The question whether or not the development of cancer must always pass through a precancerous stage, recognizable as such by histologic or clinical examination, is not merely academic; it is of paramount practical importance. The possibility of early treatment is naturally bound up with that of early diagnosis. If it can be shown that cancer is always preceded by a precancerous condition, and if this latter condition can be diagnosed as such, then the question of early cancer diagnosis becomes naturally the question of diagnosing precancerous conditions; at the same time the general prognosis must be enormously improved. If, on the other hand, it is shown that only a comparatively small number of cancers are preceded by a precancerous condition, that in the majority of instances such a condition cannot be diagnosed, and that of the diagnosable cases only a very small proportion lead eventually to cancer, the term precancer loses its importance and the chances of treatment suffer accordingly. The cancer specialist is then in the position of King Herod, who killed all infants in order to be sure that his enemy, the Messiah, would be one of his victims. Unfortunately for Herod, even such a drastic measure was of no avail, and if the most prevalent and most fatal types of cancer are not preceded by precancer, or if such precancerous condition as may exist is not amenable to diagnosis, then these dangerous forms of the disease will continue escaping early diagnosis, even if the detectable forms of precancer are all radically treated.

The less we know about the nature of a pathological process, the more we are naturally inclined to establish a cause-effect relationship, taking certain frequently associated conditions as actual causes, though they may be merely chance coincidences or parallel processes. The danger of a post hoc, ergo propter hoc conclusion is nowhere greater than in cancer. On the other hand, if it is shown that certain conditions or processes are in a comparatively large number of cases followed by cancer, we are entitled to call such lesions precancerous, not because they are the necessary intermediary stage between normal and cancerous, but because from the purely practical point of view they are often associated. Such conditions may, to some degree, be compared
with Pawlow's conditioned reflexes: if a dog is shown a piece of meat and at the same time a bell is rung, the dog's stomach secretes digestive juices; if, after a certain number of such experiments, the meat is left out, and the bell alone is rung, the dog's stomach will secrete without the presence of meat. Nobody will draw the conclusion that under any condition the ringing of a bell causes stomach secretion; nevertheless, from the practical point of view it would be the logical conclusion if one were not acquainted with the history of the case. In the same way it seems likely that certain processes may appear to be precancerous only because the intermediate relationship is unknown. It may be that a probable precancerous condition is followed by actual cancer because the factor responsible for the precancer is also able to cause cancer. Benzpyrene, for example, in the great majority of cases causes at first papillomata of a benign character and then malignant epitheliomata. The papillomata are a precancerous condition, in most cases followed by cancer, because the same drug which produces them also causes them to become malignant. On the other hand, where the etiological factor is unknown, a lesion which is sometimes followed by cancer cannot be designated as necessarily and essentially precancerous, since we cannot say that the same factor which caused this suspicious lesion is also able to induce cancer. Thus liver cirrhosis is in a minority of cases followed by liver-cell cancer. Neither the cirrhosis nor the cancer, however, can be traced back to any constant etiologic factor. Are we, then, entitled to call liver cirrhosis a precancerous condition? Only if it can be shown that in every case of cancer cirrhosis has preceded malignancy; then we may say that, for practical purposes, cirrhosis is a precancerous condition, even though little or nothing is known about the etiology of either condition. The fact that cirrhosis affects the connective tissue predominantly, while cancer originates from the epithelial cells, should not really prevent us from calling cirrhosis a precancerous condition, or the fact that many cases of cirrhosis are not followed by cancer, because, in the latter case, it can always be argued that, had the patient lived long enough, cancer might have developed. But if cancer is found in livers in which histologic examination shows no sign of pre-existing cirrhosis, then the latter cannot possibly be called precancerous.

In view of the somewhat confused state of opinion about the meaning of precancer generally, it was thought that some light might be thrown on the subject by animal experimentation. We have, thanks to the great amount of work done by experimenters of many countries, sure means of producing cancer in animals which, so far as we know, were perfectly normal before the initiation of the experiments. Thus we can watch, in their different stages, normal cells or normal tissues becoming cancerous and we can then determine at what stage a cell or a tissue can be called precancerous. In these experiments, we shall call precancerous any cell or any tissue which, left to itself, without further application of any cancer-producing agent, becomes definitely cancerous.

Four different groups of experiments will be described in this paper; the results of these experiments will then be discussed, in connection with available literature on each subject; and finally an attempt will be made to draw conclusions concerning precancer and the causes of cancer in general.
Briefly, the experiments comprise the following items:

1. The development of sarcomata and allied tumours in rats and mice treated with cancer-producing chemicals.
2. The production of benign and malignant skin tumours in mice.
3. The developmental stages of fowl sarcomata after the injection of tumour filtrates.
4. The development of spontaneous mammary carcinoma in mice of a tumour-susceptible strain.

Development of Sarcomata and Allied Tumours in Rats and Mice Treated with Cancer-Producing Chemicals

The literature dealing with the production of malignant tumours, both carcinomatous and sarcomatous, by chemical compounds, including hormones of known composition and their chemical derivatives, has been collected by Cook, Kennaway and their co-workers in two articles, published in 1936 and 1937. Only those papers which are of importance for our particular investigations will be briefly mentioned here.

Burrows, Hieger and Kennaway produced transplantable tumours of the connective tissue, chiefly spindle-cell sarcomata, in mice and rats by subcutaneous injections of $1:2:5:6$-dibenzanthracene dissolved in lard. Burrows then injected a $1:1000$ emulsion of the same substance (in gum acacia) intraperitoneally into rats. After an average period of 40 weeks tumours were found in various parts of the abdomen, invading adjacent organs. The first sign of tumour formation was a patchy opacity here and there, with subsequent nodule formation. At that stage cells already showed malignant characters; they were mostly spindle cells, with occasional giant cells. No metastases were found. Control injections with olive-oil did not produce tumours.

Berenblum and Kendal obtained spindle-cell sarcomata in fowls following intramuscular injections of a colloidal solution of dibenzanthracene. Some of these tumours could be grafted into other fowls and produced metastases.

Boyland and Burrows produced sarcomata in rats and mice by injecting them with colloidal aqueous solutions of dibenzanthracene.

Andervont injected dibenzanthracene subcutaneously into mice; tumours thus obtained were transplantable into mice of the same strain.

The histology of tumours produced by dibenzanthracene in mice, rats, and rabbits has been studied by Haagensen and Krehbiel. The animals were killed as soon as tumours were detected; of 52 tumours examined, 3 were squamous-cell epitheliomata (all in mice); the others were sarcomata (fibrosarcoma, leiomyosarcoma, rhabdomyosarcoma, and unclassified sarcoma). A few of these tumours could be grafted through several generations.

Shear injected dibenzanthracene, dissolved in lard, subcutaneously into 40 rats and obtained 11 tumours in 18 survivors. Pellets made by dissolving the same substance in cholesterol were inserted under the skin of mice and

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1 A third article bringing this review up to the end of 1939 has appeared since the present paper was submitted for publication. See Am. J. Cancer 39: 381, 520, 1940.
produced tumours in the great majority of survivors. The smallest amount of dibenzanthracene with which a tumour was produced was 0.0004 mg.

More recently, very powerful carcinogenic agents, such as 3:4-benzpyrene and methylcholanthrene, have been used by many workers. Shear introduced these substances subcutaneously into mice; in many cases ulcers developed which then became malignant; the tumours resembled spontaneous sarcomata. When definitely malignant, tumour tissue could be grafted into other mice.

Ilfeld produced visceral tumours in mice and rats by implanting pellets containing dibenzanthracene or methylcholanthrene in various organs; thus he obtained kidney carcinomata, which could be grafted, a rapidly-growing spleen sarcoma, two primary liver-cell carcinomata, and a large uterine carcinoma. The results in the kidney indicate that the first reaction of the epithelium is a protective mechanism, followed by stratification and malignant degeneration.

Haagensen and Krehbiel obtained by means of benzpyrene liposarcomata in guinea-pigs and in a mouse, which were thought to arise from adult adipose tissue.

Dunning, Curtis and Bullock injected dibenzanthracene or benzpyrene into 688 rats and 720 mice, using 1 to 12 injection sites per animal. Forty-four rats had 76 dibenzanthracene tumours after an average period of 317 days; 348 rats had 849 benzpyrene tumours after an average period of 148 days; 657 mice had 1,018 benzpyrene tumours after an average of 107 days. As a rule, the time interval decreased with the increase in the number of foci or doses. Tumour development did not depend on the genetic constitution, nor on age or sex. The majority of tumours originated from the connective tissue; a considerable number developed from muscle tissue; only a small proportion were of epithelial origin. In animals with multiple tumours the nature of subsequent tumours was independent of the type of the first tumour. The results obtained showed that the potency for malignancy is a universal cell characteristic and that the histogenesis of the tumours is determined by the fortuitous exposure of the cells to the irritant.

Hval studied the reaction of the subcutis to the application either of non-cancer-producing substances or of dibenzanthracene. While lard injections produced hyperaemia of short duration combined with an accumulation of lymphocytes, macrophages and fibroblasts, followed by the formation of a cavity in which the fat was deposited, dibenzanthracene produced hyperaemia of long duration, combined with telangiectasis and degeneration of connective tissue with accumulation of macrophages, fibroblasts, and giant cells. A cavity formed which was lined with syncytial cells and surrounded by fibrous tissue. After three injections of dibenzanthracene the vascular changes became irreversible, the degenerative changes were more pronounced, the cellular response was more polymorphous and, combined with this degeneration, small foci of regeneration appeared. The regenerative cells took on more and more atypical forms; mitoses became frequent and sarcoma formed. Developmentally the cells which became sarcomatous were intermediate between macrophages and fibroblasts.

In view of the frequent use of fats as solvents for carcinogenic agents,
Burrows and his co-workers carried out experiments which showed that, enough time being allowed (one year or more), these fats were able by themselves to produce spindle-cell tumours in 8 out of 193 rats.

Rowntree, Steinberg, Dorrance and Ciccone found that crude wheat-germ oil, administered orally to albino rats, produced sarcomata in a large number of animals.

The importance of the tissue for the development of tumours as a consequence of the application of carcinogenic substances was demonstrated by Woglom, who introduced into rat organs threads soaked in benzol solutions of benzpyrene. These threads were first passed through cutis and subcutis; the tumours arose only in the subcutis and not in the internal organs.

Quite recently, sarcomata have been produced in mice and rats by injection of oestrogenic hormones, which are chemically related to one of the most potent carcinogenic chemicals, methylcholanthrene.

Lacassagne discovered that mice which had been injected for a considerable period with large doses of oestrogenic hormones developed spindle-cell sarcomata at the site of injection in the subcutis. He is of the opinion that sex hormones act not merely as "ordinary" irritants but, to some extent at least, as directly cancer-producing substances, though the response of the subcutaneous connective tissue was far less specific and far less intense than that of the mammary gland and other sex organs. Burns, Suntzeff, and Loeb made similar experiments. Among 375 mice, injected with various sex hormones, 9 developed spindle-cell sarcomata and one a myosarcoma. With one exception, all the tumours developed at or near the site of injections.

From this survey of literature the impression is gained that the conditions described are nearly all beyond the threshold of presarcoma—if such a condition can be recognized—and that we have to deal with sarcoma proper. It was, therefore, thought advisable to start a series of experiments in which animals would be sacrificed at regular intervals and their tissues histologically examined at all stages, even before anything of a tumour condition could be macroscopically detected intra vitam. This necessitated the use of a fairly large number of animals. Both rats and mice were employed, but since mice are very delicate animals and their mortality is high at the most favorable of times, the best results were obtained with rats.

Experiments

The most potent chemical carcinogens known, 3:4-benzpyrene and methylcholanthrene, were used. As the purpose of the experiments was to study the changes produced by these chemicals, no figures will be given concerning the number of takes or the average time required for tumours to develop. When tumours, more especially intra-abdominal ones, become palpable, they must have been in existence for some time and it cannot be determined from palpation when the tumour cells made their first appearance or when they became actually malignant. As animals had to be killed at regular intervals, preferably those which appeared to be more or less near death were chosen, though occasionally healthy-looking ones were also sacrificed.

Three hundred rats and 400 mice were used for the experiments. They were divided as follows:
FIG. 1. RAT: AREA OF LIVER FIFTEEN DAYS AFTER INTRAPERITONEAL INJECTION OF METHYLCOLANTHRENE IN LARD

The needle apparently injured the liver, which is invaded by a loose connective tissue containing lymphocytes, macrophages, and fibroblasts. The liver cells are partly damaged, showing fat infiltration.

FIG. 2. MOUSE: TRANSITIONAL STAGE BETWEEN GRANULATION TISSUE WITH A RICH BLOOD SUPPLY AND A CELLULAR FIBROTIC TISSUE, SIX WEEKS AFTER SUBCUTANEOUS INJECTION OF BENZPYRENE IN LARD; NO SIGNS OF MALIGNANCY

[Legend continued on next page]
Doses: For rats 0.5 to 2.5 c.c. of a 1 per cent solution in lard was used, for mice 0.25 to 0.50 c.c. of a 0.5 per cent solution.

The pellets were made as follows. The chemical (methylcholanthrene or benzpyrene) was dissolved in molten cholesterol, care being taken not to overheat but to maintain the temperature just high enough to keep the cholesterol in a liquid condition. The concentration of the chemical was in all cases 1 per cent. The solution was sucked up into a glass pipette, in which it immediately became solidified. Bits of the pipette with its contents were then broken off. Sometimes the glass was completely removed; sometimes the pellet was inserted with the glass, thus allowing only the ends of the pellet to come into direct contact with the animal's tissues. In still other cases the pellet was inserted together with the glass and then broken up by crushing; in such instances there were in the animal's tissues (or peritoneal cavity) numerous bits both of glass and of the chemical in pellet form. When the animals died, glass could often be detected in the tumours, but the pellets could only be seen when death occurred not more than two months after treatment.

The dose, measured in milligrams of pure chemical, for mice varied from 0.25 to 1.0 mg., for rats from 0.5 to 10.0 mg.

The adding of glass particles to the pellet was done for the purpose of producing a condition of non-specific irritation in the tissues in addition to the specific irritation caused by the pellet itself. The glass, obviously, provided a permanent source of irritation, while the irritation by the chemical naturally tended to diminish with its gradual transformation and absorption. Even the glass particles, because of encapsulation by macrophages, giant cells, and fibrous tissue, would become less "irritating" in the course of time.

**FIG. 3.** Rat: Fibrous Tissue Rich in Blood Vessels and Fibroblasts, Two Months after Subcutaneous Injection of Methylcholanthrene in Lard

Some of the fibroblasts show a tendency to enlargement with irregular nuclei. This is the picture of a presarcomatous cellular fibroma.

**FIG. 4.** Rat: Section through a Nodule about 3 mm. in Diameter, Three Months after Intraperitoneal Insertion of a Benzpyrene Pellet

This section shows cellular fibrous tissue, with some normal cells (small fibroblasts) and some enlarged, with large irregular nuclei. The latter are suggestive of beginning malignancy and the nodule as a whole is in a presarcomatous condition.
Date of Death. The 200 rats treated intraperitoneally died or were sacrificed at the following periods, dated from the day of injection: During

1 month: 8 rats  9 months: 11 rats  17 months: 11 rats
2 months: 6 rats  10 months: 17 rats  18 months: 4 rats
3 months: 5 rats  11 months: 17 rats  19 months: 3 rats
4 months: 9 rats  12 months: 14 rats  20 months: 8 rats
5 months: 14 rats  13 months: 14 rats  21 months: 3 rats
6 months: 11 rats  14 months: 7 rats  22 months: 4 rats
7 months: 4 rats  15 months: 5 rats  23 months: None
8 months: 14 rats  16 months: 8 rats  24 months: 3 rats

The 100 rats which were given a subcutaneous injection of a solution of the chemical died or were sacrificed as follows: During

1 month: 4 rats  10 months: 7 rats  19 months: 6 rats
2 months: 5 rats  11 months: 7 rats  20 months: None
3 months: 3 rats  12 months: 4 rats  21 months: 4 rats
4 months: 2 rats  13 months: 2 rats  22 months: 1 rat
5 months: None  14 months: 3 rats  23 months: 2 rats
6 months: 7 rats  15 months: 6 rats  24 months: None
7 months: 8 rats  16 months: 5 rats  25 months: 1 rat
8 months: 4 rats  17 months: 2 rats  26 months: None
9 months: 10 rats  18 months: 6 rats  27 months: 1 rat

The 200 mice given intraperitoneal injections died or were sacrificed as follows:

During 1 month: 80 mice
  2 months: 52 mice
  3 months: 33 mice
  4 months: 20 mice
  5 months: 7 mice
  6 months: 6 mice
  7 months: 2 mice

The 200 mice given subcutaneous injections died or were sacrificed as follows:

During 1 month: 74 mice
  2 months: 77 mice
  3 months: 25 mice
  4 months: 7 mice
  5 months: 9 mice
  6 months: 4 mice
  7 months: 3 mice
  8 months: 1 mouse

Number of Injections: Most workers have given several injections of hydrocarbon. In no case did we give more than one, as we considered it
Some cells are definitely suggestive of malignancy, showing large, irregular nuclei and absence of intercellular fibrils. This presarcomatous tissue would probably be called “early sarcoma” by many workers.

FIG. 6. MOUSE: EARLY SPINDLE-CELL SARCOMA, SEVEN MONTHS AFTER INTRAPERITONEAL INSERTION OF METHYLCHOLANTHRENE

An attempt to graft this tissue into mice of the same strain failed.

undesirable to disturb the processes induced by the first injection. If, for example, some granulation-tissue-like reaction, the sequel of the first injection, receives a second dose of hydrocarbon, it remains questionable whether further changes represent the natural consecutive development of those first observed or whether they are attributable to the subsequent treatment.

**Insertion of Pellets:** The insertion of pellets into the abdomen, with or without glass, was made through a laparotomy incision of about half to three-quarters of an inch. The material was inserted with the aid of a small forceps and pushed into the abdomen in different directions. For this operation the animals were anesthetized with ether. No animals were lost during or immediately after the operation; but during the first week several deaths occurred, mainly among the mice. These cases are included in the figures given above.

**Results:** Tumours developed in a greater number of animals in the pellet series than in the lard-solution series. This was to be expected, since in pellet form the active chemical only gradually becomes dissolved, absorbed, and transformed, especially when the pellet is still contained in the glass capillary so that only the two small ends are in contact with the body fluids. Under these conditions the cancer-producing chemical acts over a long period. In lard solutions, on the other hand, most of the injected hydrocarbon comes
into immediate contact with the body fluids and is rapidly taken up or disintegrated.

The accompanying photomicrographs show some of the tumours produced. The types varied within the species (rats or mice). There were no particular types for either one. On examining sections it was impossible to say whether a given tumour was more likely to be from a mouse or a rat. There was, however, a considerable difference in the time taken for tumour development. While in mice tumours appeared within five or six months, a much longer period was required in rats, *i.e.* twelve or more months. In each case the time period is reckoned up to the definite beginning of malignancy and the presence of a palpable tumour. It is impossible to state, even approximately, the time required in rats or in mice for the establishment of a premalignant, *i.e.* presarcomatous, condition.

![Photomicrograph](image)

**Fig. 7.** *Rat: Osteochondromatous Area in an Intraperitoneal Spindle-cell Sarcoma, Thirteen Months after Insertion of Methylcholanthrene Pellet*

The first effect of the injection of a lard solution of a carcinogenic hydrocarbon or of the insertion of a pellet is, as might be expected, the same as that of any mechanical injury. There may be some blood extravasation with formation of a fibrin clot with blood cells or there may be merely some destruction of tissue cells by the needle or the pellet. In all cases this first injury is followed by a tissue reaction which differs in no way from the ordinary tissue reactions described in text-books on pathology. Like any foreign body, the injected or inserted material is walled off by a layer of foreign-body giant cells which are formed from the existing and multiplying tissue macrophages to which are added the different types of blood cells (lymphocytes, monocytes etc.). A granulation tissue is gradually formed which corresponds in most respects to any other granulation tissue developing around foreign bodies or necrotic material. The only difference is that in many instances, though not
The grafts consisted of purely spindle-cell sarcoma tissue.

**Fig. 9. Mouse: Subcutaneous Sarcoma, Produced by Injection of a Methylcholanthrene-Lard Solution Six Months Previously, Showing Myxomatous Degeneration of Stroma; Necrotic Cell Debris Interspersed with “Healthy” Tumour Cells**

There are cells with distorted nuclei, many giant cells, and some tumour cells with fat globules.

**Fig. 10. Rat: Subcutaneous Polymorphous-cell Sarcoma, Arising Fourteen Months after Subcutaneous Injection of Methylcholanthrene in Lard**

There are cells with distorted nuclei, many giant cells, and some tumour cells with fat globules.

**Fig. 11. Rat: Subcutaneous Spindle-cell Sarcoma with Giant Cells, Which in Some Instances Give the Impression of a Possible Origin from Sarcolemma**

This tumour was induced by the subcutaneous insertion, twelve months previously, of a benzo-pyrene pellet. Note the invasion of muscle and the nuclei of various shapes and sizes.
The cells are rather small but the nuclei are large and in some instances multiple. In shape the cells vary greatly, from round to an elongated spindle form. Mitoses are numerous. This tumour was transplantable both intraperitoneally and subcutaneously, but for only three generations.

**FIG. 13. RAT: INTRAPERITONEAL SARCOMA, SIXTEEN MONTHS AFTER INJECTION OF METHYLCHOLANTHRENE IN LARD**

The cell boundaries are poorly defined. The nuclei are irregular both as to shape and chromatin content.

**FIG. 14. RAT: INTRAPERITONEAL SARCOMA, EIGHT MONTHS AFTER INSERTION OF BENZPYRENE PELLET**

The cells are of all sizes and shapes, including giant cells. Necrotic foci are present with clear débris.

[Legend continued on next page]
by any means in all, the number of capillary vessels is increased and the blood supply generally is greater than in average aseptic granulation tissue.

As a further step towards tumour development one notices that, while normally the granulation tissue soon becomes limited to the immediate surroundings of the foreign body and is early encapsulated by a dense connective-tissue capsule, which is poor in cells, in our experimental cases the tissue remains rich in cells even where thick connective-tissue fibrils form. In the subcutis and the subperitoneal tissue the cells thus affected are the fibroblasts; in the peritoneal cavity and its organs, the flat mesothelial surface cells. This state of pre-tumour irritation seems to have appeared in all cases thus far examined during the various periods preceding malignancy. The fibroblasts tend to be larger than normal; the nucleus also enlarges and tends to become irregular in outline. Often several nuclei are formed, or there is a very large irregularly shaped nucleus. The chromatin in the nucleus is frequently increased and forms irregular masses. Mitoses begin to become more numerous and are often, even at this stage, irregular. Other cells of the mesoderm series also show a state of irritation if lying within the injected area or its immediate surroundings. The polyvalence of mesenchymal cells of the undifferentiated type is well known. Thus we can witness, far from any existing bone or cartilage, the formation of such tissue by cells which, as a consequence of long-standing irritation, become differentiated out of non-differentiated mesenchymal elements. Such a phenomenon is known to occur in other circumstances, as, for example, arteriosclerosis. The cartilage- or bone-forming cells may be said to be in a state of irritation or of early benign-tumour formation. We have not, in our experiments, been able to witness the transformation of such chondromatous or osteochondromatous tissue into actual sarcoma. In the sarcomata which contained such tissues, the malignancy was carried on by the sarcoma cells, which originated from fibroblasts or other cells of mesenchymal origin, but not from cells which had become differentiated and taken on the particular function of cartilage or bone formation. When sarcomata associated with such cartilage or bone tissue were grafted into animals of the same strain, the tumours developing from the grafts were pure sarcomata (usually spindle-cell), containing neither cartilage nor bone.

Tumours developing intraperitoneally often contained large numbers of cells which were apparently of either endothelial or mesothelial origin. Such cells, under the influence of irritation, multiplied and tended to differentiate in their own direction; they easily formed epithelium-like structures, including acini, thus giving the particular focus the appearance of an adenoma or adenocarcinoma; often lumina were present, or cells became hyperplastic and filled up the existing lumen. It was difficult, in the majority of such cases, to determine whether such cells were actually malignant or whether, like the cartilage and bone-forming cells just mentioned, they were multiplying only under the influence of a local irritant, and dividing only as long as this irritant was present.

**FIG. 15. RAT: INTRAPERITONEAL ENDOTHELIOSARCOMA ARISING NINE MONTHS AFTER INTRAPERITONEAL INJECTION OF BENZPYRENE IN LARD**

The cells are largely of endothelioma type, partly lining acinus-like structures. Other parts of the tumour are more distinctly sarcomatous, with round and spindle-shaped cells.
Briefly, these experiments show that a malignant tumour does not arise suddenly or within a short period when it is induced by an agent which, however powerfully cancer-producing it may be (leading to almost 100 per cent successes), is not in a living or semi-living condition. It will be seen that in the transmission of a filtrable fowl sarcoma by a cell-free filtrate, the conditions are totally different, not only quantitatively but also qualitatively. In the case of the sarcomata induced by chemicals in mice and rats, the preparatory period amounts to a considerable portion of the life-span of the animal. To lead to exactly the same result the preparatory period is twice as long in rats as it is in mice. Whether this means that to become sarcomatous a strain of cells (fibroblasts or other mesoblastic elements) must undergo a certain number of mitotic divisions, and that in mice cells divide twice as often as rat cells in the same period, or whether it means that even under the most favourable conditions (powerful cancer-producing agent) cells, to become cancerous, must metabolize and divide during a certain minimal part of the life-span of the animal, cannot be determined from the results of these experiments. If, however, the latter are compared with the lessons derived from human oncology, one is inclined to accept the second of the two explanations as the more probable one. From many forms of industrial cancer we have learned that, in man, the lag period between the application of the cancer-producing agent (chemicals, radiation etc.) and the appearance of cancer amounts to many years, possibly to a part of the life-span not very different, relatively, from that in mice or rats submitted to the action of chemical carcinogens; and we have no evidence at all which would allow us to conclude that in small mammals, such as mice and rats, the time between two consecutive cell divisions is much shorter than in the larger species, i.e. man.

Our experiments show once more that cancerization by agents such as chemicals proceeds gradually, by degrees, and not suddenly after a long lag period during which the cells meanwhile remain normal. There is in every instance a presarcomatous period during which cells are constantly showing themselves to be under the influence of some irritating factor. At first granulation tissue appears which does not differ in any particular way from any other granulation tissue; but this "irritation tissue," instead of subsiding after a time and producing ordinary scar tissue, persists while certain cells (fibroblasts or other mesoblastic cells) begin to divide more rapidly and gradually take on a tumour character. The behaviour of some of these cells, such as those which produce cartilage or bone, would suggest that, pathogenically, they pass through a stage of activity, differentiation, before becoming tumour cells, or at least before becoming malignant. One is led to believe that, in this process of cancerization, the cells either become gradually more sensitive to a certain stimulating agent, so that the same or even gradually diminishing amounts produce gradually increasing effects, or that, in the absence of any particular agent, they have suffered, as a consequence of the temporary action of the primary cancer-producing agent (chemical), such an alteration in their constitution that the ordinary hormonal or other "every-day" stimulating substances transmitted through the general circulation are taken up in increased quantity and thus produce a state of hyperplasia gradually leading to cancerization. On the other hand, it may be that the cells do not take up
more of these stimulating substances than normal cells, but that their response is different because their constitution has been altered by the action of the cancer-producing chemical. If one is a protagonist of the virus theory of cancer, he can say that what the chemical does is to change the dormant symbiotic cancer virus, present in any cell, into a virulent or pathogenic agent which, once stirred to activity, continues to increase in virulence even after disappearance of the original cancer-producing chemical. The latter explana-

![Image](https://example.com/image.jpg)

**Fig. 16. Multiple Rat Tumours, Eleven Months after Insertion of a Benzpyrene Pellet into the Abdominal Cavity**

Histologic examination of several of these tumours shows different types. One is a typical spindle-cell sarcoma; another has the appearance of an endothelioma with acini formation, while other small ones still fail to show definite malignancy; they are fibromata or combinations of granulomata and fibromata. This clearly indicates the multicentric origin and the varying length of time of development of these various tumours.

But it is just as easy to postulate some hitherto unknown intrinsic cell alteration, not of a virus-stimulating nature, which would gradually lead, in an autocatalytic fashion, to cancerization of the cell. In view of the fact that recent work on certain viruses leaves doubts as to their living nature, there is possibly, after all, not much choice between the two explanations, the only difference being that in one case we still think of a possible extrinsic virus
carried by the cell from birth to death, while in the other the cancer character is carried by an autogenous cell-product. For the time being the problem does not appear to be near solution.

As will be seen again in the series of mice painted with solutions of carcinogenic hydrocarbons, not only does the normal cell gradually become transformed into a malignant cell, but it is not really possible, from histological examination, to tell the moment when this occurs. Rats are known to be highly susceptible to intra-abdominal sarcoma formation and there is no doubt that this inborn susceptibility is of great importance for the production of sarcoma in practically all rats which survive long enough. This susceptibility appears to be not only species- and strain-specific, but also organ-specific. This rule applies probably to all forms of cancer in experimental animals and possibly also in man with his different racial particularities.

If we compare the results of our experiments with those of other investigators previously mentioned, we find that they agree in many respects. Some differences, however, are noticed. The lag period was, on an average, longer than that usually recorded, apparently because we gave, in the case of lard solutions of the hydrocarbon, only one injection; the reasons for this have already been given. Then, unlike Haagensen and Krebs, we did not, with certainty, diagnose leiomyosarcoma or rhabdomyosarcoma. Many of the cells seen in our tumours we were not prepared to designate as actually malignant. There is in such cases always the possibility that cells are not only carried by the tumour, but that they may, for some time at least, multiply under the influence of either the same stimuli which were responsible for the cancerization of the tumour cells proper, or of irritating metabolic products originating from the malignant cells in the neighbourhood. Since we know that the presence of tumour cells stimulates the host to provide for them a nourishing stroma, however unreasonable this appears to be from the point of view of the well-being of the organism as a whole, it does not appear illogical to assume that other than stroma cells may be similarly stimulated to hyperplasia. In one case it was found that, while the original tumour contained, apart from the obviously malignant spindle cells, many islands of chondromatous or osteochondromatous tissue, the grafts contained spindle cells only, thus suggesting that the cartilage cells were not malignant and for that reason were unable to survive transplantation. It cannot be over-emphasized that, from the point of view of surviving, a cancer cell, even though it is undoubtedly a diseased cell with a short duration of life, is more resistant than a normal or benign-tumour cell. The process which causes a cell to become cancerous at the same time creates conditions which protect it from being destroyed by the somewhat uncongenial tissue fluids produced by the organism, while normal cells, when transplanted, usually die after a short period of survival. A number of our tumours were successfully transplanted, though in no case did a tumour survive more than five subsequent graftings; it made no difference whether tumours produced intraperitoneally were grafted into the abdomen or into the subcutis or whether subcutaneous tumours were grafted again into the subcutis or into the abdomen.

Fig. 16 is an average picture of abdominal tumours in rats. Tumours are multiple, and may be due either to metastasis or to primarily multiple growth.
Apparently both types occurred. Examination of several tumours in the same animal often showed that some of the smaller ones were of a harder consistency and were made up mainly of dense connective tissue. In some cases dense fibrous tissue was found together with typical sarcoma tissue. This may represent either a secondary invasion of a simple fibroma by sarcoma, or a secondary malignant change in a primarily benign neoplasm.

Advanced conditions in the abdomen led to the formation of numerous tumours which were partly adherent, covering all the organs and penetrating into them. The omentum was always involved in such cases. Of the parenchymatous organs, the most frequently involved was the liver.

Our results do not support the statement by Burns, Suntzeff, and Loeb, that the growth of sarcomata (produced in their case by sex-hormones) starts suddenly while that of epithelial tumours (see our next chapter) is preceded by a precancerous condition. As the latter occur on the surface of the body they are more easily observed, but the principle remains the same in both cases: malignant-tumour formation in the subcutis or abdomen of rats is always preceded by a presarcomatous condition.

The subcutaneous tumours were always single; they were observed first as small tumours about the size of a pea; at that time some of them were still in the precancerous state, while others were definitely sarcomatous. In two cases in which early biopsies were done the remaining part of the tumour eventually developed into typical sarcoma, though the true nature of the excised specimen had remained in doubt. Large subcutaneous tumours usually involved the skin and eventually led to ulceration. Sometimes parts of the tumours sloughed off, but the remaining portions continued to grow and the animals usually died from sepsis.

Production of Benign and Malignant Skin Tumours in Mice

Since Yamagiwa and Ichikawa succeeded, in 1914, in producing precancerous changes and eventually typical epitheliomata in the ears of rabbits by prolonged painting with coal-tar, the number of publications concerned with tar cancer has been enormous. After Kennaway and Cook found it possible to produce skin cancer by the application of hydrocarbons of known constitution, the use of tar for such experiments almost ceased; but though far more rapid results are obtained with these known chemical substances, they are not essentially different from those observed with tar. Tar apparently owes its carcinogenic capacity to the presence, in varying quantities, of highly carcinogenic hydrocarbons; in both cases the development of cancer is preceded by precancerous changes, and it is with the study of the latter and their relation to the definite establishment of malignancy that we are concerned. We want to know through what stages normal epithelium (or normal connective-tissue cells) must pass finally to become malignant, and we are particularly interested in the elucidation of the question: At what stage of precancer has the progressing cell alteration reached the stage where the action of the cancer-producing agent is no longer needed and where cancerization continues "spontaneously," there being no other stimulation than that to which each living cell is subjected owing to its being part of the organism, which involves function and metabolism?
Leitch said, in 1922, that while mice develop tumours (warts) three months after the beginning of tar-painting, there are no definite specific cell characteristics which would indicate precancer. At a certain stage the benign warts become cancerous. Once that stage is reached, even if tarring is suspended as soon as infiltration into the subcutis is felt by the fingers, the tumour continues to grow at an undiminished rate. When tarring has been applied for a long period (four or five months) and is then stopped, at a time when there are either only a few benign papillomata or even no papillomata at all, in a certain number of cases the existing papillomata will continue to grow and eventually become malignant, or papillomata and cancers may develop at places where there were no tumours and no tar. Leitch adds: "It must be that the irritation produces in the normal cells . . . some profound change, undetectable by the microscope, so that they eventually proliferate in an unrestrained and in a harmful fashion," a change, in other words, which leads them into malignancy without the addition of further irritation.

That such an alteration of the cell constitution can be brought about by a single application of tar, has been stated by Findlay (1925) on the basis both of clinical records and experiments on mice.

A question much discussed in connection with the amount of irritation required for the cancerous transformation of skin cells in mice is that of the part played by injuries given in addition to the specific cancer-producing irritant. On this point there exist considerable differences of opinion, all based on experimental results.

A number of workers found that scarification shortened the latent period preceding cancerization by tar; to this group belong Deelman, Teutschlaender, Reding, Raposo, Babes and Serbanesco. Truffi found that in mice trauma hastened the appearance of papillomata, but not the onset of malignancy. Choldin applied test tubes with hot water (65–85°C.) to tarred areas in mice and in this way obtained carcinomata in about half the time required in controls.

Deelman (1927) tarred mice until some early papillomata appeared; tarring was then stopped and most of the papillomata disappeared. But if, a few days after cessation of tarring, incisions were made into the skin at a site where there were no papillomata, there developed in several cases in the healed or nearly healed incision wound two, three, or even more papillomata. The cells which became part of the papillomata had never been in actual contact with tar; nevertheless, they became not only papillomatous, but eventually malignant. In another series of experiments the same author tarred mice until a few small papillomata appeared; tarring was then stopped and a few days later two scarifications were made, of which one was in contact with the papilloma and the other at a distance. These wounds healed in a few days; the cells of the healed scarification in the tumour region changed into tumour cells, while those of the distant scarification remained normal.

Raposo (1928) scarified the ears of rabbits; the proliferative scars thus formed, as well as the surrounding, non-scarified area, were then painted with tar. Epitheliomata developed only in the scarified area.

Reding applied magnesium in various forms to the skin of mice and thus prevented tar from producing tumours.
Other workers found that additional irritation did not hasten the development of tar cancer. To this group belong Roussy, Leroux and Peyre, Ludford, Daëls. Daëls implanted tar-soaked threads or strips of tarred skin and applied the "dull" cautery bi-weekly; this procedure did not increase the carcinogenic activity of tar. Parodi stated that preliminary burning of the skin did not favour the development of tar cancer. Derom applied lead shields heated to 60-70°C to the skin; the skin thickened and showed increased resistance against cancer development.

Ludford (1929) found, as a result of two series of experiments, that previous scarification slightly retarded cancer development in the skin of mice.

Brunschwig, Tschetter and Bissell combined benzyrene painting with burning by radium or cauterization. Tumours formed only at a distance from the scarred areas resulting from healing of the burns.

Several workers have tried the effect of diluting or altering in some way the tar used for painting. Hieger found that dilution to 1 per cent both prolonged the induction period and reduced enormously the total number of tumours. He also found that more concentrated solutions of dibenzanthracene had to be applied less often than dilute solutions to give the same results.

Skin hyperplasia caused by many irritants does not, as a rule, lead to malignancy unless the irritant possesses carcinogenic power.

Berenblum found that mustard gas has a definite anti-carcinogenic action when applied in connection with tar or carcinogenic hydrocarbons. Alone it produces a moderate degree of hyperplasia, which, however, never leads to cancer.

Reimann and Hall experimented with parathiocresol (sulhydryl) and found that this substance, when applied to the skin, increases the rate of cell division. When, however, it is combined with the carcinogenic dibenzanthracene, fewer tumours develop than with dibenzanthracene alone.

Rondoni and Corbellini combined the application of a feeble carcinogenic substance, dibenzacridine, with repeated light cauterization and found that the latter procedure stimulated tumour formation.

Cirio observed considerable differences between different strains of mice in susceptibility to tumour formation after tarring. There was no relation between this particular susceptibility and the colour of the skin.

Boyland and Warren found differences in liability to cancer formation following the application of certain drugs.

According to Twort and Twort the skin of the scapular region is more sensitive to cancerization than the skin of the sacral or abdominal region; most resistant are the soles of the feet. There is a parallelism between liability to the formation of benign warts and liability to malignant degeneration. The occurrence of malignancy in one area reduces the number and size of benign tumours elsewhere; this, however, may be due to the debilitating influence of the cancer, as it is known that animals must be in a good enough general condition to allow the development of tumours generally. Application of oleic acid to the skin diminishes the carcinogenic potency in that area.

Mottram found that the application of suitable gamma radiation to a skin area painted with benzyrene hastened tumour production. Burrows, Mayneord, and Roberts applied x-rays to inflammatory areas in rabbits (due
to injections of kaolin and silica), and obtained an appreciable number of tumours, of which several were sarcomata.

Lacassagne obtained sarcomata in rabbits by inducing bacterial abscesses after irradiation.

Appel, Strauss, Kolischer, and Necheles inoculated rabbits with the Brown-Pearce carcinoma and gave them also subcutaneous injections of dibenzanthracene; this caused the carcinoma to grow more rapidly and to metastasize more extensively.

**Experiments**

Pursuing our aim, that of studying the precancerous state under varying conditions, we used the new, powerful carcinogens synthesized by Kennaway, Cook and their collaborators. In these experiments we employed first benzpyrene and then methylcholanthrene, the latter substance being well known for its chemical relationship to the naturally occurring sex hormones.

In a first series 100 mice were painted with a 0.3 per cent solution of 3:4-benzpyrene (originally called 1:2-benzpyrene), obtained from the Union Chimique Belge “Meurice,” Brussels. Fifty of these mice were painted three times a week in the scapular region, the hair having been clipped short; painting was continued for three months. At the end of that time 43 mice were alive, of which 35 had tumours, most of them being papillomata; 8 were early epitheliomata and 4 more were in the transitional stage, leaving 23 benign tumours. After this three-months period painting was continued for another three months, but only twice a week. During this time the 8 mice which had epitheliomata died or were killed; the 4 in the transitional stage all developed epitheliomata and were killed or died. Of the 23 mice with benign papillomata, 18 were alive at the end of the second period; 12 of the tumours had become epitheliomata, 5 were transitional, and only 1 remained apparently benign; of the 5 mice which had died (of the 23 just mentioned) 3 had epitheliomata and 2 died of intercurrent disease, with benign papillomata. Of the 8 mice which at the beginning of the second three-months period had no tumour, all survived; tumours developed in 6—4 epitheliomata and 1 in a transitional stage. To summarize, of 50 mice, 43 survived for three months or more and 41 had tumours, of which, within six months, 31 were definitely malignant and 6 in the transitional stage. The last 6 would probably have become malignant in the course of time.

The second 50 mice of this series were painted for only three months with 3:4-benzpyrene, applied three times weekly. At the end of this period there were 40 survivors; 29 had tumours, of which 9 were epitheliomata and 2 were in the transitional stage; the remaining 18 were papillomata. No further paintings were given. During the following three months the fate of the 40 mice was as follows: The 9 with epitheliomata died or were killed; the 2 transitional tumours became epitheliomatous and the hosts died. Of the 18 lesions classified after the painting period as papillomata, 9 became epitheliomatous and 3 more were transitional cases. All these animals were killed or died. Of the remaining 6 mice with papillomata, 2 died of intercurrent disease; in 1 the tumour remained a papilloma; in the other 5 regression occurred, the warts fell off, and a small scar took the place of the tumour. Of
the 11 mice which survived at the end of the three-months painting period without having tumours, 5 subsequently developed tumours; 3 were small papillomata which regressed, the other 2 were papillomata with secondary malignant changes. The mice were killed. The remaining 6 mice did not develop tumours and remained healthy.

From this series of 100 mice treated with benzpyrene one fact emerges clearly: in a certain number of cases—the figures are too small to allow of a percentage of any value being given—the "impetus" towards cancerization via papillomatosis continues for a considerable time after the cessation of painting with this highly carcinogenic substance. While after each painting some of the substance may remain in the skin for a few days, it appears reasonable to state that tumours continue to be formed—not only to grow—in the absence of benzpyrene. The latter substance is thus responsible for the initiation of a certain cell alteration which, continuing to develop at an increasing pace, eventually leads the affected cell into malignancy. Of the papillomata remaining in the benign condition at the end of the three-months painting period, more than half became spontaneously malignant. There is a difference of degree only, not of quality, between the mice which continued to be submitted to painting during the second three-months period and those which were left untreated: those painted developed tumours more rapidly and in a greater proportion; none of the tumours regressed during that period. Though all mice were of a strain which was sent to us from America, where it showed a high susceptibility to breast cancer formation in the females (the incidence of breast cancer, however, being much lower in this country), considerable differences in individual susceptibility occurred; certain mice, though painted in exactly the same way as others, did not develop epitheliomata, some not even papillomata. This fact illustrates the importance of individual predisposition, well known from human pathology, and will not be stressed further.

This series of mice treated with benzpyrene suggested a further study to determine whether the impetus towards cancerization given by the application of the carcinogenic chemical and persisting after the cessation of painting, could be increased by a different procedure, i.e. by irritation of a different nature. Moderate scalding of the affected skin with hot water was chosen, because it does not involve any chemical action of a possibly unknown character. The question we set ourselves to solve was then simply this: For the second period of three months, i.e. that which follows the first period of painting, can the physical, non-specific irritation of scalding replace the specific irritation provided by continued painting?

For this series of experiments methylcholanthrene was chosen. The cancer-producing potency of this drug is at least equal, possibly superior to that of benzpyrene, when applied to the skin of mice. In addition to this, methylcholanthrene, as mentioned above, is related to substances naturally occurring in the organism, i.e. the sex hormones. Results obtained with this drug might therefore be of greater importance for the elucidation of so-called "spontaneous" carcinogenesis in animals and in man.

For this series of experiments 600 mice were divided into three groups:
(1) The 300 mice forming the first group were painted with a 0.3 per cent solution of methylcholanthrene in benzene, three times weekly, for three months. Those having no tumours at the end of that period were then either painted with methylcholanthrene three times a week for another three months, or scalded three times a week, or left alone.

(2) The second group, of 150 mice, was submitted to a combination of methylcholanthrene and scalding; each of these procedures was applied three times a week, but on alternate days, *i.e.* on Monday painting, on Tuesday scalding, on Wednesday painting, and so on. The scalding was done in the following way. A small cotton plug, mounted on a forceps, was plunged into boiling water, left in the air for about two seconds, and then applied to the skin by simple contact for about a second. The temperature of the cotton is difficult to assess; it was well below boiling heat, probably about 60–70°C. The mice which survived at the end of three months without having tumours were then either painted again with methylcholanthrene three times weekly for another three months, or scalded three times weekly for three months, or left alone.

(3) A final batch of 150 mice was submitted for a period of three months to scalding three times a week, no methylcholanthrene being applied during that period. Of the surviving mice, none of which had tumours, some were not touched, others were painted with methylcholanthrene, and still others were painted and scalded for three months.

The effect of scalding after epilation was to cause a reddening and blister formation and, in many cases, a superficial necrosis of the skin. When scalding was combined with painting (methylcholanthrene) there were more extensive ulcerations than with painting alone; also a higher mortality.

Following are the results of this treble series of experiments:

(1) Of the 300 mice painted for three months thrice weekly with methylcholanthrene, 254 survived; 61 had no tumour; 193 had tumours, of which 49 were epitheliomata, 25 transitional lesions, and 119 benign papillomata. In the following three months all the mice with epitheliomata died or were killed; of those with transitional growths, 3 died of intercurrent disease before the cancer stage was reached, while the other 22 all had epitheliomata before the three months were over. The remaining animals, 119 with benign papillomata, were divided into 3 groups.

(a) In 40 painting three times a week with methylcholanthrene was continued. Of these 9 died before the end of the second three-months period without a change in tumour type; of the 31 survivors 21 developed epitheliomata; in 6 the tumours entered the transitional stage, and in 4 the lesions remained benign. There were no regressions.

(b) Another 40 animals with papillomata were scalded three times a week for the second three-months period; no methylcholanthrene was applied. Of this group, 8 died before the end of the period, without a change in their tumour condition; of the tumours in the remaining 32 mice, 17 became epitheliomatosus and 3 transitional; 7 remained papillomata. There were 5 regressions, *i.e.* ulcers were present but the papillomata disappeared and the ulcer margins did not become cancerous.
(c) The remaining 39 mice with papillomata were left untreated during the second three-months period. Three died without a change in tumour type. Of the remaining 36 mice, 4 developed epitheliomata and 2 transitional tumours; 11 still had benign papillomata and there were 19 regressions.

(2) Among 150 mice painted with methylcholanthrene and scalded there were, after three months, only 89 survivors, of which 45 had tumours, while 44 had none. Of the 45 tumours 14 were epitheliomata, 5 were transitional tumours, and 26 ordinary papillomata. The animals with epitheliomata all died or were killed during the next three-month period; of the 5 with transitional tumours 2 died; the other 3 developed epitheliomata and were killed. Of the 26 mice with papillomata, 13 were treated during the next three months with methylcholanthrene (paintings), while the other 13 were treated by scalding only, each procedure being applied three times a week. Of the 13 painted mice, 2 died still having papillomata; 8 epitheliomata developed, 2 tumours became transitional, and 1 remained a papilloma. Of the 13 mice treated by scalding, 3 died, still having papillomata; 7 developed epitheliomata; in the other three the lesions regressed and the papillomata fell off.

Of the 44 mice without tumours at the end of the first three-month period, one half received for the ensuing three months the same treatment they had received during the first three months, i.e. they were painted with methylcholanthrene and scalded, while the other 22 mice were only scalded. Among the 22 mice which were both painted and scalded there were 15 survivals; those that died had no papillomata, but in one mouse with a fairly large ulceration an epithelioma formed in one of the margins; the animal was then killed. Of the 15 survivors, 2 had epitheliomata, 2 transitional tumours, 3 papillomata, and 8 no tumours, though most of these had ulcers. Among the 22 which were only scalded during this second period of three months there were 20 survivals; the 2 animals which died before the end of the period had no tumours, but rather extensive ulcerations; of the 20 survivors, 4 had epitheliomata, 2 transitional growths, 5 papillomata, and 9 no tumors, though ulcers were present.

(3) Among 150 mice treated during the first three-month period by scalding only, there were 135 survivors, some of them with superficial ulcers. None of the 150 animals had any tumours. Of the 135 survivors, 20 received no treatment in the following three months; one died of intercurrent disease; the other 19 did not develop any tumours and their scalds healed. Fifty-seven mice were painted for three months with methylcholanthrene three times a week. At the end of that period there were 49 survivors, 8 animals having died before the end of the period without tumours. Of the 49 mice surviving, 10 had epitheliomata, 3 had transitional tumours, 18 had papillomata, and 18 had no tumours. Fifty-eight mice (the rest of the 135 surviving for three months) were painted with methylcholanthrene and scalded on alternate days, as described above. After three months 13 animals had died without tumours; 45 survived the three-month period. Of these, 8 had epitheliomata, 1 a transitional tumour, 14 had papillomata, and 22 had no tumours.

Discussion of Results: Scalding with hot water, as described, did not induce nor accelerate the production of either benign or malignant skin
FIG. 17. "Sessile" Papilloma in Skin of Mouse Painted for Four Months with Methylcholanthrene in Benzene

The section is from the base of the tumour. The cells are in large masses, but regular in shape and arrangement; they do not penetrate deep into the subcutis and are definitely benign.

FIG. 18. Mouse Skin, Painted with Methylcholanthrene for Three Months, Showing Transition between the Slightly Thickened but Otherwise Normal Skin and the Down-growing Cell Masses, Mostly Spindle Cells Arising from Undifferentiated Basal Cells

This is an early stage of a malignant condition.

FIG. 19. "Sessile" Papilloma of Mouse Skin Induced by Methylcholanthrene Painting for Three Months

In the area marked with a cross there are numerous mitoses (see Fig. 20). This, combined with the knowledge of the powerful carcinogenic action of methylcholanthrene, is sufficient reason for designating the lesion precancerous, though the epithelial cells themselves are still of benign character. The boundary line between epithelium and subcutis is sharp and regular.

FIG. 20. Higher Magnification of Area from Fig. 19

Mitoses are numerous. Otherwise, the cells have not yet lost their benign character.
Several papillomata have formed, some of which have begun to invade the subcutis. In the lower part of the picture is a sharp boundary line between a benign papilloma and the subcutis, while the rest of the picture shows early epithelioma: large cell masses with large irregular nuclei and some mitoses. Here the boundary line between epithelium and connective tissue is not sharply drawn.

tumours in the mice of our experimental series. If anything, the scalding diminished the number of tumours produced by painting with methylcholanthrene. This shows that for tumour production there is an optimum degree of specific irritation and that additional irritation applied simultaneously has certainly no positive and possibly a negative effect. It also shows the fundamental difference between specific and non-specific irritation. No mouse among 135 survivors, treated by scalding only, had any tumour during a three-month period. This period, it is to be admitted, is rather short for an irritant with little cancer-producing effect, but of the 20 mice given a second three-month period of scalding, none developed a tumour, and it may be added that, though the surviving mice have been observed (no treatment being given) for nearly three more months, no tumours have developed. The scalds are healed and nothing remains of the previous state of irritation.

It is unnecessary to insist on the powerful carcinogenic action of methylcholanthrene. All those who have experimented with the chemical are unanimous on that point. Though differences exist between the various strains of mice used, it can be said that within six months after the beginning of painting with a solution of this substance, 85 to 95 per cent of the survivors have tumours, and that eventually the great majority of these tumours have become, or are about to become, malignant.

The accompanying photomicrographs show some of the stages leading from hyperplasia to papilloma and to epithelioma. In view of the great im-

**Fig. 21. Mouse Skin Painted with Benzpyrene for Eleven Weeks**

Several papillomata have formed, some of which have begun to invade the subcutis. In the lower part of the picture is a sharp boundary line between a benign papilloma and the subcutis, while the rest of the picture shows early epithelioma: large cell masses with large irregular nuclei and some mitoses. Here the boundary line between epithelium and connective tissue is not sharply drawn.
portance attached by many workers to the changes occurring during the painting period in the connective tissue which underlies the affected epithelium, it may not be out of place to state that, however significant some of these changes (infiltration, hyperaemia, fibrosis etc.) may be as concomitant manifestations, they cannot be said to explain the mode of cancerization of epithelial cells. It is with the changes of that particular tissue that we are concerned. Here we find that the changes from normal to tumour condition occur gradually. It is not possible, from either histologic or naked-eye examination, to state exactly, and in every case, at what stage the cell alteration has reached that

**Fig. 22.** Infiltration of Muscular Layer in Mouse by Epitheliomatous Masses, Accompanied by Numerous White Blood Cells and Some Connective-tissue Cells, Six Months after Painting with Methylcholanthrene Was Begun and Three Months after Painting Had Been Discontinued and Replaced by Scalding (Hot Water)

**Fig. 23.** Stroma Very Rich in Cells, Surrounding Nests of Epithelioma Cells or Isolated Cells, after Five and a Half Months' Painting with Benzpyrene

It is admitted that the irregular shape of many connective-tissue cells makes this picture suggestive of carcinosarcoma; but it is thought that the richness in cells of the connective tissue is due to long-standing ulceration.

point where cessation of any irritation, specific or non-specific, will fail to prevent the affected cells from proceeding, apparently "spontaneously," on their way towards development of papilloma and epithelioma. On the other hand, cases occur in which, after cessation of irritation (painting or scalding or both), existing papillomata instead of progressing towards the malignant stage regress and disappear completely. Such cases were comparatively common in the days when tar was used as the cancer-producing agent; they became less common when the first specific chemical, 1:2:5:6-dibenzanthracene, was introduced, and are rarer still with 3:4-benzpyrene and methyl-
cholanthrene; but they occur, nevertheless, and may be explained on the basis of local cell immunity acting against the products of cell alteration—or, as some will say, against the "virus"—combined with that unknown and unmeasurable factor called predisposition.

Our experiments show that when cells have been for a certain period under the effect of methylcholanthrene—supplemented or not supplemented by scalding—it is of almost no importance whether additional irritation is produced by continuance of the painting with the specific substance or by a non-specific procedure, such as scalding with hot water. The latter measure, by itself, never produces tumours; employed concomitantly with the specific treatment it does not hasten tumour production; but given at a time when only some of the animals have reached the cancer stage, it accelerates tumour production and cancerous change almost as effectively as the continuation of the specific treatment. The position may be summarized as follows:

A specific carcinogenic substance sets in action certain unknown processes which tend to end in cancer. When these processes have brought about certain changes, cancer formation in some cases proceeds "spontaneously," without requiring further irritant action; in many cases, on the other hand, the processes terminate prematurely and tumours fail to develop. These processes, however, can be kept alive, once they are established, by any procedure which has the effect of increasing the rate of cell division in the tissue affected. However active a cancer-producing substance is, it cannot alter cells suddenly; it can only act gradually, and for this action several cell
generations are required. The more rapidly cell generations follow one another, the more swiftly cancerization proceeds. By what process the increase in cell multiplication is brought about is not of material significance if only a certain critical cell alteration has been produced. Unspecific scalding has almost the same effect as specific painting with a powerful cancer-producing chemical.

In this discussion the expression "spontaneous" has been used. It is obvious that it is impossible to keep any living cell in the organism completely free from "irritation." When a cell, after having been for a certain period under the influence of a cancer-producing agent, proceeds towards tumour formation apparently of its own accord, the application of the carcinogen having been stopped, it does so apparently "spontaneously." This does not mean, however, the total absence of any stimulation to activity. Cells receive constantly, through the blood stream, products originating from outside (food) or in other organs (hormones, etc.) which represent sources of activity for the receiving cells. This action leads, after a time, to cell division, and, if an increasing quantity of such substances is received, a state of hyperplasia may ensue. Thus, even if the application of a specific cancer-producing agent to the cells is stopped, activity and internal "irritation" still proceed and, if the specific cell alteration produced by the temporary action of the cancer-producing substance has gone far enough, the every-day "irritation" connected with metabolism may be all that is needed for ultimate tumour production.

In a previous section, which dealt with the production of sarcoma in rats and mice by benzpyrene and methylcholanthrene applied subcutaneously or intraperitoneally, mention was made of the work of several experimenters who had found that extremely small amounts of these chemicals, applied once, sufficed to induce malignancy. Our own experiments support these findings. From the examination of tissues injected with such substances and examined after a certain period, it was found that, though the presence of the cancer-producing hydrocarbons could no longer be demonstrated, cancerization still proceeded. In the case of painting, the amount of the substance taken up by the skin cells cannot be ascertained; most of it is licked or rubbed off; how much remains we do not know, but we can well say that, when application has ceased, the remaining amount quickly disappears both from the surface and from the affected cells, just as it does from the abdominal cavity and subcutaneous tissues of rats and mice which at a later stage develop sarcomata.

This delayed reaction is of considerable importance from the point of view of cancer development in man. We know that certain industrial cancers develop years after cessation of the activities which we consider to be the responsible factor. In the case of other cancers of man, which are the great majority, and for which we know of no particular cause, there is also reason to believe that whatever the agent or agents which started the cells on their way towards cancerization, these may have ceased to be present long before cancer formation began and longer still before cancer was detected.

So far as treatment is concerned, it may be said, generally speaking, that if a person has anywhere in his body a condition which from experience deserves to be called precancerous, two things must be done:
(1) If possible that precancerous condition must be cured or removed.

(2) The affected part, if not removable or actually curable, must as far as possible be kept in a condition of rest, i.e., its activity must be reduced and anything causing irritation to this part must be avoided.

From a general point of view, and as long as we are not able to give more specific advice as to what to avoid to prevent cells from preparing themselves for neoplasia, we may say that probably anything that increases their vitality and their resistance against disease factors ultimately helps in the prevention of cancer, since it helps the cells and organs to remain in a normal healthy condition, instead of being chronically in a precancerous state, into which cells of lesser vitality are brought by certain agents, whatever their nature may be.

**Sarcomata Due to Skin Painting with Benzpyrene and Methylcholanthrene:** A small number of our mice developed sarcomata either simultaneously with or apart from epithelial tumours. In all these cases ulcerations were present, which were being continually painted with either benzpyrene or methylcholanthrene; in one instance the painting was combined with scalding. Caution must be observed in diagnosing sarcoma or carcinosarcoma in such cases. The tissue reaction in a case of long-standing ulceration is very intense and the number of cells accumulating in such a stroma may be as great as in a true sarcoma. Only if the character of the cells, the constant growth of the nodule at a distance from the actual ulcer, invasion of neighbouring tissues, or other characters of sarcoma tissue are present, should sarcoma be diagnosed. Altogether 5 cases were observed in which the diagnosis of sarcoma appeared justified; in a few other cases a diagnosis of presarcomatous lesions could be made.

**Developmental Stages of Fowl Sarcomata after Injection of Tumour Filtrates from Fowls**

It is not intended to give a full account of all the papers which have been written on the histological aspect of the chicken sarcoma and allied tumours since Peyton Rous published his description in 1910. It is obvious that a study of the histogenesis of the tumour is bound up with its filterability. If transmission is accomplished by grafting pieces of tumours, or even isolated cells, the neoplasm in the new host develops from some of the transplanted cells which survive and are taken into the "household" of the animal. We learn nothing about the origin and development of the tumour from its original normal prototype. When, however, we inject a cell-free filtrate, the active element must penetrate certain cells and transform them into tumour cells; then only can tumour growth begin.

For a long time it was thought that only fowl tumours could be transmitted by means of filtrates. Experiments which seemed to indicate that occasionally, and under special conditions, mammalian tumours could be induced by cell-free material have not been generally recognized by the majority of workers as conclusive. In recent years, however, it has been shown beyond doubt that at least one mammalian neoplasm, the Shope rabbit papilloma, is transmissible by cell-free material, so that there is no justification for establish-
ing a differentiation in principle between mammalian and avian tumours, though the great majority of the former are still, from the practical point of view, refractory to cell-free transmission.

The Rous sarcoma as well as several other fowl tumours is easily transmissible by tumour filtrates; these filtrates can be injected into any part of a fowl and local tumours will result in the majority of cases; the significance of temporary refractoriness towards filterability of these tumours does not need to be discussed here.

Not all workers seem to realize that the difference between carcinogenesis by the injection of a tumour filtrate and carcinogenesis by a chemical substance is one of principle and not of rapidity of action only. A chemical substance such as benzpyrene or methylcholanthrene is specific in the sense that it has a particular action on cells, changing them gradually into tumour cells; but even in the case of the mouse, which appears to be the animal with the shortest preparatory period, cancer production requires many weeks and often months. This is a measurable part of the animal's life. In the case of man the lag-period between the initial action of a known cancer-producing chemical or physical agent and the occurrence of cancer extends to many years. In the fowl, with a life span intermediate between that of the mouse and of man, many months are required to produce cancer by such means, but with tumour filtrates only days are necessary, often as little as a week, to produce a palpable tumour.

Another important difference must be noted. We do not yet know all the animal species which respond to the action of the chemical and physical tumour-producing agents; animals of all orders and kinds appear to be more or less susceptible, and in a given animal many tissues can be made neoplastic by one and the same agent. With tumour filtrates, on the other hand, new growths are obtained only in a particular species. Though temporary growth may be obtained in allied species (we grafted the Rous tumour into guineafowls and turkeys for three generations; Andrewes into pheasants, etc.) permanent growth occurs only in that species from which the filtrate originates. Also, the tumour type is highly specific. Though we do not inoculate cells, we obtain but one type of tumour. If we inoculate a filtrate from a filterable endothelioma we always obtain an endothelioma; the result is the same as if we had introduced actually differentiated tumour cells of a particular type. We may compare the organism to a large building and the body cells to rooms furnished in any given style. The appearance of one or several of these rooms can be changed either slowly or rapidly. The householder may go into the neighbouring forest, cut down trees, and make countless kinds of furniture; he may then search for raw materials from which he fashions hangings, wallpaper, lamps, crockery, cutlery, etc. This will take time; but in this way the changes need not be limited to a particular room; articles suitable for any room can be provided. Let us assume, on the other hand, that there is in the neighbourhood another building with rooms similar though not identical to those of the first. The contents of one of these rooms can be removed, one piece after another, and transferred to the corresponding room in the first building. The pieces of furniture are not identical; but they fit the room and, in any case, save trouble and time. Such a shortcut is possible, how-
ever, only if furnishings are taken from a similar room; kitchen utensils cannot be transferred to a lounge. The various articles have a specific character. This rapid mode of transfer is what happens when a tumour filtrate from one fowl is injected into another, while the first or slower method is comparable to treatment with a carcinogenic chemical or physical agent. This fundamental difference is apparently not appreciated by those who, having studied the histogenesis of the Rous sarcoma developing after filtrate injection, would apply their findings to the origin and development of spontaneously occurring sarcoma in animals and in man. In the transmission of Rous sarcoma we inject the ready-made product of cancerization, which consists probably of modified cell contents, i.e. of heavy protein complexes which, like enzymes, are in a subliving condition and able, when inoculated into cells of the type from which they originated, to combine with the normal cell contents and transform them, in autocatalytic fashion, into tumour-cell substance. In the case of cancerization by chemicals, the carriers of the tumour character, these heavy protein complexes, have at first to be gradually created, and this is a comparatively slow process.

Recent papers by Mauer and by Levine contain ample quotations from previous workers on the histology and histogenesis of the Rous and allied fowl sarcomata, so that a review of the literature here is unnecessary. For the sake of completeness, however, references are included in the bibliography. As will be seen, we disagree on two important points from Mauer. (1) The participation of muscle cells proper in the formation of tumour elements is not substantiated by our experiments. (2) We entirely disagree with the statement that there is only a difference in time between the development of a fowl tumour after filtrate injection and the production—presumably by chemical or physical agents, though Mauer does not mention this in his paper—of mammalian sarcomata.

Experiments on the Histogenesis of Fowl Sarcoma by Injections of Tumour Filtrates

Twenty-four Leghorn fowls, aged four to six months, were used for these experiments: 12 received intramuscular injections of tumour filtrate, while in the other 12 the filtrate was introduced into and under the skin. In addition, 4 fowls received control injections of saline 2 intramuscularly and 2 intracutaneously and subcutaneously. Filtrate was produced from active tumours in the manner described in a previous paper, 2 l. c.c being used for each injection. Each fowl received on each side into the breast muscle—or into and under the skin, as mentioned above—8 injections. The sites of injection were marked with dye. Care was taken to give the intramuscular injections exactly perpendicularly to the surface, so that, when the injected areas were excised, one could be sure of having the whole area. The areas were then imbedded and sectioned.

Areas were excised after the following periods: fifteen hours, twenty-four hours, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, eighteen, and twenty days. Two fowls were used.

2 South African Institute for Medical Research, Publ. XXXI, 1934.
Fig. 25. Section Thirty Hours Injection of Rous Sarcoma Filtrate into Fowl's Breast; Accumulation of Nucleated Red Blood Cells in Subcutis

Fig. 26. Section Five Days after Subcutaneous Injection of Rous Sarcoma Filtrate

There is an accumulation of white and red blood cells, many of the latter being contained in newly formed capillaries. The mesenchymal cells are increased; some resemble the macrophage-polyblast type, while others have the appearance of fibroblasts. This development has apparently taken place in the fibrin clot and it is not possible to decide whether the accumulations of mesenchymal cells originate from white blood cells or from fixed connective-tissue elements.

Fig. 27. Section Six Days after Intramuscular Filtrate Injection; Destruction of Muscle Fibres, Which Are Replaced by Connective Tissue of the Loose Type with Beginning Mucoid Degeneration of the Matrix

This is a very early stage of tumour formation.  

[Legend continued on next page]
each time for excision, one area per fowl being removed at a time. Care was taken, when choosing areas for excision, not to remove any material from the neighbourhood of a recent wound, so that the picture might not be obscured by inflammation or injury. In all, 38 areas injected intramuscularly and 38 areas receiving intracutaneous and subcutaneous injections were examined histologically. As compared with Mauer's results, tumour development was much slower, due perhaps to a weaker filtrate. For our purpose, i.e. the search for precancerous conditions, this slower development was rather welcome.

Results of Experiments: It may be stated at once that no appreciable difference was noted between the development of intracutaneous and subcutaneous tumour nodules on the one hand and intramuscular tumour formation on the other. The consistency of the stroma differed slightly. Intramuscular tumours showed the classical myxomatous character, while skin nodules, meeting with more resistance, tended to be harder and more fibrous. But this does not affect the question of either histogenesis or of precancer. As regards the histogenesis of intramuscular tumours, in no case could the formation of tumour cells from the muscle cells themselves be observed, as claimed by Mauer. The differentiated muscular tissue does not lend itself to sarcoma formation, which is the "privilege" of undifferentiated cells only, such as blood monocytes and tissue macrophages. It does not matter into what part of the animal's organism the filtrate is injected, the result is always the same. Though the original Rous tumour was found in the muscles, its biological status, after more than a quarter of a century, is such that the site of injection is totally immaterial; even the brain, as Vázquez-López has shown, is no exception to the rule, as the microglia cells, which in the brain tissue give rise to the tumour after injection, are analogous to monocytes and macrophages elsewhere.

Only two stages can be differentiated in the formation of tumours after filtrate injection: the first stage is the non-specific reaction, which is the same as that following the injection of pure saline or an inactive tumour filtrate. The second stage, the specific tumour stage, begins suddenly; no pretumour stage is noticeable. The long preparatory period observed in mice and rats treated with cancer-producing chemicals is absent.

The photomicrographs reproduced here show some of the appearances of the tissues injected with tumour filtrate. The time taken for tumour development varied considerably, as did the reaction set up by the tissue into which the injection was made.

The first appearance is of the damage done by the injection, whether of filtrate or of saline: haemorrhage along the needle track, formation of a clot which includes red and white blood cells, and the usual steps towards scar formation—increase of mononuclear cells in and around the clot, removal of blood pigment by phagocytosis, gradual change of macrophages into fibro-

**FIG. 28. SECTION EIGHT DAYS AFTER FILTRATE INJECTION INTO SUBCUTANEOUS TISSUE**

The periphery of this focus is occupied by red blood cells; but the central part consists of a loose connective tissue with mucoid degeneration of the intercellular matrix and must be classified as early tumour tissue. The fact that it is surrounded by blood extravasation is in favour of an origin of the tumour cells from white blood cells, which were included in the clot.
cytes, and formation of connective-tissue fibrils. This goes hand in hand with a dilatation—usually slight—and new formation of small blood vessels and capillaries. When the injection had been made into the muscle, the muscle fibres which were damaged showed some proliferation of nuclei; but there was no sign of new formation of muscle fibres from these nuclei and the damaged tissue was always replaced by connective tissue. These stages of repair cannot be called precancerous, for they occur whether or not a tumour agent is present in the inoculum. After about four or five days, or more, there suddenly appear small foci bearing all the characteristics of early tumour tissue: a great increase in cells, sometimes with numerous mitoses and sometimes with scarcely any mitotic figures; cells rapidly assuming an irregular spindle-cell shape, while others remain small and round, so that they are easily taken for lymphocytes. The intercellular stroma is very loose from the beginning and shows liquefaction and mucoid degeneration. When the tumour tissue is established within muscle, it rapidly invades the spaces between the muscle fibres and breaks up the fine interfibrillar septa and the fibrils themselves with their sarcolemma. There was no indication that the muscular nuclei within the sarcolemma entered into the formation of tumour cells.

It is well known that, even with a potent filtrate, some fowls are more or less refractory to tumour formation. Some of our photomicrographs are suggestive of this. When, a week after inoculation, cellular material along the needle track consisted only of cells of the lymphocyte or macrophage type without cell multiplication or tumour formation, it must be considered questionable whether such areas would ever have become neoplastic even if they had been left untouched in the bird. Our previous work on immunity phenomena in Rous sarcoma has shown that antibodies form rapidly following the period of tumour formation; if the tumour agent has not succeeded in establishing itself within cells, changing them into sarcoma cells, it will, if still present, be destroyed or at least held in check by the antibodies present in the body fluids. While during the first week following the filtrate injection tumour foci may arise in any number within the injected area, after that period tumour tissue grows from its own resources through multiplication of cells which act as a protection to the tumour agent, so that antibodies circulating in the blood cannot reach and destroy it.

The controversy raised by the work of Carrel and that of Ludford as to the origin of the cells giving rise to sarcoma is probably not to be solved by tissue-culture methods; on the one hand, it is difficult and perhaps impossible to obtain in vitro a true change of blood monocytes into full-fledged fibroblasts; on the other hand, the fibroblasts in tissue culture are derived from non-differentiated mesenchymal elements and remain permanently in an unripe condition; this allows them to live indefinitely, as shown by Carrel's classical fibroblast strain of twenty-five or more years' duration. Thus neither monocytes nor fibroblasts in tissue culture are living the same life as they do in vivo. There is no doubt that in vivo blood and tissue monocytes (macrophages) eventually turn into fibroblasts or fibrocytes and it is certain that these cells become malignant at an early stage of their development. It is therefore comparatively immaterial how we name them; once malignant,
they may assume any shape, from the elongated spindle to the round form, but once malignant, they are different from their parent cells. They are now neither monocytes nor fibroblasts; they are sarcoma cells. Much discussion has arisen in the past as to what constitute the various elements of the reticulo-endothelial system. If, as seems justifiable, we use the term to include all the cells which, owing to their function may be called trephocytes,\textsuperscript{9} then we are entitled to call the Rous sarcoma, as McGowan has done, a malignant neoplastic disease of the reticulo-endothelial system.

As this study of histogenesis of the Rous sarcoma has not been undertaken as an end in itself, but to search for evidence of conditions which might justifiably be called precancerous, we can only state that we have found no evidence of such conditions. The cell proliferation observed before sarcoma formation sets in is not precancer; it is merely repair after injury. If the tumour agent is present, then cells in a state of multiplication, which are susceptible to cancerization, become “infected” with the tumour agent; if the tumour agent is absent or has lost its virulence, then these cells will eventually help form scar tissue. There is nothing comparable to the prolonged precancerous stage observed, for example, in the skin of mice painted with a cancer-producing chemical, where after a time the changes inaugurated in the cells continue to progress of their own accord, even if the original culprit has ceased to be present.

Many workers have used silicious earth, lycopodium spores, or similar material to cause a fixation of Rous agent circulating in the blood; or they

\textsuperscript{9} See South African Institute for Medical Research, Publ. XXI, 1928.
have set up a local injury and at this spot a tumour has formed. This same result is obtained by injection into skin or muscle of the fowl. These procedures simply cause an accumulation of susceptible cells, but these cells are normal repair elements; if they are injected with tumour filtrate, they become malignant; there is no intermediate stage.

**Development of Spontaneous Mammary Carcinoma in Mice of a Tumour-susceptible Strain**

All the tumours produced in the experiments described in the preceding sections were artificially formed. The animals used were susceptible in the sense that tumour-producing agents, injected into them or painted on their skin, found a ready response because the animals were potential cancer-bearers. If no artificial means had been employed, probably none of them would have become cancerous "of its own accord." We now turn to tumours arising "spontaneously" in mice, *i.e.* under ordinary conditions of life, there being no stimulant present, as far as we know, other than those provided by the normal functions of the individual. The strain of mice used for these studies was sent to us from the New York State Institute for the Study of Malignant Disease in Buffalo. While in America the percentage of tumour bearers among the females of this strain which had passed through several pregnancies was 70-80 per cent, in our experience it never exceeded 5 per cent. The reason for this difference is not known and need not be discussed here. Though tumours arose comparatively rarely, however, they all terminated, if the mouse lived long enough, in carcinoma (mammary carcinoma). The question then arises: Were these cancers preceded by a precancerous state or did they arise suddenly, like the Rous sarcoma developing after tumour filtrate injection? Before the results of our own investigations are given, the literature dealing with this subject may be briefly cited.

Lathrop and Loeb first established the fact that in a strain of mice with a high incidence of breast cancer early castration prevented the development of the disease, the more so the earlier in life the animal was castrated. The effect thus took place at a time when no visible tissue changes had occurred in the breast. Cori confirmed these results. Loeb and Genther's experiments showed that the hereditary tendency to breast cancer formation resided in the cells themselves and not in an increased ovarian activity.

Gibson found certain differences in the development of nipples, milk ducts, and alveoli between a tumour-susceptible and a non-susceptible strain of mice. Gardner and Strong, on the other hand, studied the development of the mammary gland in 10 strains of mice with varying degrees of tumour susceptibility and found no single structural factor associated with cancer disposition or refractoriness. Fekete's recent investigations on the morphology of the mammary gland in a high- and a low-tumour strain showed that, while in the non-susceptible strain the cell multiplication characteristic of the first part of pregnancy ceases and secretory activity begins to show itself, in the susceptible strain certain cell groups continue to show cell division during the second half of pregnancy. At a later stage, *i.e.* in the post-lactation period, all alveoli in the non-susceptible strain regress uniformly and
cease to secrete, while in the tumour-susceptible strain small groups of alveoli may persist and continue secreting. Fekete is of opinion that the anomaly leading to cancer formation may be due to an inadequate balance, within the cells concerned, between oestrin and luteal hormone on the one hand and the anterior pituitary hormone, prolactin, on the other.

Loeb, Burns, Suntzeff and Moskop examined the mammary glands of mice from various strains after castration and administration of oestrin. Their results led these investigators to believe that hormones, such as oestrin, act like carcinogenic hydrocarbons by inducing in susceptible mice growth processes which then lead to cancer formation. These hormones, however, act only on certain tissues and not indiscriminately like hydrocarbons. Hormones stimulate without injuring, while hydrocarbons first injure. Loeb and his associates believe that the cancerous state of a cell, following the embryonal and the differentiated state, is induced by stimuli acting excessively on cells with inherent constitutional characteristics. In certain cases, they say, specific agents may induce the cancerous state directly, without the interposition of preparatory stages through which normal cells have to pass when submitted to carcinogenic irritation.

Lacassagne, in various series of experiments, was able to produce breast cancer in males of cancer-susceptible strains and, after a longer lag-period, even in males of non-susceptible strains, by treatment with oestrogenic substances. He believes that the degree of reactivity of the cells to hormone constitutes the hereditary factor and not the amount of oestrin production by the ovary. Histologically the glands of tumour-susceptible strains show permanent (non-regressing) ducts and alveoli with stagnant secretion, and this condition ends in cancer. Burrows and other workers have confirmed Lacassagne's results. Both Burrows and Lacassagne also obtained hypertrophy and metaplasia of the prostate in oestrin-treated male mice. Burrows found that organs other than sex organs did not react to oestrogenic substances.

Bonser observed very little difference in the time of onset of oestrus between tumour-susceptible and tumour-resistant strains of mice. In males large doses of oestrone produced, after forty weeks, localized but active proliferation of acini, which led to cancer in the tumour-susceptible strain. In the cancer-resistant strain there was only distension of ducts. Allen, Diddle, Strong, Burford and Gardner studied the genitals and the oestrus cycles of mice during the development of breast cancer; the cycles diminished in frequency and the genital organs became extremely atrophic.

Kreyberg showed that there is no relationship between the susceptibility of mice towards the formation of breast cancer and their readiness to develop skin cancer after tar-painting.

Suntzeff, Burns, Moskop and Loeb obtained not only an increase of cancer incidence, but a lowering of the age at which cancer occurred, by administering oestrin to mice of a high-tumour strain; such treatment also raised the tumour rate of non-breeding females to that of breeders. Fekete and Green found that duct ligation was influential in determining site and time of cancer occurrence in tumour-susceptible strains.

In a paper on hormonal pathogenesis of breast cancer, Lacassagne describes the action of oestrin on the vagina, uterus, and breasts of mice both
of tumour-susceptible and tumour-resistant strains. In all three organs there was a marked difference between the two types of strain. Lacassagne mentions several possible explanations for his findings: unequal elimination of hormones from the cells; transformation (in the susceptible strains) of oestrone into a cancer-producing substance; production of a gene mutation by oestrone (similar to the action of radiations). Heiman and Krehbiel studied the influence of endogenous and exogenous hormonal factors on breast hyperplasia and the character and growth of grafted fibroadenomatous tumours in white rats.

Bagg found that rapid breeding and prevention of nursing increased the incidence of breast cancer in low-tumour strains of mice.

The Staff of the Roscoe B. Jackson Memorial Laboratory studied the constitutional factor in the incidence of breast tumours. Cross-breeding experiments showed that it was more important for the mother to belong to a high-tumour strain than for the father, if as many tumours as possible were to be obtained. Burns could find no parallelism between any feature of the sexual cycle in female mice and the frequency of cancer occurrence. Pierson obtained benign tumours and even some infiltrative down-growth of the uterine mucosa in rabbits treated with oestrogens. Overholser and Allen obtained similar results in the monkey. Gilmour observed an increased number of skin tumours (benign and malignant) in mice painted with benzpyrene if oestrone were applied at the same time.

While Michalowsky obtained teratomata in roosters by injecting zinc chloride into the testis in the spring, when gonadal activity is at its height, Bagg was able to prolong this period until the end of summer by injecting the roosters with an alkaline extract of the anterior pituitary gland of sheep.

Cramer and Horning found that the injection of the thyrotropic hormone from the anterior pituitary lobe into mice of high-cancer strains prevented the occurrence of mammary cancer.

Several authors (Cori; Gardner, Smith, Allen and Strong; Burns, Suntzeff and Loeb; Lacassagne) who injected mice with oestrogenic hormones obtained as a by-product, independently of the results in the breasts, rapidly growing sarcomata at or near the site of injection (in the subcutis); some of these could be grafted into mice of the same strain. Lacassagne sees in these sarcomata a specific action of oestrin on the connective tissue. There is no indication that this sarcoma production has any hereditary character.

Geschickter, Lewis and Hartman investigated the presence of oestrin in tumours of man and monkeys. These authors observed an increased oestrin content in breast lesions of several types such as gynecomastia, fibroadenoma, general hypertrophy, and cystic disease. Connective-tissue tumours, such as keloids, were found to contain an excess of both the gonadotropic hormone of the anterior pituitary gland and of oestrin. Enlargement of the breast and uterus in female monkeys could be produced by oestrin injections and was found to be due to an increase of periductal connective tissue.

The main results of the foregoing investigations dealing with the relationship between mammary cancer and other tumours on the one hand, and hormones, more especially sex hormones, on the other, may be summarized as follows:

The susceptibility of certain mice to the development of mammary cancer
is due in the first instance to a hereditary character of the cells involved. According as these take up large or small quantities of the hormone (oestrin, etc.) which forms their natural stimulant, breast cancer will develop more or less frequently, there being no difference in this respect between males and females. In normal life males fail to have breast cancer simply because of the absence of oestrin, not because the male breast cells are less liable to become cancerous than the female breast cells. Males given sufficient oestrin for a sufficiently long period invariably develop breast cancer if they belong to a cancer-susceptible strain; in a cancer-resistant strain, only a few will develop cancer, and that after a much longer period. This susceptibility of the breast cells to cancer is a purely local tissue particularity. There is no convincing evidence that it bears a relationship to particularities of the sexual cycle or to other manifestations of sexual life. Cancer production in the breasts of susceptible mice depends on the activity of the breasts; thus frequent breeding and lactation bring about an increase of cancer incidence.

The work of several investigators who have tried to find a histological basis for the liability to cancer, or might we say to precancerous conditions, in susceptible mice, has not yielded very definite results. Fekete's investigations, it is true, show that in the cancer-susceptible strain certain parts of the mammary gland, or certain cells in these parts, behave somewhat differently from normal cells, tending to divide instead of developing into an actively functioning state. But these cells remain normal in appearance until, rather suddenly, they behave in a malignant fashion, infiltrating tissues, etc. There is thus in these susceptible mice no condition comparable, for instance, to the papilloma stage of skin cancer produced by painting with certain hydrocarbons.

The question of a specific or non-specific irritating action of oestrogenic compounds on other tissues, as the subcutaneous connective tissue, in which sarcoma occasionally developed at the site of oestrin injection, is not definitely solved. Apart from the oestrin, there is a vector—oil—and usually also some microbial infection. Compared with benzpyrene or methylcholanthrene, the specific action of oestrin on the connective tissue is certainly much weaker and, on the whole, doubtful.

In view of the painstaking work from many countries on the development of breast cancer in susceptible mice, it appeared improbable that new light would be thrown on this subject by experimental and histologic examination. Since, however, our mice from an American susceptible strain showed a much lower incidence of breast cancer when established in Johannesburg, it was thought worth while to examine the breasts of some of them for any possible anomaly in development, more especially for the occurrence of a precancerous condition comparable to that obtained with the aid of carcinogenic hydrocarbons.

The examination of apparently normal, i.e. non-tumour-bearing, female mice gave a completely negative result. No difference was found between the development of the mammary glands in these cancer-susceptible animals and in some mice of local origin, of unknown pedigree and with an apparent absence of tumours. Some mice of the American strain subsequently developed tumours, which usually became noticeable after a period of lactation, i.e. when the breast gland would normally regress. However meagre these
results, they are nevertheless in accord with those of the investigators quoted in detail in this chapter. No definite precancerous state could be found in this cancer-susceptible strain.

The tumours developing in the mammary gland were either of the adenocarcinoma or of the carcinoma simplex type. In some tumours the cells were very small and the tumour resembled a sarcoma or lymphosarcoma; but even in these cases some portions of the tumour contained rudimentary alveoli and thus showed the transition from the more differentiated adenocarcinoma to the more anaplastic small-cell carcinoma simplex. In no tumour was there any considerable amount of connective tissue; several tumours were partly cystic.

Conclusions

The main question put at the beginning of this paper—Does the cancerization of a normal tissue occur more or less suddenly or is it always preceded by an intermediary stage, the precancerous condition?—does not allow of a single answer.

When we are dealing with the ready-made product of cancerization, originating from the cancerous tissue of an organism of the same species, or with a tissue having such a pronounced tendency towards cancer formation that nothing more than physiological stimuli is needed to obtain malignancy, then the transitional stage is either non-existent or so brief that the expression precancerous condition appears unjustified. No resistance is exerted by the cell against malignant transformation.

If, on the other hand, we are dealing with a tissue which, though liable to become cancerous, does not, for all practical purposes, do so "spontaneously," i.e. under ordinary physiological circumstances, we require two conditions to obtain malignancy: (a) an agent capable of effecting a malignant transformation in the cell which it enters; (b) a greater or less period, the precancerous stage, during which this malignant transformation takes place. The more liable to cancerization a cell is, or the more powerfully cancer-producing a substance or agent is, the shorter and less pronounced will be the precancerous period; on the other hand, the less liable to cancerization a tissue is, or the less powerfully cancer-producing a substance or agent is, the longer will be the precancerous period. It seems justifiable to see in the precancerous condition an attempt at combating the cancer-producing factor. The adherents of the exogenous-virus theory will have no difficulty in comparing this precancerous period to the incubation period of an infectious disease, during which both attacker and defender engage in an armament race. If, on the other hand, we favour the conception of cancer as the product of an endogenous cell alteration, of which the malignant stage is the ultimate result, we will see in the precancerous stage the preparatory or transitional period, during which normal cell substance gradually becomes malignant.

None of the modes of cancerization dealt with in this paper can possibly bring a definite solution to the question of exogenous virus or endogenous transformation. Curiously enough the case in which one feels most inclined to favour the exogenous-virus theory is at the same time the one in which an "incubation" period would most readily be expected. This is the trans-
mission of the Rous sarcoma, or a similar filterable tumour, by a cell-free filtrate. If the filtrate is nothing more than a suspension of a living exogenous tumour virus, we would expect the attacked cells to put up a fight. A tumour, after all, is made up of cells which have acquired characteristics different from those previously in existence; that this transformation should occur simply at the magic touch of an extrinsic virus seems, to say the least, unexpected. If, on the other hand, the tumour filtrate consists of semi-living cell products originating from a cell of exactly the same type as that which is now to be entered, it is easier to understand that no resistance is offered and that, as soon as the agent has entered congenial conditions, it is able to multiply and to transform the cell practically at once into a tumour cell. From this point of view the theory of Gye according to which the tumour agent would consist of a combination of an extrinsic virus and an intrinsic cell constituent, seems more acceptable than the theory of an extrinsic virus pure and simple.

The experiments on cancerization of skin cells in the mouse with potent chemicals show distinctly that the mode of action of a cancer-producing agent is to effect a cell alteration, not recognizable at first, but nevertheless present and of such a character that ordinary cell activity accompanied by a certain number of cell divisions will eventually bring about definite malignant transformation. This result is compatible with either of the two cancer theories mentioned above.

If we try to apply the results of all the experiments (our own as well as those of others) described in the foregoing pages to human cancer, we seem justified in drawing a certain number of important conclusions:

The rôle of heredity in the occurrence of cancer in man has been much discussed. In the majority of cases, evidence in favour of hereditary susceptibility is rather lacking and most examples would not survive the criticism of a trained statistician. On the other hand, it must be said that it is practically impossible to trace the hereditary factor in human cancer back for an adequate number of generations. Human breeding is so haphazard that it is impossible to obtain anything like the clear evidence procured from mouse breeding. There are, nevertheless, a few selected examples of familial cancer, such as that in the family originally described by Warthin (see Hauser and Weller), as well as statistical studies of small stable populations, as in Norway, which suggest that in human cancer heredity may play an important rôle. Such predisposition may be compared with cancer susceptibility in mice. In these cases we would not expect to see much of a precancerous condition. There is every reason to believe that in a strongly predisposed tissue cancerization occurs suddenly, with little warning.

There are other cases of neoplasia in man where the hereditary factor tends to create, not a ready-made cancer, but either a condition of benign tumour formation, such as rectal and colonic papillomatosis, or a semi-inflammatory, semi-neoplastic condition such as xeroderma pigmentosum. In the former case ordinary irritation, such as fecal stasis, may transform the papillomatosis into a carcinomatous condition; in the latter exposure to sunlight may lead to the development of epithelioma.

A totally different problem in human cancerology is that of industrial cancer. Here we have the condition represented in our animal experiments
by mice and rats submitted to treatment by powerfully cancer-producing chemicals, like benzpyrene and methylcholanthrene. As suggested above, probably not one in a million mice would have skin cancer spontaneously, while as many as 100 per cent may develop this disease after treatment with the cancer-producing drug. Similarly the various forms of industrial cancer, such as the lung cancer of the radium miners in Czechoslovakia, paraffin and soot cancer, aniline cancer, x-ray cancer and others, occur in individuals who otherwise would probably not have had spontaneous cancer, at least not in the particular organ affected.

There remains for consideration the great bulk of "spontaneous" cancer in man, in which the responsible factor has not been found. It is in such cases that we are looking for precancerous conditions. Industrial cancer is practically always preceded by a well marked precancerous state, an indication of a lack of susceptibility towards spontaneous cancerization. In the ordinary "spontaneous" cancer we expect to find in the great majority of cases a more or less pronounced precancerous stage, the more pronounced the less cancer-susceptible is the particular tissue involved.

If our interpretation of the precancerous state is correct, we will naturally not expect that cancer follows in every instance. It seems probable that in innumerable cases there occur chronic conditions of a more or less precancerous nature which never lead to cancer because the organism is victorious in the fight against cancerization. Whether this means a fight against a virus eager to become active, after having laid dormant for an unknown period, or a fight against the tendency towards that particular type of cell degeneration or cell dissociation which constitutes cancer, a fight there apparently is. The result depends mainly on two factors, as in any conflict between organism and micro-organism: the resistance of the organism and the virulence of the attacking agent or factor.

If this view is correct, we should be on the alert for any condition which experience has shown to be liable to become cancerous. It seems probable that no exact definition can be given, at least in our present state of knowledge, of what constitutes a precancerous condition. It is probable that any chronic condition of irritation may occasionally lead to cancer, some lesions more easily than others; and in all cases the constitutional factor undoubtedly plays an all-important rôle. Of two people with exactly the same precancerous lesion, the same age, the same mode of living, etc., one will develop cancer and the other not. Our task then is to remove or to heal any potential precancer before cancerization sets in. But the fight against cancer may go further than that. In view of the fact that the majority of precancerous conditions will probably, for a long time to come, escape our methods of detection, we must aim at strengthening the resistance of the organism generally; if the organism can overcome an infection before it becomes chronic, there will probably be no cancer. Thus we may conclude by saying that, however unsatisfactory our methods of detecting precancerous conditions may be, we must combat the onset of precancerosis by all the methods which tend to improve the health of the organism.

At the same time we will continue to investigate separately every particular form of cancer; for it seems quite reasonable to expect that for each of them
we shall find that some particular agent is responsible, just as in those forms of industrial cancer where the responsible agent is already known.

**Summary**

1. In four series of experiments the changes preceding the actual development of cancer have been observed and the attempt has been made to discover the stage at which a cell or tissue can be called precancerous.

2. In rats and mice treated with carcinogenic chemicals (3:4-benzpyrene and methylcholanthrene) the development of intraperitoneal and subcutaneous sarcomata follows a fairly long preparatory, precancerous period. Different types of tumours are produced according to the tissue on which the carcinogens act. Many of the tumours thus obtained are transmissible to other animals of the same strain, for a limited number of generations.

3. The production of skin tumours in mice with these same cancer-producing hydrocarbons is always preceded by a precancerous state, papillomatosis. When, as a consequence of the application of the cancer-producing chemical, the cell has reached a certain stage of constitutional alteration (though this stage may not necessarily be accompanied by microscopically recognizable changes), cancerization may proceed without the aid of further specific carcinogenic action. Non-specific irritation (scalding) has at this stage the same effect as the specific action by a cancer-producing compound. On the other hand, such specific stimulation cannot be replaced, in early stages, by non-specific stimulation (scalding). No increase in the rate or frequency of cancerization is obtained by the simultaneous application of a specific and a non-specific stimulant.

4. When a filtrate of a Rous tumour is injected intramuscularly or subcutaneously or intracutaneously into a new fowl, local tumours appear which have the same appearance whatever the site of injection. The cells from which the tumour develops are the blood and tissue macrophages and fibroblasts of undifferentiated type. The Rous sarcoma is thus a malignant tumour of the reticulo-endothelial system. The transformation of a normal cell into a tumour cell under these conditions occurs suddenly, there being no precancerous state. Theories concerning the nature and transmission of the Rous sarcoma agent are discussed.

5. The development of spontaneous mammary carcinoma in mice is described and its dependence on hormone administration (natural or artificial) and on a hereditary factor is discussed. There is no evidence of a clearly defined precancerous state in the development of these tumours.

6. The relation between the animal tumours described here and human cancer is discussed, and the significance of precancerous conditions in the development and early treatment of cancer is considered. The importance of treating and, if possible, preventing the establishment of precancerous conditions is stressed.

**Bibliography**

The bibliography is arranged in accordance with the four sections into which this paper is divided.
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