HEREDITARY AND ENVIRONMENTAL FACTORS IN THE ORIGIN OF DIFFERENT CANCERS[1]

By N. DOBROVOLSKAIA-ZAVADSKAIA

Institute of Radium, University of Paris

(With Seven Text-figures)

The enormous efforts made by geneticists in the investigation of hereditary transmission of cancer susceptibility in mice have resulted in the accumulation of a substantial body of evidence in favour of such a transmission.

It is a well-established fact that there are strains in which mammary cancer appears in a high proportion of females, and there are others in which this malignancy type is more or less completely lacking. This difference can hardly be accounted for by environmental conditions which, for mice colonies, are in general similar for all strains. But frequently the behaviour of a strain, as regards cancer, is determined by the origin of this strain from a cancerous or non-cancerous mother. This may be illustrated by the following observation.

A pregnant cancerous female was brought to the laboratory a few years ago by a dealer who complained that his mice stock had been devastated by frequent occurrences of tumours. This female gave birth to three young of which one male and one female attained adult age. Being crossed brother to sister, these two animals bred many times and died without cancer at an advanced age, the male when 16 months old and the female at the age of 31 months. The progeny of this couple (strain XLIV) recorded here consists of 75 males and 88 females, of which only two females developed a mammary cancer (2·3 %).

This result was disappointing, as we expected to establish a high cancer strain. However, it may be easily explained by the fact that the real female ancestor of this strain was not the cancerous female brought pregnant to the laboratory, but her daughter which was born from an unknown father, and which died at advanced age without cancer.

The incidence of mammary cancers in various strains is very different; for example, in our mice colony, it fluctuates, according to the strain, from 0 to 75 %. In strains submitted to a prolonged inbreeding (dilute-brown strain of Dr Little, "A" strain of Dr Strong, etc.), this incidence is

[1] This paper was presented to the Seventh International Congress of Genetics, August 1939, Edinburgh.

considered as more or less constant. We tried to verify this constancy in our high cancer strain R III.

If the incidence of mammary cancers does not change with the passage of time, any sample of animals born in a certain period should present the same percentage of cancers, as does the total number of animals belonging to this strain. According to this, we divided the 8-year period of observation of strain R III into six separate periods and the percentage rate of mammary cancers was calculated for each period. Only those females (465 ♀♀ in all) which survived at least 5 months were taken into consideration. We plotted the time periods as abscissae and the percentage rate of tumours as ordinates. The graphic representation of the results obtained shows a curve with two peaks (periods I and V) and

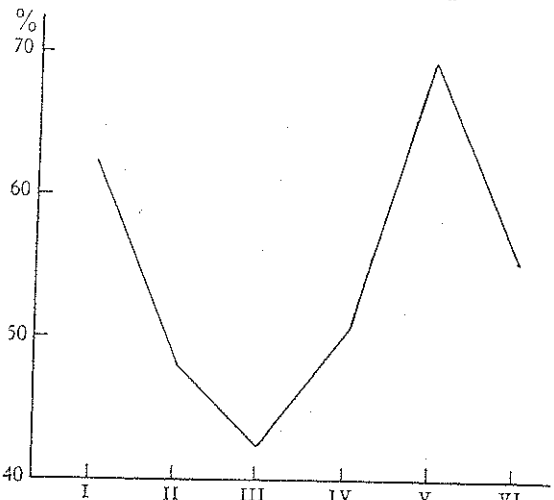

Fig. 1. Strain R III, variation of the percentage rate of spontaneous tumours (mammary adenocarcinoma) during 8 years of observation divided in six periods (abscissae).

fluctuation of the percentage rate of tumours from 42·3 (period III) to 69 % (period V). The difference between two extremes is 26·7 %, which is significant and must be taken into account (see Fig. 1).

The average percentage rate of mammary cancers in strain R III is 53·5, which approaches the simple Mendelian proportion (1 : 1) for a backcross. Such a high proportion of malignancy (in a few extinct strains it even reached 75 %) was obtained only for mammary adeno-carcinoma.

The proportions of spontaneous sarcomas and squamous cell epitheliomas which occurred in our stock were too small to be considered as depending on a simple Mendelian mechanism. However, the hereditary background of these histological forms cannot be excluded. We had a

strain (strain IV) derived from a female which died of a molluscoid tumour of Borrel-Haaland. Her son, the only male parent of the whole second generation, died of sarcoma. In this strain, there were observed five sarcomas (4·1 %) and two squamous cell epitheliomas (1·7 %) in 121 males and seventeen sarcomas (14·3 %) and four squamous cell epitheliomas (3·4 %) in 119 females, i.e. many more than in any other of our strains, but not enough for monofactorial heredity. We did not succeed in perpetuating this condition in subsequent generations.

Maud Slye also mentioned (1931, 1937) strains with high incidence of sarcoma, namely, 28·5 % in one strain and 32·9 % in another one.

Lymphadenoma, a kind of pseudoleukemia, gave as the highest proportions 22·4 % of malignancy in males and 18·4 % in females. But this was only in strain III which does not exist any more. These proportions are lower than those obtained for similar neoplastic conditions by Mercier (1937) (50–70 %) and MacDowell (1937) (90 %).

Consequently, only *the mammary cancer* has given us a sufficient proportion of tumours to serve as a test for experimentation upon the *relative part of hereditary and environmental factors in the pathogenesis of various cancers.*

The environmental influences were studied on *five non-cancerous strains*, that is to say, strains free from spontaneous mammary cancer, and on many cancerous strains, especially on our high cancer strain R III. Tar, 1 : 2 : 5 : 6-dibenzanthracene, and radon, used as extraneous pathogenic agents, answered our purpose most satisfactorily (Dobrovolskaia-Zavadskaia & Adamova, 1938, 1939).

Out of a total of 1547 animals (♂ 772 and ♀ 775) belonging to *non-cancerous strains*, 692 were kept as controls, 103 males were treated with tar, 445 animals (♂ 187 and ♀ 258) with dibenzanthracene, 198 (♂ 92 and ♀ 106) with radon and 111 (♂ 82 and ♀ 29) we infested with *Sp. morsus muris*. Sixty of the infested animals also had a tube of radon introduced under the skin of the left groin.

The following percentage rates of tumours were obtained: 59·2 % (+10 % of precancerous lesions) with tar, 19·3 % in males and 26 % in females with dibenzanthracene; 8·8 % in males and 5·7 % in females with radon. Among the infested animals, only one developed a sarcoma surrounding the tube of radon, and seven animals died with more or less pronounced lymphatic hyperplasia (7·2 % on the whole).

In respect to histological types, the local reaction was nearly the same in all these strains, namely, squamous cell epithelioma, very rarely a sarcoma, with tar; sarcomas of various structure, very rarely a squamous

cell epithelioma, with dibenzanthracene; sarcoma and squamous cell epithelioma with radon, and practically *no mammary adenocarcinoma* at all.

Two of these strains, XVII *nc* and XXXIX (our branch of Dr Strong's C.B.A. strain) were used by Dr Lacassagne for folliculine injections. Although the folliculine proved to be a very powerful stimulator for cancerization of the mammary gland, not one case of mammary cancer was obtained either in males, or in females.

Tar, 1 : 2 : 5 : 6-dibenzanthracene, and radon were also used in the animals belonging to *our cancerous strains*. These animals reacted in similar way to the animals of non-cancerous strains, with the exception that in females there appeared, in addition to sarcomas and squamous cell epitheliomas, some mammary cancers on the point of carcinogenic application, and much more frequently all over the body.

In order to investigate whether these local mammary cancers were of genetical or environmental origin, we proceeded to a detailed study of the topographical distribution of tumours in females of strain R III (Dobrovolskaia-Zavadskaia & Adamova, 1939). In 465 control females there were 249 cancerous animals with a total of 384 tumours. These tumours were scattered all over the body of the animals; we determined their percentage in different anatomical areas. Two of such areas were concerned in our experiments: (1) in experiments with dibenzanthracene, the left axilla and the surrounding parts of the scapular girdle, we found there 17·7 % of all the tumours and (2) in experiments with radon, the left groin and the surrounding parts of the pelvic girdle, 15·9 % of tumours were located in this area.

Out of sixty-two mammary cancers developed by the females treated with dibenzanthracene, only ten (16·1 %) were located at the point of carcinogenic application. Out of fifty-seven mammary cancers obtained in females treated with radon, only four (7 %) were located near the radon tube at the left pelvic girdle. The number of mammary cancers then was not increased at the points of carcinogenic application. It was slightly diminished with dibenzanthracene, 16·1 % instead of 17·7 % in control animals, and it presented with radon less than a half (7 % instead of 15·9 %) of what was determined for non-treated animals.

Consequently, the environmental factors proved to be unable to produce any glandular cancer not only in non-cancerous strains, but also in a high cancer strain R III. *Therefore, mammary cancers which appeared in treated areas were not induced but were spontaneous tumours.* They have arisen in these areas according to the genetical predisposition of their carriers.

The existence of such local predisposition is also evidenced by the fact that the great majority of mammary tumours (84 % in the dibenzanthracene series and 93 % in the radon series) were developed not on the point of environmental carcinogenic activity, but in their habitual locations (see Figs. 2, 5).

The mammary cancers developed on the point of carcinogenic application very often presented a tendency to squamous cell metaplasia of glandular epithelium and to sarcomatization of the stroma. It may be

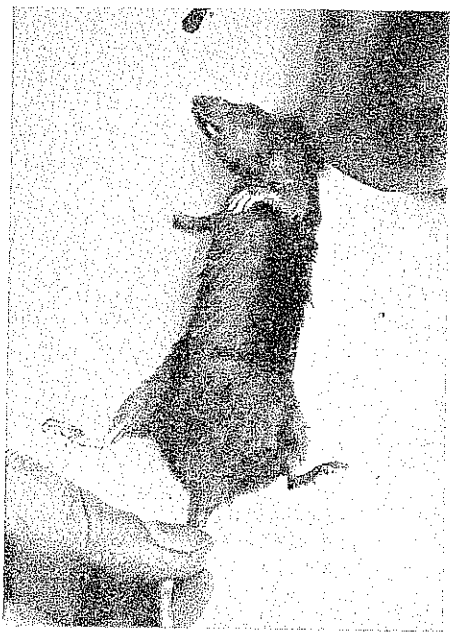

Fig. 2. ♀ 38010 XL treated with dibenzanthracene. No tumours at the point of injection, left axilla. Spontaneous mammary cancer in the left groin.

supposed that missing mammary tumours in the radon series were also replaced by sarcomas or squamous cell epitheliomas. This shows that the two latter types of malignancy may be actually produced by environmental factors. As a matter of fact, none of the tested strains proved to be completely resistant against external carcinogenic influences, and many sarcomas and squamous cell epitheliomas were obtained in cancerous as well as in non-cancerous strains.

This brings us back to the once expressed hypothesis (Dobrovolskaia-Zavadskaia, 1933) that there exists a factor of general neoplastic pre-

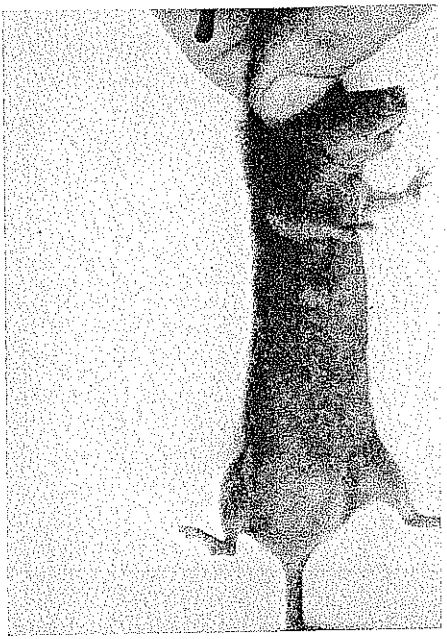

Fig. 3. ♀ 38909 XL. Nothing at the point of injection of dibenzanthracene, spontaneous mammary gland tumour in the right groin.

Fig. 4. ♀ 25108 R III, treated with radon, left groin. No tumour on the scar from radon tube, mammary adenocarcinoma of the nape.

disposition, we called it *no* (neoplasm). It seems to be widely spread in mice, and is perhaps responsible for the frequently observed appearance of cancerous animals in the first hybrid generation after crossing representatives of cancer and non-cancer strains. The assumption that spontaneous mammary cancer is dominant is opposed by the fact that it may be transmitted by non-cancerous animals.

The general factor of malignancy *no* is probably present in all non-cancerous strains, only it does not operate for want of a modifier for

Fig. 5. ♀ 38735 XL treated with radon, left groin. No tumour at the point of radon tube, mammary gland cancer in the right axilla.

"tissular" or organic localization. This modifier may be dominant since it operates in the above-mentioned hybrids in one dose. Such a mechanism may be operating for tumours in *Nicotiana* hybrids (Kostoff, 1930).

The modifier for mammary cancer seems to be closely linked with *no* and thus determines the monohybrid attitude of this cancer. The proportions which we obtained in the above-mentioned strain IV for sarcomas and squamous cell epitheliomas resulted rather from a free segregation.

The experiments with carcinogenic environmental factors have

yielded evidence in favour of the possibility of replacing this morphogenic modifier by an external agent. The comparative data obtained in non-cancerous and cancerous strains suggested that external agents may be operating so only for sarcomas and squamous cell epitheliomas but not for the mammary gland cancer. Further investigations are needed to elucidate whether or not the other glandular cancers behave in the same way.

Previous observations (Dobrovolskaia-Zavadskaia, 1934) of the hereditary transmission of mammary cancer at a specially rare location in our strains, i.e. the nape of the neck, brought us to the hypothesis of localizing modifiers. The existence of such modifiers did find its confirmation *in the extreme perseverance with which mammary cancers have kept on appearing at their usual sites outside of the zone of carcinogenic application.* On the other hand, sarcomas and squamous cell epitheliomas, at least as induced tumours, appeared only at the points of carcinogenic irritation.

The full genetic verification of the suggested interpretation is extremely difficult. The genetic constitution in the matter of cancer becomes in general clear only in advanced age when the animal is in most cases no longer capable of breeding. Still, we had a chance at genetic verification of a sarcoma developed on the point of a chronic inflammatory irritation, a circumscribed peritonitis, in a female two years old (Fig. 6). This female had nine generations without cancer before her, and she left numerous progeny (seventy-five offspring) after crosses with her brother, her son and her grandson. This mouse was the only female ancestor of our selected strain XVII *nc*, and not one case of spontaneous sarcoma was ever found in non-treated animals of this strain. This observation confirms our hypothesis that an environmental carcinogenic factor may play the part of the morphogenic modifier in the origin of a sarcoma.

The manifestation of the factor *no* in the strain XVII *nc* may be seen in many sarcomas and squamous cell epitheliomas provoked by carcinogenic agents. The genic background of this manifestation has been betrayed by the disclosure in treated animals of many resistant individuals which lived sometimes longer than those developing tumours and which died without cancer.

We come thus to the general conclusion that *heredity controls the pathogenesis of mammary cancer in mice whereas the environment dominates the origin of sarcomas and squamous cell epitheliomas.* The latter part of this conclusion opens the way to some optimistic prospects of preventing, at least some cancers, by an adequate change of environmental conditions. But what can be done with hereditary types of malignancy?

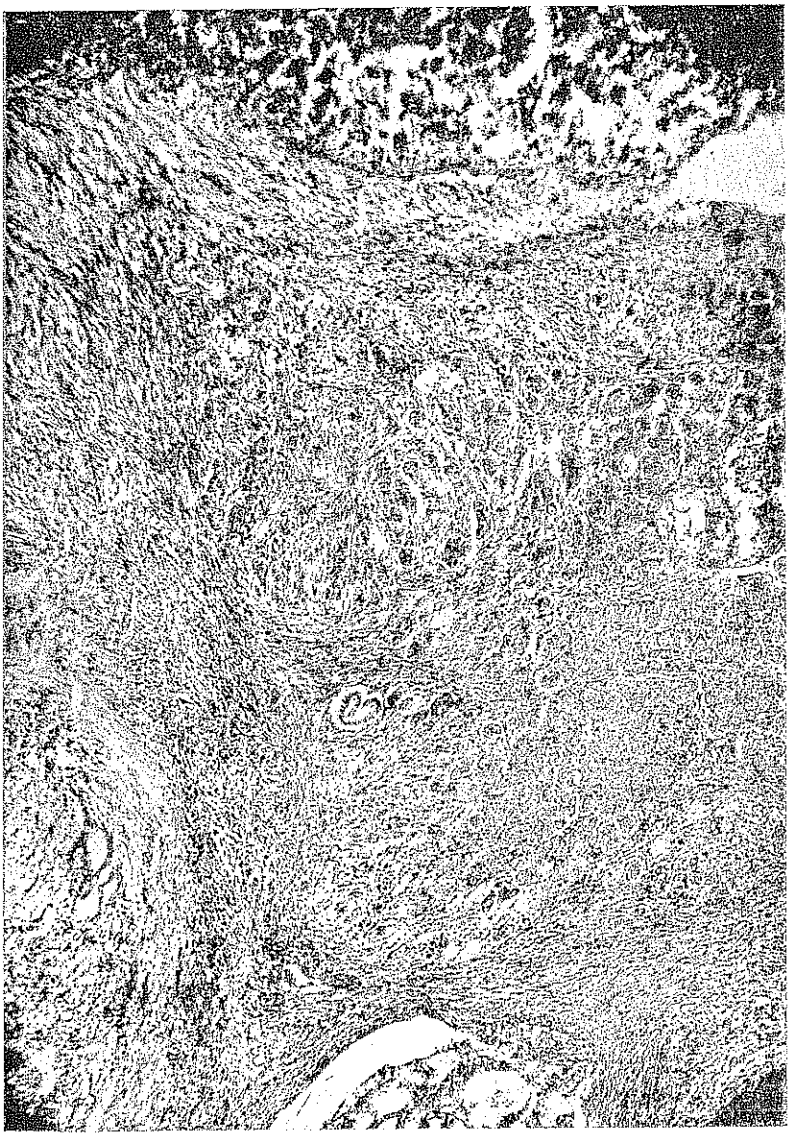

Fig. 6. ♀ 33977 XVII *nc.* Sarcoma developed at the point of a chronic intraperitoneal inflammation. The tumour involves the spleen (upper part) and the uterine tromp (lower part) and infiltrates the pancreas (in the middle of the preparation).

The animals descending from the strain R III were used by Lacassagne (1932) and by Cramer & Horning (1937) for the experiments with oestrogenic substances. A very surprising feature is that these experiments have given practically 100 % of mammary cancers both in males and in females. However, as we mentioned before, the same treatment failed to produce any such cancer in animals of non-cancerous strains XVII *nc* and XXXIX (Lacassagne, 1938). This statement emphasizes the importance of constitutional factors in the origin of mammary cancer. At the same time, it makes conclusive that all animals of the strain R III are susceptible to this cancer, that is to say that the strain R III is obviously homozygous for cancer. Why then do not all animals living long enough develop it spontaneously?

The underlying mechanism by which genic control is brought about is as yet unknown. The term "inhibition" has been used in the mendelian literature to designate the general situation in which one genetic factor prevents another non-allelomorphic factor from showing its effects. This inhibition, as stated in *Drosophila*, may be due to the presence of a sectional duplication carrying the wild type allelomorph of the gene "suppressed" the term now in use; or to a gene "specific suppressor" which gives a positive reversal of the effect of another non-allelomorphic gene, so that the double mutant type appears like the unmutated wild type (Bridges, 1919, 1923, 1932; Stern, 1929).

In the case of cancer, it is extremely difficult to establish a sufficient basis of facts for an analysis of such "normal overlaps" in terms of genes. Let us dwell a little on what is called "internal environment", by which we mean "genic environment" (Lebedeff, 1935) on the one hand, and "somatic environment" on the other.

The genic environment is certainly extremely various in our strains. Side by side with traits which seem to have nothing to do with cancer—coat colour, taillessness, waltzing, etc.—there are some pathological conditions, other than cancer, which are frequently encountered in some strains, and are practically lacking in others. To illustrate this, we can refer to a modification of the reticular zone of the adrenal gland described by Cramer & Horning (1937) under the name of "brown degeneration".

This degeneration is extremely frequent in strain R III (Fig. 7), nearly 100 % of the cancerous females develop it, and it is practically lacking in five non-cancerous strains. However, this degeneration is not absolutely necessary for cancer development. We have a cancerous strain (strain XIX) in which about 90 % of the cancerous females proved to be free of it (Dobrovolskaia-Zavadskaia & Pezzini, 1939). The kidney also pre-

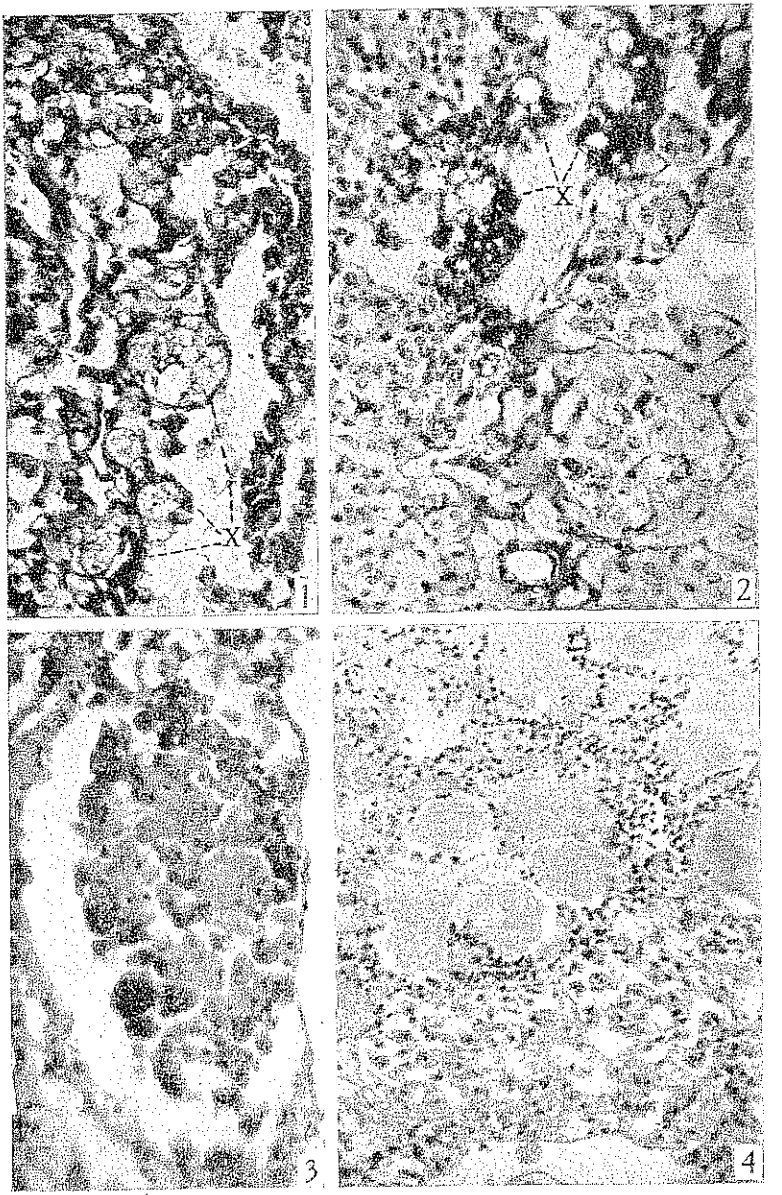

Fig. 7. Different aspects of degeneration in the reticular zone of adrenal glands: (1) vacuolated blocks (*X*), (2) vacuolated and pigmented blocks and isolated cells (*X*), (3) "brown degeneration", cells with coagulated cytoplasm and pycnotic nucleis in the way of fusion, (4) completely degenerated blocks, appeal of leucocytes.

sented some constitutional traits—hyalinization, cyst formation—peculiar only to some strains and completely absent in others. Lesions of the liver and of the spleen are frequent in cancerous mice, and it is possible that some of these lesions, and especially functional peculiarities of endocrine glands, also depend on hereditary constitution. We know very little about all this. However, the above-mentioned observations show that the genic environment of the cancer gene is different in different individuals.

The somatic aspect of the internal environment has become evident from the experiments with oestrogenic substances. Folliculine injections started at an early age change the medium of the growing organism in such a way that every animal, male as well as female, genetically susceptible to mammary cancer, actually develops it. Thus, normal overlaps practically disappear.

There is evidence now at hand, i.e. Bittner's experiments with milk, which if proved will open a possibility of changing the somatic environment in the opposite sense. An offspring hereditarily predisposed to cancer may perhaps be transformed in a normal overlap by substituting, immediately after birth, the cancer-susceptible mother by a cancer-resistant foster-mother. Will it not be the way to prevent at least cancer of the breast?

Summary

1. Animals belonging to eighteen strains of established genetic constitution were used for investigation of environmental influences as represented by tar, 1 : 2 : 5 : 6-dibenzanthracene, and radon.

2. Not one strain proved to be completely resistant, but resistant individuals were observed in all strains.

3. Sarcomas and squamous cell epitheliomas appeared as local reactions in animals of all strains. This is an evidence in favour of environmental factors as a provocative cause of these tumours.

4. Mammary cancers occurred at the points of carcinogenic application in animals of cancerous strains. These tumours, according to the proportions in which they occurred, were not induced but were spontaneous tumours.

5. Many more mammary cancers appeared in animals of cancerous strains outside of the zone of carcinogenic application, i.e. in their habitual locations.

6. The importance of the hereditary factor in the origin of mammary gland cancer in mice was thus confirmed. In what degree is it true for

other glandular cancers may be elucidated by a similar investigation on adequate strains.

7. As regards sarcoma and squamous cell epithelioma, two kinds of pathogenesis may be distinguished: (*a*) one hereditary, as illustrated by an increased occurrence of sarcomas in strain IV the male ancestor of which died of a sarcoma, and (*b*) one environmental, as stated by a frequent appearance of sarcomas and squamous cell epitheliomas in treated areas in animals of all strains. A sarcoma surrounding an inflammatory focus was verified genetically and proved to be not hereditary.

8. Not all susceptible animals actually develop cancer. These "normal overlaps" practically disappear in susceptible animals treated with some oestrogenic substances; this may be explained by a change of "internal environment".

9. The early fostering of a cancer susceptible offspring by a cancer resistant foster-mother seems to be able to change this "internal environment" in the opposite sense, i.e. to transform such an offspring in a "normal overlap". This statement if confirmed may open a way of preventing mammary cancer and perhaps some other pathological conditions.

This work was carried out with the aid of grants from the International Cancer Research Foundation, President Mr William H. Donner.

REFERENCES

BITTNER, J. (1929–30). "The experimental determination of an invisible mutation." *Mich. Acad. Sci. Arts Lett.* **11**, 349–51.

—— (1939). "Breast cancer in mice." *Amer. J. Cancer*, **36**, 44–50.

BRIDGES, C. B. (1919*a*). "Duplication." *Anat. Rec.* **15**, 357.

—— (1919*b*). "Vermilion deficiency." *J. gen. Physiol.* **1**, 645–56.

—— (1923). "The translocation of a section of chromosome II upon chromosome III in *Drosophila*." *Anat. Rec.* **24**, 426.

—— (1932). "The suppressors of purple." *Z. indukt. Abstamm.- u. VererbLehre*, **60**, 207–18.

CRAMER, W. & HORNING, E. S. (1937). "Adrenal changes associated with oestrin administration and mammary cancer." *J. Path. Bact.* **44**, 633–42.

DOBROVOLSKAIA-ZAVADSKAIA, N. (1933). "Heredity of cancer susceptibility in mice." *J. Genet.* **27**, 181–98.

—— (1934). "Über den Erblichkeitsfactor bei der Entstehung des Krebses." *Mschr. Krebsbekämpf.* Heft 6, 161–8.

—— (1936). "Facteur constitutionel (héréditaire) dans certains maladies des reins." *III Internat. Cong. compar. Pathol. Athènes*, **2ª**, 241–47.

DOBROVOLSKAIA-ZAVADSKAIA, N. & ADAMOVA, N. (1938). "Réaction, à différents agents cancérigènes, de souris appartenant à des lignées exemptes d'adénocarcinome de la mamelle." *Bull. Cancer*, **27**, pp. 308–41.

—— —— (1939). "Réaction, à différents agents cancérigènes, de souris appartenant à la même lignée cancéreuse (lignée R III)." *Bull. Cancer*, **28**, 76–106.

DOBROVOLSKAIA-ZAVADSKAIA, N. & PEZZINI, Z. M. (1939). "Dégénérescence des capsules surrénales chez les souris de différentes lignées cancéreuses." *C.R. Soc. Biol., Paris*, **131**, 240–3.

KOSTOFF, D. (1930). "Tumours and other malformations on certain Nicotiana hybrids." *Zbl. Bakt.* **81**, 244–60.

LACASSAGNE, A. (1932). "Apparition de cancers de la mamelle chez la souris mâle, soumise à des injections de folliculine." *C.R. Acad. Sci., Paris*, **195**, 630–2.

—— (1938). "Statistique des différents cancers constatés dans des lignées sélectionnées de souris, après action prolongée d'hormones oestrogènes." *Bull. Cancer*, 27, 96–116.

LEBEDEFF, G. A. (1935). "Further studies on factor interaction in *Drosophila virilis*." *Genetics*, **20**, 223–9.

MACDOWELL, E. C. (1937). "Genetics of mouse leukemia." *Occ. publ. Amer. Ass. Adv. Sci.* no. 4, pp. 42–4.

MERCIER, L. (1937). "Hérédité du lymphosarcome de la souris dans les croisements d'hétérozygotes pour le couple de facteurs cancer-non cancer." *C.R. Soc. Biol., Paris*, **124**, 403–4.

MERCIER, L. & GOSSELIN, L. (1936). "Essais en vue de retarder l'apparition du cancer (lymphosarcome) dans une lignée de souris." *C.R. Soc. Biol., Paris*, **121**, 125–6.

SCHULTZ, J. (1932). "The behaviour of vermilion-suppressor in mosaic." *Proc. nat. Acad. Sci., Wash.*, **18**, 485–6.

SCHULTZ, J. & BRIDGES, C. B. (1932). "Methods for distinguishing between duplication and specific suppressors." *Amer. Nat.* **66**, 323–34.

SLYE, M. (1931). "The relation of heredity to the occurrence of spontaneous leukemia, pseudoleukemia, lymphosarcoma and allied diseases in mice." *Amer. J. Cancer*, **15**, 1361–86.

—— (1937). "Heredity in the occurrence of cancer." *IIe Congrès Intern. de la lutte contre le cancer, Bruxelles*, 1936, **2**, 128–33.

STERN, C. (1929). "Über die additive Wirkung multipler Allele." *Biol. Zentr.* **49**, 261–290.