known as aminophenols, could produce bladder tumours if implanted. In particular, 3-hydroxyanthranilic acid and 3-hydroxykynurenin produced tumours frequently. Both these compounds have been isolated in considerable amounts in the urine of patients who have or have had bladder tumours.

Although 2-naphthylamine applied locally rarely produces tumours, yet, given by mouth experimentally or in workmen exposed to this substance, the incidence of bladder tumours approaches 100 per cent. This suggests that the effective carcinogen is produced in the body by a metabolic process.

The mode of action of this metabolite has been studied in various ways. It was originally shown by Hueper that if the ureters are transplanted before administering 2-naphthylamine no tumours would develop in the bladder subsequently. Where, however, stasis was present in the urinary tract after transplantation of the ureters (where a hydronephrosis or hydroureter developed after the transplantation), then tumours would form at these sites where stagnant urine was in contact with urinary tract epithelium (Scott, 1953). McDonald (1954) took this investigation further and was able to show that, when the bladder of a dog is divided in such a way that only half of the bladder is in contact with urine, administration of 2-naphthylamine by mouth will result in tumours only in that portion of the bladder in contact with urine. Accidentally, this finding was also confirmed by the observation that, where a fistula developed between the two halves of the bladder, tumours were produced in both halves. Strombeck (1946) transplanted portions of the bladder wall of rats to the liver and then fed the animals on azotoluene and rice flour. The majority of these rats developed papillary tumours, metaplasia or hyperplasia in the portion of the bladder which remained in contact with the urine, but in none of the 16 animals where the transplant survived did any abnormality appear in the transplanted portion of the bladder, that portion which was not in contact with the urine.

The experimental animals' work would suggest that bladder tumours will develop when certain chemicals of the aminophenol group come into contact via the urine with the urinary epithelium.

Enzymes

As these aminophenol compounds are normally present in minute amounts in the free form in normal human urine, but in greater quantities as esters, the presence of an enzyme which will split the conjugated compound is a necessary part of the process of liberation of free aminophenols. The two enzymes that could act in this way are glucuronidase or sulphatase—the first acting on 3-hydroxyanthranilic glucuronide, the second on the 3-hydroxyanthranilic sulphate ester to release free 3-hydroxyanthranilic acid.

The first confirmation that the urine of patients with bladder tumours differed from the urine of normal patients came with the work of Boyland and Williams (1954), which demonstrated that both the glucuronidase and sulphatase were raised to an appreciable extent in nearly every case of bladder tumour. Unfortunately, a raised urinary enzyme level, whether it be glucuronidase or sulphatase, is not as specific for bladder tumours as a raised acid phosphatase may be for prostatic cancer. There are several other possible causes of a raised glucuronidase value, including pyrexia, liver or renal failure, certain infections and, occasionally, tumours of other organs in the body.

Endogenous Carcinogens

As this observation on the urine of bladder tumour patients was the first to suggest that the urine was chemically different from normal urine, not only in experimental or industrial tumours but also in the apparently spontaneous tumours, the question as to the probable nature of the chemical was immediately raised. It would be logical to begin the search by investigating possible aminophenol compounds. The metabolism of the amino-acid, tryptophan, has only been investigated fully in recent years, but in the process of its metabolism it is broken down into two aminophenol compounds. These compounds, 3-hy-

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Experimental diagrams illustrating urogenous theory.

droxykyneurenin and 3-hydroxyanthranilic acid, are formed as transition metabolites before the final breakdown to nicotinic acid. Although both these substances are found in normal urine, urine of patients with tumours contains a considerably greater amount of both these compounds. This is what would be expected if the enzymes present were in fact acting on the conjugated glucuronide to release the free aminophenol.

Tryptophan has also been suspect as a possible precursor of bladder carcinogenic substances since the work of Dunning (1950) and Boyland (1954). Both were able to show that, while dogs fed on acet-amido-fluorene developed tumours at many sites, the addition of tryptophan resulted in the majority forming tumours in the bladder. A recent report also suggests that indole, with a very similar chemical composition, can also effect this carcinogenesis.

Boyland and Watson (1956) modified the Bonser Jull technique of implanting pellets into the bladders of mice. The modification consisted of closing the bladder by means of a ligature rather than meticulous suturing and consequently larger numbers of mice could be used. These mice subsequently developed bladder tumours, both papillary and solid infiltrating carcinomata, and also areas of epithelial hyperplasia similar to

those changes so frequently found elsewhere in the bladder in operative specimens of bladders removed for tumour.

Urogenous Theory

The urogenous or waterborne theory of the origin of human bladder tumours has been supported on purely clinical grounds for many years, but has been regarded as an alternative theory to that of the implantation of exfoliated cells. There is no doubt that 'seeding' or implantation of exfoliated cells can occur especially on an abraded or traumatized mucosa or into an open wound. It has also been shown that tumour cells will grow on the intact bladder mucosa, but in these experiments the bladder was kept empty. The urogenous aetiology of tumours and the seeding theory are not mutually exclusive. There are, however, certain characteristics of bladder tumours which could be better explained by the mechanism of a urinary carcinogen than by seeding.

Bladder tumours are frequently multiple either in *space*, i.e. multiple tumours present throughout the bladder simultaneously, or in *time*, i.e. single tumours arising in differing portions of the bladder mucosa over a prolonged period. These tumours, multifocal in time, may have latent periods of several years when the bladder is apparently clear

and then the process of tumour formation apparently becomes more active. Although these tumours are usually termed 'recurrences,' they may be in no way connected with the primary tumour, but may be, in fact, new tumours developing as a consequence of a continued carcinogenic stimulus. This is confirmed by the follow-up of cases of the most benign of papillary tumours, the histologically confirmed papilloma. Marshall (1956) reported that of 42 cases only 16 had remained tumour-free at the end of five years, the remainder developing either further papillomata or carcinomata.

Areas of abnormal mucosa, so frequently seen if the non-tumour-bearing portion of a bladder wall is examined, cannot be explained by seeding. Cystitis cystica, cystitis glandularis, epithelial hyperplasia, 'the mossy mucosa,' when found in the presence of a sterile urine, represent a stage prior to the proliferative phase of a tumour. There is also a type of tumour which presents as a 'cystitis,' but where the urine is sterile. On cystoscopy the bladder wall is red, mossy and can be readily mistaken for an inflammatory reaction. However, these cases frequently present as a frank infiltrating carcinomata six months or a year later.

Tumours arising over a period of years are frequently of different histological types and it is not uncommon to find tumours of obviously different histological types existing simultaneously. As the experimental tumours are frequently of different types it is more likely that this dissimilarity in type is due to a different response of the mucosa to a stimulus than to 'seeding' followed by tumour mutation to a different type.

Stasis in the bladder would appear to be a contributing factor to the development of tumours. Miller (1957) showed that in a series of 100 diverticula 14 had tumours within the diverticulum. Wallace (1956) showed that 20 per cent. of male patients with bladder tumours had or had had a definite obstructive lesion or other evidence of urinary stasis. It is tempting to suggest that the increased sex incidence 3:1 male to female could be accounted for by the increasing tendency to urinary stasis occurring in the elderly male.

Tumours of the renal pelvis, generally papillary carcinomata, usually precede the formation of tumours in the bladder, but occasionally they may develop some years after the bladder lesion. The seeding theory postulates retrograde spread which, if it occurs at all or in the absence of ureteric reflux, must be rare indeed. There are recorded cases of renal papillary tumours, treated by nephroureterectomy, which have developed a tumour in the opposite kidney without there

being at any time a bladder lesion. Both these types of lesion can be better explained on the basis of a urinary chemical carcinogen than on the seeding theory.

What is the practical significance of these biochemical investigations?

Firstly, every care must still be taken to prevent wound implantation, either at cystoscopic procedures or when the bladder is opened. At diathermy treatment all efforts should be made to effect as complete tumour destruction as possible. To leave a raw, granulating area in contact with active tumour is to invite implantation.

Secondly, every effort must be made to remove the abnormal chemical constituents from the urine by such inhibitors as 1:4 saccharolactone. This compound acts by inhibiting the enzyme glucuronidase and in this way prevents the breakdown of the conjugated aminophenol into the carcinogenic agent. It is, however, not yet a practical form of therapy for general use as it is still in the controlled experimental stage. There are three possibilities that should be considered at this point:

(i) Once the mucosa has been stimulated by a carcinogen there may be a long, latent period before a tumour develops. Removal of the carcinogen when a tumour has developed may be utterly useless as therapy or prophylaxis.

(ii) It may be possible, when a tumour has formed as a result of a stimulus, to treat the tumour and remove the stimulus in order to prevent further tumour development—the so-called recurrences.

(iii) It may be that the tumours are in part dependent on the continual supply of certain chemicals from the urine similar to some of the hormone dependent tumours. The fact that there are some cases where the tumour has either regressed or even disappeared completely after transplantation of the ureters would support this possibility.

Thirdly, the effect of a chemical carcinogen will depend on the length of time over which it may act and the concentration in the urine. Pending more effective therapy, the elimination of stasis and a maximum daily fluid intake would appear to be two ancillary modes of therapy that would conceivably minimise the risk of new tumour formation.

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androgenic action may be associated with signs of acute adrenal collapse due to deficient glucocorticoids. Diarrhoea and vomiting accentuate the loss of salt and dehydration becomes severe with depression of plasma sodium and chlorides and a raised blood urea. In milder cases signs of virilism may appear in later life and, compared with normal values at that age, urinary 17-ketosteroids are elevated with normal or low 17-hydroxycorticoids. Confirmation that hyperplasia and not carcinoma is responsible can be sought by administration of cortisone, which suppresses the output of 17-ketosteroids, and by analysis of the 17-ketosteroids, which shows a normal percentage of dehydroisoandrosterone. In an adenocarcinoma cortisone fails to reduce the 17-ketosteroid output and the percentage of dehydroisoandrosterone is high.

Conn's Syndrome (or primary hyperaldosteronism)

Soon after the discovery and isolation of aldosterone Conn described a case in which he deduced the symptoms were caused by an excessive production of aldosterone. Subsequent surgery revealed the presence of an adrenal tumour and recovery followed its removal. Clinically, the syndrome is characterized by thirst, polyuria, intermittent tetany, muscular weakness and hypertension. Oedema is not significant. There is a persistent loss of potassium in the urine and a consequent fall in the level of blood potassium. Contrariwise, sodium excretion is low and blood sodium levels are raised. A low ratio of sodium to potassium in saliva and sweat indicates increased activity of salt-retaining factor, since it shows that the abnormality of excretion is not confined to the kidney. Assays of aldosterone in the blood and in the urine can be determined by the method of Simpson and Tait, though at present this is only performed at a few special centres.

Although the majority of cases so far described have been due either to an adenoma or carcinoma of the cortex, the syndrome has also been described in association with cortical hypertrophy. Although undoubtedly rare, Conn's syndrome is a possible cause of unexplained hypertension, especially where the electrocardiograph shows evidence of potassium deficiency with inverted T waves in leads I, II, aVL and chest leads. If electrolyte

studies are consistent with the diagnosis, confirmation should be sought in blood and urinary estimations of aldosterone. Since the tumours so far described have been small, insufflation and tomography are unlikely to be helpful.

It must be stressed that, although clear-cut syndromes certainly exist, mixed syndromes are perhaps as common. Thus cases of Cushing's syndrome may show androgenic effects with raised values for 17-ketosteroids, while patients with the adrenogenital syndrome may often show features of Cushing's syndrome. This is not surprising, since there is not only an overlapping of hormonal production in disease states, but there is also some repetition of effect in the hormones themselves. Hydrocortisone is a glucocorticoid, but it is also a salt retainer and also exerts an androgenic effect. Aldosterone is a mineralocorticoid, but it can cause deposition of glycogen in adrenalectomized animals, suggesting as well some cortisone-like action.

Finally, many of the biochemical estimations discussed have been in use only for a few years and the normal range is not always fully established, so that interpretation of findings in disease states must be accepted with caution until further experience accrues.

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