Observations

ON

THE EFFECT OF CESSATION OF THE IRRITANT
ON THE DEVELOPMENT OF EXPERIMENTAL
TAR CANCER.*

BY

ARCHIBALD LEITCH, M.D.,

DIRECTOR OF THE CANCER HOSPITAL RESEARCH INSTITUTE, LONDON.

[With Special Plate.]

IN the development of tar tumours, as well in man as in laboratory animals, a long time elapses before there are any visible signs of tumour formation. In the case of mice that have had frequently repeated applications of coal tar it is not till near the end of three months that we see the beginnings of the neoplastic reaction. During this period the area affected has lost its hairs, temporarily or permanently, and the skin has become harsh and scaly: microscopic examination of that particular part of skin in those mice which have died shows irregularity and hyperplasia of the epithelium, destruction of the hair follicles, disorganization of the papillary processes with their superficial elastic felting, and evidences of chronic inflammatory reaction in the corium. But these features are inconstant, and, as I pointed out in January last when communicating to the Pathological Society the results of a year's observations on experimental tar cancers, there are no specific characteristics which we could label as pre-cancerous. As the time goes on, definite hyperplastic plaques make their appearance in the epithelium and small warty growths are formed. The latter generally start as upgrowths from the surface and are found in their early stages microscopically to consist of a branching stalk of connective tissue covered by proliferated epithelium which on the surfaces is excessively keratinized. The connective tissue stalks are well vascularized, but as the wart grows they become more encroached upon by infoldings of the epithelium, and there is downward proliferation of the epithelium below the bases of the tumours: such ingrowing buds, as a rule, exhibit central keratinization. The papilloma grows, too, by further implication of the neighbouring surface epithelium. The cells of the ingrowth may occasionally lack the usual differentiation. How far this ingrowth must proceed before we can call the tumour malignant is, of course, a matter of opinion. There are no hard and fast lines between simple and malignant tumours at any time, and in these particular tumours where the progress from the one to the other is almost certain no one can draw a sure distinction. When, however, the panniculus carnosus is penetrated few will hesitate to pronounce the tumour malignant. In the living mouse deep infiltration can be appreciated by the fingers after a time, and, if we can exclude inflammatory processes, we may be sure that the deep tissues are penetrated. The tumours may increase to a considerable size on the surface, covered by hard horny material, or they may break down. Metastases occur in the lymph glands, the lungs, or other organs. Occasionally the stage of benign wart formation is omitted, and the first sign of neoplasia is an ingrowth with a superficial ulcer; and in such instances it is difficult to date the commencement.

In some of our former experiments we stopped the applications of tar as soon as basal induration was evident, and we found that nevertheless the tumours went on growing with undiminished rate: the removal of the irritant did not arrest the process when once the malignant stage was reached. The failure therefore to detect a causal agent in a cancerous growth cannot be held to be a proof against the previous existence of such.

Now, what would happen if the irritant were suspended before the tumour had reached a malignant stage? Clinical observations in human cases point to various possibilities; some warts disappear, some remain simple for many years and would presumably continue so indefinitely, and some progress to malignancy: instances of all three are known in the soot warts of chimney-sweeps, in the warts of tar and pitch workers, and in the warts of paraffin workers.

* A paper communicated to the Pathological Society of Great Britain and Ireland, July 15th, 1922.

It would seem merely to be a question as to how far the development of the wart had gone when the irritation ceased. Yamagiwa and Ichikawa,¹ who were the first to produce tar tumours experimentally, found that when they stopped the applications of tar in rabbits some of the papillomata went on increasing whilst others diminished and disappeared. In my own experiments with rabbits I noticed in several cases that papillomata disappeared though the tar applications were not suspended; and the same thing happens in mice, though apparently less frequently. It cannot therefore be said that the diminution or disappearance of warts is wholly due to the cessation of the irritant. In order to answer the question posed we took two batches of mice which had undergone repeated tar applications for periods of four and five months respectively; the treatment was stopped, and they were examined every few days. Some of these mice already showed evidence of tumour formation, and in all cases we had fairly accurate information regarding the date of their first appearance; in other mice there was no sign whatever of papillomata or even hyperplastic plaques. After the cessation of the tar applications the hair grew again, except where a wart was actually present, and the skin, previously rough, became smooth in some cases and in others remained rough, but the succeeding events could not be correlated with the degree of the return to the apparently normal condition.

Result when Tumours were Present.

When the tar was discontinued there were 15 mice, of which 14 had small warts varying in size from a pinhead to a hempseed at most, the warts having been in existence for different periods from a few days up to a month, and another mouse which had a small subcutaneous tumour with superficial ulceration. Judging from our histological observations on numerous similar growths in animals which died, we would have no hesitation in pronouncing all of them simple, with the possible exception of the one mentioned. The subsequent history of these 15 mice showed that in 5 cases the papillomata disappeared, spontaneously or otherwise, in 3 cases the warts remained simple, 1 had a doubtful ending, and 6 became malignant.

Temporary Tumours.

1. A wart was present, small and flat, less than one-eighth of an inch in diameter, which had arisen within 13 days. It disappeared within a fortnight after the tar was stopped, leaving roughened skin. At the end of 22 days another small flat wart was seen to the right of the site of the former, but after another week it too disappeared, and a rough irregular condition of the skin without further neoplasia continued till the death of the mouse, 71 days after cessation of tar painting. Histological examination of the skin showed irregular hyperplasia of the epithelium, with small nodules of epithelial cells isolated in the corium and surrounded by round-cell and fibroblastic reaction. Many of the epithelial masses had degeneration of the central keratin, and appeared as pseudo-cysts. The panniculus carnosus was not encroached upon. (Five months' treatment previously.)

2. A small wart the size of a pinhead was present, which increased slowly in size till 70 days after the tar was stopped, when it appeared as a conical horny wart a quarter of an inch in height. Pulled off, it left merely a small area of abraded skin, which showed no sign of tumour when the mouse died a week later. (Four months' previous treatment.)

3. After tar had been applied for 4 months a minute wart was observed which had grown to the size of a hempseed one month later, when the applications were suspended. It had not increased 13 days later, but a pinhead-sized wart had appeared in front of it, and a scab had formed immediately behind it. At the end of 22 days the scab had taken the place of the larger wart. It was difficult to determine whether there was horn formation or merely a scab. However, it seemed to increase in size and to show induration beneath. This proved to be a septic condition; an axillary gland became infected and the mouse was killed, but no sign of tumour could be detected, merely acute inflammation.

4. Tar had been applied for 5 months. When it was discontinued a minute wart 1 mm. in height, which had not been there a week previously, was found. For a month afterwards it increased slowly in extent, though remaining flat. At the end of 2 months it seemed to show a deeper area of induration, and another tiny wart had appeared near it. A week later it was found that the induration was inflammatory, and pus was evacuated. The animal died at the end of 71 days, and the wart came away at autopsy, leaving no trace of tumour formation underneath.

5. Tar had been applied for 5 months, and a small wart, which had been in existence for a month, showed commencing ulceration, when the tar was stopped. This ulceration had increased, when the animal died 13 days later, but histological examination showed only the necrotic remains of the wart.

Simple Papillomata.

1. Tar had been applied for 4 months, and had produced a single wart of the size of a hempseed at the end of that time, when the applications were stopped. A fortnight later two others smaller warts appeared in the neighbourhood. Progress was slow: at the end of 48 days there were still three warts, two of the size of hempseeds and the third pinhead size. The mouse died at the end of 63 days, the tumours having shown little advance.

2. Tar applied for 4 months. A small wart appeared in the last 20 days, and when the tar was stopped there were two growing warts. One of these disappeared 20 days afterwards, but the other continued growing. The mouse died in 29 days with a papilloma histologically simple.

3. Tar applied for 4 months: in the last 20 days a wart appeared which had formed a small horn a week old when the tar was stopped. It measured then a quarter of an inch high by one-eighth of an inch thick, and a tiny wart had just appeared in front of it. Twenty days afterwards four warts were found. At the end of a month two of these had disappeared, but another was beginning. In 43 days a papilloma, the size of a large pea, had formed from the coalescence of two neighbouring warts. The warts diminished in number and size, and the mouse died 86 days after the cessation of tar applications. The papillomata were histologically simple.

Doubtful Case.

Tar applied for 5 months. A wart was noticed at the end of the fourth month, two warts in the last 18 days, and when tar was stopped there were four closely set warts all about the size of pinheads. They gave place 15 days later to a flat irregular plaque surrounded by hair. This disappeared, and at the end of 22 days a small pinhead-sized wart appeared in front of this. This in turn gave place to a shallow ulcer which seemed to be healing. At the end of 2 months there was very doubtful superficial induration, and this did not become more definite when the mouse died in 78 days after the tar had been stopped. The carcass had been eaten by the other mice and no histological examination was possible.

Malignant Tumours.

1. Tar applied for 5 months. During the last month the skin was very rough, and in the last 13 days two warts made their appearance. When the tar was stopped the larger was the size of a hempseed and the smaller less than half of that. Another papilloma, hempseed in size, appeared 20 days afterwards. At the end of a month there were three horns with doubtful induration beneath. The horns became flatter but the induration proceeded, and at the end of 43 days it seemed definitely to be malignant. The tumour became fixed to the deeper tissues. When the mouse died after 60 days it had a horn, five-eighths of an inch high, set on a broad indurated base. Histological examination proved the tumour to be a squamous-cell carcinoma spreading laterally in the subcutaneous tissues and infiltrating the muscles (Plate I, Figs. 1 and 2).

2. Tar had been applied for 5 months. A tumour arose during the last month, and formed a horny pedunculated wart about the size of an oatseed, with a tiny wart behind it, when the tar was stopped. Fifteen days afterwards three or four minute warts appeared in the neighbourhood. Progress was slow. At the end of 2 months there was an area of induration detectable at the edge of the warty masses. The growth thereafter was rapid, and the mouse died in 81 days with a warty mass which on the deep side was infiltrating the muscles of the back (Plate I, Figs. 3 and 4).

3. Tar had been applied for 4 months, and on its cessation there was found a hemispherical subcutaneous tumour with a central eschar measuring a quarter of an inch in diameter. It had not been observed 8 days previously. It reached a diameter of nearly half an inch 20 days after the tar had been stopped, and it showed central ulceration. The mouse died in 25 days, and microscopic examination showed the tumour to be an epithelioma infiltrating the muscles. This tumour was of rapid growth, extending over little more than a month, and may have been malignant even at the time when the tar was stopped.

4. Tar had been applied for 4 months. Within the last 20 days a wart appeared, and by the time the tar was stopped it had reached the size of a hempseed. It had increased 20 days later, and two smaller warts appeared in the neighbourhood. Progress was rapid. The mouse died in 24 days, and microscopic examination showed an epithelioma infiltrating the muscles (Plate I, Fig. 5).

5. Tar had been applied for 4 months, and a wart the size of a milletseed had then been in existence for 8 days. It decreased somewhat 20 days after the tar was stopped. At the end of a month the top of the wart was accidentally removed. In 43 days there was a flat ulcer with slight induration. Progress was then slow. In 76 days there was a red ulcerated area with exuberant growth in the centre, and the condition was now considered to be malignant. In 86 days the ulcer had scabbed over, the induration seemed less marked, and two small warts had appeared in the neighbourhood. The mouse died next day. Histological examination showed an epithelioma penetrating the deep muscles (Plate I, Fig. 6).

6. Tar had been applied for 4 months; a small wart appeared during the last 8 days and had grown to the size of a small hemp-

seed when the tar was stopped. Twenty days afterwards there were two small warts and two flat scab-covered areas measuring three-eighths and a quarter of an inch in diameter respectively, and beneath these there was some irregular induration. This induration by the end of a month had spread underneath the adjacent warts. These warts lost their prominence as the induration extended, so that in 43 days a raised indurated area obscured their position. At the end of 7 weeks there was an extensive indurated area nearly three-quarters of an inch in greatest diameter, with two ulcerated patches on its surface, and it was now considered indubitably to be malignant. The mouse died in 63 days, and the carcass was eaten by the other mice, and therefore the histological confirmation is awaiting.

These experiments show that some warts will disappear, some will increase for a time and then diminish and disappear, some will increase but still remain simple, and others will go on to malignancy probably as rapidly as if the irritant had continued. We can make three deductions from this: (1) that the production of the cancerous state is not necessarily the result of the action of the irritant on neoplastic, as contrasted with pre-neoplastic, tissues; (2) that the bias towards malignancy is given to the cells probably during the pre-neoplastic stage; and (3) that the successful transplantation of a tumour in the histologically doubtful stage is not a sure proof of the malignancy of the tissue at that particular date, seeing that left alone in its natural environment it will tend to declare itself as an epithelioma.

Such conclusions would be rendered much more certain by observations in cases where the irritant had been suspended before any neoplastic reaction had taken place—that is, during the prodromal period. Cases are known where men have been exposed to the action of soot, paraffin, or aniline products for a length of time and have given up such employment, and yet years afterwards they have developed the particular kind of epithelioma associated with these irritants. These cannot be lightly dismissed as strange coincidences, and they would seem to show that the essential damage to the tissues, finally resulting in malignant proliferation, is done at a comparatively early stage.

A second batch of mice was taken which had been painted thrice weekly with tar for four or five months without any sign of tumour formation resulting, and the tar applications were stopped.

Result when Tumours were not Present.

At the time the tar painting was suspended there were 20 mice with no visible sign of neoplastic reaction. Of these, 6 remained negative till they died, 4 developed temporary warts, 4 developed simple papillomata, and 6 developed malignant tumours. The 6 negative cases lived for 29, 42, 43, 48, 65, and 75 days respectively after the tar was discontinued, and no sign of tumour was at any time observed.

Temporary Tumours.

1. Tar applied for 4 months. Twenty days afterwards a pinhead-sized wart was seen. This had disappeared a week later, and the mouse died in 63 days without further wart formation.

2. Tar applied for 4 months. A month afterwards a wart the size of a hempseed was found. This made slow progress for another month and then disappeared. The mouse died in 83 days without further wart formation.

3. Tar applied for 4 months. Two months later a tiny wart was found amidst the regrown hair. This persisted unchanged for nearly a month, then diminished in size and disappeared completely at the end of 100 days. The mouse died in 110 days without further wart formation.

4. Tar applied for 4 months. Twenty days afterwards a wart the size of a pinhead was found. It did not increase appreciably in size, and had disappeared when the mouse died in 48 days.

Simple Papillomata.

1. Tar applied for 4 months. The hair grew again. Forty-three days afterwards a small, flat, pinhead-sized wart was noticed. For another month its progress was slow. In 86 days it was just palpable, having diminished for 10 days. A fortnight later it developed into a cutaneous horn. The mouse died in 112 days. Histologically a typical papilloma.

2. Tar applied for 4 months. Twenty days later a wart the size of a large hempseed was observed. This increased slowly till the mouse died in 42 days.

3. Tar applied for 4 months. Twenty days later two small warts were seen, one the size of a hempseed, the other of pinhead size. A week later the smaller had disappeared and the larger had grown no bigger. Progress was very slow. The mouse died in 63 days.

4. Tar applied for 4 months. Twenty days later four minute warts were found. Two of these coalesced a week later. Progress of all very slow. The mouse died in 42 days.

Malignant Tumours.

1. Tar applied for 5 months. The skin was rough when the tar was stopped, but the hair grew again. A month afterwards the skin was very rough and scabby. In 38 days there was doubtful growth under a scab which was pulled off. In 45 days there was definite horn formation, with doubtful basal induration. By the end of 2 months tumour formation had progressed, but the induration was still doubtful. The horn was broken off in handling. The mouse died in 71 days, and histological examination showed the tumour to be a large cornifying epithelioma definitely infiltrating the deep muscles of the back.

2. Tar applied for 4 months. Twenty days later a tiny wart was noticed. At the end of a month another wart appeared, and the first had grown to the size of a hempseed. In 43 days several warts were seen, one of them growing very well. At the end of 2 months progress had been maintained and induration of the base was suspected. A week later this induration was marked and the diagnosis of epithelioma was practically certain by 76 days. The mouse died in 98 days. Microscopic examination showed an epithelioma (Plate I, Figs. 7 and 8).

3. Tar applied for 4 months. Twenty days later there was found a wart the size of a hempseed with two tiny warts in front of it, all surrounded by hair. At the end of 27 days the larger had reached the size of a small pea. For another week it was stationary, though the base seemed somewhat indurated. Superficial ulceration commenced in 43 days and the basal induration extended. Another small wart now appeared. Five days later necrotic material was expressed from under the larger wart, and by the end of 63 days there was merely a flat scab half an inch in diameter, and it was thought that the greater part of the process must have been inflammatory and not malignant. The mouse died in 70 days, and histological examination showed an epithelioma of atypical cells deeply infiltrating the muscles.

4. Tar applied for 4 months. Twenty days later a hempseed-sized wart was observed. It increased in size and in 38 days another wart appeared beside it. Progress was then slow. In 70 days it was thought that the base of the larger showed signs of induration, but as the growth was very slow and the induration did not extend doubt remained until the mouse died in 90 days. Histological examination proved it to be an epithelioma with epithelial cell masses penetrating beyond the panniculus carnosus.

5. Tar applied for 4 months. The skin was then bald but smooth. From the 20th to the 34th day afterwards the skin was rough but again became smooth. In 63 days a small wart was detected. This increased in size and the skin grew rough. In 76 days there were two warts in this rough area. These progressed in size and the whole skin showed a verrucose appearance. The mouse died in 96 days. Microscopic examination revealed an epithelioma just penetrating the panniculus carnosus (Plate I, Fig. 9).

6. Tar applied for 4 months. One month afterwards minute raised points, hardly to be called warts, were found. A week later they might have been described as tiny warts set closely in a rough scaly skin. In 43 days they had coalesced to form a flat wart. Five days later serous fluid exuded from it and a crusted area took the place of the flat wart. In 63 days blood-stained serum exuded from this, and three small warts made their appearance. Sepsis became evident, and the resulting ulcer was taken to be merely a septic condition until the mouse died in 86 days, when microscopic examination revealed a typical epithelioma invading the deep muscles of the back (Plate I, Fig. 10).

These experiments clearly show that when tar has been applied repeatedly for a certain length of time and the irritant is then removed, tumours, even carcinomata, may make their appearance at a later date. It must be that the irritation produces in the normal cells subjected to its influence some profound change, undetectable by the microscope, so that they eventually proliferate in an unrestrained and in a harmful fashion. We may conclude that the neoplastic response to an irritant is a slow tissue reaction that exhibits no defensive property, that subserves no useful function. The internal changes in the cells in the earliest stages may some day be analysed: at present they are quite unknown. In the skin epithelium we have a tissue which we have been taught to believe is constantly being shed and constantly regenerated, and one would imagine that the damaged cells would soon have been shed leaving a new and healthy race to take their place. Of course, in man the processes take a much longer time to evolve. In mice we can discover the minimum duration of exposure to a tissue irritant that will be necessary to produce cancers, and thus form an idea of the length of the latent period before they are evident, and it is possible that this will vary with different agents.

REFERENCE.

¹Journ. of Cancer Research, 1918, vol. 3, No. 1, p. 1.

NINETIETH ANNUAL MEETING

OF THE

British Medical Association.

Held at Glasgow, July, 1922.

PROCEEDINGS OF SECTIONS.

SECTION OF PATHOLOGY (HUMAN AND COMPARATIVE).

Professor ROBERT MUIR, M.D., F.R.C.P. Edin., F.R.S.,
President.

THE PRODUCTION OF CANCER BY SPECIFIC FORMS OF IRRITATION.

BY

J. A. MURRAY, M.D.,

Imperial Cancer Research Fund, London.

THE modern experimental study of cancer etiology begins with Fibiger's experiments with the Spiroptera cancer of rats. The specific irritant is introduced by feeding rats with cockroaches carrying the larval nematodes. The cycle is completed by feeding cockroaches with the faeces of infected rats. Squamous carcinoma of the rat's stomach and tongue follows in a variable percentage of animals. Some races of rats are apparently quite resistant, and never show any lesions graver than papillomatous thickening of the gastric mucosa. Analogous results have been obtained by feeding rats with ova of *Taenia crassicolis* from infected cats. In this case sarcoma of the liver is obtained in as many as 50 per cent. of young rats (Bullock and Curtis). Both methods are convenient in application, but have the disadvantage that the animals have to be killed to observe the result.

The external application of coal-tar, first successfully utilized by Yamagiwa and Ichikawa on the rabbit's ear, though much more laborious, permits continuous observation and experimental variation in the degree of irritation. The extension of this method to the mouse by Tsutsui, and later by Fibiger and Bang, Murray and Woglom, Bloch and Dreyfuss, Deelman and others, has put us in possession of a certain method of producing undoubted malignant growths (squamous-cell carcinoma) in a high percentage of the animals used. This is evidenced by progressive growths, local infiltration, and lymphatic and vascular dissemination. In addition, Murray and Woglom have shown that artificial metastasis can be carried out with these growths. In this way very strong evidence of their malignancy can be got at an early stage, before even local infiltration has had time to occur.

So far an interval of at least three months has elapsed before true malignant growth occurs, and in some animals tarring must be kept up for as long as eighteen months to produce this result. No one has succeeded with the rat or guinea-pig, and it is probable that success with the mouse is, in part at least, to be attributed to the short life cycle of that animal. The surmise is borne out by the fact that in tar distillers and pitch workers the interval is never less than ten years, and may extend to thirty and forty years before malignant disease is set up. These observations furnish a rational explanation of the characteristic age incidence of cancer, since it is probable that the irritational factors responsible are neither so continuously nor so energetically applied in these industrial forms of the disease as in the experiments with mice.

Bloch and Dreyfuss are of opinion that the active substance is contained in the anthracene fraction of coal-tar boiling at over 300°C. I have found that an extremely active extract can be prepared from tar by successive extractions with water, alcohol, and ether. The ethereal extract gave 50 per cent. malignant growths in twelve months, reckoned on the total number of animals which survived for four months.

Result of an experiment in which unaltered tar, alcoholic extract, and ethereal extract of tar were applied to separate areas of the dorsal skin of each of 60 normal mice. After four months, when the first tumour appeared, 50 mice survived, and

LEITCH: EFFECT OF CESSATION OF IRRITANT ON DEVELOPMENT
OF EXPERIMENTAL TAR CANCER.

FIG. 1.—Large horny wart with basal induration. Tar had been applied for 5 months, and for the last 13 days a wart was present. Photo on day before death, 60 days after cessation of treatment.

FIG. 5.—A very cellular epithelioma with little keratinization, spreading laterally beneath the panniculus carnosus, which may be seen crossing obliquely upwards from the middle of the left margin. Age of tumour 44 days. Tar applied for 4 months, and tumour started 20 days before cessation of applications.

FIG. 7.—Tar applied for 4 months. Tumour started 20 days later. Photo a week before death. A large cornified mass with basal induration.

FIG. 2.—Section of No. 1. An epithelioma showing lateral subcutaneous spread. Muscle fibres were easily identified amongst the epithelial cell masses. Age of tumour 75 days.

FIG. 8.—Section of No. 7. Epithelioma with moderate keratinization. Age of tumour 78 days.

FIG. 3.—Multiple cornified warts, the larger with deep infiltration. Tar applied for 5 months; tumour formation started at end of 4 months. Photo on day before death, 80 days after cessation of applications.

FIG. 6.—An epithelioma infiltrating muscle, part of which is seen below. Age of tumour 95 days. Tar applied for 4 months, and tumour started 8 days before cessation of applications.

FIG. 4.—Section of No. 3—an epithelioma with large cornifying cell masses infiltrating the deep muscles of the back. Age of tumour 111 days.

FIG. 9.—An epithelioma just penetrating the panniculus carnosus. Tar applied for 4 months. Tumour started 2 months later. Age of tumour 33 days.

FIG. 10.—An epithelioma. Epithelial cell masses can be seen amongst the fibres of the deep muscles of the back. Tar applied for 4 months. Tumour started one month later. Age of tumour 55 days.