SOLAR CARCINOGENESIS

An Epidemiological, Experimental and Histochemical Study of Tumours Induced by Ultraviolet Radiation.

A Thesis submitted for the Doctorate of Medicine of the University of Adelaide.

by

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December, 1963

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DECLARATION OF AUTHORSHIP

The subject matter of this thesis is compiled from my own original studies. I have indicated in the text whenever assistance has been received, and by the use of references when I have relied on the published work of others. The surveys of skin cancers and hyperkeratosis were planned and executed by me. Dr. R. Silverstone, Reader in Medical Statistics in the University of Queensland, gave some mathematical and statistical advice, and contributed the mathematical part of our joint paper. A copy of this is included at the end of the thesis. The histochemical observations are all my own, with the conclusions drawn from them.

The composition and authorship of this thesis are entirely my own.

ACKNOWLEDGEMENTS

The work for this thesis has been carried out at the University of Queensland and the Institute of Medical and Veterinary Science, Adelaide.

The histochemical studies were carried out in the Institute, and I am greatly indebted to Dr. J.A. Bonnin, Director, for his encouragement and support in making available the facilities for experimental pathology. I am grateful, also, to Dr. W.E.R. Hackett, Head of Division of Medical Research, for his interest and co-operation. These studies were financed by a grant from the University of Adelaide Anti-Cancer Foundation, which provided for technical assistance and the purchase of chemical reagents. During his stay in Adelaide, I had many helpful discussions with Dr. R.M. Fullmer, of the National Institute of Dental Research, Washington. He also made the gift of many histochemical reagents.

The epidemiological studies were carried out during the tenure of a Medical Research Fellowship at the University of Queensland. I must record my gratitude to Dr. A.G.S. Cooper, Director of the Queensland Radium Institute, on whose initiative the project was planned. The collection of the material in the Cane Farmers' survey was made possible by the active assistance of the Queensland Cane Grower's Council. Mr. K.W. Blyth took charge of many of the administrative details, with the support of the late Mr. R.J.S. Muir, past General Secretary of the Council. Mr. D.F. Robertson, Reader in Radiation Physics, gave me advice on many of the physical aspects of ultraviolet radiation. Dr. J. Tonge, Director of the Laboratory of Pathology and Microbiology,

Brisbane, gave me access to the coronial autopsy material.

SYNOPSIS

A statement indicating in what respects I consider the contents of this thesis to contribute to the knowledge and practice of medicine.

The epidemiological studies of solar lesions reported in Sections 2 and 3 are the first systematic investigations undertaken in Australia, and one of the few in the world literature. The combination of the high incidence of skin cancer and the uniform centralised method of treatment is unique, and permits systematic study of the frequency of lesions over a wide geographical range. The hospital figures indicate that the incidence increases towards the Equator, but that the increase does not follow a simple relationship with latitude. Reasons for this are discussed in relation to the climatological factors. In calculating the incidence curves a method of estimating the population at risk is used, which gives a better estimate than that based simply on the total population. The significant feature of the incidence curves in relation to experimental pathology, is that their slopes are similar on straight line transformation.

The cane farmers' and graziers' survey provided a population that was documented with respect to the physical characteristics that are normally associated with predisposition to development of solar lesions, or protection against them. These populations were also analysed according to national origin, and as the occupational factor was constant, itwas possible to study the prevalence of skin lesions in homogeneous groups. Significant correlations of prevalence of lesions with latitude were obtained, and so far as I am aware, this type of correlation has not been reported before.

The histochemistry of the ultraviolet tumour in the mouse has not been published by any other worker. It is shown that the spindle cell tumours are, in fact, carcinomas. Histochemical and microscopical evidence is presented that these tumours may be due to clones of cells having undergone a mutation. They lose enzymatic characteristics of normal epidermis, yet retain in an enhanced manner those of an actively metabolising tissue. This concept of enzymatic dedifferentiation has not recieved wide attention, but is germane to the subject of tumour biology.

In the section on the histochemistry of human skin cancer it is shown that collagen degeneration is a simple ageing process, probably accentuated by tropical conditions and infrared radiation.

In Appendix 3 an entirely new histochemical method for demonstrating lipoproteins in fresh tissue, and amino and disulphide groups in fixed tissue is reported, for the most part unpublished. This is the cobalt-MTT-hydroquinone reaction. Various experiments to demonstrate its specificity are described and discussed. In the final section of Appendix 3 the adaptation of this histochemical reaction to quantitative estimation of dehydrogenase reactions in tissue sections is proposed. This could have wide application in estimating cellular metabolic activity.

1. THE EPIDEMIOLOGY OF SKIN CANCER.

1.1 Introduction and historical review.

Cancer of the skin is a widespread disease in Australia, and in the tropical regions reaches very high proportions. It is a serious problem in Queensland, where it must be considered in relation to the adaptability of white populations settling in the tropics.

This high frequency of the disease has been recognised to be due to excessive exposure to solar ultraviolet radiation for many years. In the earlier part of the century both dermatologists and pathologists have commented on this in private and hospital practice (e.g. Molesworth, 1923, 1927; Duhig, 1932). However, apart from occasional papers in the literature such as these, the problem never received much attention, as the disease was considered to be relatively trivial provided treatment was efficiently carried out. During the second decade of this century the question of white man's ability to live and work productively in northern Australia was under more active investigation, as the rate of development of northern Australia was less than that of the whole Commonwealth. The Australian Institute of Tropical Medicine was the centre of physiological research in North Queensland from 1913 to 1920 under the Directorship of Dr. A. Breinl. During this period extensive studies were made to see if there was any serious or permanent physiological impairment that might suggest that white people should not expect to be able to settle satisfactorily in the tropics. Although the results were not as elaborate as they would be nowadays, it is significant that Breinl (1921) and Breinl and Young (1919) stressed that no climatological

factors could be regarded as so severe that, given the right adaptations in housing and clothing, white people should not be able to populate the north.

These authors made no mention of skin cancer being a serious disease, although previously Breinl (1911) had written a paper on keratosis and skin cancer. In this, the lesions described were evidently in a late stage of premalignancy or early malignant degeneration, although they were all called keratosis. It is possible that he did not use the same diagnostic criteria as are used nowadays. He made the surprising statement that keratosis hardly ever occurs among the coastal population, but that it is common in the dry parts. If there is any truth in this, there may well have been a shift in incidence pattern of solar skin lesions in the past forty years. Discussions I have had with long established practitioners in North Queensland have, however, indicated that advanced skin cancer was much more commonly seen than nowadays.

Since the first observations of Findlay in 1928 that ultraviolet radiation could cause malignant tumours in experimental animals, the way has been open for correlating experimental and epidemiological research in a manner that is perhaps more satisfactory than in many other fields of tumour pathology.

The requirements for comparative epidemiology are rather stringent, as population characteristics vary from one country to another. It is necessary that incidence and prevalence rates should only be compared in properly defined and similar populations. The epidemiological study presented in this thesis represents the first

attempt to gather systematic incidence figures for skin cancer in Australia. Overseas the only comparable study is that of Auerbach (1961), who analysed the data for skin cancer given by the Cancer Morbidity Series (1950-2) of the National Cancer Institute. He calculated incidence rates for ten large cities in the U.S.A., and found that the figure doubled for every 3°48' the city was nearer the Equator. Auerbach's (1961) paper will be discussed in more detail in a later section.

Also in the United States, MacDonald (1959) compared the incidence rates for skin cancer between Anglo Americans in Connecticut and El Paso, Texas, and for lesions on particular parts of the body between Anglo and Latin Americans at El Paso. In Africa, Cohen et al (1952) have estimated the incidence of skin cancer in the white population on the basis of the numbers of cases treated at the radiation therapy departments of the Johannesburg group of hospitals. They observe that their data is almost certainly incomplete, but arrive at a figure of 8.05 per 10,000 per annum (my calculation from their data).

It must be pointed out, however, that comparisons between Australia, America and Africa should only be made with caution, as the populations differ considerably in their mode of life. There is no coloured labour in Australia, and no reason to suppose that the population of a North Queensland city is in many respects similar in racial constitution or habits to that of the white population of a large American inland city.

That ultraviolet radiation is a cause of skin cancer is supported by studies showing that country dwellers are more prone

to acquire the disease than city dwellers (reviewed by Blum, 1955) and the anatomical distribution of the lesions has been shown repeatedly to show a marked preference for the exposed surfaces (e.g. Allison, 1957; Belisario, 1959; Ward, 1952).

Previous attempts to equate the incidence of skin cancer with climatological factors have also been made with death rates in populations analysed according to occupation.

Thus, in the United States and Canada, Apperly (1941) found a correlation between increasing skin cancer mortality and proportion of the population engaged in farming, for states with a mean annual temperature between 42 and 62 degrees. In England and Wales, Atkin et al (1949) have shown that more agricultural workers die of skin cancer than professional workers, and have calculated indices of the numbers in certain groups required to produce one death. It takes three times as many professional workers as agricultural workers to produce one death. Conrad and Hill (1939) showed an excess (1.09 : 1) of observed to expected deaths from skin cancer in farmers in the British Isles, a greater excess (1.17 : 1) amongst labourers, but a much greater excess (3 : 1) amongst civil engineers and surveyors.

Although mortality figures are a fairly good guide to the incidence of major and fatal forms of cancer (Armitage and Doll, 1954, 1957), it is quite certain that from skin cancer they can only be misleading. Even squamous cell cancer is of a relatively low malignancy, and consideration of factors related to death from this condition introduces complications that cannot be assessed statistically.

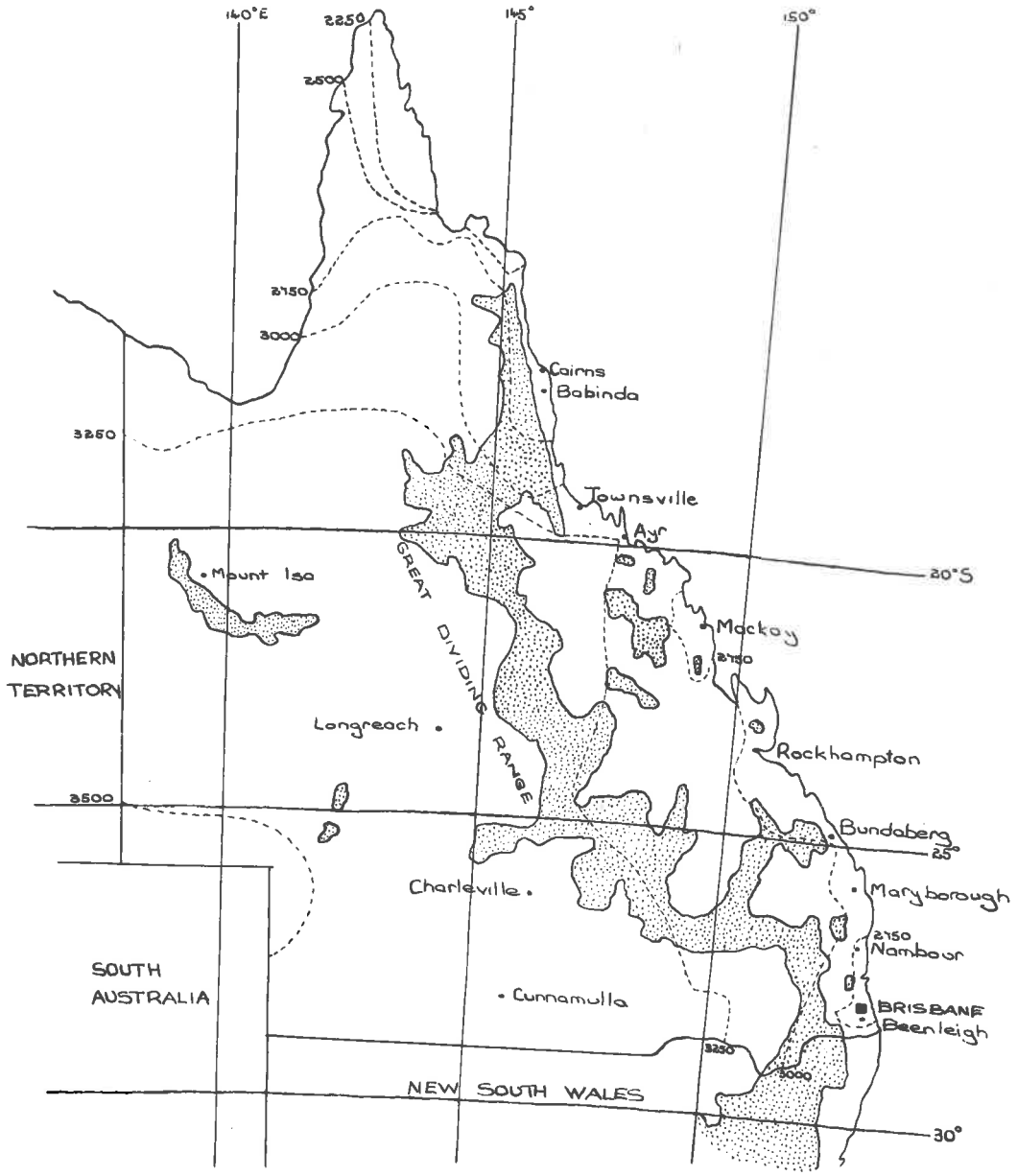


Figure 1.1. Map of Queensland showing survey areas, principal cities and contours, and average annual hours of sunshine.

1.2. Geographical and population features of Queensland.

The State of Queensland covers an area of some 670,000 square miles and lies between latitudes 29 and 11 degrees south. The geographical features are of two main types. The subtropical to tropical humid coastal strip, which includes most of the closely settled areas, has in general a high fertility and a productive primary industry. Cane growing is by far the most important agricultural activity. About half the total population lives in the main coastal cities.

The other main geographical division is the low latitude steppe to the west of the Great Dividing Range, which is dry, arid and has quite marked day to night temperature variations. The country is sparsely settled, except for the towns, and is given over to cattle and sheep grazing. The towns are all small, the largest being Mt. Isa which had a population of 7,400 in 1954. Mt. Isa is a mining town, however, and the people follow a different mode of life from those in the other western towns. The largest of these is Charleville, with a 1954 population of 4,500, and this, like the remainder, is dependent principally on the grazing industry.

A map of Queensland is given in Figure 1.1, showing the main towns and cities, contours and average annual hours of sunshine.

2. ANALYSIS OF HOSPITAL DATA.

2.1. Outline of the survey based on Queensland Radium Institute records.

The plan to be adopted when designing an epidemiological survey depends on the complexity of the disease and its manifestations, the variability of the population, the amount of documented information about the population available and the resources of the investigator. For general guidance in survey methods Yates' "Sampling Methods for Censuses and Surveys" (1953) was consulted, but none of the standard plans was suitable for the project.

The problem was to estimate the incidence of a relatively trivial condition that occurs and recurs with a remarkably high frequency in a white population exposed to a tropical climate. To estimate morbidity figures is a difficult task for most diseases unless the condition is notifiable, as there is usually no systematic collection of cases, and patients may be treated either in private practice or hospital. However, in Queensland the difficulty is overcome as skin cancer patients are referred to and treated by the Queensland Radium Institute, in the large majority of cases.

The information which is required about the populations under study includes the age distribution, length of time of living in Australia, original nationality in the case of immigrants, and occupation. The census figures fulfil most of these requirements.

There are points which are of interest in the incidence of skin cancer that cannot be investigated by analysis of any existing data, so the epidemiological study was carried out in two parts. First the incidence rates were calculated from the records of the

Queensland Radium Institute and, secondly, the prevalence of skin lesions in relation to certain physical characteristics was estimated in a sample type census, conducted in selected well defined rural communities and reported in a later section.

In planning a survey and interpreting the results it is essential that the investigator should be familiar with every stage of the collection of the data, the conditions under which the populations live and work and the reliability of the source material. Under the "Health Acts Amendment Act of 1945" the Queensland Radium Institute has the function of providing radiotherapeutic services for the whole of the State. This it does from the Main Centre in Brisbane and from a series of Sub-centres in the larger country towns of Maryborough, Bundaberg, Rockhampton, Mackay, Townsville and Cairns. The practical result of this is that there is very little private radiotherapy with equipment outside the hospitals. The preference for treatment of skin cancer is overwhelmingly in favour of radiotherapy, which is due, amongst other things, to the great frequency of the disease. Dermatological specialists play only a restricted part in this, and the radiotherapists are often consulted even if the treatment of choice is not primarily radiotherapeutic.

For reasons which will be discussed below, the incidence rates of skin cancer were calculated for Brisbane, Rockhampton, Townsville and Cairns. To get further information about the reference rate of patients to the Queensland Radium Institute, discussions were held with all the general practitioners and the specialists who would be concerned in Rockhampton, Townsville and Cairns. With a few exceptions, it was the custom of the practitioners to

recommend radiotherapy for their cases of skin cancer and more serious hyperkeratosis. They even agreed to assist by taking part in a project by recording specified details of their new cases for a trial period. Many of the practitioners were as good as their word, but it became apparent that the number of cases seen varied, and that those who saw (or reported) most cases were also in the habit of referring them to the Institute. There is a limit to what can be done in this respect and no statistics worth reporting eventuated.

Another factor is that the Institute does not use any form of appointment system, and many patients present themselves without first having been seen by a private practitioner. In addition, it is by no means certain that patients necessarily always consult the same private doctor. Not being able to construct any weighting system to take account of the patients who might be missed by being treated elsewhere, it was decided that the best estimate of the incidence would be that based on the numbers of cases seen at the various centres of the Queensland Radium Institute. These rates should, however, be regarded as minimum.

Both parts of the epidemiological study have been published, the incidence rates in the British Journal of Cancer (Carmichael and Silverstone, 1961; Carmichael, 1961), and the prevalence rates in the rural communities in the Medical Journal of Australia (Carmichael, 1962).

2.2. Collection of the data.

To provide against undue fluctuation in the numbers of cases in each age group in the samples it was felt that only Sub-centre

TABLE 2.1

Details of Survey Areas.

	Latitude in degrees S.	Population, 1954 Census	Average annual sunshine (hours)	Average rainfall	Mean daily temperature	Average index of Mean relative humidity
Brisbane	27°30'	502,320	2850	40.09	69.0°	68
Rockhampton	23°28'	40,670	2925	37.36	73.2°	66
Townsville	19°15'	40,471	2975	43.06	76.0°	70
Cairns	17°0'	21,020	2750	86.35	76.3°	76

cities with large populations should be studied. A preliminary study was carried out in Brisbane to test the method of analysis and to give a guide as to what might be expected in the major systematic study.

This first study consisted of an analysis of all the patients who presented for treatment for the first time in the year 1948. The results were essentially the same as for the later samples, so they will not be considered at present and are given in Appendix 1.

Maryborough, Bundaberg and Mackay were not included in the survey because their populations fell below 20,000 in the 1954 census.

Samples of about 1,000 records were taken from the Main Centre and the three Sub-centres given in Table 2.1. The records of the Queensland Radium Institute are kept alphabetically, separately from the main hospital records, and the classification and filing systems are similar in each place. As most cases receive radiotherapy for skin cancer and many for hyperkeratosis, the nature of the lesion and its exact site are clearly stated in the notes and instructions to the technician operating the X-ray apparatus. An exact record of each patient is thus available.

A systematic sampling method of the records was used. The first record in each sample was chosen on a random basis and thereafter every nth. record was included in the sample. These samples were taken in 1958, so that the decennial period 1948 - 1957 formed

a convenient period of observation of the patients. As Yates (1953, page 29) points out, systematic samples of this nature are not strictly random, but they are unbiased provided the factor n is not so large that some letters of the alphabet have no chance of being represented. The records that commenced prior to 1st January 1948 were not used as it was thought desirable that a period of adjustment should be allowed after the policy of the Radium Institute for the country areas was delineated, after its incorporation in 1945. If an nth. record commenced outside the sampling period it was ignored, and the next record starting within the correct dates was substituted.

The samples as originally collected contained records of patients wherever they lived, but an analysis of the patients from districts outside the metropolitan and Sub-centre cities showed that probably the numbers were not as great as they should have been. As it was not possible to collect collateral information from all the smaller country towns, particularly those far afield, it was decided to restrict the analysis based on the hospital records to the main cities served directly by the various centres of the Institute. The possibility of including the nearer and larger of the outlying towns was explored, and enquiries were made at Gordonvale, Mareeba, Atherton, Herberton and Innisfail in the Cairns district, and at Charters Towers and Ayr in the Townsville district. However, for a number of reasons, including bias in racial distribution, it was considered that studies in the rural areas would be more satisfactory in the sample type censuses reported later.

2.3. Tabulation and classification of the data.

The information in each patient's record was copied out in detail, coded and transferred to Hollerith 80 column punch cards. These were then sorted and classified according to age, sex and address of patient, and type, site and date of lesions. The presence and absence of each type of lesion is given by the symbols in the following classification:

A - Presence of basal cell carcinoma
a - Absence of basal cell carcinoma.

B - Presence of squamous cell carcinoma
b - Absence of squamous cell carcinoma.

C - Presence of hyperkeratosis
c - Absence of hyperkeratosis.

The classification of lesion types and age are given in Tables 2.2 to 2.5, and the lesion types are summarised in Table 2.6.

Lip cancer and malignant melanoma have not been included because surgery claims a greater proportion of cases.

TABLE 2.2

Classification of lesion types in quinquennial age groups.

	Brisbane males.								Brisbane females.							
Age	Abc	ABc	abC	ABc	AbC	aBC	ABC	Total	Abc	aBc	abC	ABc	AbC	aBC	ABC	Total
Age under 20	-	-	1	-	-	-	-	1	1	-	-	-	-	-	-	1
20 - 24	1	-	1	-	-	-	-	2	1	-	3	-	-	-	-	4
25 - 29	2	-	5	-	1	1	-	9	-	-	2	-	-	-	1	3
30 - 34	-	1	9	-	1	2	-	13	2	2	6	-	3	-	-	13
35 - 39	4	4	17	-	6	-	-	31	2	-	15	-	2	-	1	20
40 - 44	9	1	15	-	2	2	2	31	5	-	14	-	3	2	-	24
45 - 49	4	2	14	-	9	5	5	39	3	2	20	-	3	1	-	29
50 - 54	7	2	14	-	2	3	-	28	9	1	8	1	4	1	-	24
55 - 59	10	5	11	1	4	1	4	36	3	-	23	-	6	2	-	34
60 - 64	8	5	13	1	6	4	3	40	3	3	10	1	4	3	-	24
65 - 69	13	4	9	2	10	2	2	42	6	4	13	-	3	2	-	30
70 - 74	2	5	3	-	3	-	2	15	7	4	6	-	6	1	-	24
75 - 79	7	-	8	-	1	3	-	19	8	-	3	-	3	2	-	16
80 - 84	2	-	3	-	1	-	-	6	3	-	4	-	2	-	-	9
85 - 89	4	-	-	1	-	-	-	5	3	-	1	-	2	1	1	8
90 and over	2	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-
Age not stated	2	-	1	-	-	-	-	3	-	-	4	-	-	-	-	4
	77	27	124	5	46	23	13	320	56	16	132	2	43	15	3	267

TABLE 2.3

Classification of lesion types in quinquennial age groups.

	Rockhampton males.								Rockhampton females.							
Age	Abc	aBc	abC	ABc	AbC	aBC	ABC	Total	Abc	aBc	abC	ABc	AbC	aBC	ABC	Total
Under 20	1	-	1	-	-	-	-	2	-	-	-	-	-	-	-	-
20 - 24	1	-	1	-	-	-	-	2	1	-	2	-	-	-	-	3
25 - 29	2	-	7	-	1	-	-	10	1	-	6	-	-	-	-	7
30 - 34	2	1	6	-	4	-	1	14	2	-	9	-	-	-	-	11
35 - 39	4	-	9	-	4	-	-	17	3	-	12	-	2	-	-	17
40 - 44	8	1	21	1	4	1	-	36	4	1	15	-	2	-	-	22
45 - 49	16	3	8	-	4	-	-	31	5	1	13	-	1	-	1	21
50 - 54	8	3	10	-	9	-	-	30	4	1	7	-	5	-	-	17
55 - 59	5	2	8	-	8	2	1	26	8	1	16	1	6	1	1	34
60 - 64	17	-	11	-	7	1	1	37	4	1	4	-	5	-	1	15
65 - 69	10	1	5	-	5	-	2	23	10	1	12	-	5	2	-	30
70 - 74	12	6	11	1	-	1	1	32	3	1	9	-	3	-	-	16
75 - 79	8	1	3	-	4	1	-	17	6	-	7	-	-	-	1	14
80 - 84	5	-	-	3	3	-	1	12	4	-	1	-	4	1	-	10
85 - 89	3	1	1	-	-	-	-	5	1	-	1	-	-	1	-	3
90 and over	1	1	-	-	-	-	-	2	-	1	-	-	-	-	-	1
Age not stated	1	-	1	-	-	-	-	2	-	1	4	-	-	-	-	5
	104	20	103	5	53	6	7	298	56	9	118	1	33	5	4	226

TABLE 2.4

Classification of lesion types in quinquennial age groups.

Townsville males.

Age	Abc	aBC	abC	ABc	AbC	aBC	ABC	Total
Under 20	-	-	2	-	-	-	-	2
20 - 24	1	-	8	-	-	-	-	9
25 - 29	2	1	4	-	3	-	-	10
30 - 34	2	-	12	1	1	-	-	16
35 - 39	5	2	14	-	5	1	2	29
40 - 44	10	1	8	1	8	-	2	30
45 - 49	11	3	12	1	10	-	2	39
50 - 54	9	3	7	2	12	2	3	38
55 - 59	15	4	20	-	5	1	3	48
60 - 64	10	5	3	2	6	3	1	30
65 - 69	8	6	3	2	9	1	-	29
70 - 74	8	1	4	1	2	1	2	19
75 - 79	2	2	1	-	-	1	1	7
80 - 84	1	1	-	-	1	-	1	4
85 - 89	-	-	1	-	-	-	1	2
90 and over	-	-	-	-	-	-	-	-
Age not stated	1	-	2	-	1	-	-	4
	85	29	101	10	63	10	18	316

Townsville females.

Abc	aBc	abC	ABc	AbC	aBC	ABC	Total
-	-	3	-	-	-	-	3
1	-	3	-	-	-	-	4
2	-	4	-	1	-	-	7
3	-	7	-	1	-	-	11
4	-	9	2	2	-	1	18
5	1	12	-	4	1	-	23
3	2	13	-	3	-	1	22
2	1	12	-	6	1	2	24
7	2	18	1	4	1	1	34
3	-	8	-	14	3	-	28
6	-	8	1	4	4	1	24
3	1	5	-	2	-	1	12
2	2	1	-	3	-	-	8
-	-	2	-	2	-	-	6
-	1	-	-	2	-	-	3
-	-	-	-	-	-	-	-
1	-	2	-	1	-	-	4
44	10	107	4	49	10	7	231

TABLE 2.5

Classification of lesion types in quinquennial age groups.

	Cairns males.								Cairns females.							
Age	Abc	aBc	abC	ABc	AbC	aBC	ABC	Total	Abc	aBc	abC	ABc	AbC	aBC	ABC	Total
Under 20	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	2
20 - 24	1	-	4	-	1	-	-	6	-	-	2	-	-	-	-	2
25 - 29	1	-	5	-	1	-	1	8	2	-	2	-	-	-	-	4
30 - 34	7	-	12	-	4	-	1	24	3	-	6	-	2	-	-	11
35 - 39	1	-	9	-	2	-	4	16	-	-	10	-	2	-	-	12
40 - 44	4	4	11	-	-	-	2	21	6	-	15	-	5	-	-	26
45 - 49	10	1	14	-	6	2	2	35	5	-	27	-	5	1	-	38
50 - 54	9	3	11	-	8	1	3	35	3	-	11	-	-	-	1	15
55 - 59	4	2	5	-	6	-	1	18	5	-	11	-	4	-	-	20
60 - 64	3	2	4	-	6	-	-	15	2	1	5	-	2	1	1	12
65 - 69	4	2	3	-	2	-	1	12	5	-	9	-	4	1	-	19
70 - 74	8	2	5	-	5	-	2	22	3	2	5	-	1	-	-	11
75 - 79	2	3	2	-	2	1	-	10	2	-	1	-	-	-	-	3
80 - 84	1	-	-	1	-	-	-	2	1	-	-	-	1	-	-	2
85 - 89	1	1	-	-	1	-	-	3	-	-	-	-	-	-	-	-
90 and over	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Age not stated	2	-	-	-	-	-	-	2	1	-	2	-	-	-	-	3
	58	20	85	1	44	4	17	229	38	3	108	-	26	3	2	180

TABLE 2.6

Summary of lesion classification.

Male. Female.

(The figures in brackets are percentages)

	Brisbane	Rockhampton	Townsville	Cairns	Brisbane	Rockhampton	Townsville	Cairns
Abc	77 (24.1)	104 (34.8)	85 (26.9)	58 (25.3)	56 (21.0)	56 (24.8)	44 (19.0)	38 (21.1)
aBc	27 (8.4)	20 (6.7)	29 (9.2)	20 (8.7)	16 (6.0)	9 (4.0)	10 (4.3)	3 (1.7)
abC	124 (38.8)	103 (34.5)	101 (31.9)	85 (37.1)	132 (49.5)	118 (52.2)	107 (46.3)	108 (60.0)
ABc	5 (1.6)	5 (1.7)	10 (3.2)	1 (0.4)	2 (0.7)	1 (0.4)	4 (1.7)	0 (0.0)
AbC	46 (14.4)	53 (17.8)	63 (19.9)	44 (19.2)	43 (16.1)	33 (14.6)	49 (21.2)	26 (14.5)
aBC	23 (7.2)	6 (2.0)	10 (3.2)	4 (1.7)	15 (5.6)	5 (2.2)	10 (4.3)	3 (1.7)
ABC	18 (5.6)	7 (2.3)	18 (5.7)	17 (7.4)	3 (1.1)	4 (1.8)	7 (3.0)	2 (1.1)
TOTAL	320	298	316	229	267	226	231	180

2.4. Calculation of the incidence rates.

Trial of several models for finding a straight line transformation of the incidence rates was made, and this has been discussed in the published work by Carmichael and Silverstone (1961). The most serviceable method proved to be the logistic transformation, fitting log (P/Q) versus log (age). The system of weights used was proportional to the original sampling data.

The actual rates were calculated from the fraction:

$$\frac{\text{Number of new cases in each decennial age group}}{\text{Number of susceptible persons in corresponding group}}$$

The denominator of this fraction is important, because with a common disease, the incidence rate would be underestimated if the total population was taken to be the population at risk. A susceptible person is one who has not previously suffered from skin cancer. The steps in the calculation of the rates are as follows:

Let W_k = number of new cases in sample in age group k in 10 year period, and g be the raising factor.

Then $g' W_k$ = actual number of new cases per year.

Let N_k = number of persons in age group k, and

$p_k = g' W_k / N_k$, and

$f_k = 10 p_k$, then

$P_k = f_1 + f_2 + \ldots\ldots f_{k-1} + \frac{1}{2} f_k$, and

$Q_k = 1 - P_k$

Q_k is therefore the proportion of susceptibles in age group k, and the annual age specific incidence rate is therefore

$$r = p_k / Q_k$$

As an example of the computation, the calculation of the incidence rates for basal cell carcinoma in the Rockhampton males is given, the columns being computed from the left.

Age	W	g'W	N	p	f	Σf
25	3	0.78	2675	.000291	.002910	.002910
35	6	1.56	2702	.005770	.005770	.008680
45	24	6.24	2509	.002487	.024870	.033550
55	13	3.38	1978	.001708	.017080	.050630
65	27	7.02	1450	.004841	.048410	.099040
75	28	7.28	731	.009958	.099580	.198620

P	Q	P/Q	Y = log P/Q + 3	log x(=log age)
.001455	.998545	.001457	0.16346	1.39794
.005795	.994205	.005828	0.76552	1.54407
.021115	.978885	.021570	1.33384	1.65321
.042090	.957910	.043939	1.64285	1.74036
.074835	.925165	.080888	1.90792	1.81291
.148830	.851170	.174853	2.24298	1.87506

From these figures are computed:

$\Sigma W = 101$, $\Sigma WY = 172.76999$, $\Sigma WXY = 309.01957$, $\Sigma WX = 177.21021$ and $\Sigma WX^2 = 312.41299$. These values are then substituted into the normal equations, and the regression constant and coefficient calculated.

$$a = \frac{(\Sigma WX^2)(\Sigma WY) - (\Sigma WX)(\Sigma WXY)}{(\Sigma W)(\Sigma WX^2) - (\Sigma WX)^2}, \text{ and}$$

$$b = \frac{(\Sigma WXY) - a(\Sigma WX)}{(\Sigma WX^2)}$$

The fitted values, denoted by circumflex accents, are then calculated from the equation to the line

$y = a + bx$, which in this case is

$y = -5.23005 + 3.95578x$

$\hat{Y}$	$\hat{P}/Q$	$\hat{P}$
0.29989	.00199	.00198
0.87795	.00755	.00749
1.30968	.02041	.01999
1.65443	.04513	.04317
1.94142	.08742	.08039
2.18727	.15392	.13350

The quantity b/t is then found, where t is the arithmetical mean age of each decennial group. The fitted value of the incidence rate $\hat{r}$ can then be computed, for comparison with the observed value ($r = p/Q$).

b/t	$\hat{r}\ (= \frac{\hat{P}_2}{t})$	r
.15823	.00031	.00029
.11302	.00085	.00058
.08790	.00176	.00254
.07192	.00310	.00178
.06085	.00489	.00523
.05274	.00704	.01169

The incidence rates have been calculated for (1) the patients who presented with basal cell carcinoma alone, and (2) the total cases less those who had hyperkeratosis only. The first shows the incidence curve in a homogeneous cancer group, whereas the second is calculated from larger numbers and might be considered as a curve showing the total effect of more serious solar lesions. These rates are given in Tables 2.7 and 2.8, and the mean values, standardised on the Rockhampton population, in Tables 2.10 and 2.11. The slope parameters for the straight line logistic transformations are given in Table 2.9. The greater range of values for the regression coefficients of the first group of curves may be attributed to sampling variation, though they are all grouped round a value of 4. The numbers of those with squamous

TABLE 2.7

Annual incidence rates, observed and fitted, of basal cell carcinoma per 10,000 susceptible population.

Males.

Mean age of decennial age group	Brisbane		Rockhampton		Townsville		Cairns	
	r	$\hat{r}$	r	$\hat{r}$	r	$\hat{r}$	r	$\hat{r}$
25	3	2	3	3	5	4	3	4
35	4	6	6	9	11	13	11	10
45	14	13	25	18	41	33	25	20
55	24	24	18	32	61	65	29	39
65	41	40	52	49	66	110	26	54
75	69	60	117	70	129	162	119	77
Females.								
25	1	1	2	2	5	4	3	3
35	4	4	5	4	12	9	5	7
45	9	8	9	9	17	17	21	14
55	16	16	15	16	23	27	20	25
65	14	27	22	25	32	38	27	39
75	70	43	44	37	58	52	59	56

TABLE 2.8

Annual incidence rates of
basal cell and squamous cell carcinoma
per 10,000 susceptible population.

Males.

Mean age of decennial age group	Brisbane		Rockhampton		Townsville		Cairns	
	r	$\hat{r}$	r	$\hat{r}$	r	$\hat{r}$	r	$\hat{r}$
25	5	6	4	5	11	10	8	8
35	17	17	15	15	30	37	27	25
45	45	39	41	34	101	92	57	53
55	60	72	54	62	172	176	91	94
65	133	114	91	100	258	269	85	143
75	118	160	163	142	412	344	329	192
Females.								
25	2	3	2	2	6	6	3	4
35	12	9	6	7	23	20	10	12
45	21	20	15	15	43	45	43	26
55	36	38	36	29	78	83	34	48
65	53	63	46	49	151	132	70	77
75	114	92	45	74	150	182	87	103

TABLE 2.9

Slope parameters for the linear equations of the logistic transformation versus log age.

(i) Basal cell carcinoma alone:

	Male	Female
Brisbane	4.15	4.39
Rockhampton	3.96	3.93
Townsville	4.67	3.40
Cairns	3.90	3.88

(ii) Basal cell and squamous cell carcinoma:

Brisbane	4.31	4.26
Rockhampton	4.31	4.36
Townsville	4.87	4.37
Cairns	4.22	4.16

TABLE 2.10

Weighted standardised mean fitted incidence rates
for basal cell carcinoma
per 10,000 susceptible population.

Male.

	Brisbane	Rockhampton	Townsville	Cairns
	16.9	21.7	44.4	24.9
Ratio	1	1.28	2.63	1.47
Female.				
	11.7	11.4	19.0	17.9
Ratio	1	0.98	1.63	1.53

TABLE 2.11

Weighted standardised mean fitted incidence rates
for basal cell and squamous cell carcinoma
per 10,000 susceptible population.

Male.

	Brisbane	Rockhampton	Townsville	Cairns
	48.5	42.4	111.8	62.7
Ratio	1	0.87	2.30	1.29
Female.				
	27.1	21.0	57.5	33.8
Ratio	1	0.77	2.12	1.24

TABLE 2.12

Observed and fitted values of the cumulative probability, P, used in the logistic transformation for the data of basal cell carcinoma alone.

Males.

Age	Brisbane		Rockhampton		Townsville		Cairns	
	P	$\hat{P}$	P	$\hat{P}$	P	$\hat{P}$	P	$\hat{P}$
25	.00154	.00127	.00146	.00198	.00232	.00207	.00153	.00236
35	.00489	.00511	.00580	.00749	.01016	.00997	.00857	.00874
45	.01347	.01434	.02112	.01999	.03525	.03137	.02622	.02298
55	.03197	.03240	.04209	.04317	.08280	.07640	.05229	.04896
65	.06276	.06269	.07484	.08039	.13897	.15257	.07773	.08993
75	.11226	.10804	.14883	.13338	.21753	.26060	.14074	.14733

Females.

25	.00049	.00063	.00086	.00102	.00241	.00308	.00148	.00169
35	.00280	.00277	.00400	.00382	.01049	.00960	.00521	.00621
45	.00886	.00831	.01071	.01018	.02433	.02225	.01789	.01630
55	.02073	.01983	.02254	.02210	.04368	.04307	.03807	.03484
65	.03528	.04044	.04033	.04174	.06993	.07355	.06048	.06458
75	.07449	.07323	.07113	.07097	.11074	.11433	.09973	.10803

TABLE 2.13

Observed and fitted values of the cumulative probability, P, used in the logistic transformation for combined data of basal and squamous cell carcinoma.

Males.

Age	Brisbane		Rockhampton		Townsville		Cairns	
	P	$\hat{P}$	P	$\hat{P}$	P	$\hat{P}$	P	$\hat{P}$
25	.00259	.00337	.00190	.00290	.00537	.00532	.00379	.00501
35	.01343	.01418	.01138	.01221	.02580	.02674	.02065	.02057
45	.04323	.04073	.03846	.03519	.08663	.08536	.06065	.05646
55	.09187	.09168	.08389	.07944	.20113	.19855	.12706	.12228
65	.17414	.17216	.14632	.15128	.35360	.35941	.20561	.22066
75	.27226	.27823	.24704	.24790	.53334	.52932	.35097	.34107

Females.

25	.00126	.00180	-.00129	.00122	.00319	.00374	.00150	.00255
35	.00755	.00749	.00477	.00527	.01681	.01607	.00823	.01024
45	.02306	.02151	.01525	.01557	.04794	.04659	.03427	.02860
55	.05041	.04911	.03958	.03646	.10320	.10492	.07096	.06333
65	.09187	.09558	.07794	.07296	.19855	.19636	.11766	.11983
75	.16380	.16254	.11935	.12782	.31073	.31321	.18395	.19746

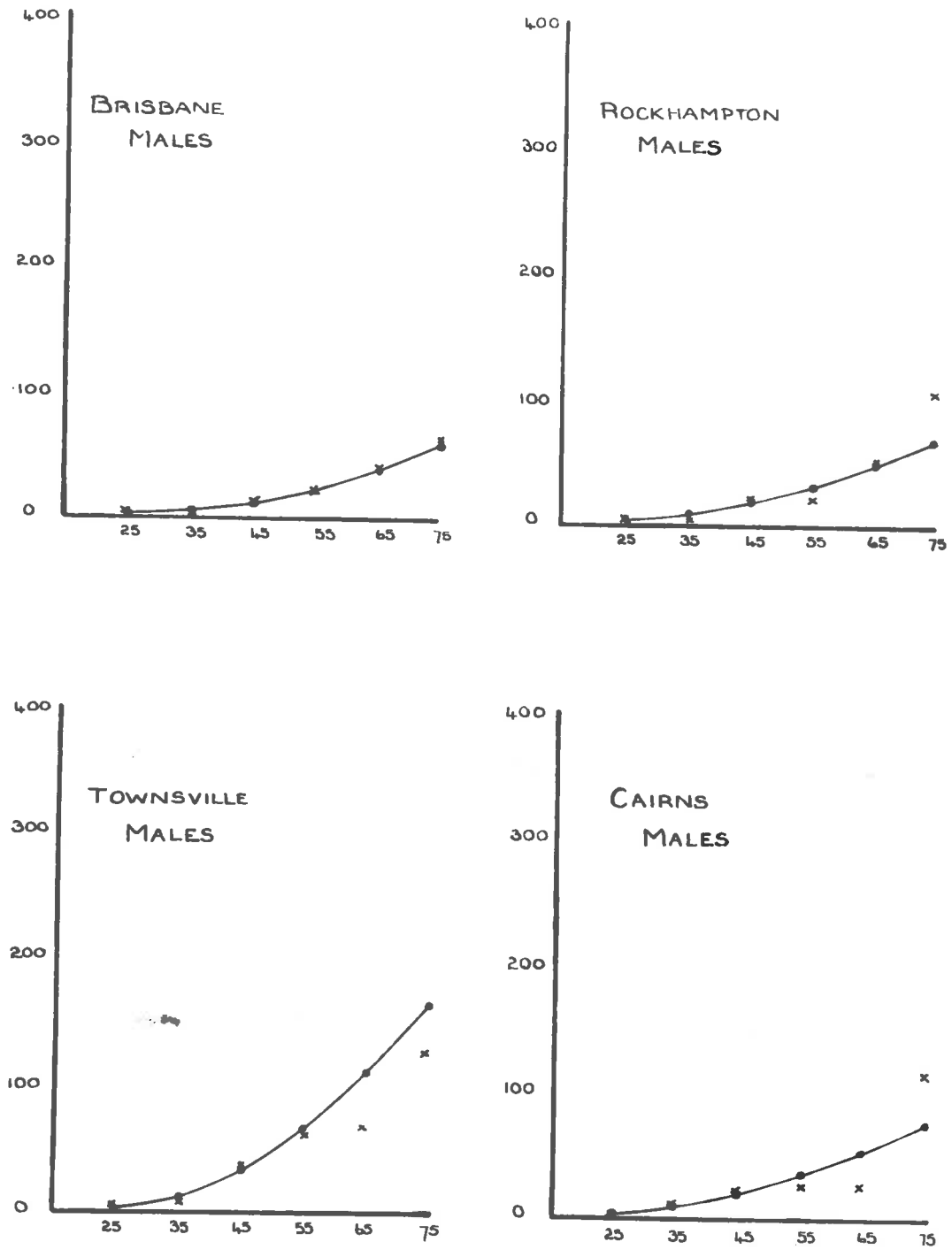


Figure 2.1. Males. Annual incidence rates for basal cell carcinoma per 10,000 susceptible population.

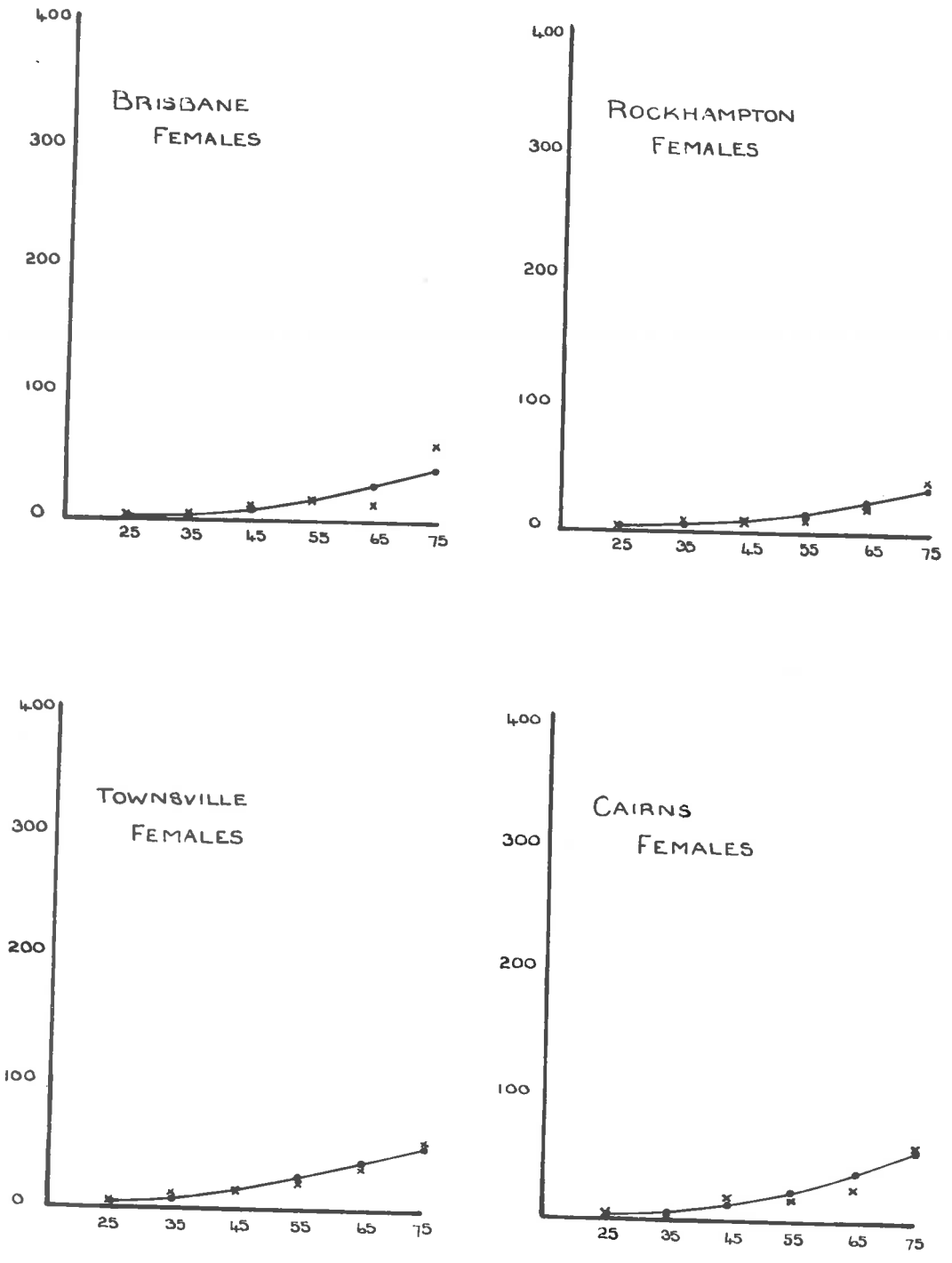


Figure 2.2. Females. Annual incidence rates for basal cell carcinoma per 10,000 susceptible population.

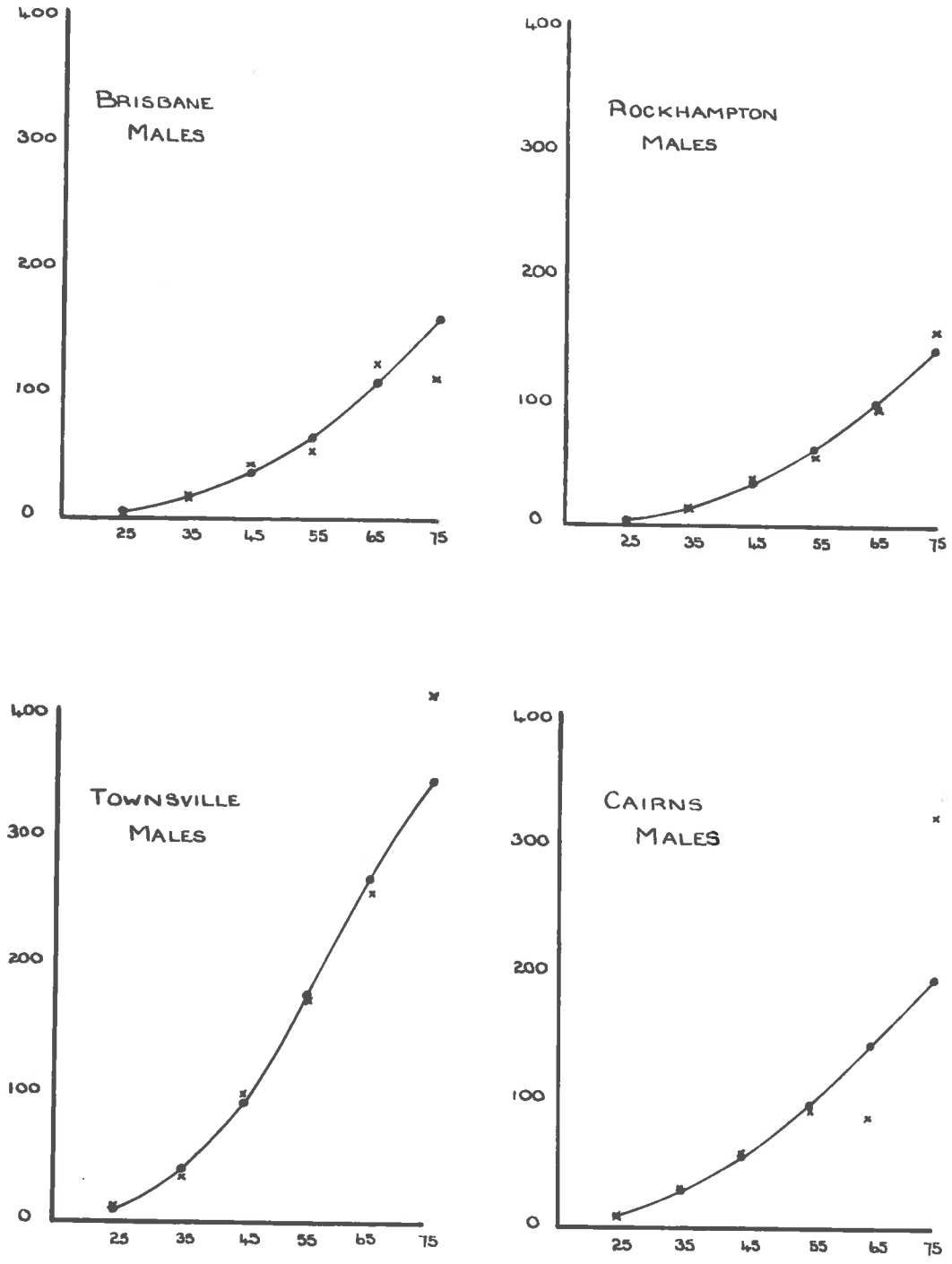


Figure 2.3. Males. Annual incidence rates for basal and squamous cell carcinoma per 10,000 susceptible population.

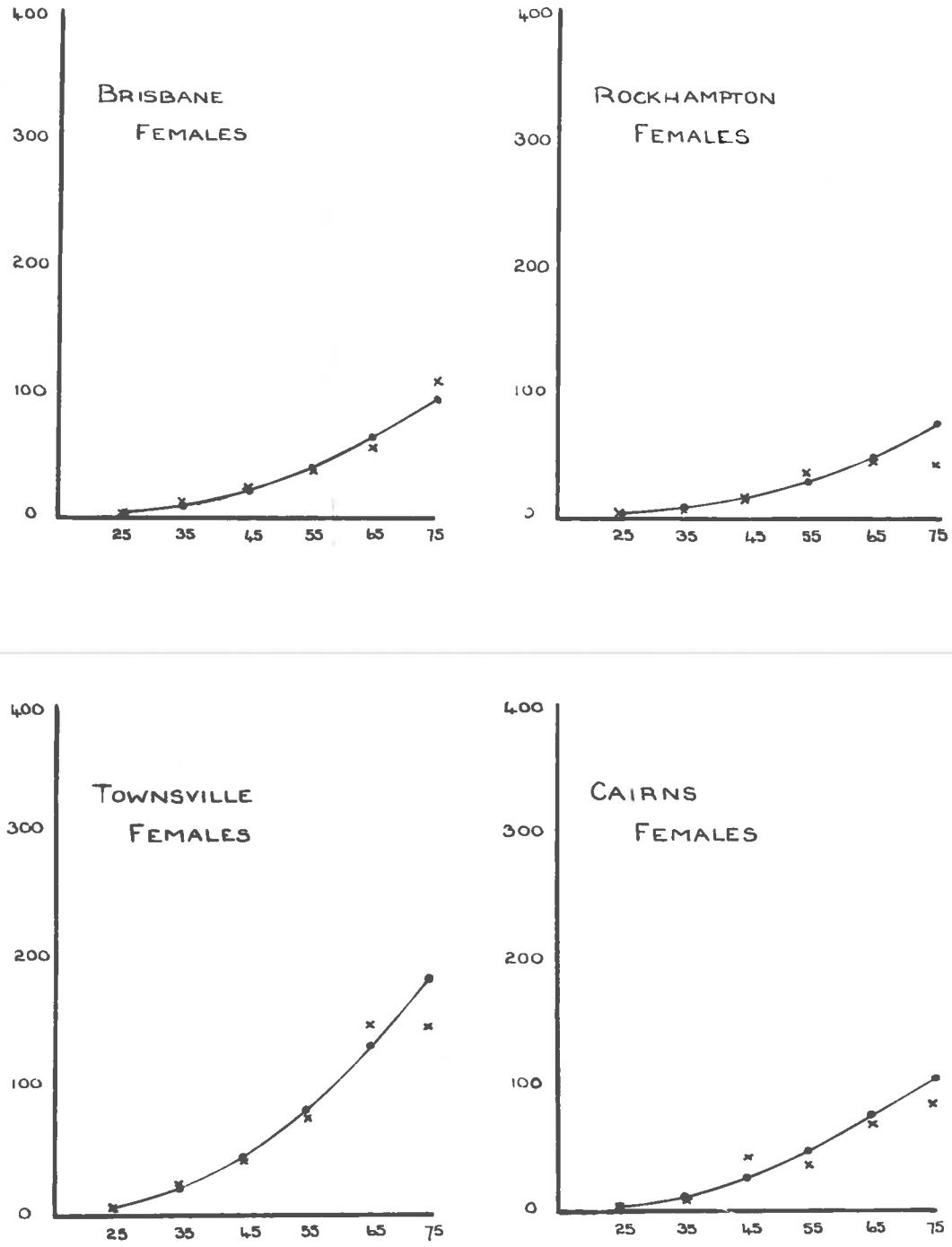


Figure 2.4. Females. Annual incidence rates for basal and squamous cell carcinoma per 10,000 susceptible population.

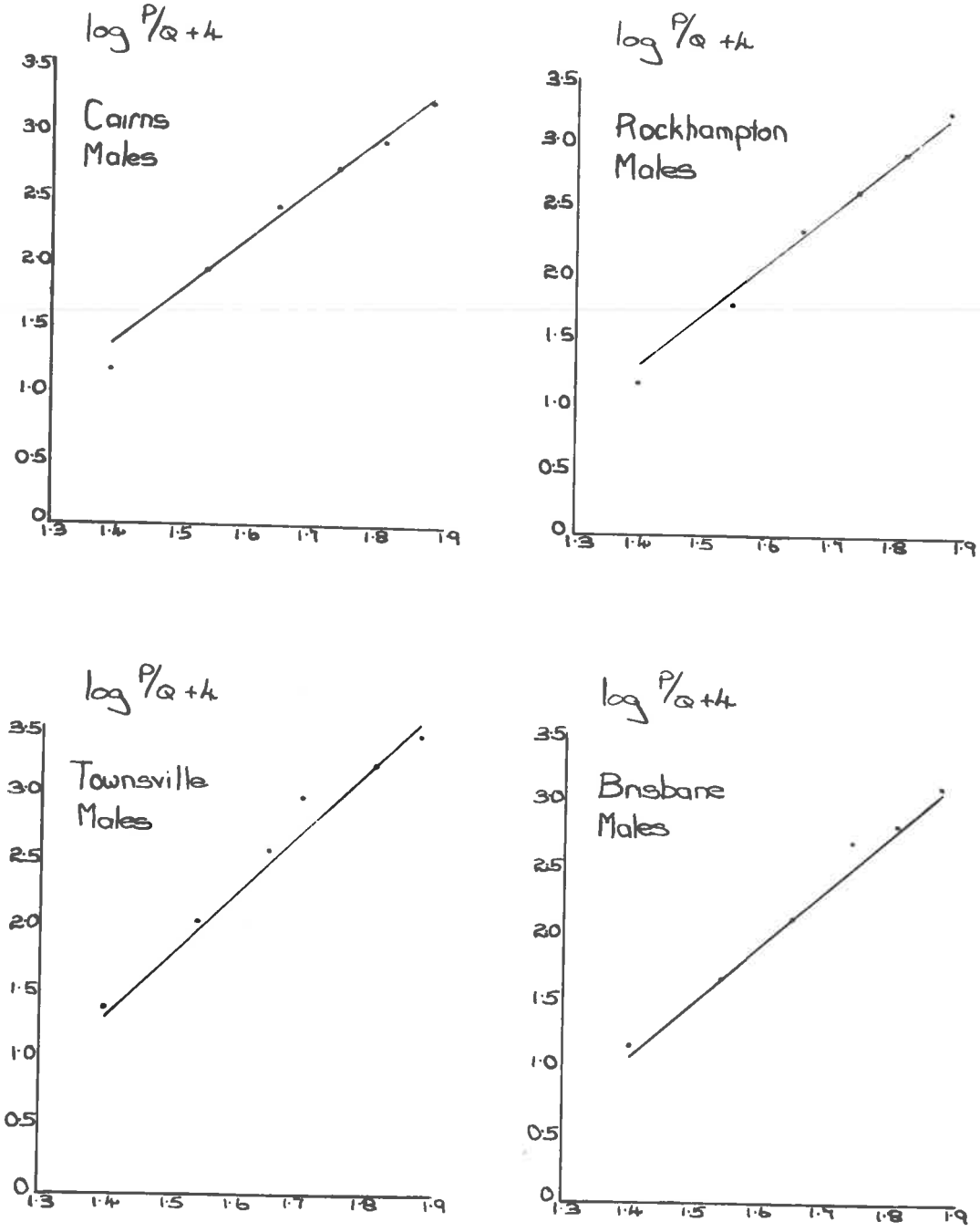


Figure 2.5. Males. Linear logarithmic logistic transformation versus log (age). Basal cell carcinoma.

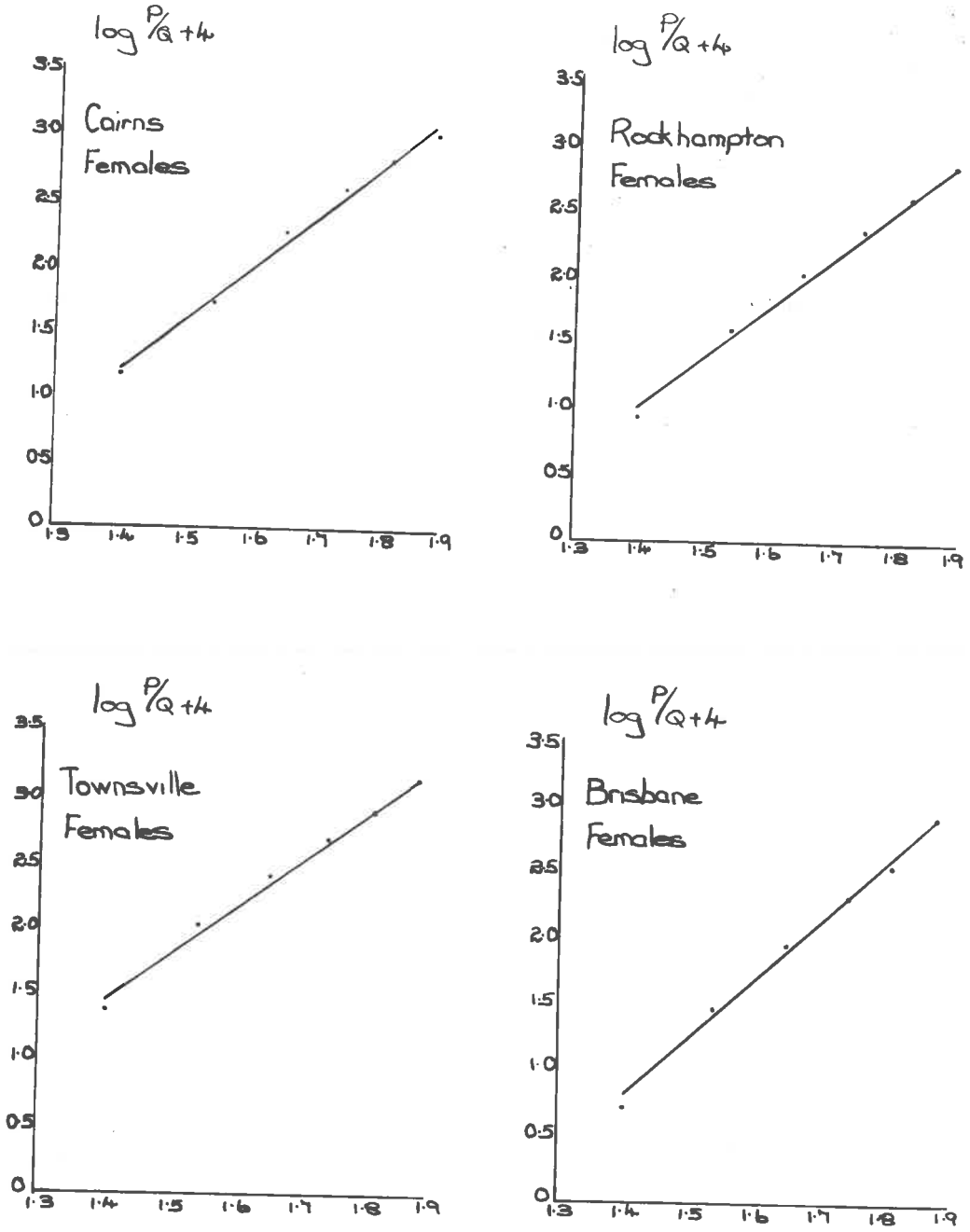


Figure 2.6. Females. Linear logarithmic logistic transformation versus log (age). Basal cell carcinoma alone.

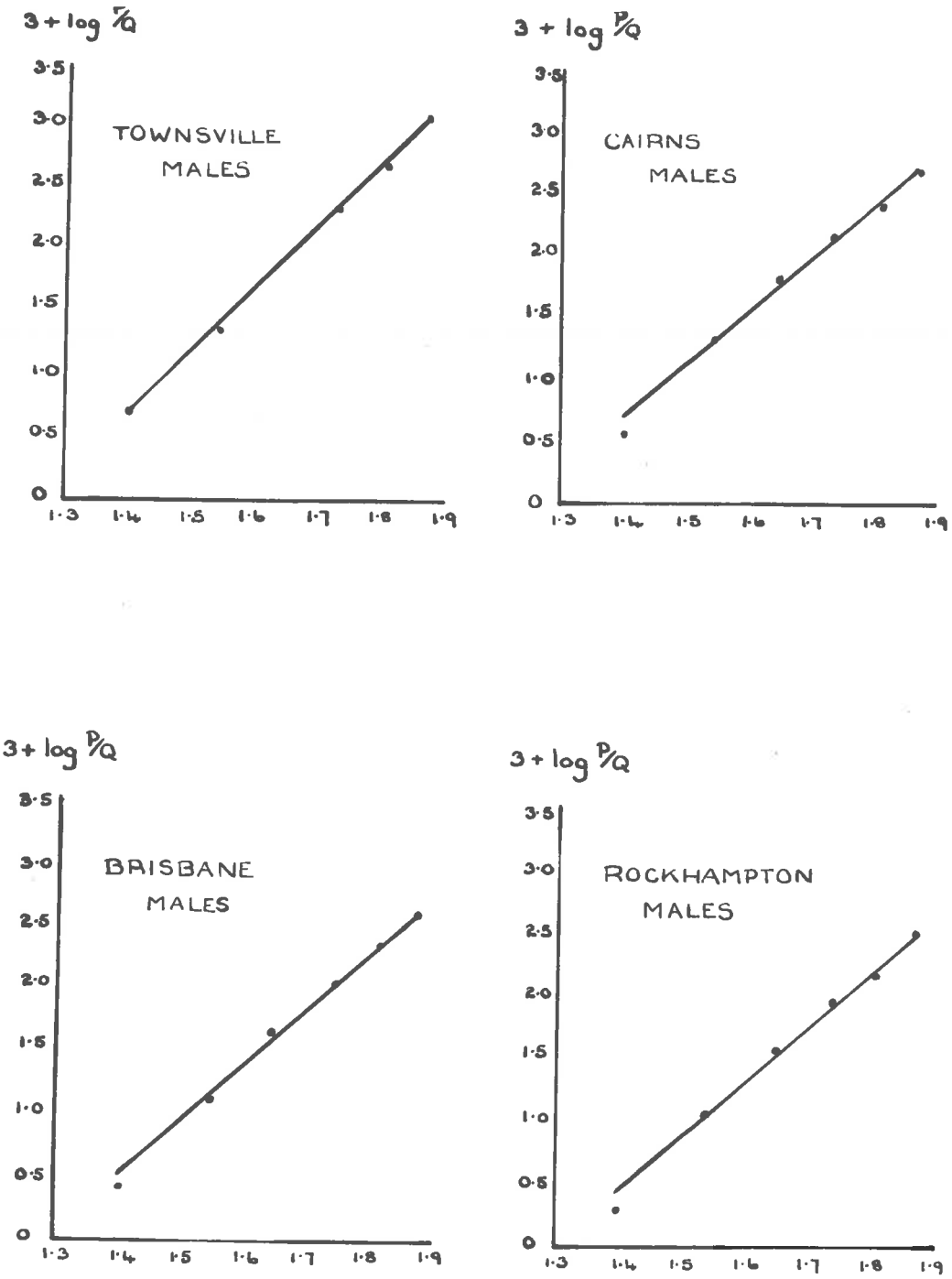


Figure 2.7. Males. Linear logarithmic logistic transformation versus log (age). Basal and squamous cell carcinoma.

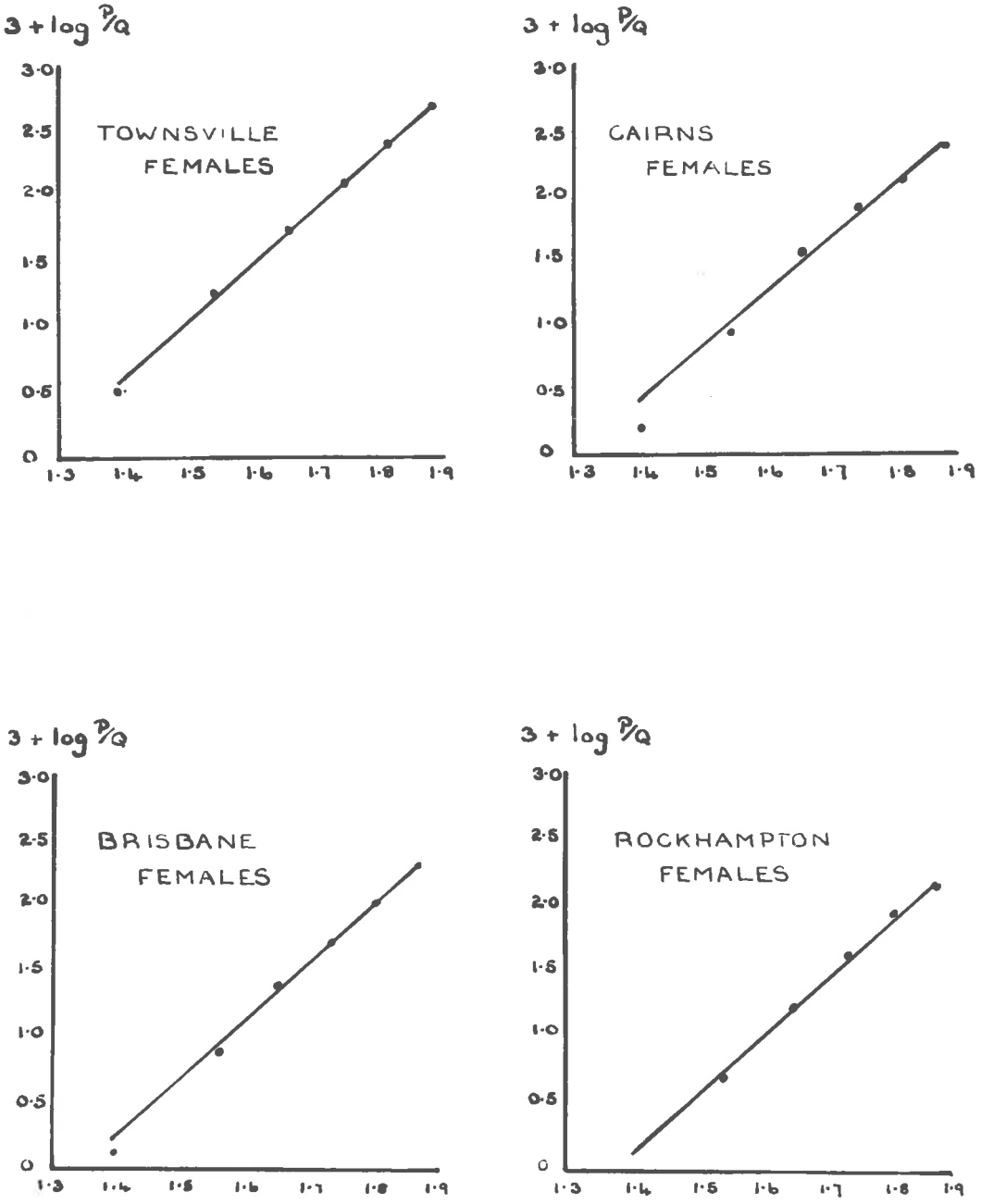


Figure 2.3. Females. Linear logarithmic logistic transformation versus log (age). Basal and squamous cell carcinoma.

cell carcinoma alone are too small to allow reliable curve fitting as there are too many zero classes, but the same slope parameter may be assumed for this group of lesions.

The cumulative probability function used in the logistic transformation is tabulated in Tables 2.12 and 2.13, and the arithmetical incidence rates and logarithmic logistic curves are plotted in Figures 2.1 to 2.8.

It has been shown mathematically by Silverstone (Carmichael and Silverstone, 1962) that there was no significant departure from parallelism in the regression curves between the sexes and between each centre for the combined skin cancer data, and the same appears to hold for the data for B.C.C. alone, except that, as mentioned above, sampling variation has more of an effect.

2.5. The proportions of lesion types and associations between them.

The classification of patients according to lesion type was given in Table 2.6. The frequencies of cases in each classification are indicated by the use of brackets. Except for the Townsville females, (Abc) > (AbC), and all (aBc) ≥ (aBC). However, all (ABC) > (ABc). From this data it is of interest to see whether hyperkeratosis can be considered to be an important premalignant condition.

The largest proportion of patients attended for treatment of hyperkeratosis alone (except the Rockhampton males), and the next largest for basal cell carcinoma alone. Likewise more patients attended on account of squamous cell carcinoma alone than for squamous cell carcinoma and hyperkeratosis. The fact that more patients

TABLE 2.14

Combined data from each of the four cities.

Percentages are shown in brackets.

Classification	Male	Female	Total
Abc	324 (27.9)	194 (21.5)	518 (25.1)
aBc	96 (8.3)	38 (4.2)	134 (6.5)
abC	413 (35.5)	465 (51.4)	878 (42.5)
ABc	21 (1.8)	7 (0.8)	28 (1.4)
AbC	206 (17.7)	151 (16.7)	357 (17.3)
aBC	43 (3.7)	33 (3.7)	76 (3.7)
ABC	60 (5.2)	16 (1.8)	76 (3.7)
TOTAL:	1163	904	2067

have rodent ulcer alone than rodent ulcer and hyperkeratosis is not surprising in view of the histological picture of the two conditions, but it would have been anticipated that of those patients who had squamous cell carcinoma, more would have had hyperkeratosis also. The small proportion of the total who have all three types of lesion may be identified as those who suffer from severe solar dermatitis and develop multiple lesions, who not uncommonly have had several lesions on all exposed surfaces.

The exceptions to the general pattern of the Rockhampton males and Townsville females cannot necessarily be put down to bias in diagnosis, as the opposite sexes in these two places conform to the general pattern.

The amalgamated data for each of the four cities are given in Table 2.14. There appears to be a pattern of negative association or independence between skin cancer and hyperkeratosis. The application of a χ^2 test may not be appropriate because there is no zero class (abc), which is unavoidable in a hospital series. However, with a common condition like hyperkeratosis, which is frequently multiple, and a less common condition like squamous cell carcinoma a greater proportion of patients with both types of lesion would be expected if one gave rise to the other. This has been discussed in the literature (Carmichael, 1961) and the conclusion disputed by Lancaster (1962). His argument starts with a false premise, in that he states that the population was divided into eight classes (sic), although it had been made plain that only the hospital population was under analysis. It would be quite

unthinkable to regard the Queensland Radium Institute patients as a representative sample of the population at large, which appears to be Lancaster's interpretation of the data.

The question of premalignancy is difficult to analyse statistically, because the importance attached to the presence of the condition, and the seriousness of the malignancy, will influence the level of association considered indicative of a precancerous state. Apart from conducting a random sample type census of the population at large, it is not possible to arrive at an unbiased estimate of the zero class. With the possible exception of the accident ward, hospital populations are almost invariably biased in one direction or another (Berkson, 1946).

The essential problem here is what is meant by hyperkeratosis. Hyperkeratotic lesions vary from thin superficial cornified scales, to heaped up lesions that may resemble the early stages of squamous cell carcinoma. The latter may certainly form squamous cell tumours, but the trivial type may be controlled indefinitely by simple treatment. Every clinical worker in this field is familiar with the multiple, even moderately raised, hyperkeratoses on the back of the hands and forearms that are essentially benign in their behaviour.

This is the crux of the matter. Hyperkeratosis is extremely common in Queensland, and the incidence in the general population is grossly underestimated in the Queensland Radium Institute figures. Lancaster's supposition of many thousand for the zero class (which he called X) may not be as true as it appears

TABLE 2.15

The Anatomical Distribution of Lesions in Selected Sites, in Patients who have been treated for only one type of lesion.

Figures in brackets are percentages.

Male.

	B.C.C.	S.C.C.	Keratosis
Forehead and temple	58 (12.1)	5 (4.8)	83 (11.2)
Periorbital region	59 (12.3)	5 (4.8)	28 (3.8)
Malar region	111 (23.1)	6 (5.7)	122 (16.5)
Nose	88 (18.3)	10 (9.5)	130 (17.6)
Ear, anterior surface	20 (4.2)	11 (10.5)	52 (7.0)
Neck	35 (7.3)	7 (6.7)	23 (3.1)
Forearm	14 (2.9)	16 (15.2)	81 (11.0)
Hand	6 (1.2)	18 (17.1)	125 (16.9)
TOTAL	391 (81.4)	78 (74.3)	644 (87.1)

Female.

Forehead and temple	38 (14.8)	5 (12.5)	72 (9.1)
Periorbital region	24 (9.3)	2 (5.0)	32 (4.0)
Malar region	43 (16.7)	8 (20.0)	172 (21.7)
Nose	67 (26.1)	4 (10.0)	197 (24.8)
Ear, anterior surface	4 (1.5)	1 (2.5)	5 (0.6)
Neck	15 (5.8)	3 (7.5)	15 (1.9)
Forearm	7 (2.7)	5 (12.5)	80 (10.1)
Hand	3 (1.2)	8 (20.0)	143 (18.0)
TOTAL	201 (78.1)	36 (90.0)	716 (90.2)

on first inspection. I consider that interpretation of the significance of hyperkeratosis can only be made in the light of the widespread prevalence of this trivial condition.

2.6. Anatomical distribution of lesions.

Some further support for the idea that hyperkeratoses are not an important premalignant condition is derived from the anatomical distribution of the lesions. This is shown in Table 2.15 for those who have had only one type of lesion, and to clarify the picture the sites of low frequency are not included.

Both squamous and basal cell carcinomas occur in some sites that show a relatively low frequency of hyperkeratosis, and hyperkeratosis occurs in some areas that show a low frequency of one or other form of skin cancer. Some sites therefore show a predisposition to develop one or other type of lesion. The anterior surface of the ear, forearm and neck are the sites of a greater proportion of the squamous cell cancers than of the hyperkeratoses. The periorbital region shows a high proportion of the basal cell carcinomas, and a lower and roughly equivalent proportion of the squamous cell carcinomas and hyperkeratoses. The nose and malar regions are sites of a high proportion of the basal cell carcinomas in both the males and females, and also of hyperkeratosis. In these two sites the males show a lower proportion of squamous cell cancers, and the females a low proportion on the nose. The neck accounts for a low proportion of the hyperkeratoses, but a higher proportion of the cancers. These data raise the suggestion that the response of the skin in different sites to the solar stimulus varies.

As these are the first and only lesions the effect of prior treatment cannot be held to influence the distribution, but subsequent treatment renders impossible an observation of their behaviour.

2.7. Occupational factors.

Occupational factors may not be as important in Queensland as in other countries in the incidence of skin cancer. By developing a tan, the outside worker may be, in fact, better protected against the effect of ultraviolet radiation than the indoor worker who runs more of a risk of recurrent sunburn from periodical exposure. Hospital records are notoriously unreliable as regards occupation, and the Queensland Radium Institute records are no exception. The census authorities also do not have much faith in occupation statistics, as was ascertained by discussion with members of the staff of the Deputy Commonwealth Statistician in Brisbane. Their experience was that workers do not always accurately describe their own occupation, so that the occupational analysis of the whole population is not known with any degree of certainty. Because of these considerations an occupational incidence of skin cancer is not attempted.

2.8. Correlation of numbers of lesions with length of time under observation.

Unlike major fatal forms of cancer, skin cancer is a recurrent disease, which is an expression of the effect of continued exposure to ultraviolet radiation. The average number of lesions suffered by patients correlates well, in most cases, with the length

TABLE 2.16

Average numbers of lesions and length of time under observation.

Years in Series	MALE				FEMALE			
	Bne.	R'ton.	T'vle	Cairns	Bne.	R'ton	T'vle	Cairns
10	1.958	2.476	2.560	3.556	2.238	1.750	2.900	2.615
9	2.680	2.400	2.536	2.750	2.000	1.800	2.733	1.928
8	2.219	2.182	3.038	2.429	2.563	2.000	2.533	2.000
7	2.233	1.742	2.484	2.238	1.950	1.682	2.357	2.100
6	1.875	1.781	2.243	2.194	1.321	1.724	2.200	1.368
5	1.893	2.086	1.933	1.682	1.474	1.630	1.947	1.368
4	2.441	1.933	2.222	1.900	2.174	1.529	1.708	1.381
3	1.710	1.452	2.182	1.559	1.607	1.852	1.708	1.357
2	2.065	1.686	1.788	1.375	1.250	1.428	1.409	1.818
1	1.704	1.444	1.742	1.400	1.250	1.571	1.250	1.192

TABLE 2.17

Correlation coefficients of numbers of average lesions and length of time under observation.

	MALE		FEMALE	
	Correlation Coefficient	Significance	Correlation Coefficient	Significance
Brisbane	0.492	Not significant	0.713	Significant at 5 per cent level
Rockhampton	0.869	Significant at 1 per cent level	0.605	Not significant
Townsville	0.825	Significant at 1 per cent level	0.996	Significant at less than 1 per cent level
Cairns	0.937	Significant at less than 1 per cent level	0.767	Significant at 1 per cent level

of time under observation. These results are tabulated in Table 2.16 and the correlation coefficients in Table 2.17. The two northern centres show a high level of significance for both males and females, but no obvious explanation for the lack of significance in the Brisbane males and Rockhampton females can be offered.

3. CANE FARMERS AND GRAZIERS SURVEY.

3.1. Introduction and collection of data.

This survey was executed to provide some data of importance in the aetiology of skin cancer and solar keratosis that could not be gained from existing statistics and census figures. The purpose was to study populations that were circumscribed and homogeneous as far as possible, and in which the occupational factor was held constant. In this part of the epidemiology the prevalence, rather than the incidence, is under investigation. Prevalence refers to the number or proportion of individuals who have, or have had lesions at the time of the survey, whereas the incidence is related to the numbers with lesions in a specific time interval.

Before deciding to concentrate on the cane farmers and graziers alone, trials were made with several other groups of workers. It was thought that useful comparisons could be made between the waterside workers in each of the main Queensland ports. With the full co-operation and assistance of the Australian Stevedoring Industry Authority and the Waterside Workers' Federation, a large number of wharf labourers were personally interviewed and examined at Cairns and Townsville. It became apparent, however, that it was quite impossible to interview them all or to get an unbiased sample. The analysis of these field studies is therefore not presented, except to record that skin cancers were very common and that hyperkeratoses, particularly of the forearms, were practically universal.

Exploratory investigations were also made with the abattoir workers at Townsville and Rockhampton, but in this case a

problem was that the work was seasonal, and that occupations varied during the slack season. Many of the workers go south. Although management and unions were helpful and co-operative, further work was not carried out because of the uncertainties.

The possibility of extending the studies to the maize growers of the Atherton Tableland was looked into, and some field work was carried out, but apart from comparison between altitudes with the coastal farmers, they had little to offer by way of comparative prevalence rates. The national groups were represented in different proportions, and comparisons between these would have involved small numbers.

The cane farmers and graziers were clearly the most suitable for survey purposes. The cane farmers form a group of closely settled farmers who do virtually the same work throughout most of the fertile coastal plains. Except for two areas where irrigation is practised, rainfall is the only source of moisture. This is known as dry farming, and irrigation type as wet farming. The latter form of farming may involve slightly different sun exposure habits, as the farmer systematically goes over his property channelling water into furrows between the rows of cane. He may, therefore, be exposed to extra sunshine during the growing season. Wet farming is carried out in the Ayr and parts of the Bundaberg districts.

The cane farmers are all members of the Queensland Cane Growers' Council, which supervises the interests of the whole industry. The cane growing districts are subdivided into mill supply areas, if there is more than one mill in the district.

The Council provided up to date lists of the farmers in particular selected mill supply areas, supplied all the necessary local information and assisted considerably with the publicity.

The populations for the graziers' survey were obtained from the Australian Pastoral Directory (1956), and lists were made of the property owners who appeared to live and work on the stations. Some assistance was obtained from the United Graziers' Association and the Graziers' Association of Central and Northern Queensland, but these organisations do not have such extensive administrative control as the Cane Growers' Council, and membership is not compulsory.

Publicity for the project was obtained through the news letters of the cane growers' and graziers' organisations, "Queensland Country Life", the local press and country editions of the Brisbane "Courier-Mail". A radio broadcast was arranged through the courtesy of the Australian Broadcasting Commission. Assistance was also given by the local secretaries of the cane growers' and graziers' organisations. The effects of this publicity, together with the knowledge and interest of the Queensland people regarding skin cancer, are reflected in the very high level of response, particularly from the cane growers. As a preliminary to the cane farmers' survey field work was carried out in the Cairns district to test the standard of knowledge of skin cancer and solar keratosis, and this was sufficiently reliable to justify the rest of the survey.

The survey was conducted by post. A form, explanatory letter and business reply paid envelope were sent to each candidate for the survey and his wife. About half the cane farmers and rather

less of the graziers replied to this first application. When the number of returns dwindled, a second form, letter and envelope were sent out. This resulted in a reply from a further 25 per cent of the cane farmers. A third application brought the level of response up to that shown in Table 3.1. The letter explained the purpose of the survey and assured the recipient that all details would be treated as confidential, and that it would not be possible to recognise any individual in published results. The business return envelope was addressed to Dr. G. G. Carmichael, and was also marked "Confidential".

The details requested of each candidate and his wife were: age, occupations previous to cane growing or grazing, place of birth, place of parents' birth, whether now or in the past affected by skin cancer or sunspots, hair colour (before going bald or grey), eye colour, complexion, reaction to the sun and familial incidence of skin cancer and sunspots. Skin cancer and solar keratoses were treated as one entity, as it was felt that distinction between the two would be unreliable and that uncertainty on the part of those surveyed might militate against achieving as good a return as possible.

The cane farmers at Ayr were interviewed by an Officer of the Cane Growers' Council, and not surveyed by post, so that the lower proportion of response in that district does not imply any less willingness to co-operate than in other districts.

The areas to be surveyed were chosen to give as wide a geographical representation as possible. Where there was a choice of mill supply area, various local factors were taken into consideration, and the area chosen was the one that seemed to have

TABLE 3.1

Mill Supply Area	Latitude °S	Total Males surveyed	Number of responders	Percentage	Number of female responders
Beenleigh	27°52'	135	115	85.2	107
Nambour	26°31'	263	224	85.2	196
Bundaberg(Bingera)	24°40'	337	295	87.5	253
Mackay (Farleigh)	21°6'	309	266	86.1	229
Ayr (Kalamia)	19°36'	199	141	70.6	112
Babinda	17°30'	275	234	85.1	198
Mossman	16°38'	183	162	88.5	133
TOTAL:		1701	1437	84.5	1228
Grazing Area					
Cunnamulla	28°0'	79	56	70.9	49
Longreach	23°29'	79	50	63.3	48
Rockhampton	23°28'	102	83	81.4	70
Cloncurry	20°38'	63	36	57.1	27

TABLE 3.2

	Beenleigh	Babinda
Neglect and no good reason	4	6
Illiterate, infirm or otherwise unable	2	2
Unco-operative	2	-
Valid reason and misunderstanding	1	1
	9	9

TABLE 3.3

The prevalence of skin lesions amongst the cane farmers.

Males

	B'leigh	Nambour	B'berg	Mackay	Ayr	Babinda	Mossman	TOTAL
Total Responders	115	224	295	266	141	234	162	1437
Number with lesions	10	44	43	38	21	30	21	207
Per Cent	8.7	19.6	14.6	14.3	14.9	12.8	13.0	14.4
Females								
Total Responders	107	196	253	229	112	198	133	1228
Number with lesions	4	19	21	6	6	11	11	78
Per Cent	3.7	9.7	8.3	2.6	5.4	5.6	8.3	6.4

TABLE 3.4

The prevalence of skin lesions amongst the graziers.

Males

	Cunnamulla	L'reach	R'ton	Cloncurry	TOTAL
Total Responders	56	50	83	36	225
No. with lesions	20	15	30	11	76
Per Cent.	35.7	30.0	36.1	30.6	33.8
Females					
Total Responders	49	48	70	27	194
No. with lesions	7	6	10	3	26
Per Cent.	14.3	12.5	14.3	11.1	13.4

the best potentiality for a full response.

The districts surveyed, latitude and proportion of response are shown in Table 3.1. It was not known how many of the cane farmers or graziers were married, but the women co-operated as readily as the men, so the proportionate response should probably be as high as that for the men.

As there is no cane growing for some distance north and south of Rockhampton, this group of graziers was substituted into the general scheme although the latitude is practically the same as that of Longreach.

The reasons for non-response were sought in two further small random samples from the Beenleigh and Babinda districts. The results are given in Table 3.2.

With regard to non-responders, compensation may be made either by considering them all to have or not to have skin lesions, and adjusting the prevalence rates accordingly, or by ignoring the contribution they make and considering them to be a random sample of the whole. As the non-responders did not show any bias relative to the proportion showing skin lesions, the latter course has been adopted. The prevalence is therefore considered in the total numbers of responders.

The numbers with lesions are shown in Tables 3.3 and 3.4.

3.2. <u>The prevalence of skin lesions in different racial groups.</u>

The cane farmers and graziers were assigned to their racial or ethnic groups on the basis of their birth-place or parents' birth-place. Those who gave Australia or Great Britain as their

and their parents' country of birth were classified as British. All the rest were classified according to their parents' country of birth, unless they themselves were born overseas. In the few cases of mixed descent, the national group was taken to be that of the non-British parent. The largest non-British groups were the Italians and Germans. That this is a reliable method of classification is substantiated by Borrie's (1954) book, "Italians and Germans in Australia". Italians migrated to Queensland in large numbers between 1921 and 1933, and many of them took up land in the cane growing districts. They were considered good financial risks by the banks during the depression years, and were encouraged to develop the cane growing industry. The pattern of migration was that the farmer arrived first and established himself economically, and then either went back to Italy to get married and bring his wife out or arranged for his fiancée to travel to Australia. There was little intermarriage between Australians and Italians. The present Italian cane growers are therefore either the original settlers or their immediate offspring.

Germans have settled in the agricultural areas of Australia since the latter part of the last century. They tended to congregate in certain areas, which in Queensland included the Beenleigh and Bundaberg districts. Going by the parents' birth-place probably accounts for the majority of the Germans, but any second generation farmers of German descent would probably be classified as British. The Beenleigh district shows the highest proportion of those of German descent, and the Bundaberg district higher than the remainder.

There is little detailed information about the other immigrant groups, though it seems very probable that they arrived at the same time as, or after the Italians.

The national origins of all the cane farmers are given in Tables 3.5 and 3.6, and of those with skin lesions in Tables 3.7 and 3.8.

The percentages with skin lesions of the total cane farmers do not show any significant trend with latitude (Table 3.3), in either the males or females. The prevalence rates for the different national groups are summarised in Table 3.9, and again there is no clear-cut correlation between prevalence and diminishing latitude. The British males approach a correlation better than the other groups, but even in this case the correlation coefficient is only 0.664 ($P > 0.1$, not significant).

The mean ages of the cane growers with and without lesions are given in Tables 3.10 and 3.11. This lack of correlation between prevalence and latitude is apparently not due to inhomogeneity regarding age distribution. There is likewise no tendency for the subjects with skin lesions to have a lower mean age towards the north.

The graziers were all of British descent, and their prevalence rates are approximately a third for each area surveyed (Table 3.4). From this data there is no evidence of either a latitude or longitude effect influencing the frequency of skin lesions amongst the graziers. The age distributions are given in Tables 3.12 and 3.13. The large standard errors in some cases are

TABLE 3.5

Male Cane Farmers classified according to national origin.

National origin	Beenleigh	Nambour	Bundaberg	Mackay	Ayr	Babinda	Mossman	TOTAL
British	78(67.8)	212(94.6)	235(79.7)	174(65.4)	73(51.8)	99(42.3)	92(56.8)	963(67.0)
Italian	1(0.9)	-	12(4.1)	41(15.1)	40(28.4)	96(41.0)	55(33.9)	245(17.0)
German	31(26.9)	4(1.8)	22(7.5)	2(0.8)	3(2.1)	7(3.0)	3(1.9)	72(5.0)
Scandinavian	-	6(2.7)	14(4.7)	5(1.9)	1(0.7)	2(0.9)	2(1.2)	30(2.1)
Dutch	2(1.7)	-	1(0.3)	-	-	-	-	3(0.2)
Greek	-	-	-	2(0.8)	4(2.8)	7(3.0)	1(0.6)	14(1.0)
Polish	1(0.9)	1(0.4)	3(1.0)	1(0.4)	-	-	-	6(0.4)
Jugoslav	-	-	1(0.3)	-	1(0.7)	6(2.6)	7(4.3)	15(1.0)
Maltese	-	-	-	39(14.7)	-	11(4.7)	-	50(3.5)
French	-	-	2(0.7)	-	-	-	-	2(0.1)
Spanish	-	-	-	-	14(9.9)	2(0.9)	-	16(1.1)
Miscellaneous and ill defined	2(1.7)	1(0.4)	5(1.7)	2(0.8)	5(3.5)	4(1.8)	2(1.2)	21(1.5)
TOTAL:	115	224	295	266	141	234	162	1437

The figures in brackets in this and succeeding tables of national origin are percentages.

TABLE 3.6

Wives of Cane Farmers classified according to national origin.

National Origin	Beenleigh	Nambour	Bundaberg	Mackay	Ayr	Babinda	Moseman	TOTAL
British	83(77.6)	182(92.8)	211(83.3)	151(66.0)	71(63.4)	101(51.0)	78(58.7	877(71.4)
Italian	1(0.9)	-	7(2.8)	19(8.3)	25(22.3)	79(39.9)	39(29.3)	170(13.8)
German	15(14.0)	6(3.1)	14(5.5)	6(2.6)	1(0.9)	1(0.5)	3(2.3)	46(3.7)
Scandinavian	2(1.9)	5(2.6)	14(5.5)	3(1.3)	4(3.6)	1(0.5)	1(0.8)	30(2.4)
Dutch	1(0.9)	-	1(0.4)	2(0.9)	-	-	1(0.8)	5(0.4)
Greek	-	-	-	1(0.4)	3(2.7)	7(3.5)	-	11(0.9)
Polish	-	1(0.5)	-	-	-	-	-	1(0.1)
Jugoslav	-	-	1(0.4)	-	-	1(0.5)	7(5.3)	9(0.7)
Maltese	-	-	-	41(17.9)	-	4(2.0)	-	45(3.7)
French	1(0.9)	1(0.5)	3(1.2)	-	2(1.8)	-	-	7(0.6)
Spanish	-	-	-	1(0.4)	3(2.7)	2(1.0)	1(0.8)	7(0.6)
Miscellaneous and ill-defined	4(3.7)	1(0.5)	2(0.8)	5(2.0)	3(2.7)	2(1.0)	3(2.3)	20(1.6)
TOTAL:	107	196	253	229	112	198	133	1228

TABLE 3.7

Male Cane Farmers with Skin Cancer or Hyperkeratosis classified according to national origin.

National origin	Beenleigh	Nambour	Bundaberg	Mackay	Ayr	Babinda	Mossman	TOTAL
British	5(50.0)	43(97.7)	34(79.1)	35(92.1)	18(85.7)	22(73.3)	17(81.0)	174(84.0)
Italian	-	-	-	1(2.6)	1(4.8)	4(13.3)	3(14.3)	9(4.3)
German	4(40.0)	1(2.3)	5(11.6)	1(2.6)	-	2(6.7)	-	13(6.3)
Scandinavian	-	-	3(7.0)	1(2.6)	-	-	1(4.8)	5(2.4)
Greek	-	-	-	-	1(4.8)	1(3.3)	-	2(1.0)
Maltese	-	-	-	-	-	1(3.3)	-	1(0.5)
Miscellaneous and ill-defined	1(10.0)	-	1(2.3)	-	1(4.8)	-	-	3(1.4)
TOTAL:	10	44	43	38	21	30	21	207

TABLE 3.8

Wives of Cane Farmers with Skin Cancer or Hyperkeratosis classified according to national origin.

National origin	Beenleigh	Nambour	Bundaberg	Mackay	Ayr	Babinda	Mossman	TOTAL
British	3(75.0)	18(94.7)	19(90.5)	5(83.3)	5(83.3)	8(72.7)	10(90.9)	68(87.2)
Italian	1(25.0)	-	-	-	-	2(18.2)	-	3(3.8)
German	-	1(5.3)	1(4.8)	-	-	-	-	2(2.6)
Scandinavian	-	-	1 (4.8)	1(16.7)	1(16.7)	-	1(9.1)	4(5.1)
Maltese	-	-	-	-	-	1(9.1)	-	1(1.3)
TOTAL:	4	19	21	6	6	11	11	78

TABLE 3.9

Prevalence rates and percentages of cane farmers and their wives with skin lesions in main national groups.

Males

	Beenleigh	Bundaberg	Mackay	Ayr	Babinda	Mossman
British	5/78 6.4	43/212 20.3	34/235 14.5	35/174 20.1	18/73 24.7	22/99 22.2	17/92 18.5
Italian	-	-	-	1/41 2.4	1/40 2.5	4/96 4.2	5/55 9.1
Remainder	5/36 13.8	1/12 8.3	9/48 18.7	2/51 3.9	2/28 7.1	4/39 10.3	1/15 6.7

Females

British	3/83 3.6	18/182 9.9	19/211 9.0	5/151 3.3	5/72 7.0	8/101 7.9	10/78 12.8
Italian	1/1 (100)	-	-	-	-	2/79 2.5	-
Remainder	-	1/14 7.1	2/35 5.7	1/59 1.7	1/16 6.3	1/18 5.6	1/16 6.3

TABLE 3.10

Mean ages and standard errors of the total Cane Farmers.

	Male	Female
Beenleigh	45.6 ± 1.12	42.05 ± 1.16
Nambour	46.78 ± 0.78	43.06 ± 0.77
Bundaberg	45.38 ± 0.70	41.60 ± 0.69
Mackay	45.13 ± 0.76	40.83 ± 0.74
Ayr	44.30 ± 0.96	40.29 ± 1.08
Babinda	45.39 ± 0.83	39.93 ± 0.82
Mossman	46.81 ± 1.15	42.89 ± 1.21

TABLE 3.11

Mean ages and standard errors of the Cane Farmers with skin lesions.

	Male	Female
Beenleigh	50.60 ± 3.99	50.25 ± 1.12
Nambour	49.44 ± 1.91	47.32 ± 2.64
Bundaberg	53.02 ± 1.60	42.38 ± 1.94
Mackay	48.56 ± 2.21	48.13 ± 3.55
Ayr	52.52 ± 2.99	42.00 ± 5.35
Babinda	47.34 ± 2.47	42.90 ± 2.54
Mossman	51.10 ± 3.25	49.73 ± 4.36

TABLE 3.12

Mean ages and standard errors of the Graziers.

	Male	Female
Cunnamulla	46.14 ± 1.44	43.66 ± 1.96
Longreach	47.92 ± 2.17	43.58 ± 1.93
Rockhampton	55.06 ± 1.29	44.89 ± 1.23
Cloncurry	48.66 ± 2.44	44.96 ± 2.42

TABLE 3.13

Mean ages and standard errors of Graziers with skin lesions.

	Male	Female
Cunnamulla	45.80 ± 2.97	46.71 ± 3.78
Longreach	51.38 ± 3.49	41.00 ± 3.03
Rockhampton	54.90 ± 3.19	51.40 ± 3.23
Cloncurry	56.82 ± 3.63	49.67 ± 12.05

due to very small numbers, and are only inserted for completeness.

3.3. Analysis of prevalence of lesions in relation to physical characteristics.

Pursuing the analysis with the British male cane farmers, trends, in some cases significant, are obtained when the prevalence is considered versus latitude in sub-groups of different physical characteristics. The data for hair colour, eye colour, complexion and ability to tan are given in Tables 3.14 - 3.17. Plus and minus signs refer respectively to presence and absence of lesions.

Inspection of the tables shows that there is a positive association between red hair, fair complexion and inability to tan and the presence of skin lesions. These associations may conveniently be summarised in the coefficient of correlation (Yule and Kendall, 1950) -

$$Q = \frac{(AB)(\alpha\beta) - (A\beta)(\alpha B)}{(AB)(\alpha\beta) + (A\beta)(\alpha B)}$$

with standard error

$$\sigma_Q = \frac{1 - Q^2}{2}\sqrt{\frac{1}{AB} + \frac{1}{A\beta} + \frac{1}{\alpha B} + \frac{1}{\alpha\beta}}$$

The symbols B and β refer to the presence or absence of skin lesions, and A or α represent presence or absence of particular physical characteristics. Brackets indicate frequencies.

These results are given in Table 3.18. When there is no association Q = 0, and it becomes positive or negative for association or dissociation respectively. Taking a deviation from zero by Q of more than three times the standard error as significant, red hair, fair complexion and inability to tan are the only characteristics positively associated with skin lesions. Conversely a dark

and sallow complexion, and ability to tan confer protection against skin lesions.

The principal factor in the development of lesions, on general and clinical grounds, is the reaction to the sun. Correlations with diminishing latitude are set out in Table 3.19.

All the red haired burn in the sun, but the numbers in this sub-group are too small for calculation of the correlation coefficient. When the red and fair haired groups are amalgamated, the coefficient of correlation does not reach a 5 per cent level of significance, and this doubtless reflects the independence between fair hair and the development of lesions. Although in the total data fair complexion is associated positively with lesions, a 5 per cent significant correlation with diminishing latitude is not reached. However, this may be contrasted with the correlation ($r = 0.441$) between blue eyes, skin lesions and diminishing latitude, where blue eyes and skin lesions are independent. Unfortunately the numbers become too small to press the dichotomies any further. There is evidence, however, that in those who cannot tan easily, i.e. a sub-group of the population selected for their well known propensity to develop skin cancer, that the prevalence increases towards the equator ($r = 0.816$, significant at the 5 per cent level).

TABLE 3.14

British Cane Farmers and Graziers classified according to hair colour.

Males.

Cane Farming and Grazing area	Hair Colour										TOTAL		
	Red		Fair		Brown		Dark		Not Stated				
	+	-	+	-	+	-	+	-	+	-	+	-	
Beenleigh	1	3	1	28	1	26	2	15	-	1	5	73	78
Nambour	3	2	16	52	7	56	17	59	-	-	43	169	212
Bundaberg	4	7	7	69	14	71	9	53	-	1	34	201	235
Mackay	3	1	12	37	9	56	11	45	-	-	35	139	174
Ayr	1	-	4	14	7	21	5	20	1	-	18	55	73
Babinda	5	2	4	15	7	32	6	27	-	1	22	77	99
Mossman	2	3	8	25	3	19	3	27	1	1	17	75	92
TOTAL:	19	18	52	240	48	281	53	246	2	4	174	789	963
Cunnamulla	2	-	9	9	6	15	3	12	-	-	20	36	56
Longreach	1	1	6	14	5	7	3	12	-	1	15	35	50
R'ton	1	1	8	16	13	23	8	13	-	-	30	53	83
Cloncurry	-	-	4	5	4	9	3	10	-	1	11	25	36
TOTAL:	4	2	27	44	28	54	17	47	-	2	76	149	225

Percentages of horizontal totals.

Total Cane Farmers	10.9	2.3	29.9	30.4	27.6	35.6	30.5	31.2	1.1	0.5	174	789	963
Total Graziers	5.4	1.3	35.5	29.5	36.8	36.2	22.4	31.5	-	1.3	76	149	225
GRAND TOTAL:	9.2	2.1	31.6	30.3	30.4	35.7	28.0	31.2	0.8	0.6	250	938	1188

TABLE 3.15

British Cane Farmers and Graziers classified according to eye colour.

Males.

Cane Farming and Grazing Area	Eye Colour										TOTAL		
	Blue		Green		Hazel		Brown		Not Stated				
	+	-	+	-	+	-	+	-	+	-	+	-	
Beenleigh	4	49	-	2	-	5	1	17	-	-	5	73	78
Nambour	29	102	2	8	4	24	7	34	1	1	43	169	212
Bundaberg	22	146	-	5	6	22	6	28	-	-	34	201	235
Mackay	26	69	2	14	1	18	5	38	1	-	35	139	174
Ayr	11	24	1	4	4	14	2	13	-	-	18	55	73
Babinda	12	40	-	3	5	14	5	20	-	-	22	77	99
Mossman	7	42	2	4	3	10	5	19	-	-	17	75	92
TOTAL:	111	472	7	40	23	107	31	169	2	1	174	789	963
Cunnamulla	16	21	-	3	4	6	-	6	-	-	20	36	56
Longreach	10	24	2	2	2	3	1	6	-	-	15	35	50
M'ton	20	37	-	-	6	3	4	13	-	-	30	53	83
Cloncurry	9	13	1	1	1	4	-	7	-	-	11	25	36
TOTAL:	55	95	3	6	13	16	5	32	-	-	76	149	225

Percentages.

Total Cane Farmers	63.8	59.8	4.0	5.1	13.2	13.6	17.8	21.4	1.1	0.1	174	789	963
Total Graziers	72.4	63.8	3.9	4.0	17.1	10.7	6.6	21.5	-	-	76	149	225
GRAND TOTAL:	66.4	60.4	4.0	4.9	14.4	13.1	14.4	21.4	0.8	0.1	250	938	1188

TABLE 3.16

British Cane Farmers and Graziers classified according to complexion.

Males.

Cane Farming and Grazing area	Complexion										TOTAL		
	Fair		Medium		Dark		Sallow		Not Stated				
	+	-	+	-	+	-	+	-	+	-	+	-	
Beenleigh	5	50	-	9	-	13	-	1	-	-	5	73	78
Nambour	29	96	3	17	9	49	1	5	1	2	43	169	212
Bundaberg	29	130	-	25	5	44	-	-	-	2	34	201	235
Mackay	26	70	3	16	6	49	-	1	-	3	35	139	174
Ayr	11	17	5	23	2	15	-	-	-	-	18	55	73
Babinda	19	41	-	5	3	27	-	3	-	1	22	77	99
Mossman	14	42	-	8	3	21	-	1	-	3	17	75	92
TOTAL:	133	446	11	103	28	218	1	11	1	11	174	789	963
Cunnamulla	17	18	-	7	3	10	-	-	-	1	20	36	56
Longreach	10	26	3	2	2	5	-	2	-	-	15	35	50
P'toon	21	32	1	7	5	13	2	1	1	-	30	53	83
Cloncurry	8	9	1	3	2	13	-	-	-	-	11	25	36
TOTAL:	56	85	5	19	12	41	2	3	1	1	76	149	225

Percentages.

Total Cane Farmers	76.4	56.5	6.3	13.1	16.1	27.6	0.6	1.4	0.6	1.4	174	789	963
Total Graziers	73.7	57.0	6.6	12.8	15.8	27.5	2.6	2.0	1.3	0.7	76	149	225
GRAND TOTAL:	75.6	56.6	6.4	13.0	16.0	27.6	1.2	1.5	0.8	1.3	250	938	1188

TABLE 3.17

British Cane Farmers and Graziers classified according to reaction to sun.

Males.

Cane Farmers and Grazing Area.	Reaction to Sun						TOTAL		
	Burn		Tan		Not stated				
	+	-	+	-	+	-	+	-	
Beenleigh	3	27	2	46	-	-	5	73	78
Nambour	27	68	16	101	-	-	43	169	212
Bundaberg	25	81	9	119	-	1	34	201	235
Mackay	24	61	11	77	-	1	35	139	174
Ayr	15	23	3	32	-	-	18	55	73
Babinda	13	22	8	55	1	-	22	77	99
Moscman	14	28	3	46	-	1	17	75	92
TOTAL:	121	310	52	476	1	3	174	789	963
Cunnamulla	15	11	5	25	-	-	20	36	56
Longreach	13	16	2	19	-	-	15	35	50
Rockhampton	23	23	7	30	-	-	30	53	83
Cloncurry	8	3	3	22	-	-	11	25	36
TOTAL:	59	53	17	96	-	-	76	149	225

Percentages.

Total Cane Farmers	69.5	39.3	29.9	60.3	0.6	0.4	174	789	963
Total Graziers	77.6	35.6	22.4	64.4	-	-	76	149	225
GRAND TOTAL:	72.0	38.7	27.6	61.0	0.4	0.3	250	938	1188

TABLE 3.18

Association between lesions and:-	Coefficient of Association	Standard error	Significance
Red hair	+ 0.68211	0.09106	$Q > 3\sigma$ Significant
Fair hair	- 0.00805	0.09156	$Q < \sigma$ Independent
Red and fair hair (amalgamated data)	+ 0.17895	0.08344	$2\sigma < Q < 3\sigma$ Doubtful significance
Brown and dark hair	- 0.17895	0.08344	$2\sigma < Q < 3\sigma$ Doubtful significance
Blue eyes	+ 0.09839	0.08669	$\sigma < Q < 2\sigma$ Independent
Brown eyes	- 0.10786	0.10698	$\sigma < Q < 2\sigma$ Independent
Fair complexion	+ 0.42447	0.07965	$Q > 3\sigma$ Significant
Dark and sallow complexion	- 0.34879	0.09581	$Q > 3\sigma$ Significant
Inability to tan	+ 0.56264	0.06190	$Q > 3\sigma$ Significant

TABLE 3.19

Male Cane Farmers of British Descent --

Proportions Affected in Groups Classified according to Physical Characteristics.

Cane Farming Area	Prevalence of Lesions			
	Red and Fair Hair	Blue Eyes	Fair Complexion	Inability to Tan
Beenleigh	2/33 (6.1%)	4/53 (7.5%)	5/55 (9.1%)	3/30 (10.0%)
Nambour	19/73 (26.0%)	29/131 (22.1%)	29/125(23.2%)	27/95 (28.4%)
Bundaberg	11/87 (12.6%)	22/168 (13.1%)	29/159(18.2%)	21/106 (19.8%)
Mackay	15/53 (28.3%)	26/95 (27.4%)	26/96 (27.1%)	24/85 (28.2%)
Ayr	5/19 (26.3%)	11/35 (31.4%)	11/28 (39.3%)	15/38 (39.5%)
Babinda	9/26 (34.6%)	12/52 (23.1%)	19/60 (31.7%)	13/35 (37.1%)
Mossman	10/38 (26.3%)	7/49 (14.3%)	14/56 (25.0%)	14/42 (33.3%)
Coefficient of correlation with decreasing latitude	0.742	0.441	0.727	0.816
Significance	0.1 > P > 0.05 5% significant value is 0.7545	Not significant	0.1 > P > 0.05 5% significant value is 0.7545	0.05 > P > 0.02 Significant at 5% level

TABLE 4.1

Country of birth for major national groups in city populations.

Percentages, from 1954 census.

Male.

	Brisbane	Rockhampton	Townsville	Cairns
Australia	84.3	92.1	89.4	86.0
Italy	0.7	0.0004	0.5	1.3
United Kingdom	9.8	6.0	7.0	7.1
Other European countries	2.0	0.8	1.0	2.2
Female.				
Australia	86.9	93.6	91.7	89.7
Italy	0.4	0.05	0.3	0.7
United Kingdom	8.8	5.2	6.2	5.9
Other European countries	1.6	0.5	0.7	1.6

TABLE 4.2

Percentages of the immigrant population, classified according to length of time in Australia.

Males.

Years in Australia	Brisbane	Rockhampton	Townsville	Cairns
< 1	4.0	3.4	3.8	3.5
1 - 2	3.4	3.5	2.6	2.2
2 - 3	6.3	7.2	6.5	4.6
3 - 4	8.0	3.4	7.7	8.7
4 - 5	11.4	4.8	8.6	11.4
5 - 6	7.3	3.6	4.3	6.4
6 - 7	2.7	2.1	2.0	1.8
7 - 8	1.1	0.8	1.1	0.8
8 -15	2.1	1.7	2.3	1.6
> 15 and over	53.0	68.9	58.9	55.5
Not stated	0.7	0.7	2.2	3.5

4. CLIMATOLOGICAL FACTORS AND SKIN CANCER.

The hypothesis under study in experimental and epidemiological observations of ultraviolet carcinogenesis is that there is a correlation between incidence of tumours and dose of radiation received. This has been substantiated by Blum (1959, page 191) in the experimental field, who found that, provided the animals lived long enough, the incidence approximated to a normal distribution. In general, it may be said that with diminishing latitude in Australia there is an increase in skin cancer, and that the incidence curves are similar.

In the data of this thesis there are, however, some exceptions which require explanation. In the published work (Carmichael and Silverstone, 1961) it was shown that Townsville had a significantly higher incidence rate than the other three cities, and that Cairns had a higher rate than Rockhampton. In the case of the females, however, Brisbane also had a higher rate than Rockhampton.

To seek the explanation, both the climatological and population characteristics must be examined. The city populations are not homogeneous, so that national differences could certainly influence the incidence rates. In Table 4.1 are given the birth places of the major national groups, and in Table 4.2 the length of time lived in Australia by the immigrant populations. The census tables do not give an analysis of the length of the time lived in Australia by the various national groups. It must be remembered also that the offspring of immigrant population forms an unknown proportion of those born in Australia. The data, however, serve as

a guide, and may help to explain some of the discrepancies. Cairns has a higher proportion of Italian-born in its population than the other three cities, which would tend to lower the incidence rate.

The climatological factors are not necessarily straightforward. The factors that influence the amount of ultraviolet radiation received at the earth's surface are:

(i) Latitude, angle of elevation of the sun and air mass.

(ii) Dust and water vapour in the atmosphere.

(iii) Ozone in the upper atmosphere; and

(iv) Degree of cloud cover.

The first three factors have been discussed by Sanderson and Hulbert (1955). The air mass is the amount of atmosphere from earth's surface to space in a vertical direction. Atmospheric absorption of ultraviolet and visible light follows an exponential law, so that the amount of radiation received at the earth's surface is a complex function of the angle of the sun and the distance through the atmosphere that the rays have to pass. Dust, water vapour and air scatter the radiation (accounting for the sky), some of which is directed downwards and some reflected back to space. Ozone is an efficient absorber of ultraviolet radiation below a wavelength of 3400 Angstrom units, and is responsible for the lower limit of the solar spectrum at about 2900 Angstrom units. The amount of ozone in the upper atmosphere varies with latitude, increasing from about 180×10^{-3} cm. at normal temperature and pressure at latitude 20°S to $220\text{-}240 \times 10^{-3}$ cm. at latitude 40°S. There is also some seasonal variation. According to Sanderson and Hulbert

TABLE 4.3

Ultra-violet radiation counts measured in thousands for one twelve-month period and average monthly sunshine hours.

Sunshine hours for Cloncurry are not available.

MONTH	Brisbane		Townsville		Cloncurry
	UVR Counts	Sunshine Hours	UVR Counts	Sunshine Hours	UVR Counts
October 1959	34.35	216.8	47.45	325.4	50.35
November	38.85	209.1	51.50	311.5	55.65
December	48.35	245.2	47.75	226.2	63.30
January 1960	51.10	283.3	51.05	250.8	54.75
February	38.30	183.8	41.20	188.6	48.05
March	37.55	250.2	39.80	221.0	43.85
April	22.90	242.8	34.90	278.0	35.05
May	17.45	198.4	25.15	248.0	23.00
June	13.20	232.2	23.00	243.8	17.85
July	13.10	206.6	24.40	266.2	22.10
August	19.70	271.1	32.90	295.4	29.90
September	26.50	251.5	35.70	296.4	35.40
TOTAL	361.35	2791.5	454.80	3151.3	479.25
Ratio UVR Counts	1.0		1.26		1.33
Ratio Sunshine Hours		1.0		1.13	

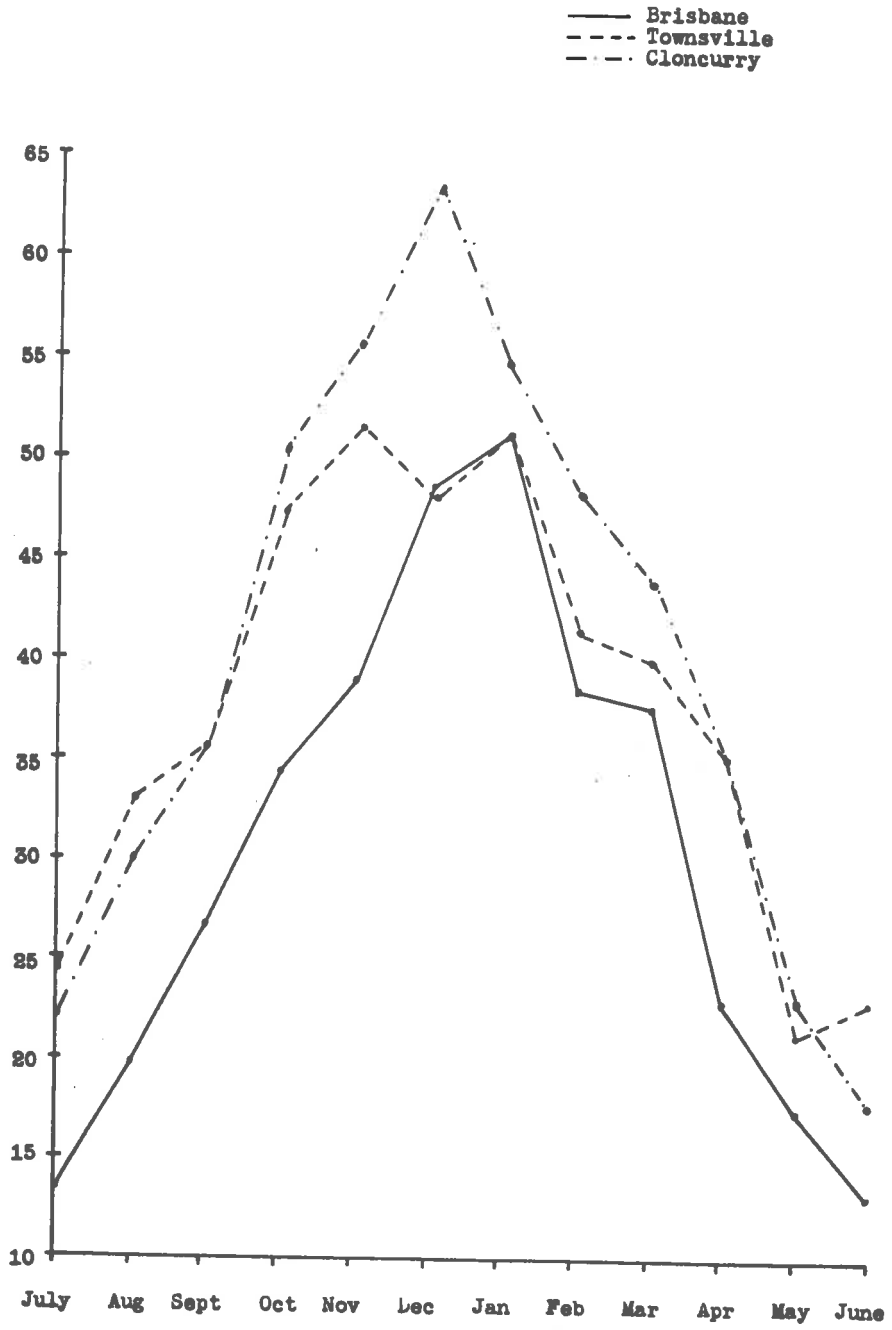


Figure 4.1. Ultraviolet radiation measurements, in thousands of counts on a photoelectric recording device (ordinate), for one twelve month period for three Queensland centres.

(1955) all these variables are so complex that there is no simple way of estimating the solar ultraviolet radiation received at the earth's surface.

The degree of cloud cover can operate to enhance or diminish the amount of radiation received at the earth's surface. A cover of thick cloud acts simply by blotting out the sunlight, but isolated cumulus type cloud increases the glare by reflection of short wave visible light and also of ultraviolet radiation.

In a parallel investigation by the Department of Physics of the University of Queensland direct measurements of ultraviolet radiation received at three places are being obtained. Measurement is by a photo-electric device that records all the solar ultraviolet radiation. The quantities (measured in counts of number of times a condenser discharges) are given for one twelve month period for Brisbane, Townsville and Cloncurry (supplied by Mr. D. F. Robertson) in Table 4.3. The shapes of the curves for Brisbane and Townsville are similar, except that Townsville has a rather higher winter and spring count. The shape of top part of the curves indicates the effect of cloud cover during the coastal rainy season. (Figure 4.1.)

In addition to the complex meteorological variables are the equally unpredictable human ones. The degree of exposure to sunlight will be determined by whether a hat is worn, the amount and type of clothing, and whether outside activities are pursued in the hottest part of the year.

The effect of sky radiation is quite potent in the tropics, and it is possible to receive an erythemal dose of ultraviolet radiation without being directly exposed. Sun exposure habits should be

interpreted in relation to this as well as the other variables.

A rough comparison may be made between the Queensland skin cancer incidence rates and those published by Auerbach (1961) for the United States. I have standardised the United States rates for New Orleans and Dallas on the Rockhampton population, but this has necessitated some approximation in both the upper and lower age groups. The rates per 10,000 are:

	New Orleans (29°57'N)	Dallas (32°47'N)
Male	22.0	29.0
Female	12.7	16.8

Comparing these rates with those given in Table 2.11, the American rates are seen to be lower than those for Queensland. Auerbach, however, used the total population for his analyses, and not the susceptible population, so that his rates would be underestimated.

He has plotted a straight line graph between incidence rates and latitude for ten cities, and found that the rate doubled every 3°48' the cities were nearer the Equator. Inspection of his graph, however, suggests that a straight line fit may not be the most appropriate, and that this relationship between latitude and incidence may be obscuring genuine variations in the incidence pattern.

In summary it may be said that the skin cancer incidence in both Australia and the United States increases towards the Equator, but that local climatic differences and population variations do not permit of a definite relationship being defined. The correlation found between diminishing latitude, prevalence of skin lesions and inability to tan found in the cane farmers' survey may be of

more significance in this regard, because many of the variables have been eliminated.

5. EXPERIMENTAL ULTRAVIOLET CARCINOGENESIS.

5.1. Introduction.

Experimental carcinogenesis with ultraviolet radiation has been carried out successfully only with laboratory mice and rats. Findlay in 1928 was the first to demonstrate tumour production by this method. Subsequently various workers have repeated the observations. Rusch and Baumann (1939) used a quartz mercury vapour lamp and achieved a 62-83 per cent tumour production in 3½ - 9 months, in albino mice of strains A and C. Black C57 mice showed a smaller proportion of tumours and required a longer time. The tumours produced were papillomas, epitheliomas and spindle cell carcinomas, with some mixing of the latter two varieties. From 1941 onwards Blum and co-workers reported the results of extensive experiments, using strain A mice (Blum *et al*, 1941, 1943 a and b; Grady *et al*, 1941; Kirby-Smith *et al*, 1942). Bain and Rusch (1943) investigated the wavelength dependence of tumour production by using a limited ultraviolet spectrum. In 1936 Putschar reported the results of experiments with both rats and mice, and stated that true mixed tumours of both mesodermal and epithelial tissue could occur. Roffo (1934, cited by Blum, 1955) induced skin cancers in rats by exposing them to natural sunlight. In 1936 Beard *et al* also showed that exposing rats to ultraviolet radiation could cause a variety of types of tumour, including carcinomas of the ears, spindle cell sarcomas of the eye, giant cell sarcoma and haemorrhagic tumours with a possible origin in the smooth muscle of the ciliary coat of the eye. Mueper (1941) irradiated congenitally hairless

rats and their haired litter mates, and found that of the hairless animals only one developed a tumour. The haired rats developed both carcinomas and sarcomas.

With hairless mice Winkelmann et al (1960, 1963) induced squamous cell carcinomas using an ordinary sunlamp. Griffin et al (1955) and Kelner et al (1956) investigated the possible effect of photorecovery in ultraviolet carcinogenesis in mice. The former found that exposing the animals to visible light at the same time as ultraviolet radiation necessitated a higher dosage of the ultraviolet radiation to produce tumours. On the other hand, animals exposed to ultraviolet radiation alone and housed in the dark required more energy for tumour production than those kept in white light. The latter authors found that photo reactivating visible light had a reducing effect on the carcinogenicity of ultraviolet radiation of wavelength 2537 Angstrom units.

There is apparently no synergism between ultraviolet and chemical carcinogenesis, the reports of experiments being somewhat inconclusive (Kohn-Speyer, 1929; Taussig et al, 1938; Epstein et al, 1961).

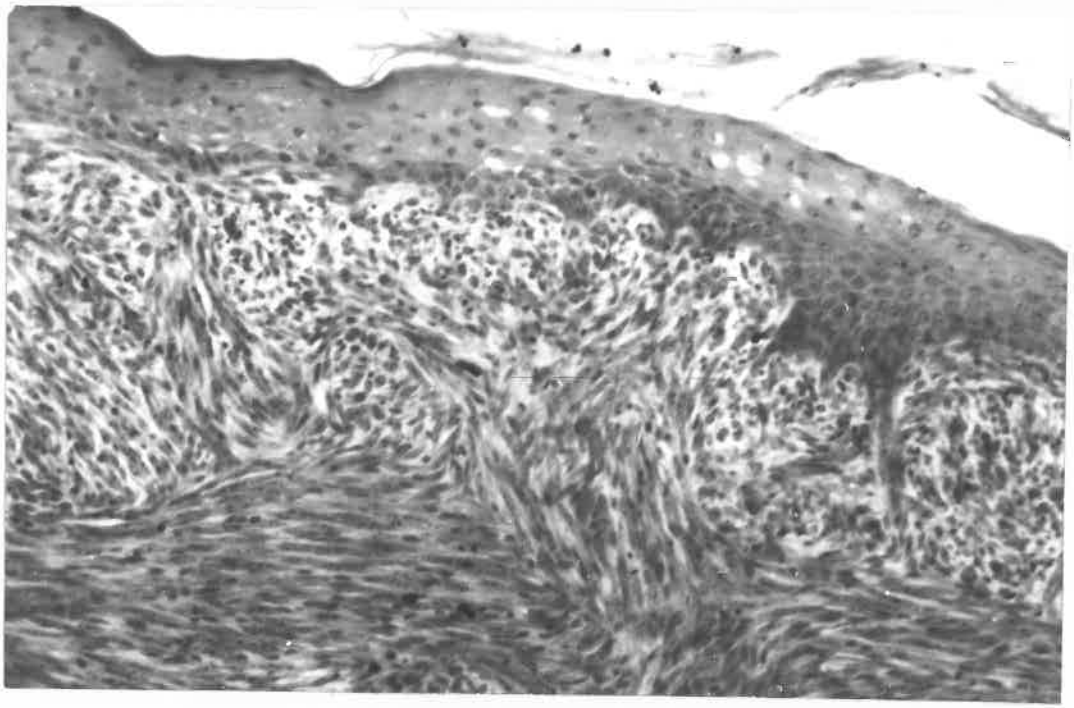
5.2. Pathology of ultraviolet tumours in experimental animals, and their possible equivalents in man.

In mice there appear to be two principal types of tumour, the obvious squamous cell carcinoma and a spindle cell tumour that has been diagnosed as both sarcoma and spindle cell carcinoma. The squamous cell tumours are usually fairly well differentiated, and resemble those due to chemical carcinogenic agents. The spindle

cell tumours are quite undifferentiated and consist of interlacing bands of elongated cells with variable nuclear size, and frequent mitotic figures in the growing phase. Findlay (1928) commented on the presence of both carcinoma and sarcoma. Grady et al (1941) considered that the majority of their tumours were fibrosarcomas, relying heavily on the appearance of the intercellular collagen and reticulin. They have published no pictures of the histology of early lesions.

The type of tumour induced appears to depend on the strain of mouse. The haired mice develop a higher proportion of spindle cell tumours than squamous. The mice homozygous for the Hr recessive gene used by Winkelmann et al (1960, 1963) all developed squamous tumours. In these animals the pilosebaceous apparatus shows regressive changes between the ages of 2 and 3 weeks. The young animals grow a normal pelage, but it is shed at about 18 days. There may be some cyclical regrowth for a time, but the adult animal has no hair. The sebaceous glands, denied the outlet of functional hair follicles, continue to secrete and the dermis becomes almost completely replaced by distended ducts containing sebaceous material.

In some experiments with hairless mice homozygous for the Naked dominant gene, I have induced spindle cell carcinomas, similar to those induced in albino haired animals (Figures 5.1, 2, 3). The Naked strain, although they have no pelage, have functional hair follicles and there is cyclical hair growth. The hair, however, is brittle and breaks off. Microscopically the skin of the Naked strain is similar to the haired animals, though the follicles may be slightly more widely separated. The other authors cited did not



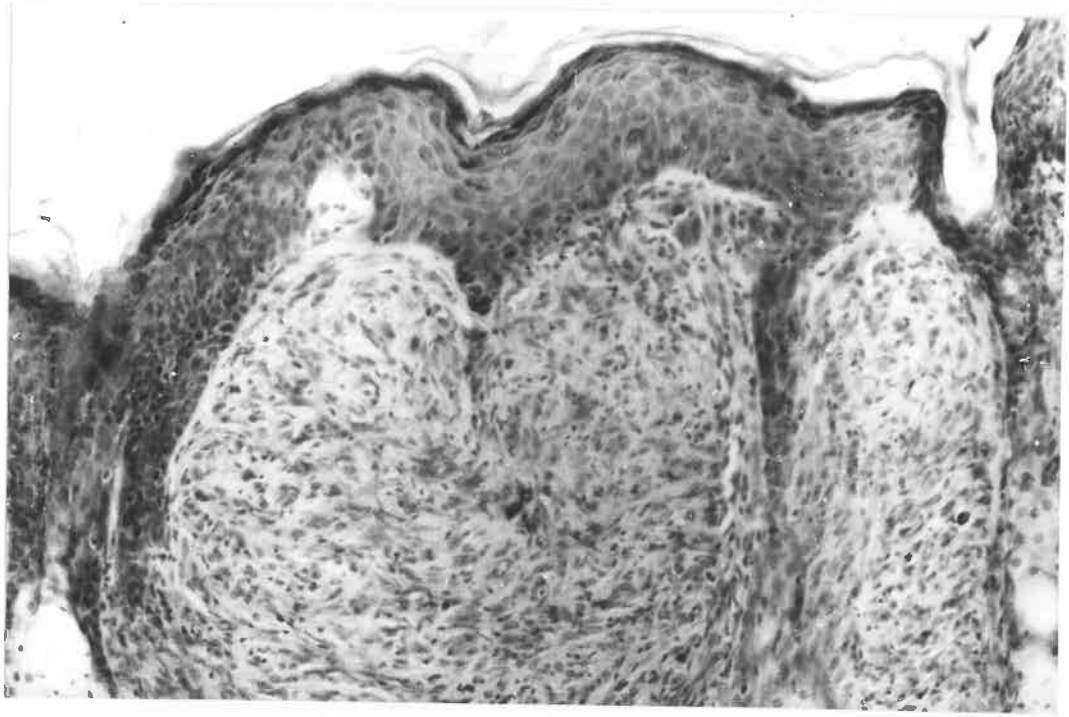


Figure 5.1. Ultraviolet tumour in white laboratory mouse, showing spindle cells arising from the epidermis. Haematoxylin and eosin. Magnification 167 x.

Figure 5.2. Ultraviolet tumour in white laboratory mouse showing origin from hair follicles (H. and E., 167 x).

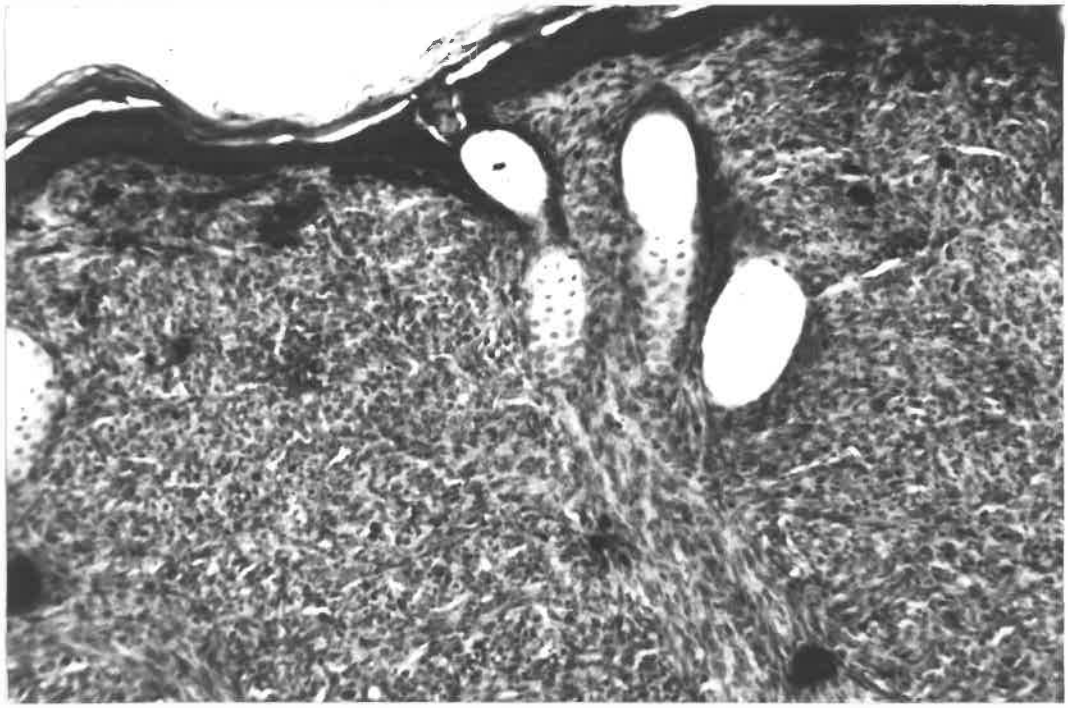


Figure 5.3. Ultraviolet tumour in naked mouse. The dark areas are sub-epidermal melanin (H. and E., 133 x).

discuss the pathology of the tumours they induced in mice. In rats the tumours seem to be principally squamous cell carcinomas, but whether the tumours designated as sarcomas are truly mesodermal tumours is not certain.

The point of origin is of importance in the consideration of the effect of ultraviolet radiation. The tumours produced in my series of experiments have been practically all spindle cell carcinomas, and appear to be identical with the pictures published by Grady et al (1941). These workers considered the spindle cell tumours to be sarcomas. On this interpretation of the lesions Blum has elaborated a theory of the mechanism of ultraviolet carcinogenesis and the penetration of the radiation through the epidermis (summarised in Blum, 1959). Figures 5.1, 2 and 3 show a spindle cell carcinoma arising from the epidermis and hair follicles. This same tumour also shows a fine fibrillary intercellular reticulin pattern that resembles that of a fibrosarcoma. As the tumours grow the intercellular fibres become more obvious and may be quite well demonstrated by a Masson's trichrome stain.

Spindle cell carcinomas also arise in human skin, and are sometimes misdiagnosed as fibrosarcomas. They usually arise in areas of inefficiently treated skin cancer or areas previously treated by depilatory doses of X rays (Martin and Stewart, 1935; Sims and Kirsch, 1948). They behave in a highly malignant fashion. In this respect they differ from spindle cell ultraviolet tumours of experimental animals, which metastasize only rarely, and grow uniformly with a well demarcated edge. Recently, Bourne (1963)

has reported a spindle cell skin tumour which has the histological appearance of a fibrosarcoma, but apparently runs a benign course and requires only relatively minor local treatment. To account for these features he has called it a paradoxical fibrosarcoma.

Experimental animals do not show any ultraviolet tumours that resemble microscopically the basal cell carcinoma of man.

5.3. The enzyme histochemistry of tumours, with special reference to skin cancers.

Enzymatic methods in histochemistry are a logical extension of conventional histological procedures. Following the development of methods for sectioning of unfixed or lightly fixed material, enzyme histochemistry has rapidly gained an important place amongst cytological techniques. It relies heavily on biochemical methods, but retains the advantage that the localisation of chemical processes is preserved, and a large part of the science is concerned with methods of visualising them. In general the methods give qualitative information, but semiquantitative estimations of enzyme activity may be made by comparing the intensities of reaction in different cells and tissues.

In cancer research studies in enzyme histochemistry have been made in the search for fundamental differences between normal and neoplastic cells. By and large, tumour cells show much the same activities as their parent tissues, and in some cases have greater activity. Two enzymes have, however, been associated with malignant disease. The glucuronide hydrolysing enzyme β-glucuronidase has been extensively studied by Fishman (Fishman, 1955; Fishman

and Baker, 1956) and is said to show a high activity in malignant disease. Phosphoamidase, a specific acid phosphatase (Gomori, 1948; Meyer and Weinman, 1955) has also been considered to have a characteristic activity in malignant disease. Both of these enzymes have a capricious and unpredictable histochemical reaction and have not proved satisfactory in routine practice.

As enzyme methods reflect a functional aspect of cell behaviour, they are likely to be more widely used in the future in studies of tumour differentiation, prognosis and response to treatment. Quantitative estimations of histochemical enzyme activity have not been used to any great extent, because the current available methods are somewhat laborious. Quantitative histochemistry will be discussed below, and a simple new method reported.

Histochemical studies of chemical carcinogenesis of the skin of the mouse have been made in a number of enzyme systems. Oka et al (1961) found "nearly the same localisation" in malignant transformation as normal skin for acid phosphatase, non-specific esterase, β-glucuronidase and β-galactosidase. Alkaline phosphatase was inactive in cancerous tissues, but its activity gradually increased in tissues adjacent to proliferating epithelium. Using 20-methylcholanthrene, Okamoto et al (1962) observed amino peptidase activity in the subcutaneous tissue adjacent to proliferating epithelium. Burstone (1956) had previously demonstrated an intense aminopeptidase activity in the stroma of various malignant tissues. Campbell (1949) found a high β-glucuronidase activity in

poorly differentiated cells of mouse squamous cell carcinoma.

In human skin cancers enzymatic studies have been reported fairly frequently. Lemon and Wisseman (1949) showed an increased acid phosphatase activity in skin tumours, and in 1954 Lemon et al confirmed this by quantitative studies. Although phosphoamidase activity cannot always be demonstrated satisfactorily (Gomori, 1948), Tomita and Takeuchi (1955) and Winter (1955) showed a characteristic increased activity in malignant tissues, including the skin. Tomita and Takeuchi (1955) used phosphocreatine as substrate. Despite the improvements in the technique proposed by Meyer and Weinman (1955), this method, although it appears to be a histochemical index of malignant degeneration, has not been widely used. Foraker (1956) studied succinate dehydrogenase, alkaline phosphatase and phosphoamidase, and found that the reactions of squamous tumours were generally similar to those of the deeper layers of the epidermis. In 1957, Reiner et al found a gradient of acid phosphatase activity towards the keratinised surface. Studying a number of the dehydrogenase enzymes and the diaphorases, Monis et al (1959) found that in tumours succinate dehydrogenase had a low activity, but that most of the coenzyme linked dehydrogenases had a high activity. Exceptions to this were glutamate and ethanol dehydrogenases. Diphosphopyridine nucleotide diaphorase was active in tumours, much more so than triphosphopyridine nucleotide diaphorase.

Studying human squamous cell cancer, including that of the skin, Kawakatsu and Mori (1963) have reported that acid phosphatase was very active in keratinising cell nests. Amino peptidase was

occasionally present in the stroma. The coenzyme linked dehydrogenases were fairly even distributed in the malignant tissue. Esterase activity was considerably enhanced in neoplastic cells.

5.4. Methods of experimental tumour production and enzyme histochemistry.

Tumours were induced in white laboratory mice by exposing them to the radiation from a Philips MLU 300 watt sunlamp. The animals were enclosed in individual wire gauze compartments round the periphery of a circular metal tray, which was mounted on a turntable on the lower shelf of a metal cabinet. The sunlamp was suspended from the under surface of the upper shelf 66 cm. from the centre of the tray bearing the mice. The animals were depilated with a barium sulphide paste, and then exposed daily, for five days a week, to the radiation, for one hour. Depilation was repeated from time to time as necessary. The first tumours appeared after seven months' radiation, and then at irregular intervals. The tumours grow fairly rapidly, and may reach a diameter of 3 cms. As they become large, the central part becomes necrotic and desiccates, so that they are covered by a hard scab that cannot be easily separated from the growth. Micro-abscess may form in the necrotic parts, but the tumours do not become grossly infected as do the benzpyrene tumours.

Estimation from the manufacturer's data indicates that the mice received approximately the equivalent of 16.4 μw per sqm. cm. per second of radiation of wavelength 2967 Angstrom units. The

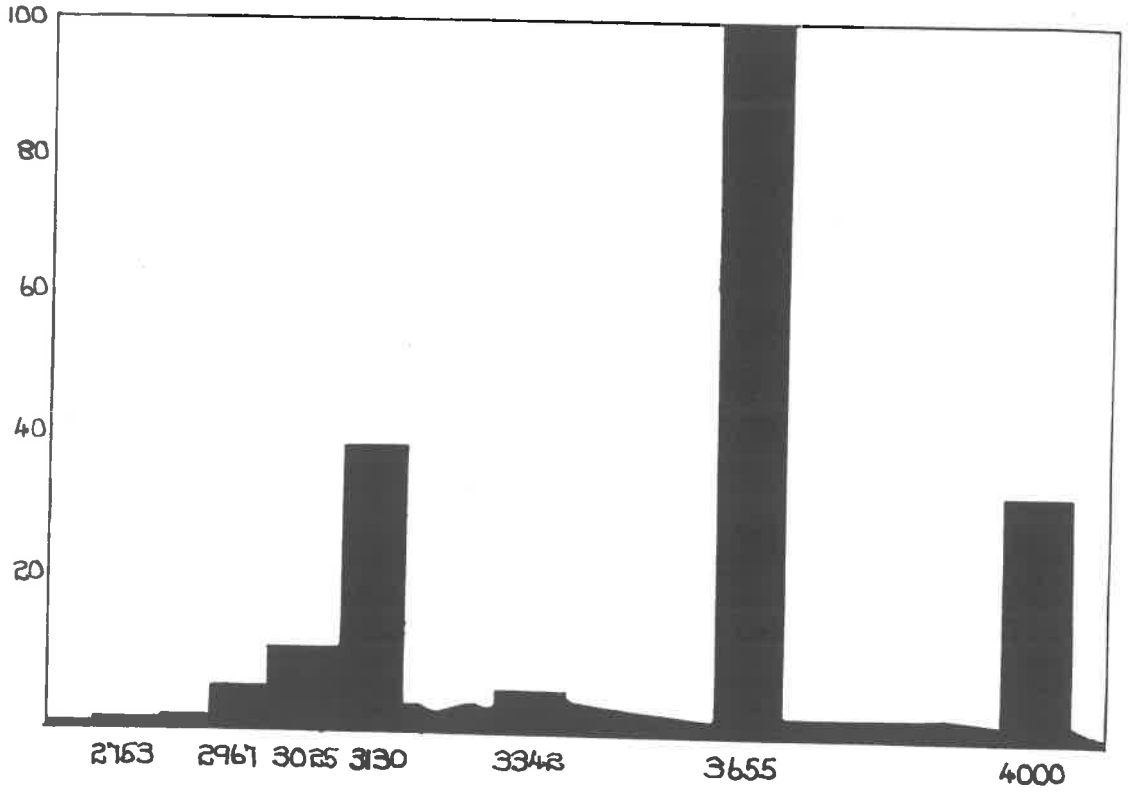


Figure 5.4. Spectral distribution of the emission spectrum of the Philips MLU 300 W sun lamp. Wavelength in Angstrom units.

emission spectrum is shown in Figure 5.4.

Benzpyrene tumours were induced by painting the depilated backs of mice with a 0.5 per cent solution of benzpyrene in acetone. Induction time was about 16 weeks. The tumours are all squamous cell carcinomas, showing all degrees of differentiation from well organised cell nests to isolated individual infiltrating cells.

All enzymatic histochemical reactions were carried out on unfixed fresh frozen, cold microtome sections mounted on slides. Where the reaction made the use of fixative necessary, this was applied to the mounted section. For comparative purposes I have used unfixed material wherever possible. It is recognised that in some cases fixation improves cytological localisation by preventing loss of some enzymes in a soluble form. The enzymes which will stand some degree of fixation are nevertheless partially destroyed (e.g. the phosphatases and esterase (Pearse, 1960, pages 68 and 70)). Whether the amount of destruction varies between different normal tissues and tumours is not known, and cannot be assumed to be constant. The cytoplasmic morphology between the ultraviolet and benzpyrene tumours and their enzyme reactivities show differences, so that the best base line for gauging differences seems to be the use of unfixed material.

All technical details of tissue preparation and histochemical methods are given in Appendix 2.

The histochemical studies were carried out on five ultraviolet and six benzpyrene tumours.

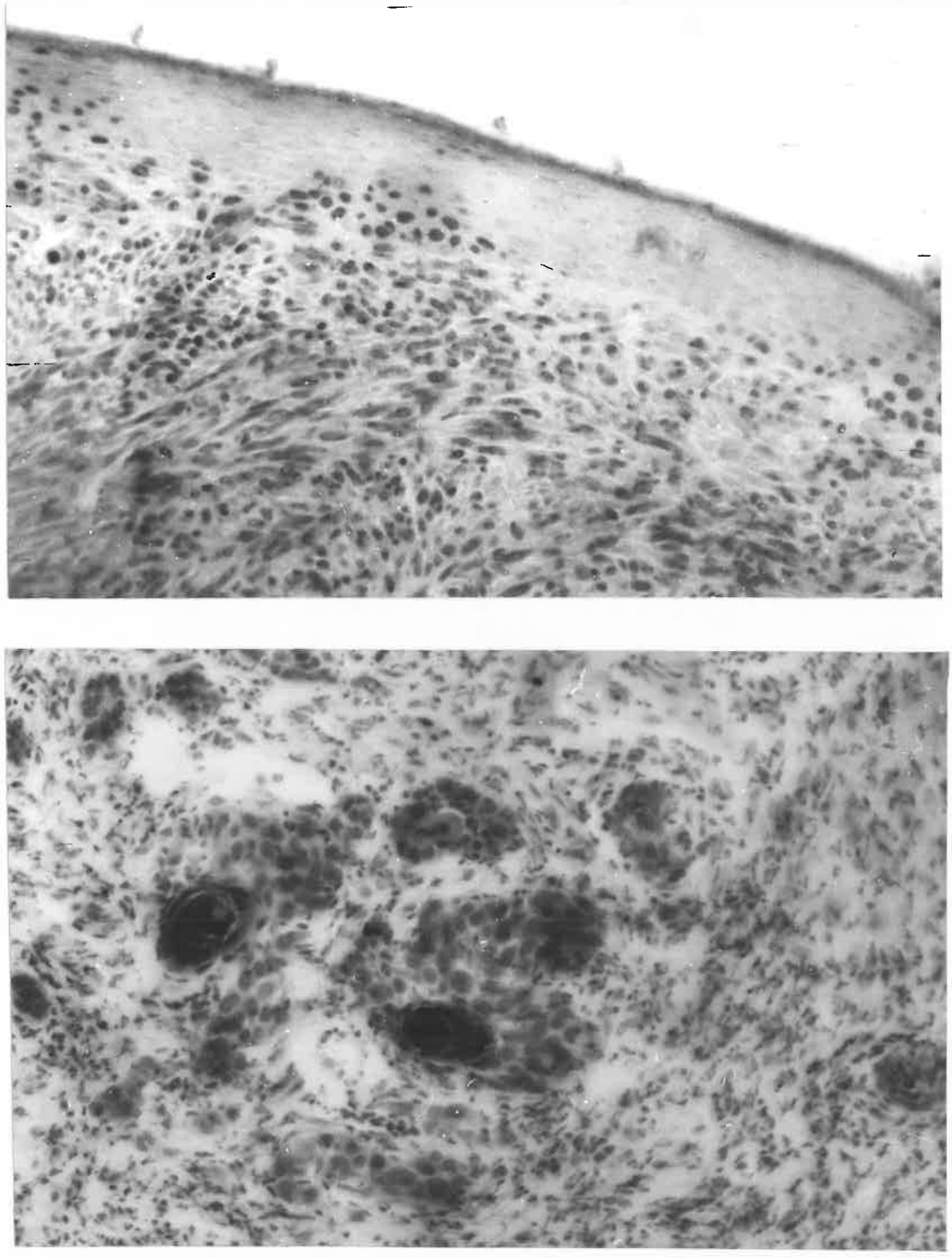
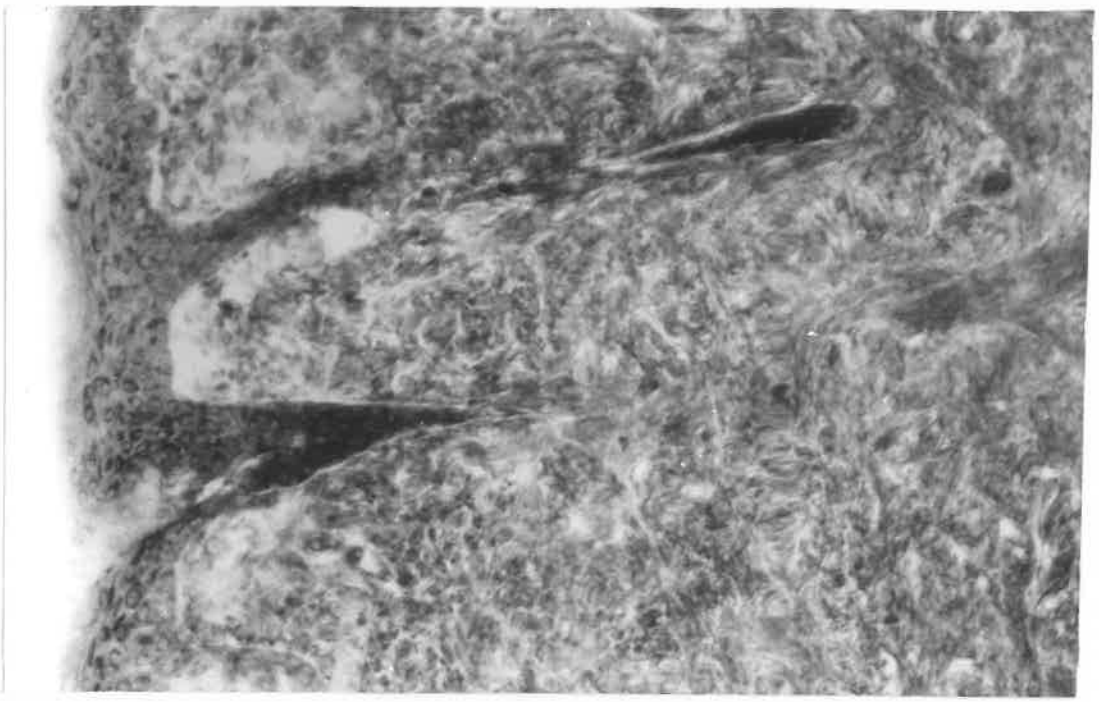
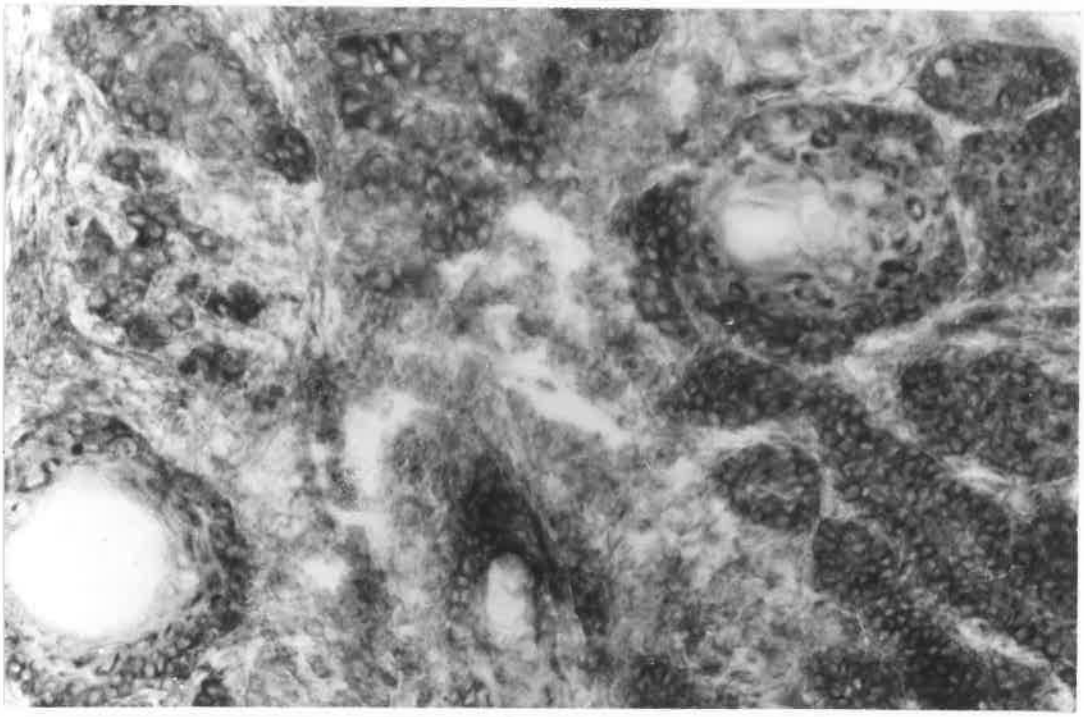


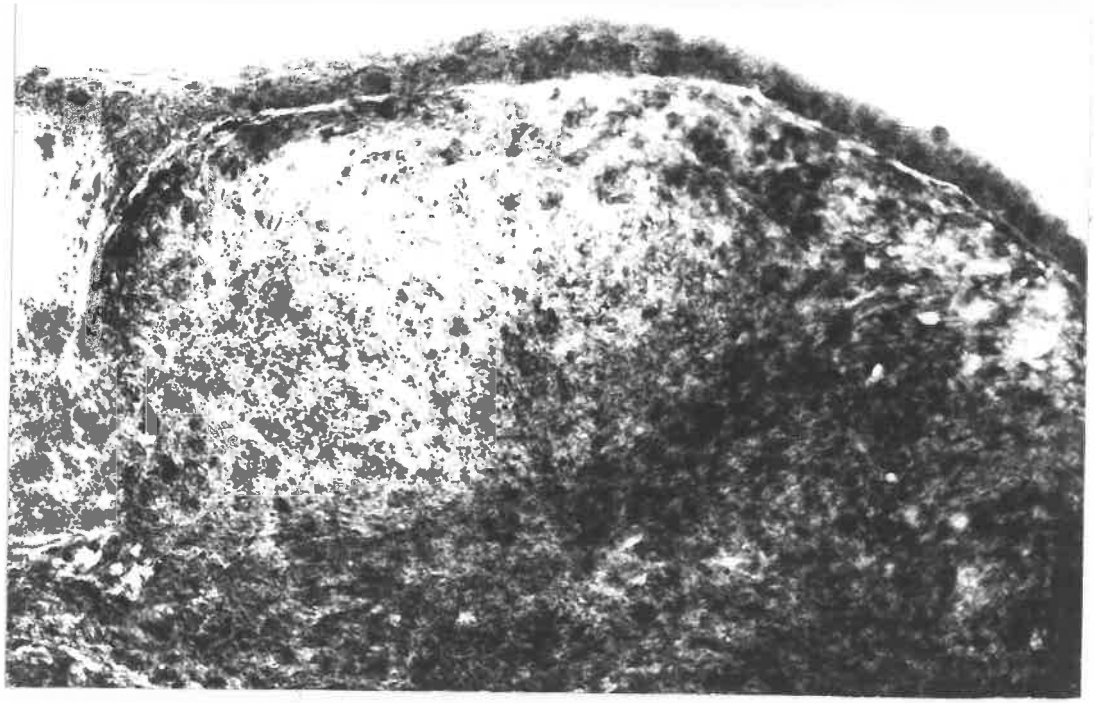
Figure 5.5. Ultraviolet tumour in white laboratory mouse. Gomori's acid phosphatase method, showing columns of active epidermal cells continuous with the underlying tumour (167 x).

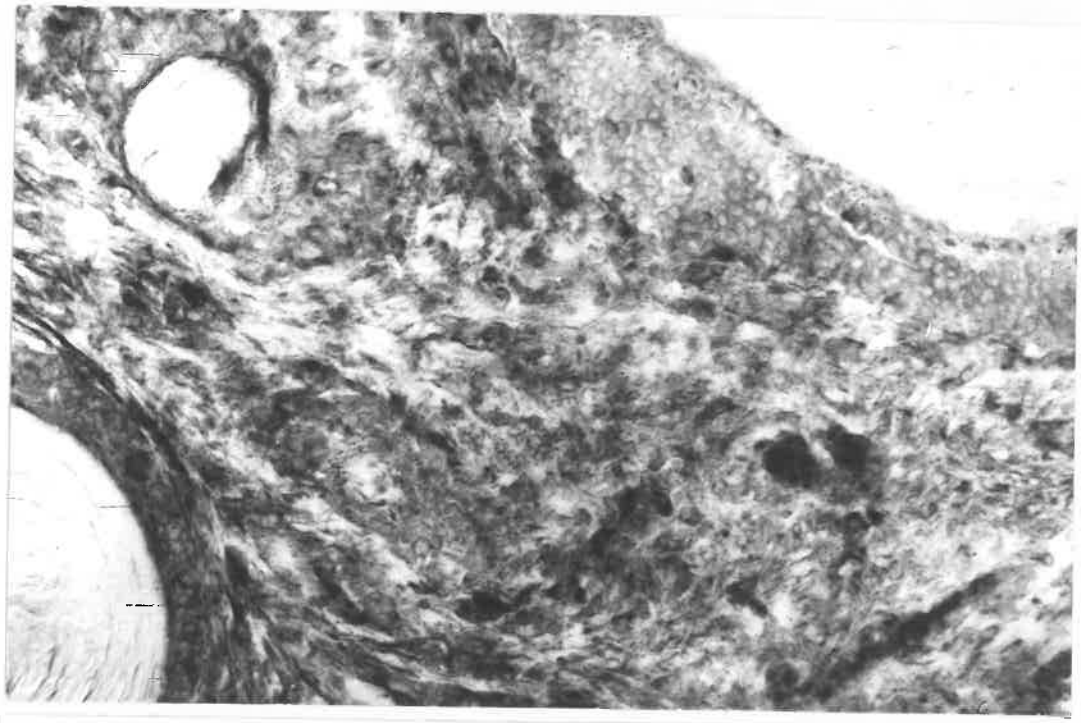
Figure 5.6. Benzpyrene tumour in white laboratory mouse. Esterase, indoxyl acetate method, with marked activity in cell nests, accentuated in the keratinous regions (167 x).



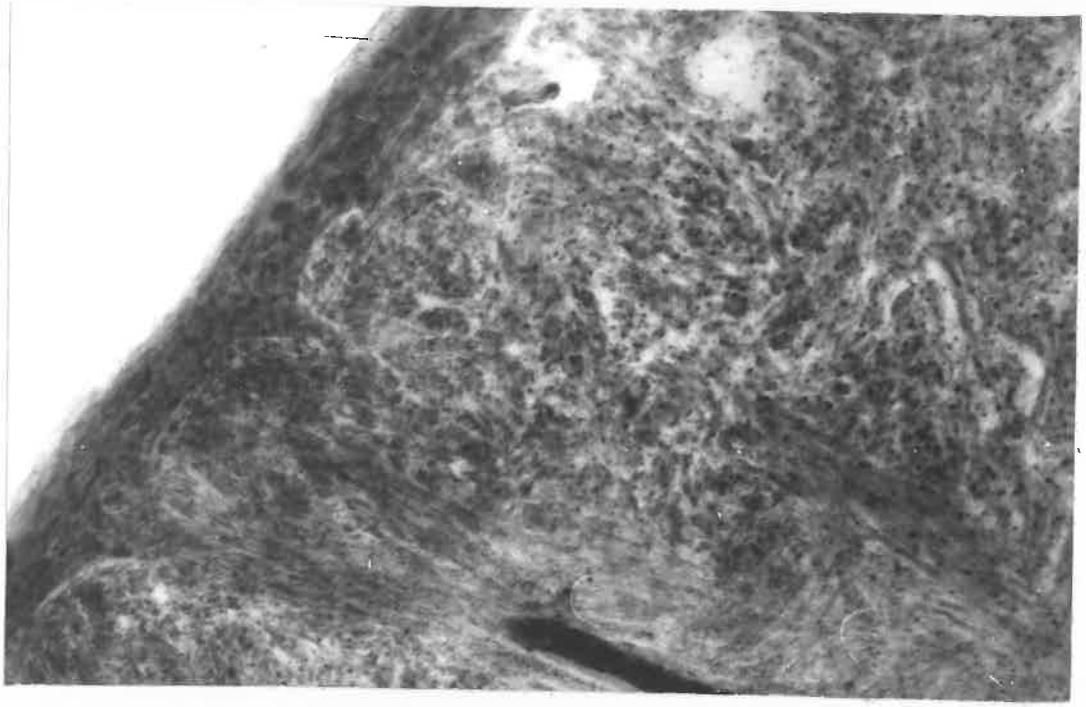


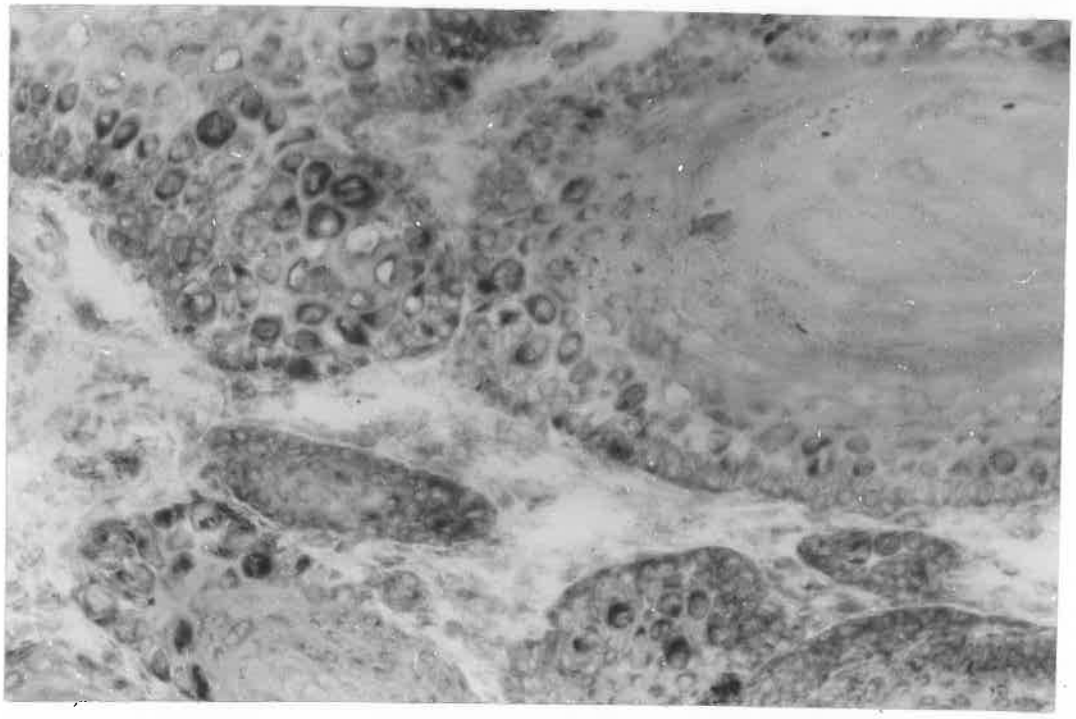
Figures 5.7. and 5.8. Lactate dehydrogenase in ultraviolet and benzpyrene tumours. Nitro BT, counterstained with methyl green (167 x).



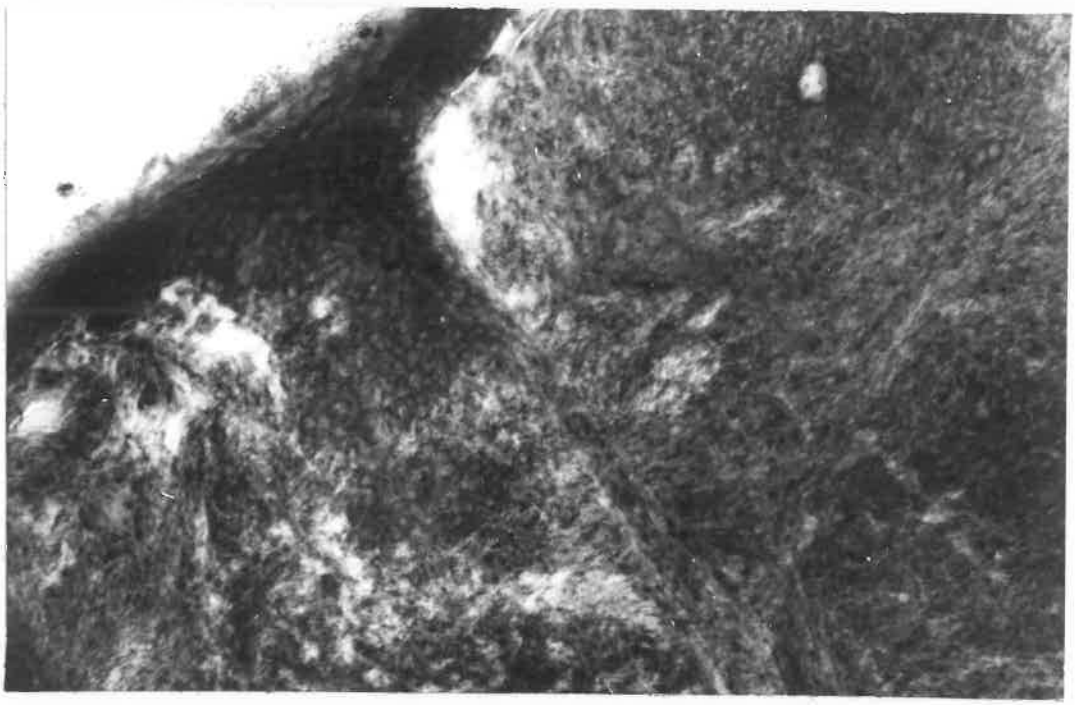


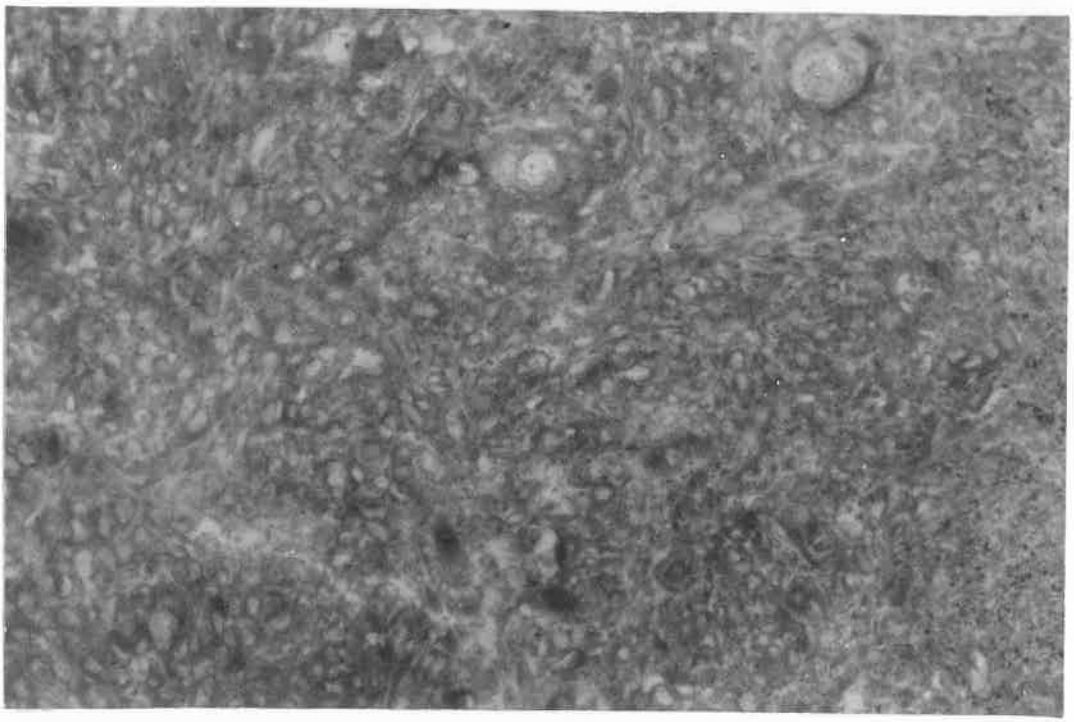
Figures 5.9. and 5.10. Glutamate dehydrogenase in ultraviolet and benzpyrene tumours. Nitro BT, counterstained with methyl green (167 x).





Figures 5.11. and 5.12. DPN diaphorase in ultraviolet and benzpyrene tumours. Cobalt-MTT, no counterstain (167 x).





Figures 5.13. and 5.14. Tetrazolium salt lipoprotein reaction. Mitochondrial stain similar to that of dehydrogenase reactions. Cobalt-MTT-Hydroquinone, no counterstain (167 x).

5.5. Histochemical observations.

1. Acid phosphatase. In the normal epidermis this enzyme reaction is very clearly localised to the stratum granulosum, with some carry-over of activity into the non-viable layer. Using the Gomori method, the lead sulphide deposit corresponds to the keratohyalin granules, but whether this is an effect of the same type as the apparent nuclear staining, which also occurs in the stratum granulosum, is uncertain. With both the Gomori and Barka methods the reaction was carried out at pH 4.5 and 5.5, with no essential difference in localisation.

(a) Ultraviolet tumours. These tumours are intensely reactive, so much so that it is impossible to make out any cellular detail in the main mass of the growth. The interesting finding, however, is at the points where the growth actually arises from the epidermis. Sharply localised columns of cells in the epidermis show acid phosphatase activity, with nuclear staining, and these are directly continuous with cells growing out of the epidermis and into the tumour (Figure 5.5). In favourable sections a number of these active areas may be seen along the epidermis, and also similar localised sites of activity may be seen in the neighbouring epidermis, not actually related to tumour. These are very probably future points of origin for cancer.

(b) Benzpyrene tumours. Many, but not all, of the well differentiated and keratinising cell nests and papillomatous parts of the tumour show acid phosphatase activity in the stratum granulosum. In addition, in these cell nests there are areas of

diffuse activity in the basal and lower spinous layers of the epidermis, but no distinctive appearances such as have been described above for the ultraviolet tumours. Some cells nests are, however, quite negative. The poorly differentiated cell clusters and infiltrating cells are fairly strongly positive.

2. Alkaline phosphatase. This enzyme is predominantly a connective and vascular tissue enzyme, and there is no significant tumour activity.

3. Adenosine triphosphatase. This reaction is very strong in all the tissues.

4. Esterase. The indoxylacetate and α naphthylacetate methods were equivalent in their general micro-anatomical localisation of this enzyme, but as the indoxyl acetate method gives a fine microcrystalline reaction product it is more suitable for identifying activity in isolated cells.

(a) Ultraviolet tumours. The epithelium over these tumours is moderately active, but shows variation in activity at the cellular level, some cells being noticeably more active than others. Activity in the tumour is decidedly faint, though some of the bands of cells are slightly more active than others.

(b) Benzpyrene tumours. The activity is fairly strong in both the well and poorly formed cell nests, and in some cases in the individual infiltrating cells. In the well differentiated epithelium of the cell nests there is no special accentuation of activity in the stratum granulosum, but there is a marked carry-over of reaction into the keratinous contents (Figure 5.6). In some cases

the form of effete cells, containing no other recognisable intracellular contents, is outlined by intracellular esterase reaction product.

5. Leucine aminopeptidase. This enzyme reaction is uniformly negative in all the tumour tissue, but there is staining of mast cells and some variable activity in hair follicles and sebaceous glands. There is only faint staining in some parts of the connective tissue.

6. Monoamine oxidase. Neither tetrazolium nor hydroxynaphthoic hydrazide method showed any significant reaction in these sections.

7, 8, 9. (i) Enzymes of the citric acid cycle and lactate dehydrogenase. The normal and hyperplastic epithelium in general shows a slightly lower activity than both the benzpyrene and ultraviolet tumours. The dedifferentiated and infiltrating parts of the benzpyrene tumours are particularly active (Figures 5.7 and 5.8).

(ii) Glutamate dehydrogenase. Both tumour types show a greater activity than the epithelium from which they arose, especially in the infiltrating regions (Figures 5.9 and 5.10).

(iii) α -Glycerophosphate dehydrogenase. The benzpyrene tumours show approximately the same activity as the normal and hyperplastic epithelium, but the ultraviolet tumours rather less.

(iv) β -Hydroxybutyrate dehydrogenase. This enzyme has a weak activity in the ultraviolet tumours, but is fairly strong in the normal and hyperplastic epithelium and in the benzpyrene tumours, particularly in the keratogenous zones.

(v) Ethanol dehydrogenase. This enzyme has moderately strong activity in the benzpyrene tumours, weak activity in normal

epidermis and weaker still in the ultraviolet tumours.

(vi) Glucose-6-phosphate dehydrogenase. Normal and hyperplastic epithelium show fairly marked activity, with accentuation at the keratinising zones. The ultraviolet tumours show slightly less activity than the normal epidermis, but the benzpyrene tumours are strongly reactive particularly at the keratinising regions of the cell nests.

In the succinate dehydrogenase reaction there is some degree of formazan deposit at the keratinising zone and in the non-viable layers, but this is not seen in the other reactions utilising Nitro-BT. This is therefore probably related to the time of incubation. With the reactions using MTT there is formazan deposition in the non-viable layers, but it is probably non-specific as it is seen also in the tetrazolium lipoprotein method.

10. The diaphorases. The actively growing areas of both types of tumour are strongly reactive for DPN and TPN diaphorase, but the formazan deposit is heavier in the DPN than the TPN reactions. The distribution of these two enzyme systems is slightly different. TPN diaphorase reaction is accentuated in the superficial half of hyperplastic epithelium, whereas DPN diaphorase is more active in the basal layers and the lower part of the stratum spinosum (Figures 5.11 and 5.12).

11. Cytochrome oxidase. In the presence of added cytochrome c all tissues, normal hyperplastic, neoplastic and connective, show intense staining. Without the addition of cytochrome c, however, the reaction is very sparse and patchy, and cellular localisation is indifferent.

12. β-Glucuronidase. This reaction was quite negative under the conditions of this investigation.

13. Tetrazolium salt lipoprotein method. This demonstrates cytoplasmic lipoprotein, which is uniform in its reaction between the epidermis and the spindle cells of the ultraviolet tumour, and homogeneous in the benzpyrene tumours (Figures 5.13 and 5.14).

5.6. Discussion.

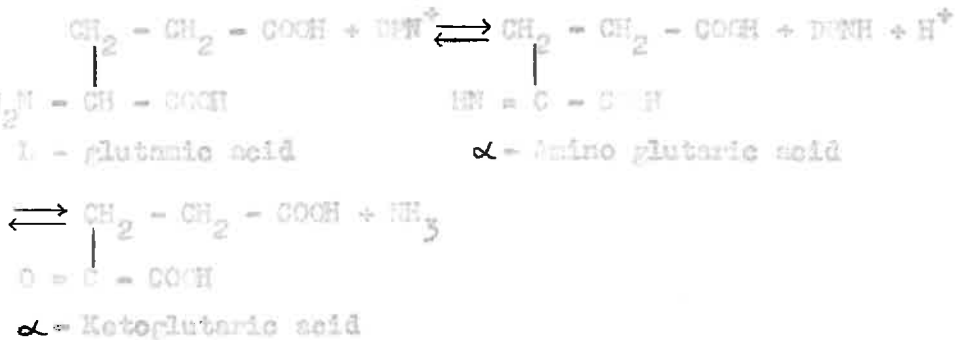
These two types of tumour are of interest because, although they both arise in the epidermis, they are totally different in their functional behaviour and differentiation. The spindle cell ultraviolet tumours show no tendency to keratinise, although a few squamous elements may be seen occasionally subjacent to the overlying epidermis. The microscopical picture shows cells growing out from the epidermis, and the acid phosphatase demonstrates columns of active cells directly continuous with those of the tumour. This appearance might well represent microscopical evidence of mutations in the epidermis, giving rise to clones of tumour cells. The benzpyrene tumours have many enzyme activities in common with the normal epidermis, and these are often accentuated. The spindle cell tumour is deficient in several of the enzyme systems, so that there is evidence of enzymatic, as well as structural differentiation.

On this basis the enzyme reactions under consideration may be classified into two groups. Firstly, those that are associated with the maturation and keratinisation of the epidermis, and are maintained in the benzpyrene tumour. Secondly, those that are related to the metabolic activity of the tumours and are active in

both types of tumour to a greater degree than in normal epidermis. Acid phosphatase does not fit into the classification unless the phosphatase of the stratum granulosum is considered to be a different enzyme from that of the main body of the ultraviolet tumour. This is very likely to be the case, as the enzyme in the two different situations almost certainly subserves different functions. There is, however, no method of distinguishing between them. Alteration in pH of the reactions does not suggest that there are different optima for the two situations.

The enzymes associated with epidermal maturation are esterase, β-hydroxybutyric dehydrogenase, glucose-6-phosphate dehydrogenase, α-glycerophosphate dehydrogenase and ethanol dehydrogenase. The enzymes concerned with cellular metabolism of the tumours are the enzymes of the citric acid cycle, lactate dehydrogenase, the diaphorases, glutamate dehydrogenase, cytochrome oxidase and adenosine triphosphatase. The last two show such widespread intense activity that they do not contribute to the study of the behaviour of the neoplasms.

Glutamate dehydrogenase is of interest because it represents the only enzyme that is significantly more active in both tumours than in the parent epidermis. It catalyses the reaction



and is concerned with the incorporation of glutamic acid into the citric acid cycle. Glutamate dehydrogenase will also act on alanine as a substrate (Fisher and McGregor, 1961). This link between protein and carbohydrate metabolism may represent one of the growth features of these tumours.

6. HISTOCHEMISTRY OF HUMAN SKIN CANCER.

6.1. Degenerative changes in the dermis.

Basophilic degeneration of the dermis is a condition that has received considerable attention since 1896, when Unna gave the first comprehensive description. The condition is characterised by an increase, primarily in the subpapillary layer of the dermis, of the connective tissue that takes up elastic tissue stains. This alteration in tinctorial reaction is only observed in areas exposed to sunlight and weathering (Hill and Montgomery, 1940; Evans et al, 1943; Lee, 1957). The best modern description of this condition is that of Percival et al (1949). The early changes are a thickening, lengthening and looping of collagen fibres. As the elastic fibres are intimately bound up with the collagen bundles, early degenerative changes may be seen by an increased angulation and apparent fragmentation of the elastic fibres. At the same time the collagen fibres start to lose their normal tinctorial properties and resemble elastic tissue in their affinity for stains. These changes steadily progress till the subpapillary part of the dermis becomes a structureless amorphous mass, without any fibrous arrangement.

These degenerative changes are very common, and may be considered to be part of the degenerative processes associated with ageing. The points to be discussed are whether basophilic degeneration plays any part in the pathogenesis of skin cancer and whether solar radiation accelerates the degenerative changes.

Gillman et al (1959) incriminated basophilic degeneration as responsible for the carcinogenic process, the mechanism of which

was unexplained. In experiments on the histological picture seen in the reaction of the skin when a donor graft site is covered with a homodermal graft, they noted proliferative epithelial changes and a conversion of the transplanted dermis to basophilically degenerated material. This transposed altered dermis eventually sloughed off as a scab. Their interpretation of this phenomenon appears to be that the active proliferation of the epidermis at the graft site was in some way akin to neoplastic transformation. It should not be forgotten in cancer studies of this nature that hyperplasia is not neoplasia, and that living cells are capable of showing quite marked reversible changes. The behaviour of the keratoacanthoma illustrates this, where severe degrees of pseudoepitheliomatous hyperplasia show complete reversal.

In an extensive analysis of the role of collagen degeneration in relation to solar skin lesions Mackie and McGovern (1958) also considered that collagen degeneration was responsible for carcinogenesis. The mechanism they proposed was that nutritional influences were the final cause of the skin cancers. These authors restricted themselves to observations on surgical biopsy material and clinical examination, and found that there was an overbearing of subjects with collagen degeneration in the fair skinned and those with skin cancer. Subjects with an olive type of skin did not show collagen degeneration clinically. Microscopically, they found collagen degeneration constantly in association with skin cancers and hyperkeratosis. By a series of deductions, they claimed to have evidence that a significant fraction of ultraviolet radiation reached

the dermis, mediated its effect on the subpapillary plexus of vessels and so gave rise to collagen or basophilic degeneration. They produced some evidence that the fair skinned had actually a thicker stratum corneum than the olive skinned, and deduced that the primary protective factor against ultraviolet radiation was the melanin pigment of the basal epidermal layer.

The optics of this situation are complex, and the biological factors are not capable of ready interpretation. Blum (1955) and Mitchell (1938) have attempted to analyse the various factors. It is known that near ultraviolet (longer wavelength) light can penetrate the epidermis and suppress the erythema of suburn, probably by direct action in the dermal blood vessels. The experimental observations discussed by Blum (1955), however, indicate that only a relatively minor fraction of ultraviolet radiation penetrates the epidermis. Direct evidence must be obtained from experiments before assumptions can be made about the effect of ultraviolet radiation on the skin. Rottier and Mullink (1952) have shown that the stratum corneum is an effective protection against erythema producing radiation. They have also shown that at wavelength 2500 - 2600 Angstrom units the erythema produced is less after stripping the stratum corneum than in the intact skin. Although this is outside the range of the solar spectrum at the earth's surface, it nevertheless demonstrates that the stratum corneum is not biologically inert, and that photochemical reactions take place in it.

Mackie and McGovern (1958) also consider that the pigment

in the basal layer is the main factor in the protection of the olive skinned against erythemal ultraviolet radiation. Simple examination of skin peelings following sunburn will demonstrate the presence of pigment in the desquamated epithelium. It is also a common observation in everyday life to see relatively well tanned people peeling, the peeled areas showing a pink untanned colour in contrast to the neighbouring tanned skin. Thomson (1955) has shown that the pigment in the stratum corneum of negro skin is an important factor in ultraviolet absorption.

There are uncertainties in relation to the interplay between the dermis and epidermis in carcinogenesis. Experimentally, using 20-methylcholanthrene, Billingham et al (1951) offered some evidence that the dermis influenced the carcinogenic process. However, the roots of the hair follicles were, of necessity, left in the donor area when Thiersch grafts were taken. Ghadially (1961) has reported experimental observations with chemically induced tumours, suggesting that they arise in the hair follicles. There appears to be no definite evidence that the dermis plays an active role in carcinogenesis, and that solar cancers are not primarily an epithelial disease. My own experimental observations support this view. The dermis of irradiated mice shows no degenerative changes or abnormal tinctorial reactions. I have made some observations on human skin that indicate that degenerative changes of the dermis are independent of skin carcinogenesis. A series of unselected forearm skins from coronial autopsies in Brisbane was studied for degenerative changes in the dermis. The subjects were of all ages and of all

TABLE 6.1

Presence of collagen degeneration in an unselected series of coronial autopsies in Brisbane.

Age in years	Male		Female	
	With degeneration	Without degeneration	With degeneration	Without degeneration
1	-	1	-	1
20 - 29	-	5	-	-
30 - 39	3	2	1	-
40 - 49	2	1	1	-
50 - 59	4	2	2	-
60 - 69	11	-	2	-
70 - 79	4	-	6	-
81	1	-	1	-
84	-	-	1	-
Total	25	11	14	3

skin types, and had followed a variety of occupations. These cases are listed in Table 6.1. The interesting finding was that collagen degeneration was present in most of the cases over forty years of age. It was also noted to be present in those who had an olive type of skin, and in those who were heavily tanned. Subjects of this type rarely develop skin cancers, and inspection of the bodies did not reveal any evidence of solar lesions. It was not uncommon to see marked collagen degeneration in the presence of a heavy collection of melanin in the epidermis. Likewise, degeneration was seen in young and middle-aged subjects without epidermal atrophy. The detailed pathology of several cases will be discussed to illustrate these points. The illustrations are of skin from dorsal aspect of forearm in each case.

Case 1. Female, aged 32 years. Fine textured, olive skin without any trace of lesions. Sudden death due to accidental lacerations of the throat. Figures 6.1 and 6.2 show moderately advanced elastotically degenerated collagen.

Case 2. Male, aged 39 years. Heavily tanned forearms with no evidence of solar lesions. Sudden cardiac death. Figures 6.3 and 6.4 show severe collagen degeneration, with a normal vascular pattern. There is no ectasia of the blood vessels or epidermal atrophy.

Case 3. Male, aged 53 years. Heavily tanned forearms with no evidence of solar lesions. Sudden death. Figure 6.5 shows collagen degeneration proceeding to an amorphous state. The vascular structure is normal and there is no epidermal atrophy.

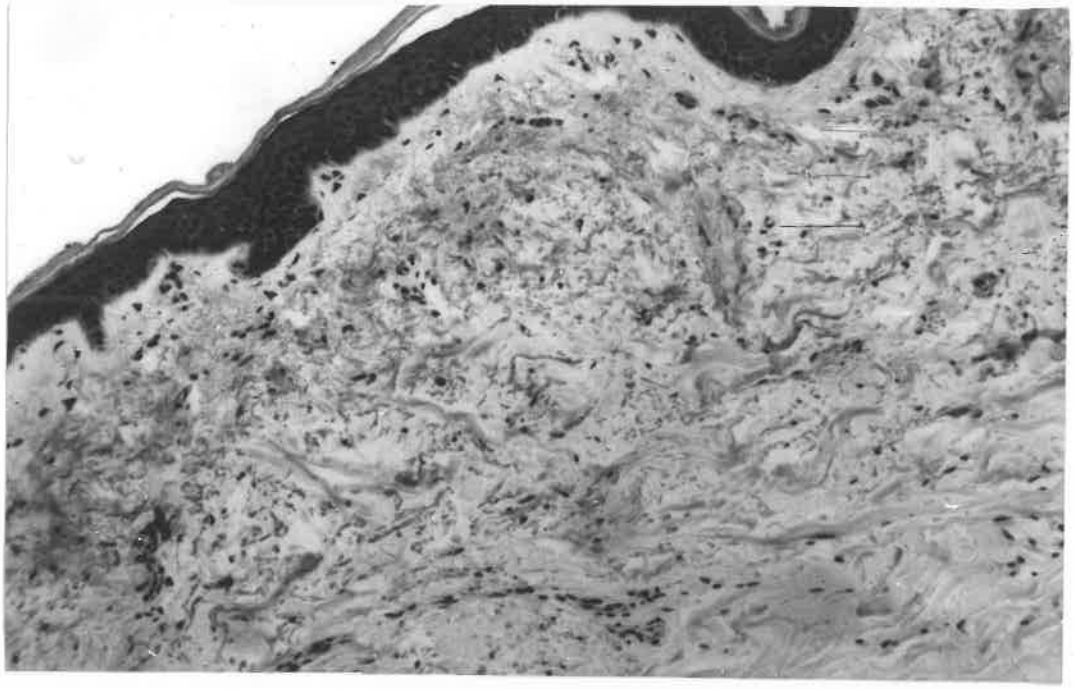
Case 4. Female, aged 38 years. Olive type of skin with no

evidence of solar lesions. Sudden death. Figure 6.6 shows early changes of increased elastic tissue staining with thickening and angulation of the fibres.

Case 5. Female, aged 34 years. Moderately tanned forearm. Sudden death. Figure 6.7 shows increased basophilia of dermal connective tissue with no epidermal atrophy.

These cases have been selected to illustrate the early changes and to emphasize that clinical inspection does not always reveal the presence of even marked degeneration changes. During the process of the degeneration the vascular pattern does not show any variation from the basic normal pattern. The capillary loops of the papillary layer and the capillaries and arterioles of the subpapillary and reticular layers are well preserved in the presence of severe degeneration (Figures 6.3 and 6.5). Figures 6.8 and 6.9 demonstrate the normal vascular pattern in a seven week old child and a 21 year old male. Figure 6.9 illustrates the basophilia of elastic tissue, but there is no excess of it or fragmentation of the collagen fibres. I have not observed the ectasia of the blood vessels noted by Mackie and McGovern (1958) and this would seem to be a late development. It may even be due to loss of support for the vessels as they traverse the altered layers of collagen. Figure 6.10 shows the vascular pattern and dermal homogenisation of the skin from the immediate surrounds of a low grade squamous cell carcinoma of the upper arm of a 62 year old man.

Figure 6.11 shows the lipid staining with Fettrot 7B of the degenerating collagen fibres from the neighbourhood of a basal cell carcinoma (reported by Percival et al, 1949).



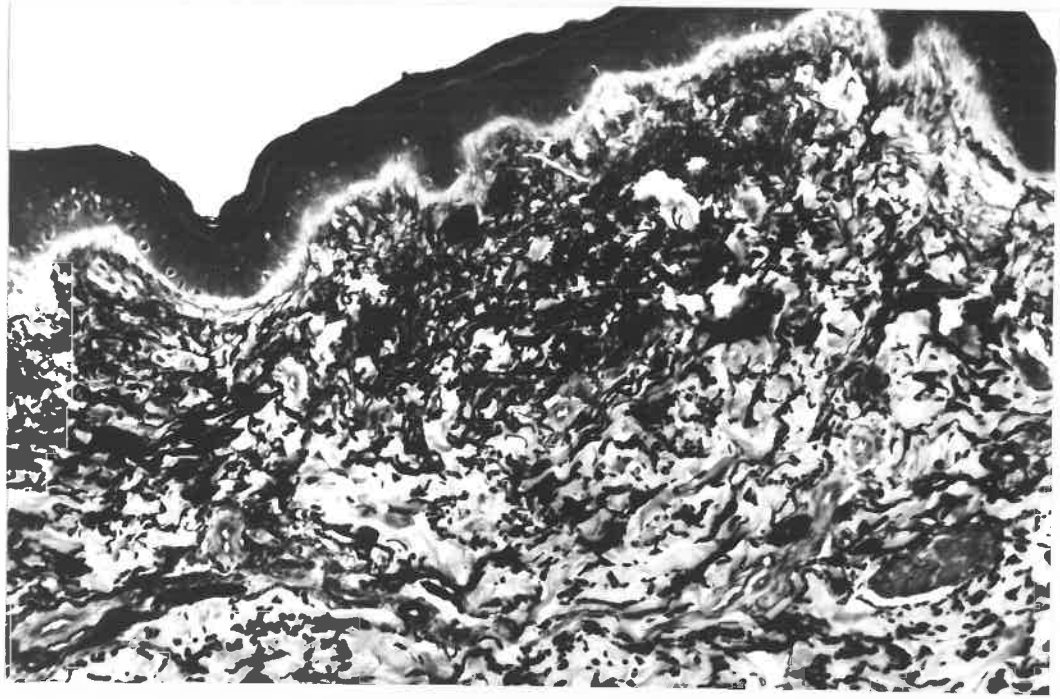
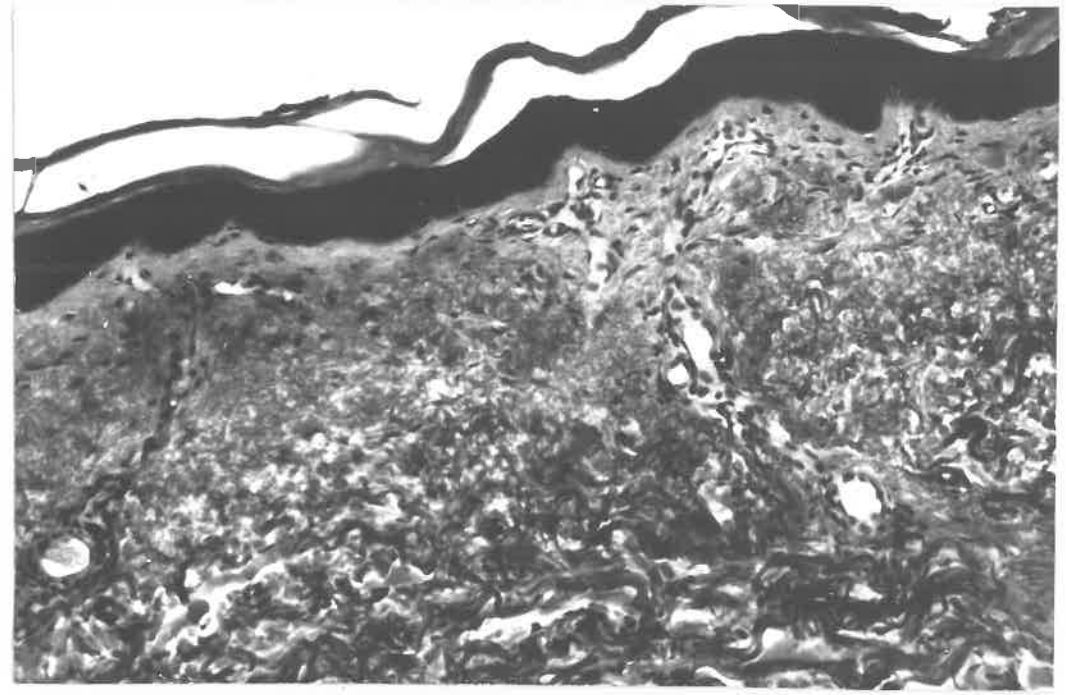


Figure 6.1. Haematoxylin and eosin (133 x).

Figure 6.2. Verhoeff and eosin.
Female aged 32 years with fairly marked collagen degeneration. All sections of skin are from the extensor aspect of the forearm, except where otherwise stated (133 x).



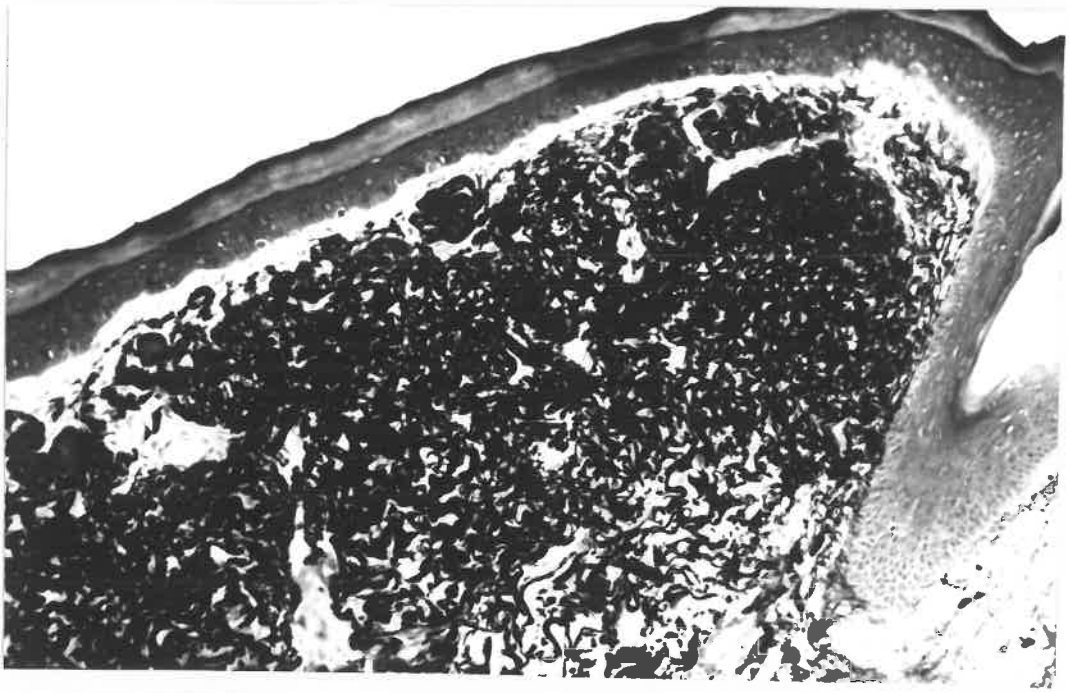


Figure 6.3. Haematoxylin and eosin (133 x).

Figure 6.4. Verhoeff and eosin.
Male aged 39 years with severe collagen degeneration (133 x).

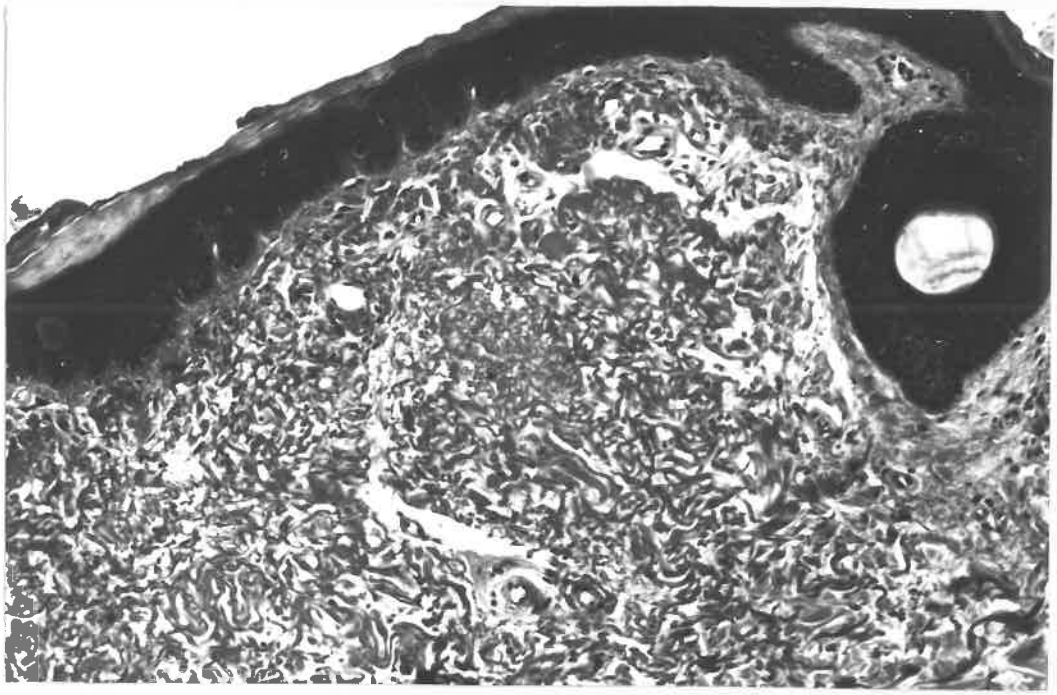


Figure 6.5. Haematoxylin and eosin.
Male aged 53 years with advanced collagen degeneration (133 x).

Figure 6.6. Verhoeff and eosin. Female aged 38 years, with early degenerative changes in the collagen (133 x).

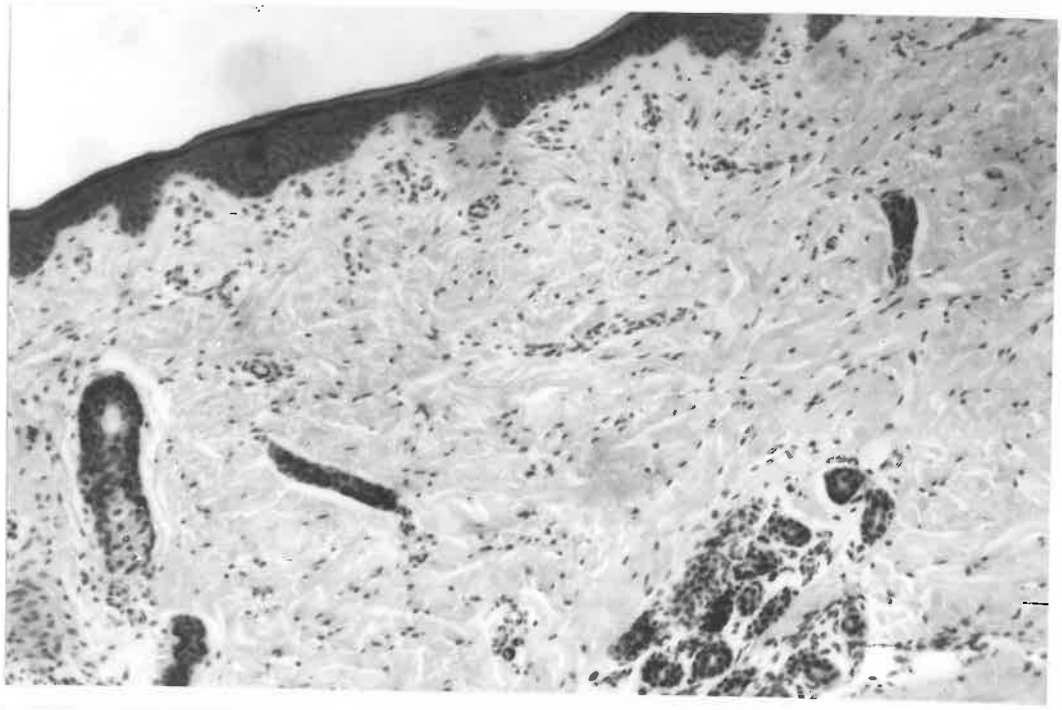
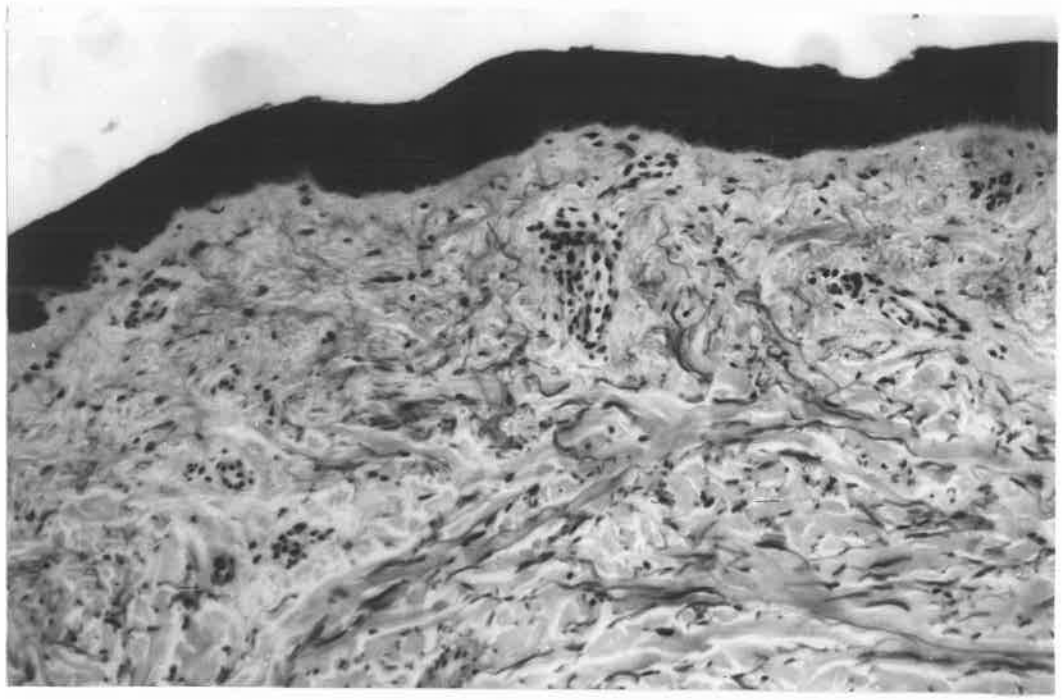


Figure 6.7. Verhoeff and eosin.
Female aged 34 years with fairly marked increase of elastic tissue staining of the dermis (133 x).

Figure 6.8. Haematoxylin and eosin.
Normal vascular pattern in a seven week old child (133 x).



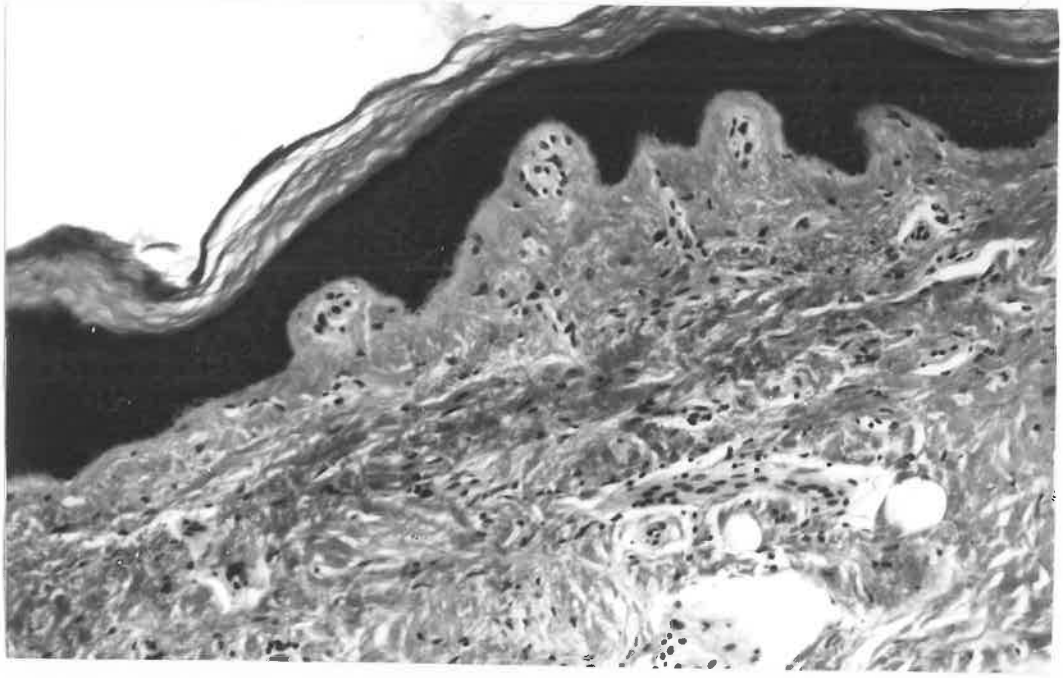


Figure 6.9. Haematoxylin and eosin.
Normal vascular pattern in a 21 year old male (133 x).

Figure 6.10. Haematoxylin and eosin.
Vascular pattern and degenerated collagen from the vicinity of a low grade squamous cell carcinoma of the upper arm of a 62 year old man (133 x).

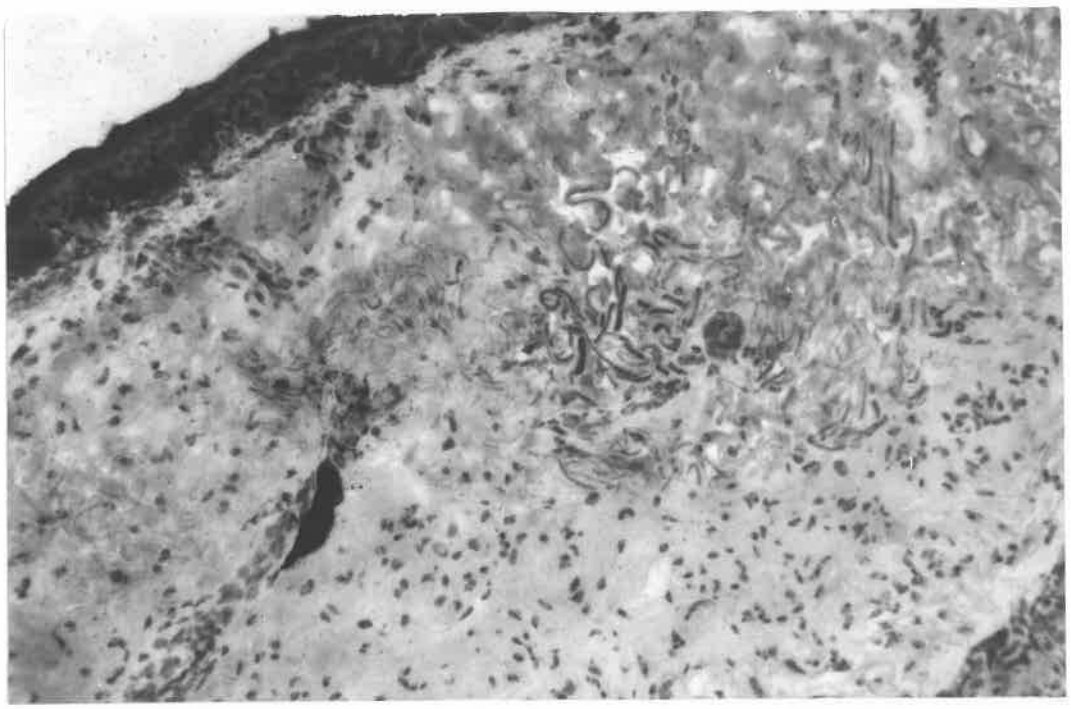


Figure 6.11. Fettrot 7B (Ciba) and haematoxylin. Frozen section of skin from the vicinity of a basal cell carcinoma. Shows marked staining of degenerating collagen fibres and lesser staining of amorphous material.(133 x).

6.2. Mechanism of collagen degeneration.

From the considerations in the previous section a direct effect of ultraviolet radiation and an effect due to local vascular disease seem to be unlikely causes of the collagen degeneration. This leaves the possibility of a nonspecific effect due to ageing and weathering, or the action of another part of the solar spectrum as causes of the degenerative changes.

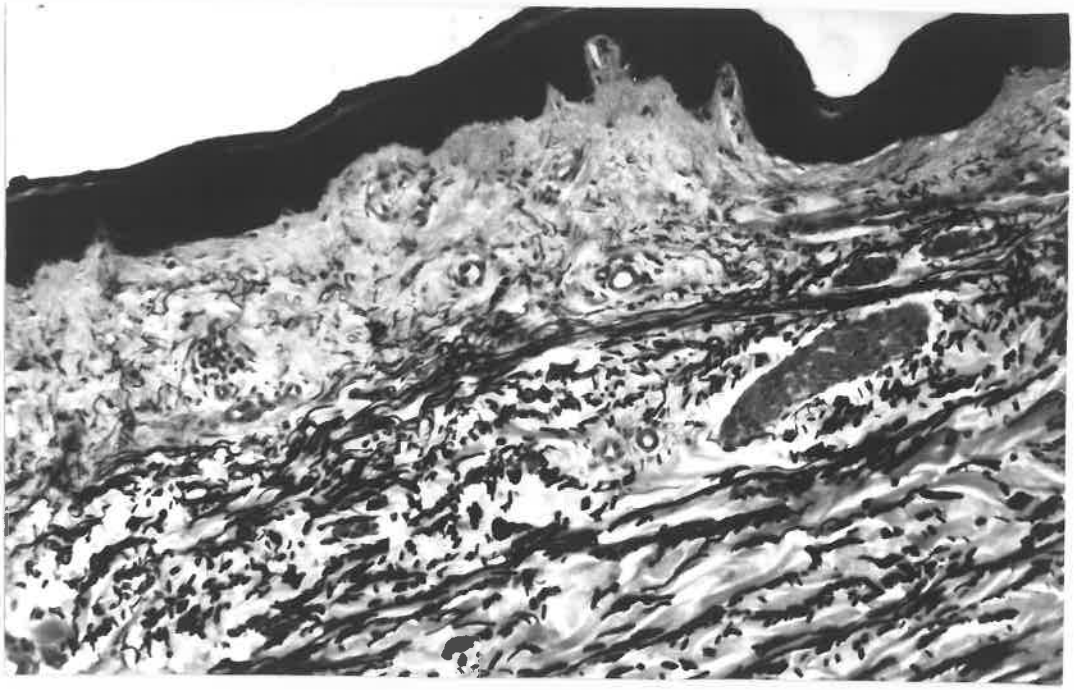
Collagen degeneration has been reported from various parts of the world where skin cancer is not a problem (e.g. Percival et al, 1949, in Edinburgh). However, there is an impression that it is more prevalent in the tropics, so that the effect of tropical conditions superimposed on a normal ageing process probably accentuates the frequency of occurrence.

There is evidence that collagen can be converted to elastin like material by various experimental manipulations, including exposure to ultraviolet radiation (Keech et al, 1956). However, in a later paper Keech and Reed (1957) revised their opinion regarding the degrading effect of ultraviolet radiation on collagen, and considered that the degenerative reaction was probably due to heat alone. The thermal degradation point of mammalian collagen is well above the physiological range (60°), and in the experiments of Keech et al (1956) treatment of the collagen with collagenase was necessary before degradation could occur. Hall (1961) suggested, in connection with vascular disease, that removal of a small amount of stabilising polysaccharide may lower the thermal transition point of collagen sufficiently for denaturation to take place at body temperature.

This argument of Hall (1961), if it may be transferred to the connective tissue of skin, would apply all the more to basophilic degeneration. Woernley (1952) has reported intense spectral absorption bands for carbohydrates in the 8000 - 11,000 Angstrom unit region. The observations of Bonner and Duncan (1962) and Hendler et al (1958) are in fairly good agreement, and fit in with the possibility that infrared radiation penetrates the epidermis in sufficient quantity to damage the dermis. The former authors measured the infrared absorption spectra in vitro of melanin from several sources and showed that the transmission fell at about 6000 Angstrom units and was then maintained at a steady 40 per cent through the longer wavelengths. Hendler et al (1958) showed that the reflectance of white human skin increased rapidly at about 7000 Angstrom units and then started to fall from about 10,000 Angstrom units. Thus the presence of melanin pigment partially protects against infrared radiation.

This property of the penetration of the radiation is, of course, used in the infrared photography of the superficial venous system. The discrepancies between the spectrophotometric and reflectance results are no doubt due to the differences in the experimental systems.

In basophilic degeneration of the dermis, the papillary layer is always spared. The structural variations could account for this. The papillary layer contains a much greater proportion of PAS positive reticular fibres than the other parts of the dermis. There is as yet no direct experimental evidence to support the hypo-



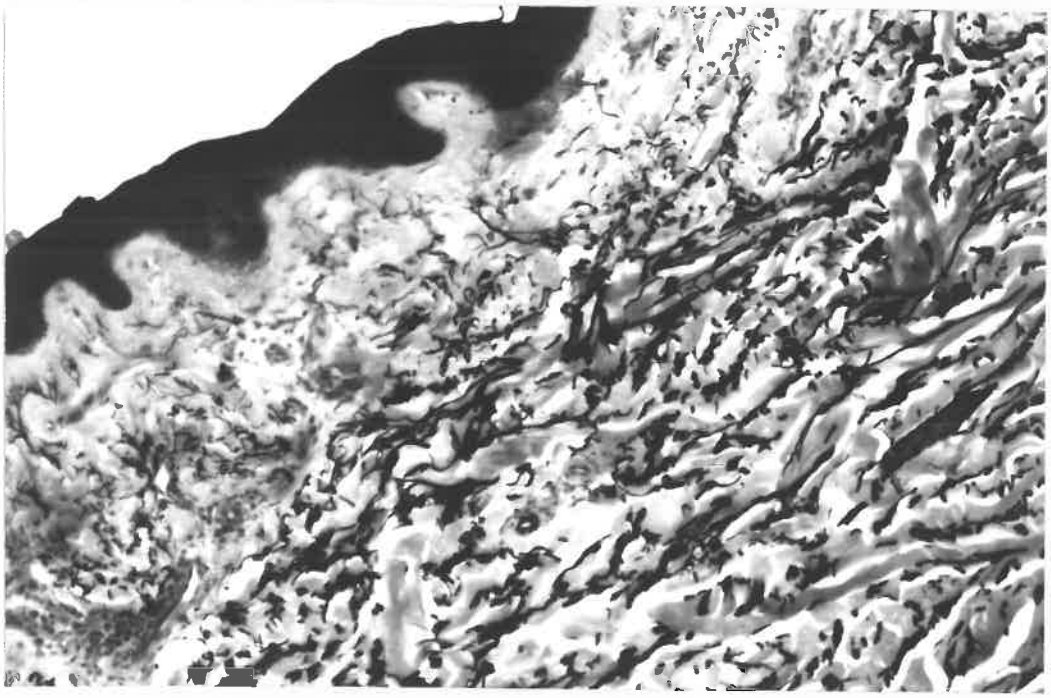


Figure 6.12. Verhoeff and eosin. Skin from the dorsal aspect of the forearm of a 42 year old Indian (133 x).

Figure 6.13. Verhoeff and eosin. Skin from the dorsal aspect of the forearm of a 41 year old Chinese (133 x).

Neither of these show any increase in elastic tissue staining.

thesis, but the requirements for a photochemical reaction in the dermis by infrared radiation are all satisfied by the available reports for white skin.

I have examined the skin of several coloured subjects and have found no evidence of increased basophilia of the cutis or elastotic degeneration. The elastic fibres remain fine and discrete (Figures 6.12 and 6.13). This may be simply due to the presence of greater quantities of pigment, or possibly to racial, genetic or other factors. This finding does not contradict the infrared hypothesis, but needs to be investigated with a wider range of material.

6.3. Enzyme histochemistry of human skin tumours.

I have examined a series of biopsy specimens of human skin tumours (obtained through the courtesy of Dr. B. S. Hanson and Dr. W. Tipping) and have found that the reactions of the neoplastic tissue of squamous cell carcinoma are similar to those in the mouse. The reactivity of basal cell carcinoma is variable and does not run to a pattern, though the neoplastic cells show generally less of an activity with all the enzymes than the parent epidermis. Figure 6.14 shows the lactate dehydrogenase activity of a rodent ulcer in an 85 year old man, the neoplastic cells show a clear cut difference from the parent epidermis, both in cytological localisation and intensity. The glutamate dehydrogenase reaction in a rodent ulcer in an 89 year old man is shown in Figure 6.15. The activity is mainly in the basal layers of the epidermis and proliferating cell masses.

The acid phosphatase activity in a basal cell carcinoma with areas of keratinisation from the neck of a 70 year old female is shown in Figure 6.16. The enzyme activity shows a similar distribution to that in squamous cell benzpyrene tumours in the mouse, and is associated with the process of keratinisation. Esterase activity in this tumour shows a similar distribution.

There do not appear to be any characteristic enzymatic features of human skin cancer, such as have been found in the ultraviolet tumour in the mouse.

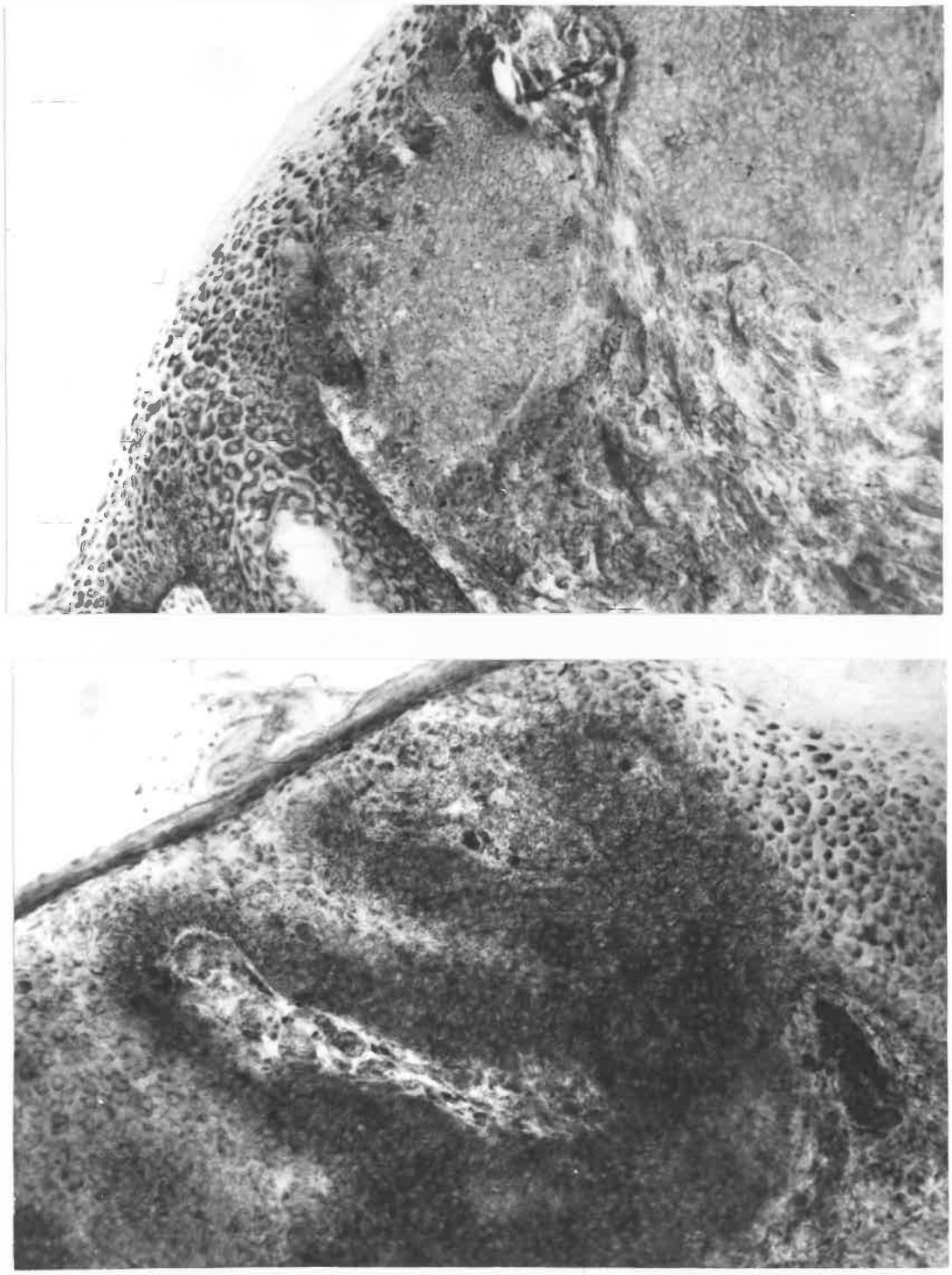


Figure 6.14. Lactate dehydrogenase. Nitro BT counterstained with methyl green. Rodent ulcer, showing marked contrast in reaction between neoplastic tissue and parent epidermis (133 x).

Figure 6.15. Glutamate dehydrogenase. Nitro BT counterstained with methyl green. Rodent ulcer, showing accentuated activity in lower epidermal layers (133 x).

Figure 6.16. Acid phosphatase, Barka method. Squamous cell carcinoma in a 70 year old female. Enzyme activity in keratinising layers and epidermal cells. Haematoxylin counterstain

Appendix 1. The 1948 sample of records from the Queensland Radium Institute.

This first sample consisted of the records of all the patients who presented for treatment of skin cancer or hyperkeratosis at the Brisbane centre of the Queensland Radium Institute in the year 1948. The purpose was to test the statistical and sampling technique, and to provide an independent verification of the analysis of the later sample, which was part of the systematic survey. The incidence rates for total skin cancer per 10,000 susceptible population are given below.

Mean of decennial age group	Male		Female	
	Observed rates	Fitted rates	Observed rates	Fitted rates
25	5	5	1	2
35	17	15	5	6
45	29	33	21	16
55	58	59	33	35
65	87	95	55	67
75	160	134	93	110

The slope parameters for the logarithmic logistic transformation versus age (as for Table 2.9) are:

Male	Female
4.21	5.07

The weighted mean incidence rates (standardised in the Rockhampton population at 1954 centres) are:

Male	Female
42.2	28.5

The male slope parameter is similar to that for the later Brisbane sample, but the mean rate is 13 per cent lower, whereas the female mean rate is similar but the slope parameter rather higher. This is unlikely to be due to any systematic error, and is probably due to sampling fluctuation, bearing in mind that the later sample was analysed for an average of ten years' observations.

The classification of patients into lesion groupings gives a result that shows no statistically significant difference from that of the later sample. The symbols are the same as those used previously (Table 2.6).

	Male	Female	Total
Abc	163 (21.3)	145 (23.9)	308 (22.4)
aBc	52 (6.8)	31 (5.1)	83 (6.0)
abC	293 (38.3)	277 (45.6)	570 (41.5)
ABc	21 (2.7)	5 (0.8)	26 (1.9)
AbC	148 (19.3)	101 (16.6)	249 (18.1)
aBC	37 (4.8)	29 (4.8)	66 (4.8)
ABC	51 (6.7)	19 (3.1)	70 (5.1)
Total	765	607	1372

Making the necessary amalgamations of classes to eliminate small numbers, χ^2 testing with the later Brisbane sample (Table 2.6) gives a value of 5.6287 (degrees of freedom = 5, $0.5 > P > 0.3$) for the males, and 1.8639 (degrees of freedom = 4, $0.8 > P > 0.7$) for the females.

Appendix 2. Histochemical procedures.

1. Preparation of sections. Experimental tumours were removed after the animal had been killed by stunning and neck dislocation. The blocks of tissue were trimmed and rapidly frozen between pieces of solid carbon dioxide. Human skin tumours were similarly frozen immediately after removal. Sections were cut at 8 - 10μ on a Cambridge Rocker microtome housed in a deep-freeze cabinet, at a temperature of approximately -20°C. The sections were mounted on chemically clean glass slides, allowed to dry at room temperature and stored in a refrigerator (0 - 4°C.).

2. Special histochemical methods.

(1) Acid Phosphatase.

a. Gomori (1950).

Preparation of Incubating Medium.

(a) in 500 ml of .05 M acetate buffer at pH 5, dissolve 0.6 gm. of lead nitrate. Add 50 ml of a 3% solution of sodium β-glycerophosphate.

(b) Keep solution in incubator at 37°C. for 24 hours and filter.

(c) Add about 5% extra distilled water to the filtrate. Incubate sections for 3 hours at 37°C., wash, and treat with dilute ammonium sulphide for 2 minutes. Counterstain with methyl green or eosin.

b. Barka and Anderson (1963), modified by Dr. H. M. Fullmer (unpublished).

Incubating medium.

20 mg. sodium α-naphthyl acid phosphate.

13 ml distilled water.
5 ml Veronal acetate buffer.
1.6 ml hexazonium salt.

Filter and add 1.5 gm. of polyvinylpyrrolidone.

Adjust pH to required level and incubate sections at room temperature for 15 minutes.

Preparation of Hexazonium Salt.

Solution A - 4% basic fuchsin in 2N HCl.

Solution B - 4% sodium nitrite in distilled water.

Place equal quantities of A and B together in a flask in an ice-bath (0 - 5°C.) for 15 minutes.

Counterstain with haematoxylin.

(2) Alkaline Phosphatase.

a. Gomori (1952).

Incubating medium.

5% sodium glycerophosphate	10 ml.
2% sodium diethyl barbiturate	10 ml.
Distilled water	5 ml.
2% calcium chloride	20 ml.
5% magnesium sulphate	1 ml.

Incubate sections for 4 hours at 37°C., wash and treat with dilute ammonium sulphide for 2 minutes. Counterstain with methyl green or eosin.

b. Azo Dye method (Pearse, 1960).

Incubating medium.

20 mg. sodium α-naphthyl acid phosphate.
20 ml 0.1 M Tris buffer (pH 10).
20 mg. Fast Red TR or Fast Blue B.

Filter and incubate sections at room temperature for 15 minutes.

c. Adenosine triphosphatase (Wachstein and Meisel, 1957).

Incubating medium.

10 ml 1.25% adenosine-5-triphosphate.
5 ml 0.2 M "tris" buffer (pH 7.2).
50 ml 0.2% lead nitrate.
5 ml 0.1 M magnesium sulphate.

Incubate sections for 2 hours at 37°C., wash and treat with dilute ammonium sulphide for 2 minutes.

Counterstain with methyl green or eosin.

(3) Esterase.

a. Indoxyl acetate method (Holt, 1958; Holt and Withers, 1952).

Incubating medium.

1.3 mg 5-Brome-indoxyl acetate.
0.1 ml ethanol

Allow to dissolve and add:

2 ml 0.1 M "tris" buffer (pH 7).
1 ml 0.05 M potassium ferricyanide.
1 ml 0.05 M potassium ferrocyanide.
1 ml 0.1 M calcium chloride.

Add water to 10 ml.

Incubate sections for 2 hours at 37 C. Counterstain with alum carmine.

b. Naphthol AS acetate method (Gomori, 1952).

10 mg α-naphthol acetate.
0.25 ml acetone
20 ml 0.1 M phosphate buffer (pH 7.4)
100 mg Fast Blue B salt.

Filter the solution and incubate sections for 15 minutes at room temperature.

(4) Leucine Aminopeptidase (Nachlas et al, 1957).

Incubating medium.

1 ml L-leucyl-β-naphthylamide (1 mg/ml of distilled water)
10 ml acetate buffer (0.1 M, pH 6.5)
8 ml sodium chloride (0.85%)
1 ml potassium cyanide (2×10^{-2} M)
10 mg Fast blue B salt.

Incubate sections for 1 hour at 37°C.

(5) Monoamine Oxidase.

a. Tetrazolium Method (Glenner et al, 1957).

Incubating medium.

25 mg Tryptamine hydrochloride.
4 mg sodium sulphate.
5 mg Nitro-blue tetrazolium.
5 ml 0.1 M Phosphate buffer (pH 7.6)
15 ml distilled water.

Incubate sections for 45 minutes at 37°C. Counter-stain with methyl green.

b. Naphthoic Hydrazide Method (Koelle and Valk, 1954).

Preincubating medium.

3.0 ml distilled water
1.5 ml hydrazine
3.0 ml buffer (0.2 M - Na_2HPO_4)
7.5 ml Na_2SO_4 (w/v, 40%, pH 8.6)

Control preincubating medium as above with 0.15 ml Marsilid (0.1 M)

Incubate sections for 1 hour at 22°C.

Sections rinsed in following medium:

4.5 ml distilled water
3.0 ml buffer (0.2 M - Na_2HPO_4)
7.5 ml Na_2SO_4 (w/v, 40%, pH 8.6)

Incubating medium.

4.35 ml distilled water
0.15 ml M-NaOH
3.0 ml buffer (0.2 M - Na_2HPO_4)
7.5 ml Na_2SO_4 (w/v, 40%, pH 8.6)

Heat to 80-90°C. and saturate with 2-Hydroxy-3-naphthoic acid hydrazide. Cool, filter and add 1.0 ml tryptamine hydrochloride (0.1 M).

Control incubating medium as above with 0.15 ml Marsilid (0.1 M).

Incubate sections for 2 hours at 37°C. (During incubation oxygen is passed through the medium.)

Developing medium.

300 mg. Fast blue B salt
10 ml distilled water
5 ml phosphate buffer (pH 7.4)

Develop sections for 3 minutes at room temperature.

(6) Succinic Dehydrogenase (Nachlas et al, 1957)

Incubating medium.

5 ml 0.2 M-phosphate buffer (pH 7.6)
5 ml 0.2 M-sodium succinate
10 ml aqueous solution of Nitro-BT (1 mg/ml)

Incubate sections for 1 hour at 37°C.

(7) Diphosphopyridine nucleotide (DPN) dependent dehydrogenases.

Substrates for dehydrogenases.

Sodium lactate; sodium malate; sodium glutamate;

sodium α-glycerophosphate; isocitric acid; β-hydroxy butyric acid; ethanol.

Incubating medium.

0.4 ml Substrate (1.0 M)
0.4 ml DPN (0.1 M)
0.4 ml Sodium cyanide (0.1 M)
0.4 ml Magnesium chloride (0.05 M)
1.0 ml "Tris" buffer (pH 7) 0.2 M
1.0 ml Nitro-BT (1 mg/ml)
0.4 ml Distilled water
300 mg Polyvinylpyrrolidone.

Adjust the pH of the solution to 7 and incubate sections for 20 minutes at 37°C. Counterstain with methyl green.

(8) Glucose-6-Phosphate dehydrogenase (Hess et al, 1958)

Incubating medium.

0.2 ml 0.5 M-cobaltous chloride
1.0 ml "tris" buffer (0.2 M, pH 7)
0.4 ml Glucose-6-phosphate
0.4 ml TPN (0.1 M)
0.4 ml sodium azide (0.1 M)
0.4 ml magnesium chloride (0.05 M)
1.0 ml MTT (1 mg/ml)
0.4 ml distilled water
300 mg polyvinylpyrrolidone.

Adjust the pH of the solution to 7 and incubate sections for 20 minutes at 37°C. Counterstain with methyl green.

(9) DPN and TPN Diaphorase (Scarpelli et al, 1958)

Stock solution.

2.5 ml MTT (1 mg/ml)
0.3 ml cobaltous chloride (0.5 M)
2.5 ml "tris" buffer (0.2 M, pH 8.0)
4.1 ml distilled water

750 mg polyvinylpyrrolidone.

The pH is adjusted to 7.2.

Incubating medium.

10 ml stock medium
60 mg DPNHor TPNH.

Adjust the pH of the solution to 7.0 and incubate the sections for 20-30 minutes at 37°C. Counterstain with methyl green.

(10) Cytochrome Oxidase (Burstone, 1960)

Substrate.

N-phenyl-p-diphenylamine hydrochloride with either p-amino-azo-benzene or 3-amino-9-ethyl carbazole as coupling agent.

Incubating Medium.

To 15 mg of chosen pair in a flask, add:
0.5 cc. ethyl alcohol and dissolve. Add:
35 cc. distilled water,
15 cc. 'tris' buffer (0.2 M, pH 7.4)
Filter into coplin jar and incubate sections for 60 minutes at 37°C.

Place sections in following solution for 1 hour.

10% cobaltous acetate in unbuffered formalin.
5 cc. acetate buffer (0.2 M, pH 5.2)

(11) β-Glucuronidase (Seligman, 1954)

Substrate solution.

30 mg 6-bromo-2-naphthyl-β-D-glucopyruronoside
5 ml ethanol
20 ml phosphate-citrate buffer (pH 4.95)
75 ml distilled water
Incubate sections for 4-6 hours in the substrate solution at 37°C.

Rinse sections and them immerse in a solution of Fast Blue B in cold (4°) phosphate buffer (0.02 M, pH 7.5), 1 mg. per ml.

(12) Non-enzymatic tetrazolium lipoprotein method (Carmichael, 1963)

Stock tetrazolium solution.

25 ml "tris" buffer (0.2 M, pH 8.2)
3 ml cobaltous chloride (0.5 M)
Filter and add:
25 ml MTT (1 mg/ml)
25 ml "tris" buffer (0.2 M, pH 8.2)
41 ml distilled water
Adjust pH to 6.

Incubating medium.

15 ml CoMTT
0.25 ml hydroquinone (0.1 M, freshly prepared)
Incubate sections for 90 minutes at 37°C.

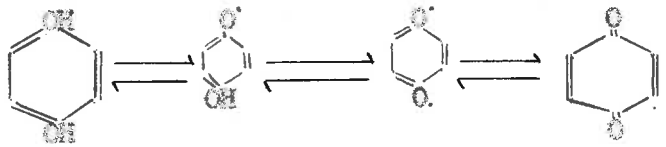
Appendix 3. Studies on the tetrazolium salt reduction method for lipo proteins.

1. The tetrazolium salt reduction method for phospholipids has been reported in the literature (Carmichael, 1963) and the technical details are given in Appendix 2.

The reaction demonstrates principally phospholipids conjugated in the form of lipoproteins and is a very ready method of showing up mitochondria. This reaction is carried out on frozen sections, either unfixed or fixed in buffered formalin.

The mechanism depends on the reaction between hydroquinone and the nitrogen of phosphatidyl choline, ethanolamine and serine (i.e. lecithin and the cephalins). The hydroquinone lipid complex then reduces the tetrazolium salt, with precipitation of insoluble formazan at the site of reaction.

Hydroquinone is a diphenol, which in solution forms a mixture of benzoquinones and semiquinones according to the equation



Hydroquinone Semiquinones Benzoquinone

The free radicals of the semiquinones are active reducing groups, and the mechanism of the reaction is probably that one of the hydroxyl groups conjugates with the nitrogen of the lipid and either a hydrogen is liberated from that site, or the opposite hydroxyl group forms a free radical, with liberation of another hydrogen.

The hydrogen is then taken up by the tetrazolium salt, with precipitation of the formazan.

The most suitable tetrazolium salt for the reaction is 3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyl tetrazolium bromide (usually known as MTT) in a buffered solution at pH 6. Above neutral pH the hydroquinone spontaneously reduces the tetrazole, but at acid pH levels the spontaneous reduction is slow and there is clearly localised precipitation of formazan in the cytoplasm, resembling that seen in dehydrogenase histochemistry. Of the other tetrazolium salts Nitro-BT(2,2'-di-p-nitrophenyl-5,5'-diphenyl-3,3'-(3,3'-dimethoxy-4,4'-biphenyl) ditetrazolium chloride) reacts rapidly at an alkaline pH, and Tetra nitro-BT may also be used. The brown formazan of the latter, however, is not a good contrasting colour for microscopical preparations. I have used MTT chelated with cobalt (COMTT) almost exclusively as it gives good cytological differentiation and is easily soluble in organic solvents, a property necessary for quantitative estimation. The chemical interpretation of the reaction is supported by a number of tests and experiments:

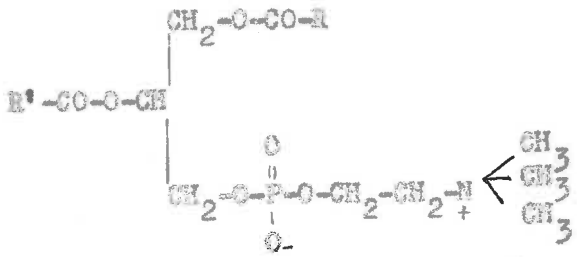
(1) Prior exposure of the sections to alcohol very rapidly prevents the reaction. Acetone and chloroform almost completely prevent it, but, curiously, ether alone does not affect it at all, apart from the distortion of the cell picture due to the dehydrating effect. The reaction may be carried out on a section, the formazan dissolved in ether and the reaction repeated. This manoeuvre may be repeated five or six times. This differential solubility serves

to emphasize that solubilities of lipids in intact tissues and in vitro are not necessarily similar.

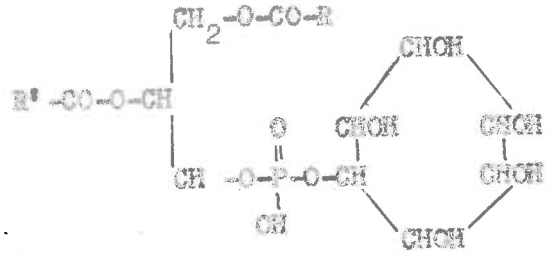
(2) The reaction works in vitro. Aqueous suspensions of lecithin, cephalin and sphingomyelin (i.e. water added to alcoholic solutions) added to the CoMTT-hydroquinone mixture cause a formazan production in about the same length of time that it takes for the histochemical reaction. This implies that the in vitro reaction is proceeding in the same manner as the histochemical. The reaction also works in vitro with the basic parts of the phosphatidyl esters. Solutions of ethanolamine (buffered to pH 6) serine and methacholine (stable derivative of choline) will reduce the tetrazolium salt in the presence of hydroquinone. Glycine, glutamine and tryptamine behave in a similar manner, and the mechanism of reduction of tryptamine is no doubt similar to that utilised in the tetrazolium salt method for demonstrating monoamine oxidase (Glenner et al, 1957).

In the absence of hydroquinone, the reaction is negative both with sections and in vitro.

(3) The reaction in vitro with a suspension of phosphoinositide is negative, which precludes the possibility that the formazan production is taking place at unsaturated bonds of fatty acids. In the formulae, R and R' indicate fatty acids.



Phosphatidyl choline (zwitterion form)



Phosphatidyl inositol.

(4) The possibility of an enzymatic reduction of the tetrazolium salt in the tissue sections must be excluded, though it is most improbable in view of the in vitro tests. The reaction works after exposing the sections to boiling water (15 minutes) and formalin (24 hours at room temperature). These exclude the possibility of a dehydrogenase utilising the hydroquinone as a substrate. Although peroxidase, with its wide range of substrates, can oxidise hydroquinone, the likelihood of this enzyme or of cytochrome oxidase being responsible for the reaction can be discounted by the observation that cyanide and azide make no difference to the amount of formazan production. Tetra nitro BT was used as the tetrazolium salt in the case of cyanide. Further evidence against a peroxidatic mechanism is that peroxide added to the medium diminishes, not enhances the formazan deposition. Catalase added to the medium

makes no difference to the reaction.

(5) Experiments with fractionated cells have also been carried out. The liver of a freshly killed guinea pig was perfused in situ with chilled 5 per cent sucrose in 4 per cent neutral buffered formalin. As soon as the blood was washed out the liver was removed, chopped up and homogenised. This breaks up most of the cells. The homogenate was fixed overnight at 4°C., with constant stirring. The material was then centrifuged at 700xg for 10 minutes giving the first sediment, which consisted of nuclei, whole cells and débris. The first supernatant was centrifuged at 5000xg for 10 minutes, and the second sediment collected consisting mainly of mitochondria. A final centrifuging of the second supernatant at 24,000xg for 10 minutes gives a sediment consisting mostly of microsomes. When the sediments and final supernatant were tested with the CoTT-hydroquinone mixture, a positive result was obtained only with the first and second sediments, even after prolonged incubation. The partial fixation by cold formalin was found to be necessary, as without it there was some autolysis, and a positive reaction was obtained with the final supernatant. Attempts have been made to visualise the formazan under the electron microscope, but results have not yet been entirely satisfactory.

(6) Parenteral administration of hydroquinone to the laboratory mouse sends the animal into a state of muscular spasm. With small doses the animal recovers fairly rapidly, but with larger doses the spasms develop into a series of tonic contractions with neck retraction. This is evidence that hydroquinone potentiates the

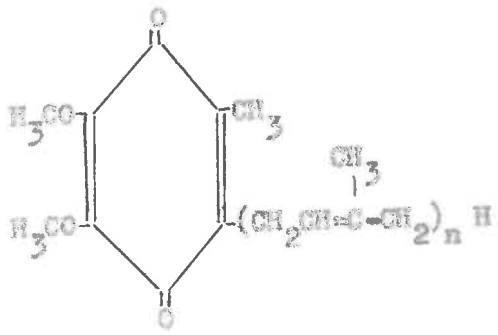
action of acetyl choline by protecting it from choline esterase, by conjugating with the choline molecule.

2. The function of quinones in dehydrogenase histochemistry.

The formazan deposition in the tetrazolium salt lipoprotein method bears strong morphological similarities to that from dehydrogenase reactions. However, evidence has been brought forward to prove that the reaction is itself non-enzymatic. Cytologically it is closely related to particles that have enzymatic functions, and this suggests that the molecular structure of the lipoprotein being stained is intimately concerned with enzymatic functions. Red blood cells do not stain by this method, but a model for their structure such as that of Mitchison (1950) indicates that the lipid is on the surface of the erythrocyte envelope, and that there is no protein present to trap the formazan. Other formazans (e.g. from nitro BT) are said to have a substantivity for protein which is necessary for efficient microscopical demonstration of dehydrogenase reactions (Pearse, 1960, page 546). Substantivity for protein by the formazan from the CoMTT chelate may exist but has not been proved. Cell and nuclear membranes are not well stained by this method, though plaques of formazan may be seen in cell membranes, particularly on longer incubation in formalin fixed material.

Since the independent discoveries by Crane et al (1957) and Morton (1958) it has been known that quinones will act as electron acceptors in biological oxidations. A series of substituted benzoquinones have now been discovered, each with their own characteristic side chain. They all consist of 2,3-dimethoxy-5-methyl-1,

4-benzoquinone with a polyisoprene side chain in position 6.



These substances have been named ubiquinone or coenzyme Q_n, where n represents the number of isoprene groups. This side chain governs the optical properties of the molecule, and this property has been used in another system of nomenclature in which each quinone is identified by its optimum spectroscopic absorption band. There have been a number of publications on this subject, which have been recently summarised by Hatefi (1963). The reduction of bound coenzyme Q of mitochondria can be brought about by the addition of pyruvate plus malate, succinate, reduced diphosphopyridine nucleotide and β-hydroxybutyrate. The significant feature of the biochemical studies in relation to the histochemical is that the activity of coenzyme Q is intimately bound up with the presence of lipid. This lipid has been named coenzyme Q lipoprotein, and consists of a very high proportion of lecithin and cephalin (Basford, 1959; Basford and Green, 1959).

Sowerby and Ottaway (1962) reported a non-dialysable, non-enzymic, heat stable factor in partially purified glutaminase preparations from kidney mitochondria. This factor raised the reduction of tetrazolium salt to 100 per cent of theoretical value in the

enzymatic oxidation of glutamate. It was replaceable by ubiquinone.

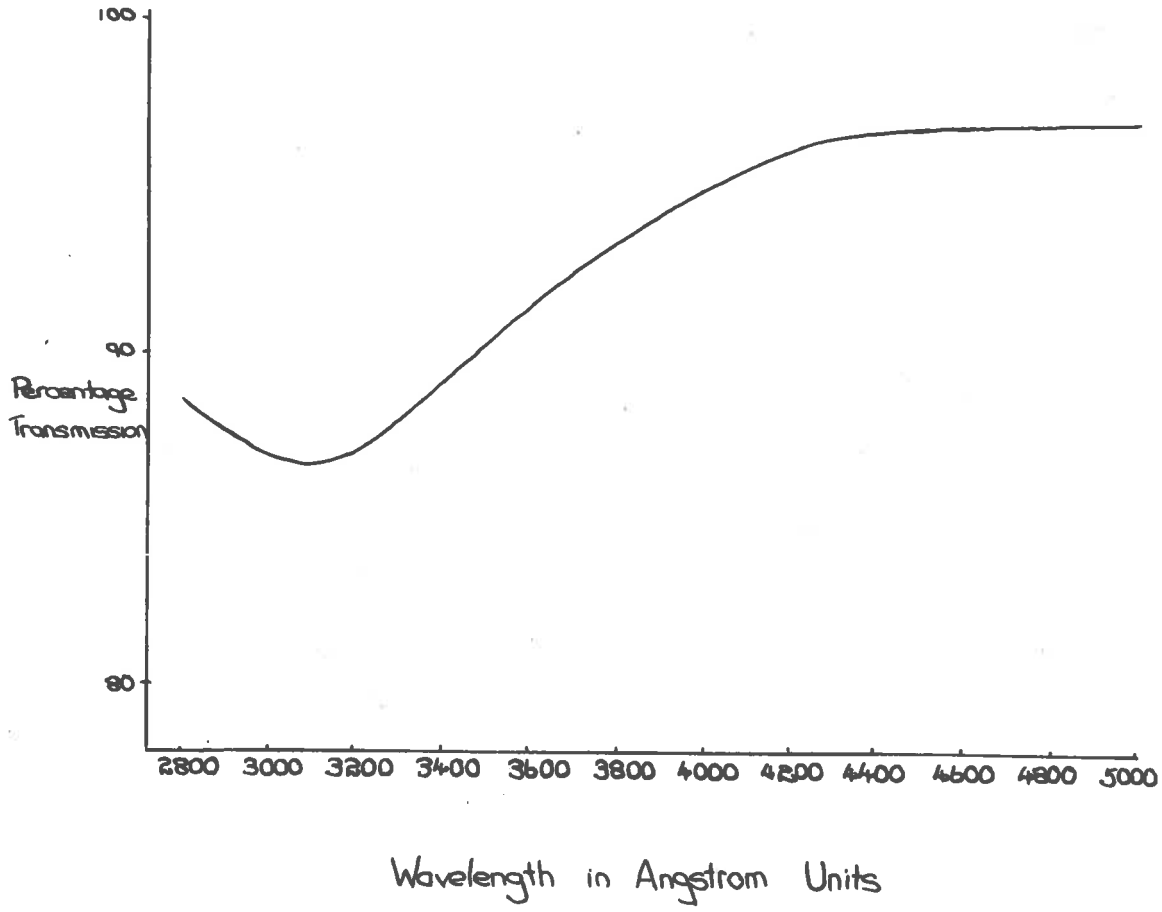
Wattenberg and Leong (1960) found that the addition of coenzyme Q and menadione to the histochemical medium for demonstrating succinic and α-glycerophosphate dehydrogenase resulted in an increased deposit of formazan. I have not been able to successfully substitute menadione for hydroquinone in the phospholipid reaction, so that these observations demonstrate the incorporation of the quinone into the enzymatic reaction.

This tetrazolium salt reduction method for lipoproteins represents the histochemical demonstration of another stage in the electron transport chain.

3. The quantitative estimation of dehydrogenase activity in tissue sections.

One of the promising applications of enzyme histochemistry is its quantitative estimation. The main drawback, at the present, is that a stable parameter of the section must be known before rates of activity can be determined. Lemon et al (1954) estimated acid phosphate activity and then analysed the sections for deoxypentose and total nitrogen. With alkaline phosphatase Davies et al (1954) used interference microscopy to assess the rate of activity in a given field.

Estimation of dehydrogenase activity is conveniently estimated in terms of formazan production, but there is still the problem of the basis of the production rate. The cobalt-MTT hydroquinone reaction may conveniently be utilised, as it is a reaction that can be easily controlled and the standard for comparison can be



Appendix. Figure 1. The spectrophotometric transmission curve of the formazan eluted from sections of mouse liver by chloroform. Cobalt-MTT-hydroquinone lipoprotein reaction.

set at any suitable level.

Procedure: A block of tissue is trimmed so that a large number of uniform sections may be cut, and is rapidly frozen in solid carbon dioxide. Allowing 15 sections for each reaction, a dehydrogenase stain utilising cobalt-MTT is carried out. In addition a standard cobalt-MTT hydroquinone reaction is performed on a further 15 sections. The histochemical conditions are exactly the same as for normal qualitative investigations, except that the time of reaction and temperature must be set to a standard. I allow the dehydrogenase reactions to proceed for 20 minutes at 37°C., stopping the reaction by washing the sections and allowing them to dry. A suitable time for the non-enzymatic lipoprotein method I have found to be 1 hour 45 minutes at 37°C. The formazan production from each reaction is then estimated by eluting it with chloroform (4 ml.) and measuring the optical transmission spectrophotometrically. The curve for optical density for the formazan from cobalt MTT is shown in Appendix Figure 1.

The maximum absorption is at 3100 Angstrom units, and this wavelength has therefore been selected for the quantitative estimations. The enzyme activity can then be expressed as a ratio, by dividing the percentage transmission of the formazan from the lipoprotein reaction by that from the dehydrogenase reaction. The ratio, multiplied by 100, then gives a convenient unit system, running on a linear scale with low values for low activity and higher values for high activity.

This work is in the preparatory stages for publication.

and I am proposing to call the units Tetrazolium units, or the abbreviated form TZ units. Some results with guinea pig tissue are given below. The advantage of this system is that only the cells active in enzyme processes are estimated. The interpretation in homogeneous tissues is obviously the most straightforward but it could have application in sections, such as malignant tissue, where there is a variable pattern and activity. With the appropriate microscopical control, there is no reason why sections containing different types of cell should not be assessed for their enzymatic activity.

Previously the occurrence of quinones in biological oxidation systems was discussed with reference to the biochemical data (Mateffi, 1963). Interpretation of the quantitative enzyme activities that utilise quinones as part of the oxidative pathway calls for no comment, but the system could be used for all enzyme activities that can be demonstrated by the use of MTT, with the reservation that the functional interpretation is not the same. The enzyme systems that can utilise MTT as electron acceptor are lactate, malate, isocitrate, glutamate, β-hydroxybutyrate and glucose-6-phosphate dehydrogenases and the di- and triphosphopyridine nucleotide diaphorases. Succinic dehydrogenase is rather variable in its demonstration with CoMTT and is not entirely satisfactory, except in tissues that have a high activity, but possibly the addition of coenzyme Q or menadione as has been done by Wattenburg and Leong might bring it into line with the other enzymes of the citric acid cycle.

The enzymes of the citric acid cycle and diphosphopyridine nucleotide diapherase in beef heart mitochondria all utilise coenzyme Q, so that, provided the same obtains in other animal tissues, the estimation of these in terms of Tetrazolium units could become a standard procedure. Glucose-6-phosphate dehydrogenase has not, apparently, been shown to utilise coenzyme Q, but using the property of the tetrazolium phospholipid reaction as a stable cell parameter, repeated estimates of the activity of the enzyme can be obtained.

Apart from the interest in malignant disease, this quantitative method could find an application in biopsy work with liver, kidney and muscle tissue.

Example of results of enzyme estimations from tissue sections expressed in terms of formazan production.

Guinea pig tissue	Reaction	Mean per cent transmission with standard error	Enzyme activity expressed as Tetrazolium units
Liver	CoNTT-hydroquinone	32.76 ± 0.69	
	Malate dehydrogenase	72.67 ± 1.07	45
	Glutamate dehydrogenase	89.19 ± 1.75	36
	β-Hydroxybutyrate dehydrogenase	87.67 ± 0.92	37
Kidney	CoNTT-hydroquinone	48.84 ± 1.39	
	Malate dehydrogenase	67.93 ± 0.63	71
	Glutamate dehydrogenase	94.60 ± 0.83	51

4. Connective tissue staining by the Cobalt NTT-Hydroquinone reaction.

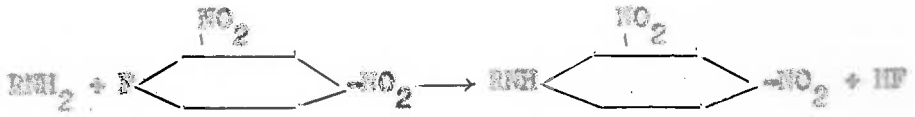
With fixed tissues the CoNTT-hydroquinone acts in quite a different way. With tissues fixed in Zenkers and Helly's fluids or formalin, dehydrated and infiltrated with paraffin wax,

collagen is stained grey-black with formazan. In skin there is also staining of the stratum granulosum, stratum corneum and keratin of the hair. Skin fixed in Carnoy's fluid shows only the staining of the stratum granulosum and keratinous structures. The staining in the two situations is due to the presence of different reactive groups.

The reaction in collagen is due to the presence of amino groups that become reactive by the process of fixation and dehydration, presumably due to the breakdown of cross-linkages. Carnoy's fluid is a protein precipitant, but the constituents do not enter into chemical combination with the tissues, which accounts for the negative collagen reaction. That the amino groups are the reactive ones in the connective tissues can be shown by blocking reactions.

(1) Blockade by sodium p-toluenesulphurchloramide (Chloramine T) (5 per cent in normal acetic acid 18 hours at 37°C.) prevents the amino group staining, but not that of the keratinous structures.

(2) Blockade by 1-fluoro-2, 4-dinitrobenzene prevents the production of formazan.



If now the protein nitrobenzene complex is reduced (as for the usual diazotisation reaction) the tissue becomes intensely reactive again, with heavy formazan staining.

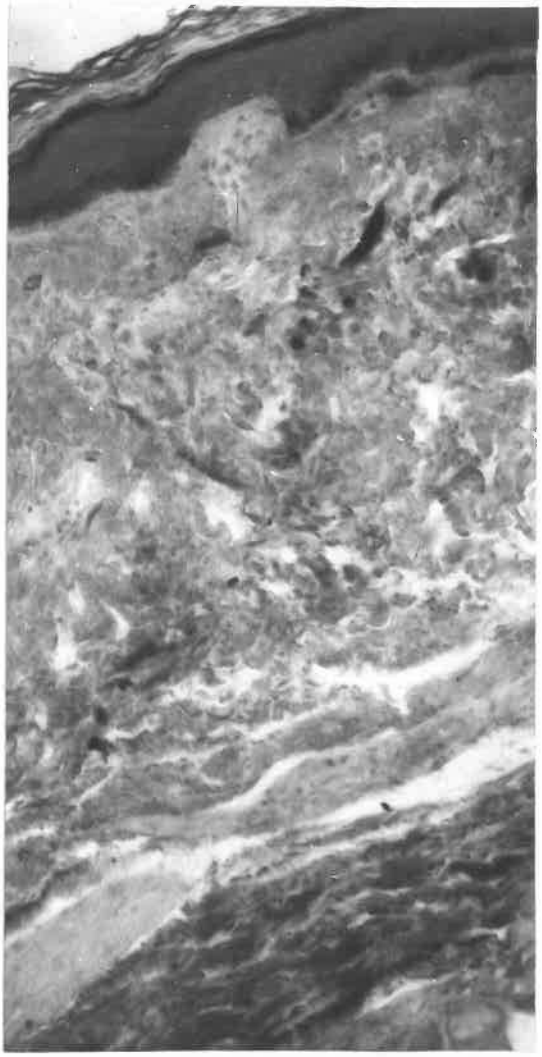
Thus artificially prepared amino groups will react with the CoNTT-hydroquinone mixture.

This reaction is not equivalent to the alkaline tetrazolium methods for connective tissues, as it is negative in the absence of hydroquinone.

The specificity of the keratin staining is also a reaction peculiar to the CoNTT-hydroquinone method. Blockade of sulphydryl groups does not prevent the reaction, but prior reduction of disulphide groups by sodium thioglycolate (0.3 M, 2 hours at 55°C.) prevents the reaction in the keratinous structures.

The degenerated collagen of skin, discussed previously, does not stain by this method, and elastic tissue cannot be identified in normal skin with this stain. The staining is due to the presence of the higher concentration of diaminomonocarboxylic acids (arginine and lysine) in collagen compared with that in elastin. Collagen also contains a higher proportion of monoaminodicarboxylic acids, but prolonged methylation (1 per cent HCl in methyl alcohol, 48 hours at room temperature) fails to prevent formazan precipitation, so that the free carboxyl groups do not participate in the reaction. Whether the reaction demonstrates the hydroxyl groups of hydroxyproline and hydroxylysine as well as amino groups cannot be determined for certain, as there appears to be no specific blocking agent for these groups.

The staining of disulphide and amino groups in skin is shown in Appendix Figure 2. This section shows both normal and elastotically degenerated collagen, the latter being unreactive.



Appendix 3, Figure 2. Skin from the dorsal aspect of the forearm of a 62 year old male, with severe collagen degeneration. Fixed in Helly's fluid, dehydrated and embedded. Stained by Cobalt-MTT-hydroquinone method. Black formazan staining indicated amino groups in normal collagen and disulphide groups in keratin and stratum granulosum. Counterstained with methyl green (133 x).

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Reprinted from
The British Journal of Cancer,
1961, Vol. XV, p. 409

THE EPIDEMIOLOGY OF SKIN CANCER IN QUEENSLAND: THE INCIDENCE

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Received for publication May 2, 1961

IT has been well known for many years that the incidence of skin cancer in Australia is very high. This is more especially true of Queensland than the other Australian States, and the same probably holds for the Northern Territory, though there are no figures readily available. There is also good evidence that other tropical and sub-tropical countries have a high incidence of skin cancer amongst the white population of North European descent. Details for the Transvaal are given by Cohen *et al.* (1952); for East Africa by Piers (1948), and for the Southern States of the U.S.A. by Auerbach (1961). Social customs, economic factors and labour conditions in these countries are different from those in Australia, so that direct comparisons of statistical data should only be made with caution. In Australia there is virtually no coloured labour, and whites do manual work in all climates.

This present study has been designed to provide exact epidemiological information about skin cancer in Queensland, with a view to correlating it with the results of direct measurements of ultraviolet radiation that are currently being made by the Department of Physics in the University of Queensland. Although previous Australian authors have drawn attention to the high incidence of skin cancer (Molesworth, 1928; Belisario, 1959) it is believed that this is the first attempt to provide systematic statistics that are comparable for different parts of the State.

Geographical and Population Features

Queensland is well adapted for this epidemiological study because the population groupings fall into fairly well defined limits, and are widely spread, so that differences in response to climatic factors should be clearly apparent.

The State of Queensland covers an area of 670,000 square miles, and has a population of 1,318,259 (1954 Census). The southern border of the State lies on latitude 29° South and its northern tip, Cape York Peninsula and the Torres Strait Islands, lies between latitudes 10° and 11° South. Broadly the population can be divided into two categories. The first and major part of the population lives in the South East corner of the State, and the coastal plain from the south to as far north as Cairns (Fig. 1). The smaller section of the community lives in the arid regions to the west of the Great Dividing Range. The coastal plain has a sub-tropical to a tropical humid climate. In the south of this strip dairy farming and small crop production predominate, and in the north sugar cane

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production is the most important primary industry. On the other hand, the climate of the vast sparsely populated districts to the west of the Great Divide is described as that of low latitude steppe and desert (Blair, 1942). Sheep and cattle grazing are the main occupations, though in the North West at Mt. Isa there is a well established mining industry with a related increase in density of population. The population of Mt. Isa was 7400 in 1954, and the next largest western town was Charleville, with a population of 4500.

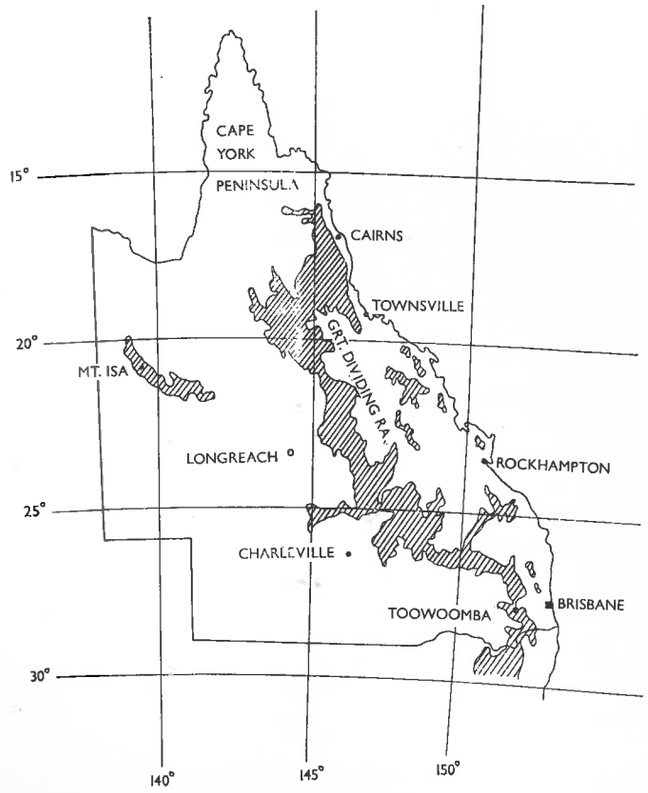

FIG. 1.—Map of Queensland, showing main geographical features and the location of the four cities surveyed.

As a result of the necessity to confine this investigation to places with reasonably large populations and with reliable hospital statistics, it is concerned only with the incidence figures for Brisbane, Rockhampton, Townsville and Cairns. It was found that in places with small populations the results were too variable, though there is a distinct clinical impression that there is a high incidence of lesions in the west. This was confirmed by a small scale postal survey carried out amongst graziers in selected western districts.

In considering cities only, bias due to exposure factors should be limited to a certain extent, though probably not entirely. In addition to this it should be borne in mind that country dwellers are less conscientious in attending hospital than city people, which would introduce further bias if they were covered by a survey such as this rather than by " on-the-spot " sampling.

Some climatological data for the four centres covered are shown in Table I.

TABLE I.—*Climatological Factors in Survey Areas*

	Latitude in degrees S.	Population, 1954 Census	Average annual sunshine (hours)	Average rainfall	Mean daily temperature
Brisbane	27° 30′	502,320	2850	40·09	69·0°
Rockhampton	23° 28′	40,670	2925	37·36	73·2°
Townsville	19° 15′	40,471	2975	43·06	76·0°
Cairns	17° 0′	21,020	2750	86·35	76·3°

Collection of Material

The estimation of the incidence of a non-fatal, non-notifiable, relatively trivial disease with a high cure rate presents a problem that is not usually met with in compiling cancer statistics, where the death rate is a good guide to the incidence rate. However, in Queensland the treatment of skin cancers is practically uniform, in that the majority of patients attend the Queensland Radium Institute. By using the records of the Institute at the Main Centre in Brisbane and at the provincial Sub-Centres at Rockhampton, Townsville and Cairns it is possible to arrive at a good estimate of the incidence of the disease. It was ascertained from general practitioners that most of the patients are referred to the Radium Institute and, in addition, quite a number report without being referred by a doctor. Using the Institute's records, it is ensured that standards of diagnosis and record keeping are equivalent in the 4 cities concerned. The estimates should, however be regarded as minimum. Basal cell and squamous cell cancers are considered together.

A random sample of about 1000 records in each of the 4 cities was taken, of patients who presented for the first time for treatment of a skin lesion, during the 10-year period 1948–57. The total number of cases of malignant disease, and the total numbers seen at each centre were known, and from this it was possible to work out the sampling fraction, and its reciprocal, the raising factor. The factors are listed in Table V of Appendix II. The numbers of cases in the samples in each 5-year age period over the age of 20 were multiplied by the appropriate raising factor, to give the age specific numbers of cases attending for treatment.

Computation of Rates

The actual incidence rates were computed for each decennial age period from the fraction :

$$\frac{\text{Numbers of new cases in each 10-year age group}}{\text{Number of susceptible persons in corresponding group}}$$

The denominator of this fraction should be noted because the incidence rates are computed on the basis of the susceptible and not the total population a " susceptible " being a person who has not previously had a skin lesion. The incidence rates are for those with first lesions only.

The method of estimating the rates and the susceptible population is as follows :

Let

n_k = number of new cases in age group k (obtained by multiplying the corresponding number of cards by the raising factor) ;

N_k = mean number of persons in age group k ;

$p_k = \frac{n_k}{N_k}$;

$P_k = p_1 + p_2 + \quad \ldots \quad + p_{k-1} + \frac{1}{2} p_k$;

$Q_k = 1 - P_k$.

P_k estimates the probability that a person whose age is that given by the mid-point of the k-th age group will have had at least one lesion by that time. It may be called the " age-specific prevalence ".

The proportion of susceptibles at this age is, therefore, Q_k.

Hence, the " age-specific annual incidence rate " for first lesions at this age is estimated by

$$r_k = \frac{1}{10} \frac{p_k}{Q_k}$$

It corresponds to the " age-specific death rate " or " force of mortality " in ordinary life-tables.

Fitting Suitable Curves

The prevalence function P and the incidence rate r are connected by a simple relationship (see Appendix I). It may therefore be a matter of mathematical convenience whether we fit the curves for r directly or fit the curves for P and deduce those for r. (Certain methods of estimating the curve may make the two methods fully equivalent, though this is not the case for the " least squares " methods used here.)

A number of different probability models of carcinogenesis have been proposed, and no doubt some of the incidence curves arising from these models would fit the data. Good use might, for instance, be made of the models proposed by Armitage and Doll (1957) or by Armitage (1959). It is doubtful, however, whether skin cancer incidence rates of the kind considered in the present survey, being, as they are, averages over different social classes and different degrees of skin pigmentation, afford suitable means of testing any given model (see, for instance, Neyman (1960), and Weibull (1951)).

Conversely, the fact that a particular probability distribution function happens to " fit " the data does not necessarily imply that the usual assumptions and models leading to that distribution are applicable to the material being studied.

The principal objects of the present survey were : (i) to show that the incidence curves varied noticeably from one district to another ; (ii) to see to what extent the factors leading to differentiation among the incidence rates were themselves age-dependent ; and (iii) to provide preliminary information necessary for the later correlation of the incidence rates with ultra-violet solar radiation in the various districts.

In deciding on questions (i) and (ii) it appeared desirable to seek prevalence curves for which, through transformations to linearity, the ordinary processes of linear regression analysis might be applied. Already, in laboratory experimental work, Blum (1955) has found the integrated normal curve to be appropriate to the production of skin cancers in albino mice by ultraviolet radiation. However, it has recently been questioned whether these observations are transferable to the situation in man. Winkelman, Baldes and Zollman (1960), who performed experiments with congenitally hairless mice, did not observe inflammatory lesions or any other skin changes before the induction of tumours. In any case, as will be seen from Fig. 2, the probit transformation of the observed values of P did not produce linearity.

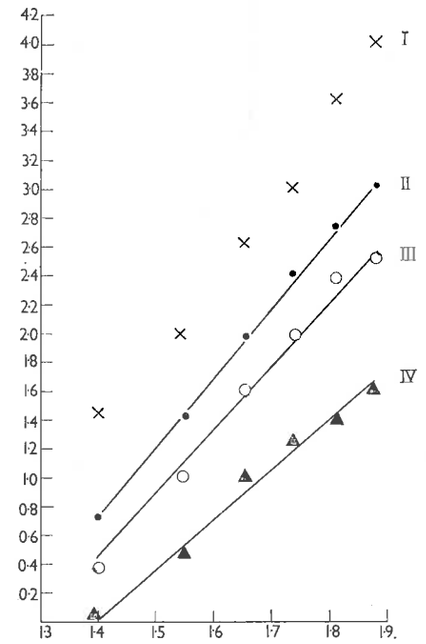

FIG. 2.—Comparison of results of various transformations seeking linearity. Townsville males.

I. Integrated normal : Y = probit $P - 1$.
II. Logistic : $3 + \log P/Q$.
III. Weibull : $3 + \log (- \log Q)$.
IV. Weibull : $3 + \log r$.

The Logistic Curve

Better results were obtained by using the logistic curve and plotting log P/Q against log (age). (In using either the integrated normal or the logistic curve it is not necessary to assume the existence of a " tolerance distribution " (Berkson, 1951).

Straight lines were fitted to the values of log P/Q, using weighted least squares. The usual weighting systems were not employed since the successive estimates of the values of P_k are not mutually independent. Weights were taken propor-

tional to the precision of the estimates (ignoring the error caused by sub-sampling from the cards). The fitted values of r were obtained from those of P by means of formula (2) of Appendix I. Chi-square tests of closeness of fit were performed by calculating the expected number of cards in each age group and comparing them with the numbers actually recorded.

Table V of Appendix II shows the observed and fitted values of r, as well as the appropriate values of chi-square. Only in one case out of the eight, namely Cairns males, was the value of chi-square large enough to indicate an apparent discrepancy from hypothesis, and this is obviously attributable to the vagaries of sampling, as can be seen from the irregularities in the sequence of observed values of r.

The equations of the 8 straight lines are given in Table VI of Appendix II.

The Weibull Curve

It has frequently been noted that certain types of age-specific death rates from carcinoma display linearity when the logarithm of the rate is plotted against the logarithm of the age. In other cases there is a marked departure from linearity, the implications of which have been discussed by Armitage and Doll (1957) and others.

In the present case linearity seemed sufficiently well marked to justify trying this system of curves. It should be noted (Appendix I) that if the r-system behaves in this way then the P-system conforms to the " Weibull distribution " (Weibull, 1951) which has many applications to life-testing data and to certain classes of biological data as well. For the Weibull P-system the graph of log ($-$ log Q) is linear when plotted against log (age).

To provide a comparison with the logistic system it was decided to fit the values of P by the Weibull distribution as well as fitting the values of log r directly. Table V of Appendix II shows the results of each process. It also gives (for comparison with the logistic system) the values of chi-square appropriate to the Weibull P-system. It will be seen that there is little to choose between this system and the logistic. If the Weibull system were accepted the logical thing to do would be to fit the values of log r directly.

Table VI of Appendix II gives the formulae for all 24 straight lines obtained by the various methods. (According to the theory of Appendix I, the slope parameters for log ($-$ log Q) should exceed those for log r by 1 when the Weibull system is used. That this does not exactly occur arises from the fact that the least squares solutions are not invariant under the transformation from P to r.)

If a set of parallel lines is fitted to all 8 districts the common slope parameter is that given in the same table.

Graphs

The following graphs are presented :

Results of fitting various curves to the data for Townsville males (Fig. 2).

Results of fitting straight lines to log P/Q versus log (age) — logistic method (Fig. 3).

Results of fitting straight lines to log r versus log (age) (Fig. 4).

Curves for r versus age obtained by fitting a set of parallel straight lines to the data for log r versus log (age) (Fig. 5).

Regression Analysis

A regression analysis was performed on each of the 3 sets of data for the purposes of : (i) testing for parallelism of the 8 curves in each set ; (ii) detecting significant differences among the prevalence or incidence rates for the various

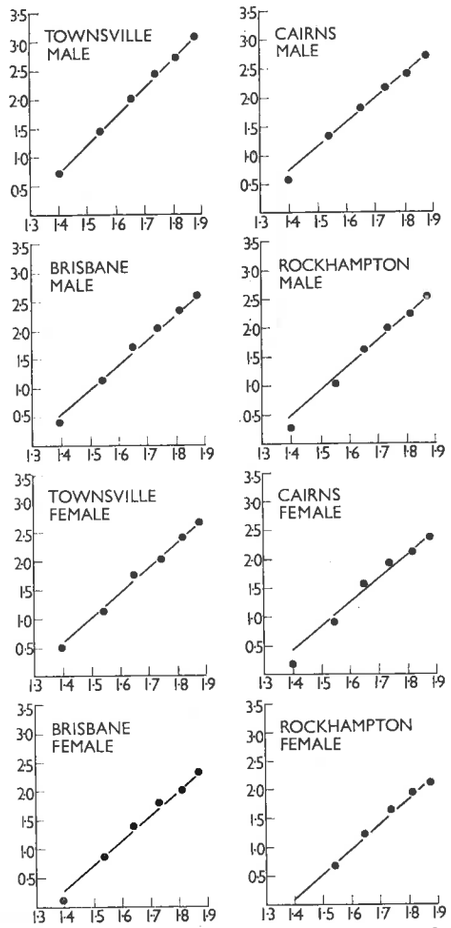

FIG. 3.—Application of the logistic curve to the prevalence rates.
Ordinates—3 + log P/Q.
Abscissae—log (age).

localities as well as differences between the sexes. Procedures appropriate to testing all contrasts in an analysis of variance were used, and the 1 per cent level of significance employed.

The somewhat lengthy numerical details of the analyses will not be presented here, but the following summary should suffice :

(i) There were no significant variations from parallelism among the 8 lines of each set under any of the 3 systems used.

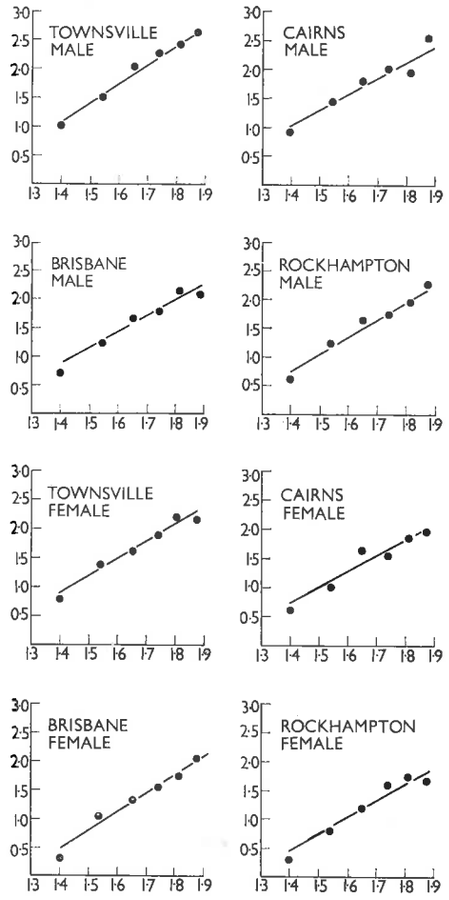

FIG. 4.—Straight lines fitted to the values of 4 + log r (ordinates) versus log (age) (abscissae)

(ii) Sex differences were of a high order of significance in all 3 cases.
(iii) For the prevalence curves, P, the following significant differences emerged (T = Townsville, C = Cairns, R = Rockhampton, B = Brisbane. The symbol " > " stands for " is significantly greater than ").

Method of fitting	Males	Females
Logistic . . .	T > C, B and R C > R	T > C, B and R C > R B > R
Weibull . . .	T > C, B and R C > B and R	T > C, B and R C > R B > R

It will be seen that the only conflict is that the contrast C > B for males appeared under the Weibull system but not under the logistic. In relation to the

direct fitting of straight lines to log r the only significant differences at the " 1 per cent level " were T > C, B and R for both sexes. It is to be expected, of course, that significant differences will be more easily established on the prevalence data than on the annual incidence data. Indeed, if a lower level of significance is admitted (the " 10 per cent level ") the significant differences for log r are exactly the same as for the logistic P-system given above.

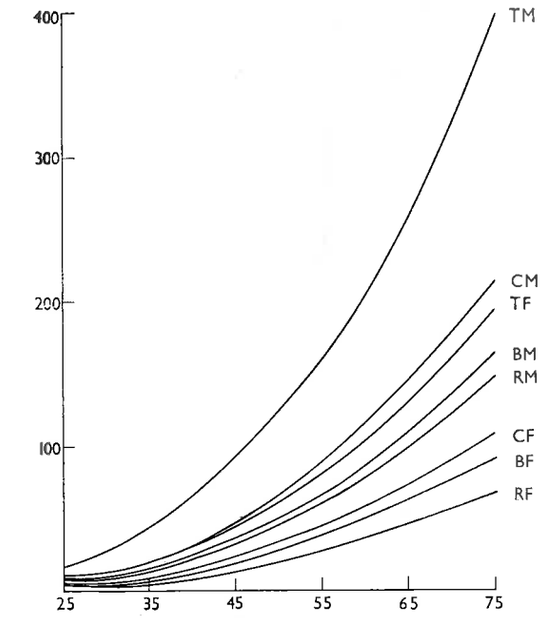

FIG. 5.—Annual incidence curves, per 10,000 population, derived by fitting parallel lines to log r versus log (age).

T—Townsville ; C—Cairns ; B—Brisbane ; R—Rockhampton.
M—Male ; F—Female.
Abscissa—Age in years.

The implications are that separate prevalence and incidence curves may be drawn for Townsville, Cairns and Rockhampton for both males and females, while there is also a likely separation between Brisbane and Rockhampton females. Some problems arising from the orderings given above are discussed later.

Estimates of Average Risk

The average annual risk of incurring a first lesion over the period 20 years to 80 years of age was estimated from the mean ordinate of the incidence curve over this range. Two sets of results are presented in Table II below, namely, estimates based on the values of r as obtained by fitting logistic curves to the prevalence data, and estimates based on the values of r obtained by fitting Weibull curves directly to the values of r.

TABLE II.—*Average Annual Risk of Incurring a First Lesion Over the Period from 20 to 80 Years of Age*

Locality	Average annual risk			
	Males		Females	
	Logistic method	Weibull method	Logistic method	Weibull method
Townsville	0·0150	0·0157	0·0074	0·0073
Cairns	0·0082	0·0080	0·0043	0·0040
Brisbane	0·0064	0·0062	0·0035	0·0035
Rockhampton	0·0056	0·0056	0·0027	0·0024

Estimates of Prevalence

Using the distributions fitted to the values of P it is possible to estimate, for for any district, the overall " prevalence " (that is, the percentage of people who, at any particular time, will be found to have at least one active or old lesion). This is best done for a population that has been standardised with respect to its age distribution and then allocated the appropriate district prevalence figure for each age group.

Table III shows such estimates for a standard population obtained by pooling the 4 district populations for the particular sex. (The assumption is, of course, that of constancy of the age-specific rates over the life-time of an individual.) Estimates are confined to that section of the population between 20 and 80 years of age. Separate estimates were obtained from the logistic and the Weibull P-distributions.

TABLE III.—*Estimated Prevalence in Age-standardised Populations in the 4 Localities among Persons between 20 and 80 Years of Age*

Locality	Estimated prevalence per cent			
	Males		Females	
	Logistic method	Weibull method	Logistic method	Weibull method
Townsville	12·84	12·77	7·70	7·67
Cairns	8·19	8·13	4·75	4·72
Brisbane	6·28	6·24	3·75	3·71
Rockhampton	5·49	5·41	2·84	2·79

Equivalent Ages

By fitting a set of parallel lines to the appropriate function of P, and employing the usual techniques of " relative potency " estimation in dosage trials one can calculate indices showing what ages in the various localities are equivalent as far as prevalence is concerned. For example the prevalence at age t years in Townsville is equivalent to the prevalence at age 1·22 t years in Brisbane, according to the logistic method, and to an age of 1·21 t years according to the Weibull method, and this is true for all values of t between 20 and 80.

Similar results were obtained, by each method, for the other centres. The agreement between the two methods and between the sets of results obtained from the data for males and the data for females is evident from the following

table (Table IV). The factor 1·22 for Townsville may be called the " relative intensity factor for ages " for Townsville as compared with Brisbane.

TABLE IV.—*Relative Intensity Factors for Age*

Locality	Relative intensity factor					
	By logistic method			By Weibull method		
	From male data	From female data	Average	From male data	From female data	Average
Brisbane	1·00	1·00	1·00	1·00	1·00	1·00
Rockhampton	0·96	0·93	0·95	0·96	0·93	0·95
Townsville	1·23	1·21	1·22	1·22	1·21	1·21
Cairns	1·08	1·06	1·07	1·07	1·06	1·06

INTERPRETATION OF RESULTS AND CONSIDERATION OF CLIMATIC FACTORS

The most noticeable feature of the curves just obtained is that their linear transforms are parallel if plotted against log (age). It appears from this survey that the cities differ only in the different incidence rates and that the factors causing the differentiation operate uniformly at all ages. The reaction patterns do not appear to differ in any way qualitatively, as is shown in another paper (Carmichael, 1961).

This observation suggests that there might be a type of dose response relationship to ultraviolet radiation. However two points need explanation :

(i) Why the Townsville rates are so much higher than those for both Cairns and Rockhampton ;
(ii) Why the Brisbane and Rockhampton rates are similar in the case of the males.

In Table I the sunshine hours recorded are shown, and it will be noticed that Cairns has less sunshine than Townsville, although it is nearer the Equator. This in itself might be enough to explain the anomaly, but population characteristics enter into the question as well. In Cairns there is a higher proportion of those Italian born or of Italian descent than in Townsville. Italians do develop skin cancer but with nothing like the same frequency as those of Anglo-Saxon or Celtic origin. Economic status probably also plays a part ; Townsville is an industrial city and is the commercial centre and port for a large area of the North West of the State. Cairns, on the other hand, is more of a tourist resort, and has little hinterland. The major industrial activity is concerened with sugar production and export.

There are several factors that influence the intensity of solar radiation received at the earth's surface :

(i) Latitude and air mass.
(ii) Solar constant (intensity of radiation outside atmosphere).
(iii) Concentration of ozone in the atmosphere, which is a very potent absorber of ultraviolet radiation.
(iv) Dust and water vapour in the atmosphere. The former absorbs and scatters ultraviolet, and the latter absorbs infrared radiation.
(v) Sky radiation as opposed to direct radiation.

Prediction of the incident ultra-volet radiation from this complex system of variables is not yet possible to a reliable degree, so it is necessary to make direct measurements. This is in progress. With these meteorological variables must be considered certain human factors. Firstly there are unmeasurable characteristics such as use of protective clothing, hats, and time of exposure to the sun. While it is generally conceded that ultraviolet radiation is responsible for this high incidence of skin cancer, it must be remembered that it is not yet possible to treat effects due to this form of radiation in the same quantitative ways that are applicable to effects due to ionising radiation.

Migratory movements of the population are also likely to affect the skin cancer incidence rate, and it is well known in Queensland that graziers tend to retire to the coastal cities. Townsville attracts the elderly population of the north west rather than Cairns, which could be a factor responsible for the high rate of skin cancer in the upper age group. In the same way, of course, a large incoming young migratory population would tend to lower the incidence rates in the earlier age groups. To collect information concerning these factors would require an extensive sample census, and the results would be of uncertain interpretation, particularly as the individual's susceptibility to solar radiation seems to alter through the years. Middle-aged and elderly people are usually a good deal more cautious about exposure to hot tropical sun than are children and young adults. In connection with exposure to the sun, it should be noted that it is quite possible to receive an erythemal dose of ultraviolet radiation in Queensland without being exposed directly to the sun, i.e. from sky radiation.

It may be that considerations such as these are responsible for the figures for the Brisbane males being similar to those for Rockhampton.

The slopes of the lines for log (incidence) against log (age) cluster about the value 3, which is in contrast to the values for death rates due to cancer, which are about 5 or 6 (Armitage and Doll, 1954). As the curves we have calculated are equivalent to death rates for lethal diseases with short survival times, this poses further problems for the multiple mutation theory of carcinogenesis proposed by Nordling (1953) and also by Stocks (1953) who used cohort death rates, unless it be assumed that neoplasms in different organs are the result of different numbers of mutations. Solar cancers might be regarded as one of the simplest and most straightforward examples of carcinogenesis that can be studied in man. However, even in this instance there is disagreement about the importance of the melanin pigment and thickness of the stratum corneum as protective factors against solar radiation (Mackie and McGovern, 1958; Blum, 1959; Thomson, 1955). Until some of the biological difficulties are resolved, the appropriateness of various theoretical models of carcinogenesis cannot be adequately assessed.

SUMMARY

The age specific incidence rates of skin cancer in four coastal cities in Queensland are calculated and discussed. On the assumption that ultraviolet radiation is the causative factor, it is suggested that the only difference in response to the stimulus by the four populations is one of frequency of incidence. This arises from the observation that the curves are parallel for each of the four cities. Several methods of fitting lines to the data are described.

This investigation was carried out while one of us held a Medical Research Fellowship at the University of Queensland. We are indebted to Dr. A. G. S. Cooper, Director of the Queensland Radium Institute, who allowed free use of the records of the Institute, and who helped with the numerous problems that arose during the course of the survey.

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APPENDIX I

Prevalence and incidence curves

If $P(t)$ is the probability that a lesion will occur before time t, the probability that a person who has not had a lesion before time t till get one in the interval $(t, t + \Delta t)$ is

$$r(t)\ \Delta t = \frac{p(t)\ \Delta t}{1 - P(t)}, \qquad (1)$$

so that

$$r(t) = -\frac{d}{dt} \ln \{1 - P(t)\} \qquad (2)$$

If Δt is taken as 1 year, $r(t)$ is the annual incidence rate.

Logistic curve

The 2-parameter logistic curve on $x = \ln t$ is

$$P = \frac{1}{1 + e^{-(A+Bx)}} = \frac{1}{1 + ct^{-b}}, \text{ say} \qquad (3)$$

We have

$$\log \frac{P}{Q} = a + bx, \text{ say} \qquad (4)$$

and

$$r(t) = \frac{bP}{t}, \text{ from (2).}$$

Weibull curve

The Weibull distribution is

$$P = 1 - \exp(-Ct^D) \quad . \quad . \quad . \quad . \quad (5)$$

so that

$$\ln(-\ln Q) = \ln C + Dx \quad . \quad . \quad . \quad . \quad (6)$$

and

$$\ln r = \ln(CD) + (D-1)\,x \quad . \quad . \quad . \quad . \quad (7)$$

Parallel lines for log (— log Q) imply parallel lines for log r, and *vice versa*.

APPENDIX II

Table V shows the observed values of r and those obtained by the use of: (i) a separate logistic curve for the prevalence data for each sex and each district; (ii) a separate Weibull prevalence curve for the same prevalence data; and (iii) a separate Weibull incidence curve obtained for each set of incidence rates by fitting the values of log r directly.

TABLE V.—*Observed Incidence Rates* (r) *per* 10,000 *Susceptibles; the Fitted Rates* $\hat{r}_l$ *Obtained from the Logistic Prevalence Curve; the Fitted Rates* $\hat{r}_w$ *Obtained from Weibull Prevalence Curves; the Fitted Rates* $\hat{r}_w'$ *Obtained by Fitting Log r Directly*

BRISBANE MALES (Raising factor = 34)

Age	No. in sample	No. in population	r	$\hat{r}_l$	$\hat{r}_w$	$\hat{r}_w'$
25	5	33,045	5	6	6	7
35	18	37,639	17	17	17	19
45	41	32,585	45	39	37	38
55	39	24,658	60	72	70	67
65	60	18,717	133	114	120	107
75	21	8,379	118	160	188	161

Chi-square*: Logistic 4·65; Weibull 6·77

ROCKHAMPTON MALES (Raising factor = 2·6)

25	4	2,675	4	5	5	5
35	16	2,702	15	15	14	15
45	38	2,509	41	34	32	32
55	38	1,978	54	62	61	59
65	44	1,450	91	100	105	99
75	35	731	163	142	167	152

Chi-square*: Logistic 4·23; Weibull 4·81

TOWNSVILLE MALES (Raising factor = 4·9)

25	7	3,156	11	10	12	12
35	19	3,136	30	37	36	37
45	49	2,615	101	92	84	85
55	59	2,109	172	176	166	163
65	53	1,559	258	269	294	282
75	21	536	412	344	477	450

Chi-square*: Logistic 1·57; Weibull 0·28

TABLE V (*Continued*)

CAIRNS MALES (Raising factor = 2·4)

25	5	1,570	8	8	9	10
35	19	1,739	27	25	24	25
45	31	1,385	57	53	50	49
55	37	1,118	91	94	91	86
65	20	714	85	143	150	138
75	25	281	329	192	228	205

Chi-square*: Logistic 14·90; Weibull 13·39

* See text.

BRISBANE FEMALES (Raising factor = 35)

Age	No. in sample	No. in population	r	r_l	$\hat{r}_w$	w
25	2	35,668	2	3	3	3
35	12	38,430	11	9	9	9
45	19	33,010	21	20	20	19
55	27	27,525	36	38	37	37
65	31	22,473	53	63	64	62
75	31	11,381	114	92	102	97

Chi-square*: Logistic 2·96; Weibull 2·34

ROCKHAMPTON FEMALES (Raising factor = 2·6)

25	2	3,012	2	2	2	3
35	7	2,857	6	7	6	7
45	15	2,633	15	15	15	15
55	28	2,110	36	29	28	26
65	29	1,750	46	49	49	42
75	14	892	45	74	78	62

Chi-square*: Logistic 4·77; Weibull 4·21

TOWNSVILLE FEMALES (Raising factor = 4·9)

25	4	3,052	6	6	7	8
35	13	3,020	23	20	20	21
45	20	2,400	43	45	43	44
55	28	1,970	78	83	81	79
65	36	1,464	151	132	136	129
75	14	666	150	182	213	195

Chi-square*: Logistic 1·13; Weibull 2·77

CAIRNS FEMALES (Raising factor = 2·4)

25	2	1,613	3	4	4	5
35	7	1,608	10	12	12	13
45	22	1,263	43	26	25	25
55	13	987	34	48	47	43
65	17	662	70	77	78	67
75	8	271	87	109	120	98

Chi-square*: Logistic 6·69; Weibull 8·84

* See text.

Table VI gives the equations of the various sets of straight lines fitted to the data.

TABLE VI.—*Straight Lines Fitted to Various Transformations of the Data: A_i ($i = 1, 2, 3$) and B_i are the Location and Slope Parameters, Respectively.* [$x = log$ (*age*).]

	Method of Fitting					
	Logistic to prevalence data $\log \frac{P}{Q} = A_1 + B_1 x$		Weibull to prevalence data $\log(-\log Q) = A_2 + B_2 x$		Weibull to incidence rates $\log r = A_3 + B_3 x$	
Data fitted	A_1	B_1	A_2	B_2	A_3	B_3
Brisbane males	−8·80	4·31	−8·63	4·16	−7·11	2·83
Rockhampton males	−5·56	4·31	−8·84	4·24	−7·49	3·03
Townsville males	−9·08	4·87	−8·67	4·39	−7·47	3·26
Cairns males	−8·20	4·22	−8·17	3·97	−6·90	2·78
Brisbane females	−8·70	4·26	−9·01	4·21	−7·93	3·16
Rockhampton females	−9·00	4·36	−9·28	4·30	−7·44	2·80
Townsville females	−8·53	4·37	−8·81	4·13	−7·20	2·93
Cairns females	−8·40	4·16	−8·57	4·03	−6·98	2·65
99% Confidence interval for common slope parameter		4·36±0·15		4·17±0·17		2·92±0·38

Made and printed in Great Britain for
H. K. Lewis & Co. Ltd., by
Adlard & Son, Ltd., Bartholomew Press, Dorking.

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