The following is a review of the studies carried out in this laboratory during the past seven or eight years with the view of (a) elucidating the effect of certain exogenous tumor-producing chemical agents and (b) ascertaining the presence within the human body of chemical substances that may call forth the formation of tumors. The present review is based in the main on the results obtained by the author and his associates; data from the literature will be only occasionally mentioned.

I. EXOGENOUS BLASTOMATOGONIC AGENTS

Virchow's conception of "irritation" as the cause of tumors has led to a long series of studies whose aim was to produce cancer experimentally through the agency of diverse stimuli. Observations on some kinds of occupational cancer in man, in particular on the skin cancer of chimney sweeps, pointed to the distillation products of coal, and an important advance was Yamagiwa and Itchikawa's discovery that cancer can be produced in laboratory animals by painting their skin with tar. The problem of tar cancer was taken up by numerous students so that we are now in a position to produce cancer systematically in a great number of cases; to study thoroughly its morphology and morphogenesis; to ascertain the presence of regularly appearing precancerous changes; and finally to raise a number of questions concerning pathogenesis and etiology.

At first the appearance of the tumor on the painted skin was regarded as conclusive evidence in support of Virchow's conception, as the growth was attributed to the "irritating" toxic action of tar, which leads to chronic inflammation, injury of the tissues, and their constant pathological regeneration. However, comparison of the carcinogenic action of different tars and their fractions with their chemical composition suggested that the tumor-producing capacity is appropriate not to the tar as a whole, but only to certain ingredients. According to Leitch (1922) coal tar may contain a "specific" cancer incitant in the form of definite chemical substances, and the long series of investigations by Twort, Bloch, Kennaway, and others contributed to an elucidation of the problem. Thus there arose the concept of carcinogenic substances, which at first was a mere hypothesis but through the discoveries of Kennaway, Hieger, Mayneord, Cook, and others later became an established fact. A new trend of study was inaugurated by the publication of the first data on cancer produced by means of 1,2,5,6-dibenzanthracene and other chemically pure substances, and a number of important facts have been brought forward within the past decade. Although these studies are a mere continuation of those dealing with tar, they have solved a number of problems in a way different from that which had been possible until then. It is only natural, therefore, that our investigation of carcinogenic tars, pursued during a number of years, should have culminated in a study of pure carcinogenic agents.

SUBSTANCES TESTED. MODE OF ADMINISTRATION.

SOLVENTS

The following substances were used: (a) 1,2,5,6-dibenzanthracene, (b) 9,10-dimethyl-1,2-benzanthracene, (c) 3-methyl-10-ethyl-1,2-benzanthracene, (d) 3,4-benzpyrene, (e) 3,4,8,9-dibenzpyrene, (f) o-aminazotoluene, and (g) p-dimethylaminoazobenzene, and others. All these were synthesized in the U. S. S. R., and some in this laboratory. A number of other compounds tested proved inactive. For skin applications benzene was generally used as solvent, and for subcutaneous administration olive, sunflower, or castor oil. Some experiments, however, showed that skin cancer may be obtained by painting the skin with the carcinogenic agent dissolved, not in benzene, but in sunflower oil. Again subcutaneous administration of the compound in crystalline form, without any solvent, likewise caused tumor formation. Apart from subcutaneous and intraperitoneal injection and skin painting, the carcinogens were sometimes administered with food or introduced as vaginal plugs (Klenitzky). Most of the experiments were carried out on mice, but in some rats, rabbits, or guinea pigs were employed. In the last named, large doses of 1,2,5,6-dibenzanthracene (25 to 48 mgm.) produced
malignant growths after 19 to 22 months (Shabad), a confirmation of Haagensen and Krebbiel, who reported the induction of sarcoma in guinea pigs with 3,4-benzpyrene.

**Malignant Tumors at the Site of Application**

These tumors are distinguished by their multiformality. Squamous cell keratinizing carcinoma is the most common in mice after skin painting, but other types of cancer occur as well, such as squamous cell cancer without keratinization, spindle-cell carcinoma resembling sarcoma, and cancer originating from the sebaceous glands. Along with epithelial tumors, painting of the skin may incidentally result in the formation of sarcoma, presumably because the carcinogen penetrates through abrasions of the skin into the subcutaneous tissue.

At the site of subcutaneous administration there usually develop sarcomas, which may vary widely in structure. Besides the spindle cell type there occur polymorphous cell sarcomas and, somewhat less frequently, fibrosarcomas. As to their origin, some are derived from muscle: the malignant leiomyomas and the less common rhabdomyomas. The source of the latter is indicated by their pronounced polymorphism, their numerous large cells, and by traces of striation (Kleinenberg). In some cases the sarcomas of mice have been particularly rich in thin-walled, dilated vessels, which imparted to them the appearance of angiosarcoma. In the guinea pig a peculiar sarcoma of adipose tissue was discovered, i.e., a malignant lipoblastoma, which metastasized to the lungs and eyes. Finally, in one case the subcutaneous administration of 9-methyl-10-ethyl-1,2-benzanthracene resulted in the formation of a malignant neurinoma.

Application of tar and dibenzpyrene plugs to the uterine cervix produced squamous cell carcinoma, either with keratinization or not.

Particular attention is directed to the fact that in a number of cases malignant tumors formed not directly at the site of administration, but close to it. Thus, after the subcutaneous injection of carcinogens a papilloma (Shabad) and a squamous cell carcinoma (Kleinenberg) appeared close to the injection sites, and the subcutaneous administration of 9,10-dimethyl-1,2-benzanthracene called forth, besides sarcoma, a mammary cancer. These were separate tumors, but in some cases such growths may fuse and present the picture of so-called carcinosarcoma.

Azo compounds elicited hepatomas in different stages of malignancy, and much less frequently tumors from the epithelium of the bile ducts, the so-called cholangiomias. It should be noted that multiple primary hepatomas occurred paralleling the great number of tumors produced in the skin and subcutaneous tissues by hydrocarbons. Azo compounds often caused peculiar proliferations of the stromal cells of the liver, producing infiltrates after the type of leukosis (Morozenskaya).

Thus carcinogenic compounds induce tumors not only at the site of application but also at a distance from it, and not only malignant tumors but most often benign tumors as well.

**Transplantation of Induced Tumors**

This was carried out in a number of cases, and five new sarcoma strains with one of liver cancer were carried through many generations. Microscopic examination of these growths showed that transplantation had not modified their general structure; even under the skin the liver cancer still retained certain features in the 67th generation characteristic of primary liver tumors (Morozenskaya).

**Remote Tumors**

Tar experiments have already shown that besides the tumors at the site of application a great number of different structures arise in remote regions. The possibility of producing remote neoplasms has been confirmed in this laboratory by numerous experiments with chemically pure blastomagenic substances. Thus in mice treated subcutaneously, intraperitoneally, per vaginam, or by skin painting, multiple primary skin tumors were often noted in nontreated regions, which frequently originated from the sebaceous glands; as well as many primary adenomas of the lung and, somewhat less frequently, tumors of the mammary glands, cancer of the buccal mucosa, and lymphosarcoma of the thymus. A critical examination of these facts suggests the following doubts:

Are these remote tumors spontaneous? It is well known that tumors of the lung and mamma may spontaneously occur in mice and, although much less frequently, sebaceous adenoma and lymphosarcoma of the thymus. This objection can be refuted, however, since our mice belonged to a strain (RV) that has been under observation for about 14 years, during which the frequency of spontaneous growths has been an object of special study. Primary tumors of the lungs, for instance, occur in 5.2 per cent of cases, or in 8.5 per cent of mice above 8 months old; cancer of the mammary glands in 1.2 to 2.0 per cent of the females, and adenoma of the sebaceous glands only in isolated instances (Shabad and Kleinenberg). After the administration of blastomagenic agents lung tumors were recorded in 22.5 to 36.2 per cent (application of 1,2,5,6-dibenzanthracene) or in 30 to 50 per cent (injection of 3,4,8,9-dibenzpyrene), etc.; i.e., in a number of cases that is far beyond the possibility of spontaneous occurrence.
If remote tumors are called forth by a carcinogen, does their localization depend on its direct penetration: into the lungs by the inhalation of particles adhering to the skin; into the mammary glands through their ducts; into distant skin areas by licking, scratching, etc.? The administration of blastomatogenic substances per vaginam showed that although in this case the dissemination of the substance to the skin was excluded, many remote tumors developed nevertheless in the skin. However, a decisive answer was obtained by injecting blastomatogenic substances under the skin and into the peritoneal cavity. In this case also there occurred cancer of the mamma, adenoma of the lungs, and sebaceous adenoma. For example, adenoma of the lungs followed the subcutaneous administration of 1,2,5,6-dibenzanthracene in 27 per cent of cases, or in 39 per cent of mice surviving 3 months after the beginning of the experiment.

Finally, comparison of the occurrence of remote tumors with the strength of the blastomatogenic substance, as estimated by the number of tumors formed at the application site, conclusively showed that the remote tumors are likewise produced by the agent. Thus with a similar mode of treatment and equal amounts of administered substance, 3,4,8,9-dibenzanthracene caused a greater number of lung tumors than 1,2,5,6-dibenzanthracene, while the still more effective compound, 9,10-dimethyl-1,2-benzanthracene, produced adenoma of the lungs in 80 per cent of mice within 8 months after the beginning of the experiment (Kleinenberg). Again, upon the administration of the same substance in different doses the number of remote tumors increased with increase in the dose (Shabad and his associates).

Accordingly there is no doubt that the agents producing tumors at the site of application may also elicit neoplasms distant from it, in particular in some internal organs. Hence, one can judge the blastomatogenic properties of a compound also by the appearance of remote tumors, in particular, lung adenoma. Their occurrence may indicate, also, that blastomatogenic agents exert a general action upon the organism within which they circulate. Circulation of these compounds, conclusively demonstrated by the fluorescence method, was suggested by the observations on remote tumors. Finally, the appearance of these neoplasms makes it very probable that blastomatogenic substances may become localized beyond the site of their administration.

Systematic study of the blastomatogenic action of azo compounds (Morozenskaya) also showed that with different modes of administration a number of remote tumors occurred in addition to the hepatic neoplasms: lung adenoma (in 28.7 per cent of mice surviving 8 months), adenoma of the sebaceous glands, or mammary cancer. These facts, confirmed by Andervont, suggest that in spite of the specificity of azo compounds, which to a certain degree are organotropic with regard to the liver, one may find some common features in the blastomatogenic action of azo compounds and the carcinogenic hydrocarbons.

The appearance of remote tumors in connection with diverse blastomatogenic substances raises the problem of localization. Despite circulation of the blastomatogenic substances, the tumors occur only at definite, so to say favorite, places. This is presumably to be accounted for in part by the circulation paths of these substances within the organism. Thus the skin as well as the mammary glands, the sebaceous glands, and the lungs may be regarded as destinations for fats and lipoids, which may act as solvents of the blastomatogenic compounds. On the other hand, a certain significance is to be attached to the structure of the substance as well. This may well be exemplified by o-aminoazotoluene: Administration of this substance by mouth as well as its subcutaneous injection and application to the skin in benzene solution, invariably resulted in the appearance of liver tumors; whereas at the site of its administration, e.g., in the painted skin, not only tumors but even initial changes were altogether absent (Morozenskaya).

Local Action of Blastomatogenic Compounds and the Role of Inflammation

Systematic inspection of the experimental animals and microscopic examination of the site of administration of the blastomatogenic substance, and of the tumors caused by it, showed that the severe acute and subsequent chronic inflammation so common with carcinogenic tars is absent. Indeed, the inflammatory changes in skin painted with 1,2,5,6-dibenzanthracene are no greater, but rather less pronounced, than with pure benzene (Shabad).

Subcutaneous administration of dibenzanthracene in vegetable oil results immediately in an acute inflammatory reaction, which, however, is not more active than that set up by the solvent alone. Several days thereafter the inflammatory reaction subsides and encapsulation of the droplet ensues, followed by partial resorption of the oil. The "oleoma" is gradually transformed into an "oleogranuloma", but the phenomena accompanying the administration of the oil with dibenzanthracene are quite similar to those following administration of the oil alone.

When oil was administered with dibenzanthracene or some other blastomatogenic substance one could note, 3 to 3½ months later, the beginning proliferation of richly cellular connective tissue in one or several areas of the oleoma wall; i.e., the beginning of sarcoma. In no case did we note any signs of in-
noted. Finally, there were no traces of maturation of the newly proliferating connective tissue, such as but slightly differentiated connective tissue cells were inflammation. The accumulations of proliferating and stance cause any inflammatory changes, in particular inflammatory proliferation such as might precede tumor formation.

Special experiments were carried out in which 1,2, 5,6-dibenzanthracene was injected at laparotomy directly into the stomach wall in rabbits so that it lay beneath the mucosa. The solution did not cause any inflammatory changes except a slight natural reaction to the oil: at least no such active inflammatory proliferations of the epithelium as those readily called forth by the injection of tar into the stomach wall (Haga, Garshin).

Of particular interest is the microscopic examination of the site at which remote tumors arise. There is absent here even the inflammatory reaction that invariably occurs at the site of injection of any substance and hence always takes place in skin painted with blastomatogenic substances and at the site of their subcutaneous injection. It is safe to conclude from numerous data that remote tumors are not preceded by any ordinary or specific inflammatory changes, and that these play no role in their genesis.

In this connection we may mention the hepatic tumors caused by azo compounds. No early inflammatory changes are found in the liver, and the peculiar proliferations of the stroma cells noted at advanced stages are modifications of the leukosis type. In our numerous experiments on mice cirrhosis never occurred, though this is supposed to take place in the genesis of primary cancer of the liver in man.

Finally, special experiments were carried out to ascertain the role of acute and chronic inflammation in the genesis of skin cancer following the application of chemically pure blastomatogenic substances (Monastyrskaya). With 3,4,8,9-dibenzpyrene in vegetable oil inflammatory changes were found in the skin at an early date, but these were not more pronounced than those set up by an oily solution of 3,4,8,9-dibenzpyrene-quinone, which is not carcinogenic at all. A turpentine solution of 3,4,8,9-dibenzpyrene called forth more extensive inflammatory changes in the skin than the same amount of this substance in benzene, although this of course was to have been expected. Yet with the benzene solution there were not fewer, but more, tumors than with the turpentine solution.

It can not be denied that some blastomatogenic substances may exert, besides their blastomatogenic action, a considerable local and general toxic effect. Thus, in guinea pigs subcutaneously treated with large doses of 1,2,5,6-dibenzanthracene there occurs a peculiar liver cirrhosis (Shabad and Urinson), while benzpyrene and dibenzpyrene, even in oily solution, cause more extensive inflammatory changes in the skin of mice than 1,2,5,6-dibenzanthracene. The most powerful blastomatogenic substance available in this laboratory, 9,10-dimethyl-1,2-benzanthracene, causes considerable degeneration and necrosis in the injected tissues. A correlation between this increased blastomatogenic effect and the increased tissue injury has not yet been established with certainty. It will be noted that some of the compounds tested, such as "sulforesol" and "emulsol", caused a pronounced inflammation in the painted skin although their blastomatogenic effect was slight. Although final elucidation of the role of inflammation in carcinogenesis must be deferred to further investigation, it may be suggested that it is less significant than so far assumed; or at least that it may have a somewhat different significance. While prior to the discovery of chemically pure blastomatogenic substances some clinical data suggested that chronic inflammation itself may be a precancerous condition, the concept of precancerous change acquires at present a more restricted specific sense. If the effect of the blastomatogenic substance is not accomplished by way of inflammation it may be assumed that we are dealing here with a more specific, more delicate, and more intimate action upon the tissues, perhaps resembling that of the hormones, for example.

**Correlation Between Chemical Structure and Blastomatogenic Action**

The correlation between blastomatogenic action and chemical structure has already been extensively studied, principally by two groups: Kennaway, Cook, and others; and Fieser, Shear, and their associates. We can supplement their observation by several examples from this laboratory. Thus in the systematic study of a number of derivatives of 1,2-benzanthracene, synthesized by Mikhailov in the laboratory of Professor Ushakov and tested by Kleinenberg, it was found that 1,12-trimethylechrysene is a very weak blastomatogenic substance. Subcutaneous administration never produced sarcoma at the site of injection. Application of an oily solution to the skin of mice throughout their life cycle finally elicited papillomas, which, however, were never transformed into cancer; in the internal organs, particularly in the lungs,
primary adenomas were detected in a comparatively large number of cases. It will be noted that in its chemical properties this substance may be regarded not only as a derivative of chrysene but of 1,2-benzanthracene as well.

The next stage in the systematic study of derivatives of 1,2-benzanthracene was the assay of 9,10-dimethyl-1,2-benzoanthracene. This substance proved a very powerful blastomatogenic agent, in that it was capable of inducing sarcoma when administered in as small a dose as 0.1 mgm. Painting of the skin of mice with a 0.1 per cent solution in benzene (Prokofieva) produced papillomas as early as the 12th day of the experiment. According to Kleinenberg, painting with 0.05 per cent solution elicited papillomas in almost all experimental animals beginning at the 40th day, and cancer after 2 to 3 months. There also occurred especially numerous remote tumors, lung adenomas in particular.

9-Methyl-10-ethyl-1,2-benzanthracene likewise proved a very strong blastomatogenic agent (Kleinenberg), but it did not reveal the toxic and necrotic properties characteristic of 9,10-dimethyl-1,2-benzoanthracene.

Another homologue of the same series was studied, in which the radical of acetic acid occupies position 10 while position 9 is occupied by the methyl group. The investigation of this substance (9-methyl-1,2-benzoanthracene-10-acetic acid) appeared of particular interest since it may be a preceding stage in the synthesis of 9,10-dimethyl-1,2-benzoanthracene. It was found that while the last link in the whole chain of synthesis is a very powerful blastomatogenic agent, the link immediately preceding it is biologically inactive. Similar relations were found with regard to methylcholanthrene, which is preceded in the course of synthesis by inactive dehydrocholanthrene. Hence it may be concluded that the blastomatogenic properties of a substance appear suddenly in connection with its structure. It will be of interest to note that the acetic acid derivative is comparatively more akin to compounds that may occur in the organism than are other derivatives of 1,2-benzanthracene.

Experiments with 3,4,8,9-dibenzpyrene showed that this is a strong blastomatogenic agent. Upon subcutaneous administration it produces sarcoma in nearly 100 per cent of cases, and applied to the skin it elicits papillomas in almost all mice and cancer in two-thirds of them. Moreover, there were (Kleinenberg) multiple lung tumors (30 to 50 per cent), cancer of the mammary glands (in one-third of the females), and other remote tumors such, for example, as lymphosarcoma of the thymus. On the other hand, 5,10-quinone-3,4,8,9-dibenzpyrene had no blastomatogenic activity at all. Of particular interest is the fact that the introduction of oxygen into the molecule of a carcinogenic substance deprives it of the capacity to produce tumors.

Our observations, as well as those of others, testify that modification of structure affects blastomatogenic properties. In our view, the facts cited above suggest that modifications of structure such as might occur within the organism may neutralize carcinogenic action; i.e., may act as antiblastomatogenic factors. In this connection we may cite another observation made in this laboratory on solutions of 9,10-dimethyl-1,2-benzanthracene. It was found that when this compound was exposed to light and air for 2 or 3 months it showed an appreciable decrease in carcinogenic activity. Hence it may be assumed that the oxidation of blastomatogenic substances may play the role of an antiblastomatogenic factor.  

**Antigenic Properties of Blastomatogenic Substances**

Our efforts to control the development and growth of neoplasms have included the treatment of transplanted tumors with spleen extracts, spleen itself, and blood. It was shown on extensive material (Chalezkaya) that spleen and blood serum reduce susceptibility to a graft and inhibit the growth of a number of experimental tumors. But what is most important, spleen and blood from animals affected with carcinomas or sarcomas, whether transplanted, spontaneous, or produced by chemical agents, were found inactive in this respect. This observation led to a study of serum from normal and tumor-bearing animals, in the course of which it was found (Petrov and Chalezkaya) that while the former inhibits the growth of the Ehrlich carcinoma, the latter inhibits in but 8 to 10 per cent of cases.

The experiments just described have to do with factors that influence the established tumor. Others are under way concerning the possibility that antiblastomatogenic factors may exist. This study is only beginning, but we may communicate here some preliminary results.

It is an established fact that some chemical substances possess antigenic properties (Landsteiner and others), and it seemed worth while to ascertain whether or not some of the blastomatogenic compounds possess them. Two methods of attack were possible: (a) to administer the substances in pure form, or (b) combined with proteins. The latter has been adopted by Creech and Franks, who introduced dibenzanthracene into the protein molecule:

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1It would appear that some distinction should be made between mere loss of carcinogenic activity and the presence of an anti-carcinogenic factor, but the difficulties of international communication made it impossible to ascertain Professor Shabad's opinion on this. Ed.
and obtained in this way 1,2,5,6-dibenanthryl-
carbamidocasein, which possessed the specificity of
anthracene.

We began with the former method. The study was
carried out on 104 guinea pigs with o-aminoazo-
toluene, dimethylaminoaobenzene, 3,4-benzpyrene,
and methylcholanthrene by the procedure of Land-
steiner. Sensitization was achieved by means of a 1
per cent solution of the substance to be tested in pure
olive oil, in doses of 0.1 cc. for 10 days in succession.
Thirty days after the first injection a single injec-
tion of 0.1 cc. of a 1 per cent solution was made
into the skin of the opposite side. All the experiments
gave a negative result; no signs of a specific reaction,
hyperergic inflammation or necrosis, were noted; there
was no difference among the injection sites of various
substances, or in the reaction of sensitized and control
animals.

The combination of a carcinogen with proteins
was then tried. o-Aminoazotoluene was tetratotized
and combined with the proteins of bovine and horse
serum. The azoprotein thus obtained was used for
sensitizing rabbits, whose serum was subsequently
used to test the presence of specific precipitins. The
latter were found to appear in the rabbit serum and
to react specifically not only with the antigen used for
sensitization, e.g., the combination of o-aminoazo-
toluene with the proteins of bovine serum, but also
with another antigen containing the same substance;
that is, the combination of o-aminoazotoluene with
the proteins of horse serum (Korosteleva). In this
way it was shown that the combination of a carcino-
genic compound with protein may possess antigenic
properties that are specific for a definite blastomato-
genic substance such as o-aminoazotoluene.

These data, of course, are only the first step in an
entirely new field, and require further elaboration.
Yet they suggest that within the organism there may
exist antiblastomatogenic factors that counteract car-
cinogenic chemical compounds. Further progress
along these lines may lead, on the one hand, to an
attempt to influence exogenous, and eventually endo-
genous, carcinogenic agents. It is possible, on the
other hand, that serological studies may contribute to
the search for the latter by identifying them as antigens.

THE BLASTOMATOGENIC ACTION OF SOME SUBSTANCES
OF DIRECT PRACTICAL SIGNIFICANCE

Study of the blastomatogenic compounds may throw
light upon many aspects of tumor pathogenesis and
at the same time of practical medicine. Thus it has
been found that the latent period varies with the
activity and dose of the compound administered, but
on the whole it is fairly long, amounting, with agents
of moderate activity like coal tar and 1,2,5,6-diben-
anzthracene, to about one-fifth the life span. It will
be noted that the latent period for occupational
cancer in man is about the same, namely 12 to 15 years.
This suggests the possibility of prophylaxis. If some
product is suspected of being carcinogenic an ex-
periment may show whether it is or not long before
completion of the induction period in man.

Some time ago we investigated the slate tars of
the U.S.S.R. and found (Larionov, Soboleva, and
Shabad) that they differ in carcinogenic power. Thus
the greatest number of tumors in mice was obtained
with slate tar of Chuvashia (Middle Volga), a smaller
number with Barsass tar (Western Siberia), while
those of the Leningrad district were wholly ineffective.

Within recent years a number of studies have been
carried out in this laboratory, which may be divided
into 3 groups: (a) those dealing with lubricating oils,
(b) with azo compounds, and (c) with hydrocarbons.

To the first group belong the studies of “sulfofresol”
(Prokofieva) and “emulsol” (Verkhovskaya), both of
which are used as refrigerating mixtures in the metal
industry and contain mineral oils. They caused a
pronounced dermatitis upon prolonged painting of
the skin in mice. Sulfofresol induced papillomas in
25 to 45 per cent of the animals and cancer in 7 to
12 per cent at the site of administration, while emulsol
produced papillomas in isolated cases only, and no
cancer at all.

Certain azo compounds are of practical interest,
since they may be used to synthesize food dyes. It
should be emphasized in this connection that o-amino-
azotoluene, and less so dimethylaminoaobenzene
(Morozenskaya), may produce tumors of the liver and
lungs upon application to the skin. This is to be
borne in mind in considering the pathogenesis of
aniline cancer of the urinary bladder in man.

Among the hydrocarbons of practical significance
are some derivatives of 3,4,8,9-dibenzyrene (Kleinen-
berg). While it is a very active carcinogen, its 5,10-
quinone compound, which is an important industrial
dye, is inactive either by itself or in the form of water-
soluble compounds.

Here belongs also an experimental study on the
properties of benzanthrene (Morozenskaya) and of
tetramethylaminophenone, Michler’s ketone (Pro-
kofieva), which may serve as either initial or inter-
mediate products in dye technology. These also were
found to be ineffective with regard to tumor forma-
tion.

EXPERIMENTAL CANCER OF INTERNAL ORGANS

Though all blastomatogenic agents elicit multiple
tumors at a distance from the site of administration,
and primarily in the lungs, this does not enable us
to produce neoplasms of the internal organs at will, since to a certain degree these are a haphazard phenomenon.

More reliable is the administration of azo compounds. o-Aminoazotoluene, for example, elicits tumors first of all in the liver, thus providing a convenient method of investigating their earliest stages. This is exemplified in a recent study made in this laboratory by Elzina, who found that the respiration of liver cells did not decrease as the malignant transformation came on, and that glycolysis did not increase even at the stage of hepatoma formation. Transplantable hepatomas, on the other hand, behaved in all respects like any other grafted tumor.

A third approach to the experimental production of internal tumors is the introduction of carcinogens directly into the various organs. In this direction a number of studies have been carried out by Klenitzky, who produced cancer of the cervix in mice by inserting coal tar or 3,4,8,9-dibenzpyrene on cotton plugs. In this way the whole course of preliminary morphological changes was followed up and concepts of precancerous lesions of the cervix were modified. The experimental production of cancer of the cervix in mice is the more significant as spontaneous carcinoma at this site is rare in them, if, indeed, it occurs at all; but it was an elaborate procedure that failed in many cases. The simultaneous subcutaneous injection of folliculin favored the appearance of cervical cancer, whereas the administration of such an irritating agent as formol in vegetable oil did not cause either cancer or precancerous changes. In this way the significance of the combined action of different agents in the genesis of cancer of the cervix was demonstrated and the results of the experiments were brought closer to clinical pathology.

It was of interest to find that the introduction of dibenzpyrene plugs into the genital tract in castrated female mice produced no cancer at all. This undoubtedly points to the significance, as an endogenous blastomatogenic factor, of the sexual cycle. The latter is directly evidenced by the fact that papilloma and skin cancer in tar-painted mice appeared sooner and in greater number in females that had spent their lives isolated from males, in contradistinction to the smaller number of tumors recorded in females kept with males (Klenitzky).

The evidence just cited demonstrates the significance that can be attached to the female sex hormones and other estrogenic substances in the development of tumors. It seemed necessary, therefore, to investigate first of all the possibility that some carcinogenic substances may possess estrogenic activity. Prokofieva showed that, contrary to the widely adopted opinion based on the studies of English authors, not one of the numerous compounds tested, including 3,4-benzpyrene, had any such capacity.

Of great interest was the examination of synthetic estrogenic compounds for carcinogenicity. Lacassagne showed that the administration of large doses of folliculin produces malignant tumors in mice, but the question remains open whether it acts as a chronic stimulus to the genital apparatus by virtue of its powerful estrogenic action, or because it is a high molecular sterol. It seemed possible to approach this problem through a chemically more simple estrogen, polianol (Maksimov). This substance, which has a pronounced estrogenic action (Prokofieva), called forth considerable heterotropic proliferation of the genital epithelium in female mice (Kazanskaya), and peculiar adenomatous changes of the prostate with pronounced keratinization in male mice (Klucharev), but no tumors of any sort have so far been noted in spite of numerous and prolonged observations. Of special interest is the fact that the changes in the prostate receded as soon as the administration of polianol was discontinued (Klucharev). Thus the data point to the conclusion that a certain role in the blastomatogenic action of folliculin is played by its sterol structure but not by its physiological action.

As to the mode of action of estrogenic substances on the genital epithelium, it may be suggested that a direct stimulation takes place. This idea led to a study of the effect produced by folliculin on the healing of fissured nipples. According to Kazanskaya, painting the nipples of nursing mothers with folliculin promotes their healing.

In concluding this chapter of our review we may point out that the logic of research has led from investigation of the exogenous blastomatogenic agents to a consideration of certain substances, such as the female sex hormones, that may arise within the organism itself. We have demonstrated the significance in carcinogenesis of the combined action of exogenous and endogenous factors, in particular of the estrogenic substances mentioned above. The evidence thus obtained naturally brings us to a search for endogenous blastomatogenic substances that presumably may be produced within the organism itself and act as the cause of "spontaneous" tumors.

II. ENDOGENOUS BLASTOMATOGENIC SUBSTANCES

The development of the concept of blastomatogenic substances, their synthetic production, and the accumulation of a vast experimental material on their action, raise the question whether or not there may arise within the organism itself blastomatogenic substances similar in a certain degree to the exogenous agents known at present.

As early as 1925 Kennaway obtained tar-like car-
cinogenic substances through distillation of a number of organic products, including human skin, at a temperature of 800° to 920° C. In discussing the results he suggested that in the human organism similar substances may form although slowly and gradually. However, Kennaway himself insisted that his experiment should not be regarded as essentially different from those with coal tar: In both cases the responsible agent was a naturally or artificially produced exogenous compound.

A consideration of the structure of blastomatogenic substances in the light of modern knowledge of the structure of sex hormones, bile acids, and cholesterol justifies the assumption that within the organism there may occur complex polynuclear compounds resembling the blastomatogenic hydrocarbons (Cook).

An important contribution to this problem was Lacassagne's finding that in mice cancer may be incited by means of folliculin. Another, although indirect, piece of evidence to support this hypothesis was the production (Cook, and Fieser and his associates) of very active blastomatogenic substances, methylcholanthrene and cholangthrene, from bile acids. The possibility of the endogenous origin of blastomatogenic substances was indicated also by some additional but still less direct evidence. Thus Burrows and Mayneord injected mice subcutaneously with a lard solution of cholesterol that had been irradiated with a large dose of x-rays. Sarcoma arose at the site of injection in 2 animals. Of great interest is Bittner's demonstration that the agent causing mammary tumors in certain strains of mice is transmitted through the milk.

The indirect evidence just cited was obviously insufficient to solve the problem of the endogenous origin of blastomatogenic substances. It could be solved only by direct experimental attack; by endeavoring to isolate from cancer patients chemical substances that would produce tumors in animals. At the present time we have information on futile attempts in this direction by several authors (Bricker, Bürger and Uiker, Rondoni, Sobotka and Block, etc.). Such failures are quite comprehensible, since direct experiment involves a number of enormous difficulties. As a matter of fact, prior to our studies, which offered the first direct evidence of endogenous blastomatogenic substances in man, it was uncertain how to search for them and where, and what is to be regarded as a blastomatogenic agent within the human body. Moreover, one had to take account of the fact that blastomatogenic substances might exist within the human body only at certain stages in malignant neoplastic disease, and hence might be absent when the attempt is made to discover them. This suggestion seems especially pertinent in view of the well known fact that tumors in animals may appear a long time after administration of an exogenous agent has been discontinued. Despite these difficulties we are able to offer considerable experimental evidence for the occurrence of blastomatogenic substances within the human body.

The first experiments, carried out by the author in 1935, consisted in painting mice with a 1 1/2 month benzene-“infusion” (in the cold) of minced liver or tumor from persons dead of cancer. The experiments were carried out on 162 mice, but could not be completed because nearly all the animals died within 3 to 5 months from the toxicity of the preparation. None of the animals developed a tumor, but it should be borne in mind that none of them lived more than 7 1/2 months from the beginning of the experiment.

This failure forced us to modify the experiment by concentrating the extracts and administering them subcutaneously. In January, 1937, malignant tumors were recorded at the injection site in 3 out of 8 mice treated with benzene extracts of the liver of a woman dead of gastric cancer. Since then a great number of experiments have been carried out in this laboratory, most of which have already been described (Shabad, Neufach, Kleinenberg). It was found that liver extracts from cancer patients actually contain blastomatogenic substances, and our pioneer results have been confirmed by others (Hieger, Des Ligneris, Steiner, and so on). Below we discuss briefly our principal results.

**Liver Extracts**

Benzene, a reliable solvent for a number of synthetic carcinogenic compounds, was used to extract blastomatogenic substances from the human body. Relying upon observations on remote tumors, we considered the possibility that blastomatogenic substances may circulate throughout the organism, and it seemed worthwhile to try to extract them from some organ that was not involved by the tumor. It was natural to attack first of all the liver, which is closely connected with the transformation of sterols and is the site of formation of bile acids. Furthermore, the liver seemed advantageous because of its large size.

It was accordingly removed from 41 persons dead of malignant growths at various sites. In most cases, 14, the tumor affected the stomach or, in 6, the lungs. All other localizations and varieties of tumor were represented by single cases. In 32 of the 41 cases the extracts were prepared from livers in which no metastases could be detected macroscopically. In the remaining 9, the livers contained large metastases, and the extracts were prepared not so much from liver itself as from the invading malignant tissue.

Each extract was used for a single experiment as a rule. The number of mice employed varied ac-
cording to the amount administered (about 1 gm.). In some instances undissolved extract was employed whereas in others the extract was diluted 2 to 3 times with olive oil because of its toxicity. Two hundred and seventy-three mice were used, of which 179 belonged to strain RV.

Livers from 26 persons dead of various diseases, and not affected with cancer, served as controls. Their ages varied from 20 to 80 years, 19 of them being above 40, in accordance with the age of the cancer patients. Twelve had died of pneumonia and 10 of cardiovascular disorders. The diagnosis, as in all other cases examined, was confirmed by autopsy and microscopic examination.

**Bile Extracts**

In order to restrict as much as possible the large number of ingredients of liver tissue, a series of experiments was carried out (Neufach and Shabad) with bile extracts. The bile was procured from the gall bladders of persons dead of gastric cancer (2 cases), lung cancer (2 cases), and sarcoma (2 cases). The bile was evaporated to dryness on a water bath and then extracted with benzene; the benzene was distilled off and the remainder dissolved in olive oil and lard and injected subcutaneously into 35 mice.

All the mice of our strain RV that served as controls lived out their natural span, and at death were subjected to careful macroscopic and microscopic examination. Of 634 that died throughout the observation period 389 were above 8 months old, and in 40 of them several kinds of tumors were detected; i.e., 6.3 per cent of the total number, or 10.2 per cent of those surviving more than 8 months. Malignant tumors occurred only 9 times, thus in 1.4 or 2.5 per cent. First place as regards frequency was occupied by primary adenoma of the lung, detected in 33 cases (5.2 or 8.48 per cent); in 4 of these the growths were malignant. Other neoplasms, cancer of the skin, hepatoma, etc., occurred only occasionally. Adenocarcinoma of the mamma was found in 4 animals, i.e., in 1.2 or 2.03 per cent of the female mice. In one female there was a large adenoma.

Comparison of these results with those obtained in mice injected with liver extracts from persons dead of cancer showed a considerable increase in the number of tumors for the latter group. Among 179 mice of this series, or among the 108 that lived more than 8 months, some variety of tumor was found in 62; in 24 the growth was malignant. Thus the percentage of mice with tumors was 34.6 or 57.4 per cent as compared with 6.3 or 10.2 per cent in the controls, and the percentage with malignant tumors was 13.4 or 22.2 against 1.4 or 2.3 per cent. That is to say, the total number of tumors was increased 5 times, and that of malignant neoplasms as much as 10 times. It will be of interest to note that the administration of bile extracts from persons dead of malignant tumors gave approximately the same results as the liver extracts of these patients. Thus with bile extracts tumors were recorded in 37.1 per cent of all animals, or in 50 per cent of mice surviving more than 8 months, while the number of mice with malignant tumors was 17.1 per cent of the total number, or 23 per cent of those that survived for more than 8 months from birth.

Analysis of the material according to the variety and localization of the tumors showed that in every category the number of tumors was appreciably greater in mice treated with liver and bile extracts from cancer patients. This was true of cancer of the skin and the mammary glands, as well as of lung tumors. Moreover, among the mice injected with the extracts there occurred tumors such as were not found in the controls; e.g., squamous cell cancer of the jaw, or cancer of the kidney.

Finally, in 6 mice (2 males and 4 females), that is, in 3.3 or 5.5 per cent, we noted malignant tumors at the site of injection of liver extracts from cancer patients. In 3 these were sarcoma, in 1 carcinosarcoma, and in 2 mammary adenocarcinoma. As a rule these growths were connected with the oleoma. As to their morphology, they resembled in every respect malignant tumors elicited at the site of injection of synthetic carcinogenic compounds. Two of the sarcomas were transplanted with success and carried to the 31st and 45th generation respectively. The adenocarcinoma failed to grow after transplantation.

In mice injected with liver extracts from persons that did not die of cancer the tumors were of significantly less frequent occurrence, although more numerous than is the rule with untreated mice of our strain. The total number with tumors was 2 to 2½ times greater than in untreated mice, and about that many times less than after the injection of extracts from cancer patients. Malignant tumors were detected after the administration of "noncancer" extracts only twice as frequently as in the untreated group, and about 4 times less frequently than with "cancer" extracts. It should be emphasized that the overwhelming majority of the tumors occurring after the injection of noncancer extracts were lung tumors, and that other varieties and localizations were represented by single cases. No tumors occurred at the injection site.

Thus it will be seen that the tumor frequency was significantly different in mice treated with cancer and noncancer extracts. The number produced by liver and bile extracts was so much above that spontaneously arising in our strain that there can be hardly
any doubt as to the blastomatogenic effect of the extracts.

**Lung Extracts**

Our attention was primarily attracted to the lungs as they appear to play a certain role in the metabolism of lipoids, which may act as solvents of blastomatogenic substances. Moreover, as has been indicated above, primary tumors of the lung are fairly frequent after the injection of exogenous blastomatogenic substances, which is to be accounted for by distribution of these substances throughout the organism.

The lungs of 19 persons dead of malignant tumors at various sites (8 of gastric carcinoma, 1 of sarcoma) were extracted. In only 3 did the lungs contain tumor tissue (2 cases of primary cancer of the lung and 1 with metastases from a sarcoma). As controls we used the lungs of 20 patients, 12 of whom died of pneumonia. The experiments were carried out on 212 mice.

In mice injected with extracts from persons dead of malignant neoplasms there appeared a great number of tumors. Thus among mice surviving more than 8 months from birth various kinds of tumors were detected in 54.3 per cent, the percentage of malignant ones amounting to 13.8 per cent. It should be emphasized that these data coincide with those obtained upon the injection of bile and liver extracts (50.0 or 57.4 per cent). Extracts from persons dead of diseases other than neoplasia produced a much smaller number of tumors; 13.6 or 23.1 per cent against 37.3 or 54.3 per cent, although this is notably greater than the spontaneous tumor incidence (6.3 or 10.2 per cent) in mice of the RV strain.

Particular attention is called to the fact that in one case we succeeded in inducing sarcoma at the site of injection of an extract of the lungs of an 89 year old woman with cancer of the gall bladder that had not metastasized to the lungs.

In this same series another mouse, which died 16 months after the beginning of the experiment, had a keratinizing squamous cell cancer of the mouth. The total number of cancers of the mouth obtained with lung extracts from persons dead of malignant tumors amounted to 3, whereas with noncancer extracts and in untreated mice of the RV strain no such tumors ever occurred.

The greatest number of tumors was always found in the lungs. Except for one carcinoma, these were adenomas. Cancer extracts produced lung tumors in 31.3 or 47.8 per cent of injected mice, and noncancer extracts in 13.6 or 23.2 per cent. Spontaneous lung tumors in the RV strain occurred in 5.2 or 8.48 per cent of the mice.

The results obtained testify to the possibility of detecting blastomatogenic substances in the liver and lungs of man, that can be extracted with benzene. This confirms all our preceding statements, and contributes further to an elucidation of the nature of the blastomatogenic agents in the human organism, since it excludes a number of products characteristic of hepatic tissue, such as a great number of pigments, bile acids, etc. Finally, lung extracts displayed a less toxic and irritating effect than those of liver and bile, though they produced as many tumors.

**Nonsaponifiable Fraction**

The crude benzene extracts of the preceding experiments contained a mixture of most diverse substances, mostly of a lipid nature. The fractionation of lipid organ extracts, with a simultaneous assay of the blastomatogenic activity of every fraction, was greatly impeded by the fact that results can be obtained only by the expenditure of a large number of animals and considerable time. Hence we had to confine our study to one fraction only. For this purpose the nonsaponifiable fraction of liver extracts from cancer patients was used.

In an experiment carried out on 50 mice we used the livers from 9 patients (2 men and 7 women) dead at from 37 to 65 years of cancer at various sites (stomach in 6 cases, liver [primary] in 1, uterus in 1, and ovary in 1). In 4 of these 9 cases a large number of metastases were seen in the liver. Thus, in a total of 5 extraction was done not so much on hepatic tissue as on invading tumor.

The administration of the nonsaponifiable fraction elicited various kinds of tumors in 31.2 per cent of the mice that lived to be more than 8 months old. Particular attention is directed to the fact that in 1 case we succeeded in obtaining a sarcoma at the injection site, while the total number of tumors was 2 to 2½ times as great as that occurring spontaneously in our mice.

**Discussion**

The total number of animals treated with extracts amounted to about 800. The material was procured from over 100 human bodies (liver in 76 cases, lungs in 39, and bile in 6).

It is concluded that the injection of benzene extracts of the liver, bile, and lungs from persons dead of malignant neoplasms produces diverse tumors in mice, either benign or malignant, and either at the injection site or, most often, distant from it.

It is to be pointed out that our extracts were prepared from the livers, bile, and lungs of persons

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2 Attention is called to the fact that the liver extract of the same patient likewise possessed an appreciable blastomatogenic capacity.
affected with various forms of malignant tumors at different sites. The results obtained should therefore be related to some special property of cancer of the stomach, bronchus, gall bladder, etc., but to some property common to all malignant tumors. At the same time, in contrast to Steiner and some others, we did not combine extracts from different patients; hence we may state with certainty that the blastomatogenic effect was produced by diverse malignant tumors; by cancer of the stomach, lung, larynx, intestine, pancreas, gall bladder, liver, and by lymphosarcoma, of both men and women.

The results obtained with the nonsaponifiable fraction from cancer patients suggest that the blastomatogenic substances detected in man belong among the nonsaponifiable compounds. Attention is therefore directed to the possible transformations of sterols and their role in the genesis of cancer, particularly under the influence of such factors as radiant energy. This is particularly important in connection with the tumors caused in both man and the lower animals by x-rays, radium, ultraviolet rays, etc. In this connection it may be mentioned that Neufach, of this laboratory, has demonstrated the transformation of cholesterol by ultraviolet irradiation into a peculiar product possessing certain new properties; i.e., change of color, melting point, absorption spectrum, fluorescence, and the capacity to affect a photographic plate. Khuletzkaya, on the other hand, showed in this laboratory that in mice painted with blastomatogenic substances there occurs during the papilloma, or initiatory, period a rise in the blood cholesterol.

The production of tumors with bile and certain organs from cancer patients was so unexpected that doubt has been aroused concerning the reliability of the result.

First of all, one might regard the growths as spontaneous, and hence not connected with the administration of the extracts. This doubt is refuted by at least two considerations: (a) In most of our experiments mice of a known strain were used, and a sufficient number of control animals (above 600) enabled us to determine the incidence of spontaneous neoplasms. (b) Different percentages of tumors were obtained with extracts derived from cancer patients and from persons dead of other diseases. It should be emphasized that tumors were recorded at the site of injection, and that these were produced only by extracts from cancer patients.

A second ground for argument is the suggestion that the blastomatogenic substance was formed during preparation of the extracts. The objection may be refuted for the following reasons: Benzene cannot be responsible for the blastomatogenic action, since it has long been known that the low-boiling tar fractions, to which benzene belongs, are not carcinogenic. Furthermore, control experiments showed that benzene does not produce either local or remote tumors, lung tumors in particular. The extracts were not heated above 80° to 140° C. and did not reach those temperatures (800° to 900° C.) at which Kennaway and others have obtained carcinogenic products from various organic materials. But the most conclusive evidence that the active principle in our extracts actually was isolated from the human body is the fact that the tumors produced by material from cancer patients were more numerous that those incited by extracts from patients dead of other diseases. The fact that similar results were obtained with liver, lung, and bile extracts seems to support the idea that neither the extraction nor the conditions under which the material was procured will explain the blastomatogenic action of our extracts.

Finally, a third possible objection is that the tumors in our experiments were not produced by specific blastomatogenic substances, but by nonspecific irritation associated with the chronic inflammation at the injection site. Against this are the numerous observations on chemically pure carcinogens, which show that there is no correlation between tumor formation and irritation. Secondly, it deserves to be emphasized that bile extracts, which are more irritating than liver extracts, produced no tumors at the injection site, whereas lung extracts, which are less irritating, elicited sarcoma. In the third place, liver extracts from persons free of cancer were no less irritating than those from cancer patients, yet they produced a significantly smaller number of tumors. Finally, and most important, nonspecific irritation through local chronic inflammation cannot account for the appearance of remote tumors. Since these have appeared in connection with tar and synthetic carcinogenic agents also, they can be explained only by a general effect of the agent upon the organism. And this points to the conclusion that the liver, bile, and lung extracts actually contained blastomatogenic substances.

Our first communication stimulated others to repeat our experiments. Butenandt did not succeed in obtaining tumors in mice, apparently because his nonsaponifiable fraction was administered only once; that is to say, probably in too small a dose. Gummel also failed to reproduce our results.

The first confirmation was reported by Hieger, from Kennaway's laboratory. Among 367 mice treated with various kinds of liver extracts from persons dead of malignant tumors or other affections, he obtained sarcoma at the site of injection in 13. Most of these (11 of 13) were produced with extracts from persons with malignant tumors; only 2 resulted from the in-
jection of extracts from Bantu natives dead of "uncer-
tain causes, but not cancer." It will be remembered
that among the Bantus of South Africa, from whom
Hieger obtained his material, primary cancer of the
liver is of comparatively frequent occurrence.

Des Ligneris carried out his study in cooperation
with Hieger, by painting the skin of mice with liver
extracts from persons dead either of cancer or
of other diseases. Among 237 mice he obtained 25
tumors in the painted area, and of these 7 proved to
be malignant.

Steiner administered subcutaneously the nonsaponifi-
able fraction of liver extracts from persons dead of
cancer, and among 37 mice that survived for more
than 6 months from the beginning of the experiment
obtained a sarcoma at the injection site in 13. He
denied the blastomatogenic activity of similarly pre-
pared liver extracts from patients free of cancer, but
subsequently encountered the phenomenon as did we,
although the number of tumors was smaller than
with extracts from cancer patients. Similar extracts of
the neoplasm itself did not produce any tumors in
Steiner's experiments, but Menke obtained sarcomas in
2 mice at the injection site of an extract of cancer from
the human body. Yet Menke did not succeed in
producing tumors with the nonsaponifiable fraction
of cancer extract.

Kinosita reported that Tanaka elicited sarcoma in
mice at the injection site of the nonsaponifiable frac-
tion of liver extract from a man dead of cancer of
the stomach. Similar results were reported also by
Sannid, Truhaut, and Guérin, in France. Finally,
Steele, Koch, and Steiner have described the produc-
tion of tumors with extracts of urine from patients
with and without cancer.

Thus the possibility of detecting carcinogenic agents
in the human body, first reported by the writer, has
been confirmed. In numerous experiments, carried
out in this laboratory as well as in other countries
and on different strains of mice, tumors have been
produced with extracts of bile, liver, lungs, and urine
from persons dead of malignant growths. It is sig-
nificant that owing to modifications in the preparation,
and particularly in the application, of the nonsaponifi-
able fraction in both this and other laboratories, it is
certain that the origin of the blastomatogenic sub-
stances is not referable to such chemical procedures
as the use of benzene, etc.

Of special interest is the blastomatogenic effect of
extracts from persons dead not of cancer, but of
other diseases. According to our experience, which
has been confirmed by Steiner as well as by Sannid,
Truhaut, and Guérin, this is possible, and it may be
accounted for in two ways. First, all livers may ac-
cumulate small amounts of blastomatogenic substances,
which manifest no activity probably because of in-
sufficient concentration. Secondly, among the controls
there may have been persons who would have devel-
oped cancer had they not died of some other disease.
This second alternative is supported in part by the
data of Hieger and Des Ligneris on the livers of
Bantus, though no decisive conclusion can yet be
drawn. In any case, our data and those of Hieger,
Des Ligneris, and Steiner leave no doubt that the
blastomatogenic activity of extracts from persons dead
of cancer is more pronounced than that of similar
extracts from those free of the disease. This means
either that extracts of the liver and lungs from per-
sons dead of malignant tumors contain more blasto-
matogenic substances than extracts from those without
cancer, or that their action is more effective.

In most of our experiments the extracts were
prepared from livers and lungs that were free of
metastases; hence it may be taken for granted that
blastomatogenic substances may be found in the human
body at a distance from the tumor. Some experiments,
however, were done with livers containing so many
metastases that we actually dealt with a tumor extract
rather than an extract of the liver itself. These were
much less active than extracts from livers without
metastases, eliciting 27 per cent of tumors against the
62 per cent obtained in mice injected with extracts of
livers that were free of metastases. It will therefore
be seen that a tumor may contain at least no more
blastomatogenic substances than the liver. This
conclusion is supported by Steiner's data and by the
unpublished data of Khaletzkaya, of this laboratory,
and of Vadova (laboratory of Professor N. N. Petrov),
which show that benzene extracts of the Ehrlich
mouse carcinoma and the Brown-Pierce rabbit car-
cinoma produce no tumors in mice or rats at the
site of administration.

Although this important questioning of the localization
of blastomatogenic substances in tumors still remains
open, it is our definite opinion that they neither pro-
duce nor accumulate blastomatogenic substances of
the type with which we are now concerned.

In considering the results of our experiments and
those confirming them, the fact deserves mention
that the number of tumors elicited was not great
in all the experiments with extracts from persons
dead of malignant tumors. It will be recalled that in
our own experiments we did not as a rule combine
extracts from different sources, and so had a better
chance to compare the presence and the amount of
blastomatogenic substances in various subjects.

The results appear to suggest that blastomatogenic
substances can be isolated from the organs of some
persons only and that not all extracts, even from
cancer patients, have considerable blastomatogenic

activity. With regard to this fundamental problem it should be borne in mind, however, that the determination of blastomatogenic effect involves great difficulties. Indeed, even preparation of the extracts is not yet standardized, while an actual estimation of their effect requires 2 years of observation on mice whose age and intercurrent diseases may affect the outcome of the experiment.

One of the most essential problems to be solved in the future is whether the blastomatogenic substances in the human body are of exogenous or endogenous origin. This cannot be settled until we have more information on their chemical nature; and, what is most important, on the conditions governing their presence in the organism. Even then it will not be easy to decide. We may recall as an example that cholesterol originates within the organism, but it can also be ingested with the food. Nevertheless, it may be suggested provisionally that the blastomatogenic substances in question are most probably of endogenous origin, in the strict sense of the term.

The last question to be discussed is that of the chemical nature of these endogenous blastomatogenic substances, and in particular of their specificity. Though their nature is still wholly obscure, it may be said at least that they are very stable; extractable by benzene, petroleum ether, and other organic solvents; and, what is most important, that they belong to the nonsaponifiable fraction of the lipids in common with cholesterol, the bile acids, and allied substances.

In this connection it might have been thought that the blastomatogenic effect noted in our experiments was exerted not by some new and still unknown product, but by certain constant constituents of the liver such, for example, as the bile acids. The suggestion might be strengthened by the fact that Ghiron, and later Cook and the Kennaways, showed that the administration to mice of desoxycholic acid may eventually elicit sarcoma. Yet it appears that there is enough evidence at present to contradict the idea that bile acids are the active principle of our blastomatogenic extracts. The amount of chemically pure bile acids which, according to Cook and Kennaway, produces malignant tumors in mice is as much as 70 mgm., or at least not less than 28 mgm. This greatly exceeds the quantity in the amounts of liver extract injected. Again, not all livers by any means contain a similar quantity of the active principle, as is conclusively indicated by our data and as has been mentioned by Hieger also. It will be of interest to note that, according to Steiner, the total amount of nonsaponifiable lipids is about the same in livers from persons with or without cancer. The appreciable difference in the number of tumors elicited by liver extracts from cancer patients and those dead of other affections testifies against a blastomatogenic role for the bile acids. If it be assumed that the bile acids of cancer patients are distinguished by some unusual properties, in particular by a specific blastomatogenic activity, we come back once more to a denial of the blastomatogenic role of bile acids from the normal liver and the assumption of special blastomatogenic substances in the livers of cancer patients. Finally, the blastomatogenic activity of the lungs, whose tissue components differ essentially from those of the liver, naturally contradicts the blastomatogenic role of bile acids.

It may thus be assumed that there exist within the human organism blastomatogenic substances that behave to a certain degree as specific chemical originators of malignant neoplasms. One may readily imagine that it is precisely with the appearance of these that the “prehistory” of cancer begins. Hence the problem of neoplasia becomes a double problem, in which not only the condition of the cell “stimulated” by the carcinogenic agent has to be explained, but the origin and character of the “stimulus” itself accounted for.

The question naturally arises whether there exist factors that counteract both the production and activity of endogenous blastomatogenic substances and thereby impair their capacity to produce tumors. The study of exogenous blastomatogenic agents may give some information here, for it is known that a change in structure may deprive them of their activity. On the other hand, certain reactions of the organism that tend to detoxicate these compounds should be investigated.

SUMMARY

The data so far supplied by the study of experimental carcinogenesis suggest certain general concepts on the origin of tumors. When the two principal hypotheses, those of Virchow and of Cohnheim, are examined from the modern point of view it will be seen that both have been about equally confirmed and discredited. Virchow’s conception of the importance of irritation has been justified in so far as it led gradually to the discovery of the chemical carcinogens, but the modern concept of blastomatogenic “stimulation” is substantially different from the old idea of nonspecific irritation. As to Cohnheim’s hypothesis, any carcinogen may elicit a tumor at any site in any animal, which makes the presence of embryonic remnants unnecessary. Yet if the blastomatogenic substance is endogenous it is only a cell product after all, and in this respect the modern point of view approaches more or less that of Cohnheim, who especially accentuated the significance of the endogenous factor in tumor genesis.
The origin of endogenous blastomagenic substances and the mode of their action remain the fundamental problem in cancer research. Although it is still obscure, the recent rapid accumulation of important information on the etiology and pathogenesis of neoplasms stimulates us to still more tenacious and thorough investigation, and encourages a belief in final success.

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Note: We have conscientiously endeavored to verify all references, in accordance with our custom, but because of war conditions many of the publications here cited are unavailable.—Ed.
On Tumor-Producing Chemical Substances

L. M. Shabad

*Cancer Res* 1945;5:405-419.