Papers and Originals

Factors Concerned in the Location of Human and Experimental Tumours*

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The lecture which I have the honour to give was founded in memory of Mrs. Ernestine Henry by her son, Sydney Alexander. She was the wife of a successful and highly individualistic general practitioner in Rochdale, Lancashire. Her wit, charm, and vivacity must have shone forth in the dark surroundings of that Northern industrial town, situated on the edge of the bleak Pennine moors, its life governed by the shrieking of the mill whistles and the sound of the clogs of the mill hands as they hurried to their work. It was a united family and there was an unusually strong bond between mother and son. We can imagine that her wish might have been that he should continue in the practice, but when it became clear that he was not happy in that sphere she gave him every encouragement to follow his bent—that is, the exercise of his tenacious and inquiring mind.

Sydney Alexander Henry

First and foremost Sydney Alexander Henry was a collector. He collected degrees, books, pictures, prints, engravings, and "cases," and as his life's work was devoted to the study of occupational cancer of the skin he collected everything he could find relating to chimney-sweeps and to the form of cancer from which they suffered—namely, cancer of the scrotum. He bequeathed this magnificent collection to Leeds, where it is housed in the way he himself planned, and has been well catalogued. I strongly recommend a visit to the Abbey House Museum at Leeds for the purpose of studying this collection.

In 1920 Henry joined the Factory Department of the Home Office, and for the next 10 years worked in Manchester, later moving to London. From 1920 onwards he devoted himself to the study of occupational cutaneous cancer in the field and in the records. Both in his father's practice in Rochdale and in his work as a factory inspector he would come in contact with cases of cancer of the scrotum in mule spinners. In his first paper (Henry, 1928–9) he analysed 300 cases of mule-spinners' cancer, showing for the first time "the actual duration of contact with a carcinogenic agent necessary in man to bring about a neoplastic reaction." This ranged from 16 to 63 years. He also showed that 14 of the 300 cases had left the industry 1 to 12 years before the disease occurred, indicating that once the neoplastic process was set in train a continuation of the stimulus which caused it was unnecessary. In 1946 his excellent book *Cancer of the Scrotum in Relation to Occupation* appeared, epitomizing his vast experience and painstaking investigations and remaining a classic to this day.

Let us summarize the facts concerning occupational cancer of which Henry would be aware in 1920 (Table I). It is to be noted that all are examples of the occurrence of tumours at the site of application of the carcinogen, except Rehn's observation and possibly that of Hutchinson, where there was certainly a local precipitating cause. S. R. Wilson's prize essay of 1910 (written while he was house-surgeon at Manchester Royal Infirmary) had not been published (Brockbank and Stopford, 1927), but Henry would be aware of this analysis of 40 cases of scrotal cancer attending Manchester Royal Infirmary between the years 1902 and 1910, of which at least 28 were cotton mule spinners and not one was a chimney-sweep. Wilson made three important observations: that other forms of surface cancer were not common in spinners; that spinners constitute only a comparatively small percentage of the total population; and that mule spinning is a limited branch of spinning. For these reasons he regarded this high incidence as all the more significant. He came very near to incriminating the mineral oil with which the men's clothes were soaked as the causative agent, as indeed it was later proved to be. Such accurate and discriminating clinical observation can still find a place in the investigation of occupational cancer.

TABLE I.—*Occupational Cancer*, 1920

Year	Author	Site of Tumours	Causative Agent	Occupation
1775	Pott	Skin of scrotum	Soot	Chimney-sweeps
1874	Volkmann	" " "	Tar	Distillers of brown coal
1887	Hutchinson	" " "	Arsenic	Oral treatment of psoriasis
1895	Rehn	Bladder epithelium	Aniline	Dyestuffs labourers
1907	Workmen's Compensation Act	Skin of scrotum	Soot	Chimney-sweeps
		Cutaneous	Pitch, tar, tarry compounds	Various
1910	Wilson	Skin of scrotum	Mineral oil	Mule spinners

Henry would no doubt also be aware of the work of Yamagiwa and Ichikawa (1918), who had induced cutaneous carcinoma on the ear of the rabbit by painting with tar. This was the starting-point of all experimental carcinogenesis.

There was no difficulty in accepting that locally applied carcinogens caused tumours at the site of application, stimulation of the affected cell to an excessive mitotic rate being envisaged. It was more difficult to understand how tumours could occur at sites distant to the point of application of the chemical—for example, bladder tumours in dyestuffs workers and liver tumours after oral administration to rats of *o*-amino-azotoluene (Yoshida, 1932). My purpose is to trace some of the steps which have led to an understanding of the factors concerned in locating tumours at many different sites in the body.

Occupational Bladder Cancer

The mystery emanating from Rehn's (1895) original observation that three workers out of 45 engaged in the manufacture of magenta in a neighbouring dyestuffs factory were attending his surgical clinic suffering from cancer of the bladder was

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enhanced by the failure of Leitch (1929) and Berenblum and Bonser (1937) to reproduce the disease in the experimental animal. I should like to quote Leitch's own words: "For the last seven years experiments have constantly been going on with scores of chemical products from dyeworks, administered by inhalation, ingestion or surface application to several thousands of animals, and when the animals have died a minute examination of the urinary system has been made, but no sign of neoplasia has been afforded. The solution of the problem would be welcome, for until the particular noxious substance or group of substances is known, no real measures can be taken to prevent dyeworkers' cancer." As soon as Hueper and Wolfe (1937) had shown unequivocally that the oral administration of crude 2-naphthylamine to dogs caused benign and malignant bladder tumours, the way was open for investigation of the factors determining the location of the tumours in man and the dog at this site, and no other. Subsequent work in Leeds showed that the administered amine itself and not a contained impurity caused the tumours.

As a result of the extensive experimental work undertaken since 1937, and of surveys of the industrial disease, seven compounds only are incriminated as carcinogens in the dyestuffs industry (Table II). Of these it is by no means certain that 1-naphthylamine is carcinogenic in its own right, the 4–7% content of 2-naphthylamine in the crude product being sufficient to account for its weak carcinogenic effect; magenta has not been proved experimentally to be carcinogenic and the evidence for dianisidine is uncertain.

TABLE II.—*Carcinogens in the Dyestuffs Industry*

Compound	Evidence for Carcinogenicity	
	Statistical	Experimental
2-Naphthylamine	+	+
Benzidine	+	+
Crude 1-naphthylamine	+	?
Auramine	+	+
Magenta	+	−
4-Aminodiphenyl	+	+
Dianisidine	?	Not tested

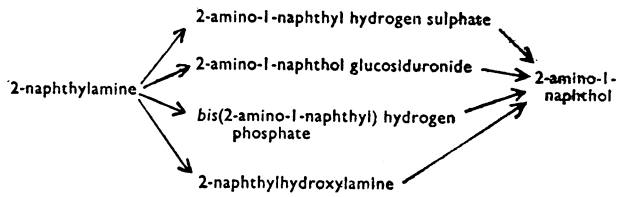
Table III summarizes some of the now known factors determining the occurrence of 2-naphthylamine tumours in the bladder. The proofs that the carcinogen is urine-borne are very strong, and there is additional evidence that retention of urine from any cause in any part of the urinary tract favours tumour development above the level of the obstruction. This may be due to the increased time available for enzyme action upon metabolites. The availability of carcinogenic metabolites in the urine is the result of a complicated but crucial mechanism. In 1948 Clayson began extensive quantitative investigations of the urinary metabolites of 2-naphthylamine in seven species of animal—dog, cat, ferret, mouse, rat, hamster, and rabbit. He found (Bonser, Clayson, and Jull, 1951) that the proportion of a dose of administered amine converted to conjugates of 2-amino-1-naphthol varied with the species, being highest in the dog (susceptible) and lowest in the rabbit (insusceptible). As conventional methods of testing 2-amino-1-naphthol hydrochloride for carcinogenicity gave equivocal results, Jull (1951) suggested that it might be possible to implant surgically a wax pellet containing the suspended chemical into the mouse bladder and thus to ensure contact with the bladder epithelium under conditions of urinary flow. This method has come to be known as bladder implantation, and though it must be used and interpreted with care it is recognized as a useful test method for determining the carcinogenic properties of chemicals. In this way 2-amino-1-naphthol hydrochloride was shown to be a carcinogen, 2-naphthylamine itself being considerably less active.

TABLE III.—*Factors in 2-Naphthylamine Carcinogenesis*

Factor	Method of Proof
Pure amine carcinogenic	Oral administration to dog
Carcinogen urine-borne	Isolated bladder pouch, dog Sigmoid loop bladder, dog Urine : plasma ratio of metabolites in dog 200 : 1
Stasis	Sigmoid loop bladder; obstructed ureters One renal pelvic tumour in obstructed ureter in 59 human industrial deaths
Availability of urinary carcinogenic metabolites	Quantitative estimation in urine

Many metabolites have been demonstrated in the urine of dogs fed 2-naphthylamine, and it is likely that most of them are non-carcinogenic detoxification products. The site of metabolism is the liver. Our concern is with the conjugates from which the ultimate carcinogen or carcinogens are liberated and with their mechanisms of liberation. Some of the possibilities are shown in Table IV. The first three compounds are *ortho*-aminophenols, the last is an arylhydroxylamine. Interest attaches to the latter because it is known that another important bladder carcinogen—2-aminofluorene—is converted to a urinary arylhydroxylamine in those species susceptible to the action of the parent compound.

TABLE IV.—*Scheme of Liberation of Actual Carcinogen*



Whatever the mode of entry of 2-naphthylamine into the animal body, metabolism takes place in the liver, but only in the mouse do tumours occur in that organ. Boyland (1963) explains this by postulating that the rate of liver conjugation with sulphate or glucuronide in the dog exceeds that of formation of 2-amino-1-naphthol tenfold, and thus the concentration of free aminophenols in the liver must be small and their presence fleeting.

The conjugated metabolites formed in the liver are excreted in the urine and bile. Some of the latter are reabsorbed from the alimentary tract and excreted again in bile and urine, so that the products circulate within the animal for some time. Even more important may be absorption through the bladder epithelium.

Experimentalists are sometimes criticized for administering to animals much larger doses than are likely to be encountered by workmen in a dangerous occupation. Goldblatt (1949) made over 300 estimations of the amount of amine present in the urine of men manufacturing 1-naphthylamine, and found that even at a time when the least precautions were taken the average daily excretion did not exceed 1–2 mg. per kg. body weight. With the methods then available he would only measure a part of the amine. This apparently low excretion might represent an intake of at least 1 g. of amine per workman per week, a not inconsiderable amount. Hueper and Wolfe (1937) and Bonser (1943) gave 500 mg. of 2-naphthylamine daily to dogs. These were explorations in an unknown field, and were designed to show carcinogenic activity rather than to determine dosage. A recent experiment by Deichmann, Radomski, Glass, Anderson, Coplan, and Woods (1965) throws light on this aspect of carcinogenesis. Four groups, each of six beagles, received 1 mg./kg./day, five times a week, of 2-naphthylamine, 4-aminodiphenyl, 4-nitrodiphenyl, or benzidine for three years. One group of six received 1 mg. of each compound—namely, 4 mg./kg. chemical a day. All six dogs receiving 4-aminodiphenyl developed tumours (four malignant); none of the dogs receiving 4-nitrodiphenyl, benzidine, or 2-naphthylamine developed tumours; and five of six dogs receiving the mixture developed malignant tumours (Table V). Thus 4-aminodiphenyl is the most potent of the four carcinogens, and is effective in as short a time as three years at a dose level of 1 mg./kg./day, a total dose of

780 mg./kg. per dog. It would be quite possible to ascertain minimum dose levels for the other carcinogens.

TABLE V.—*Comparison of Carcinogenic Potency of Four Compounds (Dog)*

Compound	Result	Range of Total Dose (g.)
4-Aminodiphenyl	Papilloma (2). Carcinoma (4)	5·4– 7·3
4-Nitrodiphenyl	Bladders normal	5·2– 7·6
2-Naphthylamine	Subepithelial haemorrhages	4·8– 9·1
Benzidine	Bladders normal	4·8– 8·5
Mixture	Normal (1). Carcinoma (5)	19·8–40·8

Bladder Cancer of Unknown Origin in Human Beings

After the clear demonstration that industrial bladder tumours are caused by exogenous urine-borne carcinogenic metabolites of absorbed chemical compounds it seemed important to look for endogenous metabolites in patients suffering from so-called spontaneous bladder cancer. Dunning, Curtis, and Maun (1950) reported that when D,L-tryptophan was fed concurrently with 2-acetylaminofluorene to rats the incidence of bladder tumours was greatly increased. They suggested that the carcinogenic action might be the result of combined elimination products of tryptophan and 2-acetylaminofluorene. Price (1966) formulated a working hypothesis which assumed that human bladder cancer was a metabolic disease in which there was a disorder of tryptophan metabolism with an increase in the urinary concentration of tryptophan metabolites. If one or more of the latter were a weak carcinogen, after a long latent period bladder tumours would appear. Price and his colleagues therefore sought to determine whether or not patients with bladder cancer excrete abnormal quantities of tryptophan metabolites or other aromatic amines and to find out whether or not these compounds could be shown to be bladder carcinogens.

Having devised suitable analytical methods, they found it advantageous to administer a loading dose of L-tryptophan to exaggerate any differences which might exist between patients and control subjects. They found that 20 of 41 patients with non-industrial bladder cancer excreted abnormal levels of tryptophan metabolites (kynurenic acid, acetylkynurenine, kynurenine, and 3-hydroxykynurenine), whereas 16 patients with industrial bladder cancer had essentially normal tryptophan metabolism. It is to be presumed that the latter would be randomly selected members of the population without any inborn disturbance of metabolism. Other authors, using different chemical methods and estimating other metabolites, have confirmed that patients with carcinoma of the urinary bladder often excrete increased quantities of certain urinary tryptophan metabolites. However, patients with other neoplastic disease often show similar metabolism, as well as those with scleroderma, porphyria, rheumatoid arthritis, and during pregnancy. Price believes that abnormal tryptophan metabolism is not a specific disorder of the bladder cancer patient, but he still thinks that in spite of the tremendous labour involved the study should be pursued.

Price found that five tryptophan metabolites were carcinogenic when implanted into the mouse bladder in a cholesterol pellet. He is inclined to accept this as positive evidence despite uncertainties introduced by the known variables, such as the action of the vehicle, the rate of elution, etc.

Prevention of Bladder Cancer

Two attempts have been made to use the theories based on the known facts in regard to metabolism, as just described, in the treatment or prevention of bladder cancer. Boyland and Wallace (Boyland 1963) gave patients who had suffered from bladder tumours repeated doses of the ammonium salt of 1→4-saccharolactone, a potent β-glucuronidase inhibitor, hoping to prevent the occurrence of new tumours, but without any success. Boyland, Kinder, and Manson (1960) dosed dogs with 2-naphthylamine and simultaneously administered capsules of the same inhibitor, hoping to prevent the development of bladder tumours. This experiment failed, but it must be remembered that it was possible to maintain a high level of inhibitor in the urine for only 12 of the 24 hours. Thus it can still be argued that more continuous treatment might have succeeded.

Ureteric Tumours

The fact that industrial and experimental urinary tract tumours are usually confined to the bladder has been a matter for surprise since the urogenous theory of their cause came to be accepted. As the carcinogenic metabolites are continuously in contact with the whole urinary tract there must be differences in the conditions in the bladder compared with those in the ureter and renal pelvis. The following factors are likely to be operating: urine remains longer in the bladder, and therefore there is more time for enzymic degradation of metabolites (Boyland, 1963); there is a peak of excretion of an administered dose of 2-naphthylamine after about four hours, so that the renal pelvis and ureter are subject to high concentrations only immediately before and after the peak, whereas human and dog bladders retain metabolites for long periods of time (Clayson, 1962); the amount of exposed bladder epithelium is greater than that of the renal pelvis and ureter, and therefore there is a greater chance of tumour development (Willis, 1960). The fact that embryologically the ureter is developed from the Wolffian or mesonephric duct and the bladder from the urogenital sinus (Willis, 1958) might eventuate in the adult in different enzymic systems in the two organs.

Occasional ureteric and renal pelvic tumours have been observed in dyestuffs workers. Scott and Boyd (1953) observed two ureteric carcinomas in dogs in which the ureters were implanted into the sigmoid colon and there was obstruction to the flow of urine. But the outstanding example of the experimental induction of ureteric tumours is by administration of 2-acetylaminofluorene to the rabbit. This finding by Bonser and Green (1950) has been confirmed by three other workers, yet no satisfactory explanation for the occurrence of the tumours is forthcoming. Bonser (1962) thought that the glandular metaplasia observed in the intravesical portion of the ureter might cause ureteric obstruction, with subsequent stasis.

Table VI shows that the induction of bladder cancer on 14 occasions by seven known carcinogens, tested on five species, was associated in two rats only (apart from the above-mentioned rabbits) with the simultaneous induction of ureteric tumours. It is known that the carcinogenic metabolite of 2-acetylaminofluorene is excreted in rabbit urine at a higher level than in other species (Irving, 1962). Possibly specific enzyme production is more abundant or of different type in this species. Such factors are open to experimental investigation.

TABLE VI.—*Induced Tumours of Urinary Tract*

Compound	Man	Dog	Rat	Rabbit	Mouse
2-Naphthylamine	B	B	—	—	—
2-Acetylaminofluorene		B	B	B+U	B
4-Aminodiphenyl	B	B	—	B	?B
4-Nitrodiphenyl		B			
3-Methoxy-2-aminodiphenylene oxide			B+U		
4-Hydroxydibutylnitrosamine			B+U		
4-Ethylsulphonylnaphthalene-1-sulphonamide					B

B = Bladder tumours. U = Ureteric tumours. — = No tumours.

Recently Discovered Carcinogens

Three occurrences, not at first having any bearing on the induction of tumours, have resulted in the discovery of new

carcinogens, the study of which has thrown light on cellular mechanisms in carcinogenesis.

Aflatoxin

In 1960 poultry breeders in this country (and later in Brazil) became alarmed by an epidemic of liver necrosis in turkey poults, the cause of which was finally narrowed down to the feeding of ground-nut meal in which a mould, *Aspergillus flavus*, was growing. The toxic material extracted from fungus-infected ground-nuts was called aflatoxin, which has been separated into four components B_1, the most toxic, B_2, G_1, and G_2, all of pentacyclic structure with one or more lactone rings. The first effect in rats, ducks, and rainbow trout is acute hepatic periportal necrosis, followed by the induction of hepatomas. The dose required is extremely small (Table VII). Aflatoxin inhibits D.N.A. synthesis in the liver by preventing D.N.A. polymerase from transcribing D.N.A. and in turn inhibiting R.N.A. polymerase. There is thus interference with the genetic determinants of the cell (Rees, 1966).

TABLE VII.—*Potency of Aflatoxin (B_1 Component)*

Species	Dietary Level	Tumour
Rat	0·1–0·05 p.p.m.	Hepatoma
Duck	0·03 p.p.m.	,,
Rainbow trout	0·0005–0·002 p.p.m.	,,

We must now consider the mycotoxin hypothesis as a cause of human primary liver cancer, especially in Africa. Oettlé (1966) has pointed out that, for *A. flavus* to grow, humid conditions are needed and that such conditions prevail where liver cancer is prevalent in Africa. Also, food-storage practices under primitive conditions predispose to mould growth. There are other candidates for the role of causative agents, such as the *Senecio* alkaloids, but the extreme potency of aflatoxin, associated with conditions favourable for its production, casts grave suspicion upon it.

Nitrosamines

Barnes and Magee (1954) investigated the toxic properties of dimethylnitrosamine in consequence of an incident when two out of three men using it as a solvent in an industrial research laboratory developed cirrhosis of the liver. In acute experiments haemorrhage and centrilobular necrosis occurred in all the species tested, and in chronic experiments in rats hepatocellular carcinomas, with metastases, occurred at an early date. Later Magee and Barnes (1962) showed that the location of the tumours in the rat could be altered by rearranging the period and amount of dosing. Continuous dosage over an extended period caused hepatomas, whereas short intensive feeding caused mainly renal tumours, either well-differentiated carcinomas or anaplastic mixed tumours, resembling nephroblastomas. They suggested that when high doses were given the excess chemical was not degraded in the liver but was spilled over to the kidney.

These observations have led to a great volume of work aiming mainly in three directions: the mode of action of nitrosamine, the long-term effect of alteration in chemical structure, and the effect of variation in dosage, especially the single-dose effect.

Schoental and Magee (1962) suggested diazomethane as an intermediary in the carcinogenicity of dimethylnitrosamine and its analogues, and showed that both rats and mice developed lung adenomas or subcutaneous sarcomas after inhalation or subcutaneous injection of this compound. Magee (1964) further brought evidence to show that diazomethane interferes with D.N.A. synthesis and in this respect resembles aflatoxin.

The biological effects of 80 or more analogues of dimethylnitrosamine have been studied by Druckrey and his colleagues. Some of these compounds induce tumours in most unusual sites—for example, the oesophagus, nasal cavity, brain, or nerve. But of all the many compounds tested only dibutylnitrosamine and its derivative 4-hydroxybutyl-butylnitrosamine induce tumours of the rat bladder (Druckrey, Preussmann, Ivanković, Schmidt, Mennel, and Stahl, 1964) (Table VIII). As the urinary compared with the plasma concentration of water-soluble nitrosamine metabolites after a single injection of a large dose of the dibutyl compound was 10–100 to 1 they concluded that the ultimate carcinogen was urine-borne.

TABLE VIII.—*Induction of Tumours by Butylnitrosamine*

Compound	Method of Administration	Tumours			
		Bladder	Ureter	Oeso-phagus	Liver
Dibutylnitrosamine	Oral	+	0	+	+
	Subcutaneous	+	0	a few	a few
4-Hydroxybutyl-butyl-nitrosamine ..	Drinking-water	+	1	0	0

Druckrey, Steinhoff, Preussmann, and Ivanković (1964) gave single doses of nitrosamines by various routes, causing tumours at latent periods no longer than when numerous doses were given (Table IX). To this list may be added N-methyl-N-nitrosourea, which induced 10 benign and 24 malignant tumours in a variety of organs in 16 rats after a single intravenous injection. The authors postulate as the essential mechanism the rapid liberation of diazomethane at the affected sites. No wonder that they issued a warning regarding the dangerous properties of this group of chemicals.

TABLE IX.—*Single-dose Tumours in Rats*

Compound	Dose (mg./kg.)	Route	No. and Site of Tumours	Latent Period (weeks)
Dimethylnitrosamine ..	37	Inhalation	Ethmoid 1/3	43
Diethylnitrosamine ..	280	Intravenous	Kidney 4/8	46
			Ovary 1/4	46
	280	Oral	Liver 1/3	70
Methyl-vinyl-nitrosamine	44	Inhalation	Nose 1/1	40
Ethyl-butylnitrosamine	370	Oral	Kidney 1/4	54

The importance of these observations needs no stressing. That a single dose of a carcinogen can set in train a process which will culminate in cancer after a latent period without the need of a further application is a new conception. Much work has already been done in formulating the idea of a single rapid specific "initiating" phase of induction of the cancer process followed by a prolonged nonspecific "promoting" phase. It is in this latter respect that chronic irritation finds a place in cancer causation. The information available on single-dose induction of cancer in such organs as the ethmoid and nasal cavities and the kidney do not yet permit the postulation of a two-stage mechanism, but they provide a starting-point for investigation of the co-factors that may be concerned in the subsequent stages of cancer development. The fact that the affected sites are so unusual also promises the opening up of new search for enzymatic or other chemical mechanisms which may be involved.

Cycad Seeds

In searching to explain the phenomenal incidence of amyotrophic lateral sclerosis in the Chamorro population of Guam and the other Mariana Islands, it was noticed that the seed of *Cycas circinalis*, a palm-like tree, was an important source of food in spite of its recognized toxicity. The local methods of preparation usually removed the toxins causing acute effects, but it was thought that a cumulative effect might occur from small residues. Two additional foci of the disease were then discovered in the Kii peninsula of Japan and the south coast of west New Guinea, both areas where the cycads grow. Today

the use of native plants is diminishing because of the long procedures needed for processing; and Whiting (1964), though living for several months in the Kii peninsula, "could not locate one resident who had knowledge of the local use of cycads as food," though she did trace their use as medicine. Kurland (1964) summarized the position as follows: "If there is a toxic ingested substance common to these three foci of ALS, it has not been recognized. The cycad does not appear to be a likely candidate."

During the investigation of amyotrophic lateral sclerosis some preliminary reports suggested that cirrhosis and carcinoma of the liver were more prevalent among Chamorros than in the U.S.A. For the two reasons stated investigation of the toxic properties of the meal obtained from the seed of *Cycas circinalis* was undertaken and attempts were made to isolate the active principle from the meal. Laqueur, Mickelsen, Whiting, and Kurland (1963) fed cycad seed meal to rats and induced many hepatocellular tumours, some of which metastasized, reticuloendothelial proliferations in the liver, renal carcinomas, undifferentiated renal tumours, and occasionally carcinomas of the large intestine. They were struck with the similarity between the cycad tumours and those induced by dimethylnitrosamine. Work in this field has gone ahead with amazing rapidity (Laqueur, 1965; Whiting, Spatz, and Matsumoto, 1966). It is now accepted that the active principle in the meal is a glycoside, cycasin, which is decomposed to its aglycone methylazoxymethanol by bacterial enzymic action. This latter fact was established because cycasin is carcinogenic on feeding but not when injected, whereas methylazoxymethanol is effective when injected intraperitoneally. In the latter circumstance numerous carcinomas of the duodenum occurred (Laqueur and Matsumoto, 1966). Cycasin is non-carcinogenic when fed to germ-free rats, and because the cycasin is not split by intestinal bacteria in these rats they tolerate experimental feeding more readily. Cycasin is a general carcinogen, being effective in rats, mice, and guinea-pigs and producing neoplastic disease at various sites.

The most exciting position is now reached when the carcinogenic effects of nitrosamines and cycad seeds can be traced to the liberation of closely related compounds belonging to the group of diazoalkanes. These and aflatoxin act by interference with D.N.A. synthesis, and therefore attack the vital mechanisms which are concerned with replication of the cell and the handing on of genetic information.

The damage caused by the administration of certain chemicals to the grown animal does not cease with the individual. The sins of the fathers may be visited on the children. Laqueur (personal communication) fed cycasin to pregnant rats from the 17th to the 19th day of pregnancy and induced nephroblastomas in the surviving offspring. Druckrey, Ivanković, and Preussmann (1966) gave a single intravenous injection of diethylnitrosamine to rats on the 15th day of pregnancy and induced malignant neurinomas and a glioma in offspring surviving to 23 weeks, though the mother survived tumour-free. Such observations may lead to an explanation of the causes of childhood tumours.

Conclusion

In the time at my disposal I have selected known aspects of carcinogenesis which seem to me to help to unravel the causes of human tumours. I would like to have heard Henry's comments on the new work which is making such rapid progress towards an understanding of the factors concerned in the location of certain tumours. He would certainly have been the first to appreciate the value of clinical observation in stimulating fruitful research. Three conclusions may be made:

1. A return to a "natural" way of living would not eliminate carcinogenic risks; it might even increase them.

2. We can expect that further carcinogenic chemicals will be introduced into man's environment, but as safeguard we can observe such restrictions in their use as are called for in the light of present and future knowledge.

3. No strict relation has yet been formulated between chemical structure and carcinogenic potency. For this reason there is justification for continuing the search for new classes of chemical carcinogen in the hope that such a relation will eventually be found.

Summary

The thesis of this lecture was to compare the state of knowledge regarding cancer induction as it was in 1920, when Sydney Alexander Henry started his life's work on cutaneous cancer in industry, with the state of present-day knowledge, when many factors are known to be concerned in locating human and experimental tumours at certain sites.

The work of the past 45 years in industrial bladder cancer is reviewed. This has demonstrated that few chemical compounds are known to be involved, that they need to be metabolized to ultimate carcinogens which are then excreted in the urine, and that stasis is probably an important factor, as most of the tumours occur in the bladder. The ease of induction of ureteric tumours in the rabbit by 2-acetylaminofluorene and the possible causes are discussed. An evaluation of the attempts to isolate urinary carcinogenic metabolites from patients suffering from spontaneous bladder cancer is also made.

Three recent observations, not at first immediately connected with cancer induction, are briefly reviewed. The occurrence of liver necrosis in turkey poults led to the discovery of aflatoxin, derived from *Aspergillus flavus* growing in ground nut meal. This is the most potent liver carcinogen known, and it is especially so in the rainbow trout. Aflatoxin may account for the high incidence of liver cancer in certain parts of Africa. An incident in a laboratory led to the investigation of dimethylnitrosamine as a liver poison, resulting in a massive amount of information about the carcinogenic properties of this and related compounds and elucidation of their mode of action through diazoalkanes. Finally, it was noticed that amyotrophic lateral sclerosis was occurring in almost epidemic proportions in certain areas of the Far East. In an attempt, still unsuccessful, to find the cause a glycoside (cycasin) was isolated from the seed of *Cycas circinalis*, a palm-like tree which grows in these areas. This was found to be intensely carcinogenic in animals, and when fed to pregnant rats induced renal tumours in the offspring. The metabolite of cycasin is methylazoxymethanol, and thus there is a close resemblance to the mode of action of the nitrosamines.

From the knowledge gained from these widening fields it is concluded that a return to a "natural way of living" would not eliminate carcinogenic risks and that we must use all the available knowledge to eliminate environmental carcinogens from contact with man. Though at present carcinogenic potency cannot be predicted from chemical structure we must aim to widen our knowledge in this field. This is the chief justification for pursuing the study of new classes of cancer-producing chemicals.

References

Barnes, J. M., and Magee, P. N. (1954). *Brit. J. industr. Med.*, **11**, 167.
Berenblum, I., and Bonser, G. M. (1937). *J. industr. Hyg.*, **19**, 86.
Bonser, G. M. (1943). *J. Path. Bact.*, **55**, 1.
—— (1962). In *Morphological Precursors of Cancer*, edited by L. Severi. Perugia.
—— Clayson, D. B., and Jull, J. W. (1951). *Lancet*, **2**, 286.
—— and Green, H. N. (1950). *J. Path. Bact.*, **62**, 531.
Boyland, E. (1963). *The Biochemistry of Bladder Cancer.* Springfield, Ill.
—— Kinder, C. H., and Manson, D. (1960). *A.R. Brit. Emp. Cancer Campgn*, **38**, 45.
Brockbank, E. M., and Stopford, J. S. B. (1927). *Brit. med. J.*, **2**, 993.
Clayson, D. B. (1962). *Chemical Carcinogenesis.* London.
Deichmann, W. B., Radomski, J., Glass, E., Anderson, W. A. D., Coplan, M., and Woods, F. (1965). *Industr. Med. Surg.*, **34**, 640.

Druckrey, H., Ivanković, S., and Preussmann, R. (1966). *Nature (Lond.)*, **210**, 1378.
—— Preussmann, R., Ivanković, S., Schmidt, C. H., Mennel, H. D., and Stahl, K. W. (1964). *Z. Krebsforsch.*, **66**, 280.
—— Steinhoff, D., Preussmann, R., and Ivanković, S. (1964). Ibid., **66**, 1.
Dunning, W. F., Curtis, M. R., and Maun, M. E. (1950). *Cancer Res.*, **10**, 454.
Goldblatt, M. W. (1949). *Brit. J. industr. Med.*, **6**, 65.
Henry, S. A. (1928–9). *J. Hyg. (Lond.)*, **28**, 100.
—— (1946). *Cancer of the Scrotum in Relation to Occupation*. London.
Hueper, W. C., and Wolfe, H. D. (1937). *Amer. J. Path.*, **13**, 656.
Hutchinson, J. (1887). *Lancet*, **2**, 1166.
Irving, C. C. (1962). *Cancer Res.*, **22**, 867.
Jull, J. W. (1951). *Brit. J. Cancer*, **5**, 328.
Kurland, L. T. (1964). *Fed. Proc.*, **23**, 1337.
Laqueur, G. L. (1965). *Virchows Arch. path. Anat.*, **340**, 151.
—— and Matsumoto, H. (1966). *J. nat. Cancer Inst.*, **37**, 217.
—— Mickelsen, O., Whiting, M. G., and Kurland, L. T. (1963). Ibid., **31**, 919.
Leitch, A. (1929). *A.R. Brit. Emp. Cancer Campgn*, **6**, 32.
Magee, P. N. (1964). In *Cellular Control Mechanisms and Cancer*, edited by P. Emmelot and O. Mühlbock. Amsterdam.
—— and Barnes, J. M. (1962). *J. Path. Bact.*, **84**, 19.
Oettlé, A. G. (1966). *Proceedings of 6th Canadian Cancer Conference*, edited by R. W. Begg, p. 224. London.
Pott, P. (1775). *Chirurgical observations relative to the cataract, polypus of the nose, the cancer of the scrotum, the different kinds of ruptures and the mortification of the toes and feet*. London.
Price, J. M. (1966). *Proceedings of 6th Canadian Cancer Conference*, p. 224. London.
Rees, K. R. (1966). *Gut*, **7**, 205.
Rehn, L. (1895). *Arch. klin. Chir.*, **1**, 588.
Schoental, R., and Magee, P. N. (1962). *Brit. J. Cancer*, **16**, 92.
Scott, W. W., and Boyd, H. L. (1953). *J. Urol. (Baltimore)*, **70**, 914.
Statistics of Compensation and Proceedings under the Workmen's Compensation Acts and the Employers' Liability Act, 1880. Published annually by H.M. Stationery Office.
Volkmann, R. (1874). *Beitr. Chir. (Lpz.)*, p. 370.
Wallace, D. M. (1959). *Tumours of the Bladder*, Vol. 2 of *Monographs on Neoplastic Disease at Various Sites*. Edinburgh.
Whiting, M. G. (1964). *Fed. Proc.*, **23**, 1343.
—— Spatz, M., and Matsumoto, H. (1966). *Economic Botany*, **20**, 98.
Willis, R. A. (1958). *The Borderland of Embryology and Pathology*. London.
—— (1960). *Pathology of Tumours*, 3rd ed. London.
Yamagiwa, K., and Ichikawa, K. (1918). *J. Cancer Res.*, **3**, 1.
Yoshida, von T. (1932). *Proc. imp. Acad. Japan*, **8**, 464.

Relation between Change of Blood Pressure and Age

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Sixty years after the invention of the sphygmomanometer we still know little about the development of arterial hypertension. Few attempts have been made to study its natural history, because this cannot be done with hospital populations, and most epidemiological studies have been cross-sectional and therefore inappropriate for this purpose.

The conflicting views on the nature of unexplained hypertension remain unresolved. Platt (1961, 1963) and his followers, who believe essential hypertension to be a disease entity, think that there are two types of person, those whose pressures do and those whose pressures do not increase markedly in middle age, and that this is determined at conception by the presence or absence of a single pair of genes acting at a single locus. Their dissenters were unimpressed by the evidence, and were unwilling to attribute a disorder so common in old age to the effects of a single pair of genes. Pickering (1961, 1965) challenges this concept of essential hypertension on the grounds that evidence derived from many sources suggests that it is a graded characteristic, its effects depending largely on the level attained. His school believes that age and environmental factors are of major importance in determining blood-pressure levels and that the relatively small contribution from inheritance is polygenic. Those who disagreed were able to draw attention to considerable sections of some communities in whom pressure does not rise with age.

Longitudinal surveys of general populations provide an opportunity to study some of the outstanding questions. This paper reports analyses of data, collected over periods of 10 and 8½ years in two such studies in South Wales, in which the relations between change of blood pressure, the pressure already attained, and age are explored.

Population Samples and Surveys

The populations of a typical Welsh mining valley, the Rhondda Fach, and a neighbouring agricultural area, the Vale of Glamorgan, were selected by the M.R.C. Pneumoconiosis Research Unit for epidemiological studies of tuberculosis and pulmonary disease in contrasting occupational groups (Cochrane *et al.*, 1955, 1956). The population of the Rhondda Fach was defined by censuses in 1950 and 1953, and a serially numbered card was prepared for each person over 5 years of age resident in the valley at either time. The cards were numbered in a geographical order, and one in every 100 was selected to give a systematic random sample of the population for the blood-pressure investigation ; the cards of those who had died or left the area were replaced by random selection from the same batch of 100 cards. The final sample included about 1 in 90 of the total population of 23,889 residents in 1953. The population of the Vale of Glamorgan, where a census was taken in 1955, included 5,691 subjects over the age of 5 years. Random samples were selected from nominal lists to give groups of 200 subjects of each sex.

The first blood-pressure surveys were undertaken in the winters of 1954 and 1956 in the Rhondda Fach and Vale of Glamorgan respectively. The random samples were assumed to be representative of the general population and were used as the propositi for genetic studies in which measurements were made also on all their first-degree relatives living within 25 miles (40 km.). Over 95% of the propositi and over 95% of their relatives were examined in the initial studies.

Follow-up studies were carried out in the Rhondda Fach in the winter of 1958 and in the summer of 1964 ; the Vale of Glamorgan sample was followed up in the summers of 1960 and 1964. Of 1,216 subjects seen in the first Rhondda survey 909 (74.8%) were examined 10 years later, the death certificates of 130 (10.7%) who had died were studied, 149 (12.3%) had left the area, and 28 (2.3%) had refused follow-up. Of the 1,464 subjects seen at the first survey in the Vale of Glamorgan 1,142 (78.0%) were still being followed up eight and a half years later, the death certificates of the 139 (9.5%) who had died were examined, 144 (9.8%) had left the area, and 39 (2.7%) had refused further follow-up. By 1964 either the blood-pressure status or the cause of death was known for 2,320 (86.6%) of the original 2,680 individuals.

Details of the techniques used have been described in previous publications from these studies (Miall and Oldham, 1955, 1958, 1963). Casual blood-pressure measurements were made by one observer using a mercury sphygmomanometer in all surveys reported here. Observations were made in the subject's own home after he or she had been sitting quietly for at least five and usually more than ten minutes. Measurements were made at any time between 9 a.m. and 10 p.m., but the majority

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