Abstracts

Reports of Experimental Research

CARCINOGENIC COMPOUNDS


Statistical methods developed for the analysis of dose-response data are discussed and applied to problems involving the responses of animal groups to the action of carcinogenic hydrocarbons. The authors feel that the application of these biomathematical methods allow: (1) a clear and concise presentation of the experimental data; (2) an estimation of the statistical significance and reproducibility of the data; (3) an interpolation of data, within the range of observed results, without further experimentation; and (4) the guidance of further experimentation by the extrapolation of the available data.—L. L. W.


The nuclear and cytoplasmic volumes of both basal and spinous cells of mouse epidermis progressively increased in size following the application of methylcholanthrene under standardized conditions. However, these values were markedly decreased in a carcinoma developing from these precancerous hyperplastic cells. Mitoses were more frequent in hyperplastic basal cells than in hyperplastic spinous cells, or in untreated epidermal cells, but were less than in malignant cells.

The cells of 17 squamous carcinomas produced by methylcholanthrene were compared with the hyperplastic, nonmalignant cells included in the same sections. No single measurement of size revealed significant differences between malignant and nonmalignant cells. Comparative measurements of the cells of human carcinomas and adjacent hyperplastic epithelial cells likewise revealed no single definite criterion that could be used to differentiate malignant and hyperplastic cells.—L. L. W.


Cancer has been induced in the skin of Swiss mice by a method of application in which the carcinogen acts on the cells infrequently and at long intervals. A 0.6% solution of methylcholanthrene in benzene was applied at intervals of 2 weeks, 3 weeks, and 1 month, respectively. Using this protracted technic, the dose of carcinogen effective in producing cancer was smaller than when it was applied thrice weekly for 14 weeks. Furthermore, the dose became increasingly smaller as the interval between successive applications was prolonged. These results are not in accord with the accepted view that the carcinogenic hydrocarbons induce cancer in the skin by stimulating directly the mitotic activity of the epithelium. They support a conception, arrived at from a histological study of the early changes in carcinogenesis, that the carcinogenic hydrocarbons produce a transient toxic effect on the epithelium, inhibiting mitotic activity, and that the epithelial proliferation which eventually leads to cancer is due to the formation in the skin of a substance stimulating the epithelial cells to mitotic activity for a prolonged period of time. The significance of these findings in relation to human skin cancer is discussed.—Authors' abstract.


In an earlier publication (Dodds, E. C., L. Golberg, W. Lawson, and R. Robinson, Nature, London, 143:247, 1948) the formula of the synthetic estrogen, stilbestrol, was shown to suggest that ring closure might produce compounds similar to estrone or to chrysene. Since all the hitherto known carcinogenic hydrocarbons show the condensed ring structure, a compound was sought for in which the carcinogenic power would be retained when the ring structure was opened. a-Ethyl-β-secondary-butyloestrol, which is related to 3,4-benzpyrene and to 1,2-dimethyl chrysene, was applied in benzene to the skin of 50 mice, and two tumors (one spindle cell epithelioma and one sarcoma, without metastases) were obtained after 12 and 15 months, respectively. The sarcoma arose on the back 2 cm. behind the painted area, and is growing in the first grafted generation.

BENZYPRENE DIMETHYLCRYSENE a-Ethyl-β-sec-BUTYLOESTROL

No tumors of the skin were obtained in periods of 9 months or less with diphenylhexane, diphenylhexadiene, diethylstilbene, 4,4'dihydroxy-α-diethylstilbene (stilbestrol), 4,4'dihydroxydiphenyl-hexane (hexestrol), or 4,4'dihydroxystilbene. Stilbestrol given by this method was toxic and none of the mice receiving it lived for more than 3 months.—E. L. K.


Blastomogenic (carcinogenic) substances were extracted from benzol from the human lung as well as from the liver. The lungs from which blastomogenic extracts were made

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were obtained from patients dying of cancer and also from patients dying of other diseases. The extracts produced a variety of tumors at the sites of injection and at other locations when injected subcutaneously into mice of the R. V. strain and in mice of a strain of unknown origin. The extracts of lungs were less toxic than extracts of liver. The results confirm and extend previous reports of these authors. The possible origin of the blastomogenic substances in the lung from exogenous or endogenous sources is discussed.—S. B-J.


General systemic effects of carcinogenic hydrocarbons (benzpyrene) applied to the skin of mice were investigated by determinations of the ratios of partially oxidized substances excreted in the urine, the oxidation-reduction potentials of the blood, and the oxygen consumption of slices of organs. Local effects upon oxidation processes were studied in tissue slices and in skin to which the hydrocarbons had been applied. The oxidation processes were not disturbed during the precancerous period of papilloma formation, but survived in a secondary manner after the appearance of carcinomas.

Incomplete investigations of proteins of induced tumors indicated that they contained an abnormally large proportion of d-glutamic acid.

The inclusion of the carcinogenic hydrocarbons, benzpyrene and dibenzanthracene, in the medium of tissue cultures of mouse fibroblasts produced changes in the morphological, biochemical, and proliferative characteristics of the cells. The data were not sufficient to indicate whether these cells had been transformed into malignant cells.

Transtumulation of the nervous system by section of the sciatic nerve and treatment of the central end with formalin or croton oil affected the incidence of induced carcinoma in mice. An accelerating or inhibiting effect was dependent upon the time in the experimental cycle at which the lesions of the nervous system were produced.

The following conclusions are suggested: 1. Carcinogenic hydrocarbons applied to the skin of mice do not affect the oxidation processes during the precancerous period of papilloma formation. 2. Changes in the oxidation processes of the organism of some organs, and of tissues occurring in connection with carcinoma induced by carcinogenic hydrocarbons are of a secondary nature. 3. The primary change caused by carcinogenic hydrocarbons may be an alteration of protein metabolism. 4. Carcinogenic hydrocarbons produce changes in cells by action directly upon the cells. 5. In the organism the nervous system seems to function as an intermediary link in the production of carcinomas induced by carcinogenic hydrocarbons.—Author’s summary as revised by S. B-J.


The length of time between the detection of induced subcutaneous sarcomas by palpation and death of the animal was studied in male mice of strains C3H and L. The mean length of survival, 4.36±0.10 weeks, was the same whether 20-methylcholanthrene or 1,2,5,6-dibenzanthracene was used, whether the dose of the carcinogen was 0.1 or 0.5 mgm., and whether palpable tumors appeared early or late following the injection of the carcinogen.—Author’s summary.


In an attempt to assess the significance of necrosis in the pathogenesis of neoplasia, 144 Dobrovolskaia-Zavadskia R III strain mice were injected subcutaneously four times at intervals of 4 weeks with 0.05 cc. of minced spleen from animals of this same strain. One week after each of these treatments 0.1 mgm. of methylcholanthrene in 0.05 cc. of olive oil was injected at the same site. An equal number of controls receiving methylcholanthrene alone were prepared. After correcting for extraneous mortality and ulceration at the site of injection, 82% of the controls bore tumors. Latent periods and rate of tumor growth were approximately the same. It is therefore concluded that the presence of necrotic splenic tissue had no effect on the incidence or growth characteristics of the tumors produced.—L. L. W.

Hormones


This paper compares and reanalyzes the data of Cramer and Horning (Lancet 1:72, 1938) and Haagensen and co-workers (Proc. Soc. Exper. Biol. & Med., 48: 820. 1940). The conclusion drawn is that both experiments, when carefully examined, show the treatment of Paris R III mice with anterior pituitary hormone to be effective in preventing the development of spontaneous mammary cancer.—L. L. W.

Viruses


Observations were made on the adrenals of two strains of mice, the Dba strain which has a high incidence of mammary tumors, and the C57 black strain in which mammary tumor incidence is low. Virgin females, males, castrated females, and castrated males were used. Castrations were performed at 1, 21, and 41 days of age. A difference between the x-zone of virgin females of the Dba and C57 black strains was noted. The x-zone of the Dba mice persists for over 200 days and its regression is accompanied by vacuolization. The x-zone of the C57 mice on the other hand undergoes complete regression by 100 days without vacuolization. The x-zone of the male and female mice castrated before puberty resembles the virgin females of the same strain. The x-zone of the hybrid between the Dba and the C57 strains resembles the JAX Dba parent. Hybrids having the C57 mother seem to show a larger x-zone than those having a Dba mother.—Author’s abstract.
GENETICS


The incidence of spontaneous tumors of the mammary gland, liver, and lung in a subline of strain C3H mice maintained at the National Cancer Institute is reported. Of 1,558 breeding females, 91.37 per cent developed spontaneous mammary gland tumors at an average age of 8.58 months. Of 350 virgin females, 97.43 per cent developed spontaneous mammary gland tumors at an average age of 10.43 months. Of 141 females over 1 year of age, 9.95 per cent developed spontaneous hepatomas and 4.35 per cent developed pulmonary tumors. Of 320 males over 1 year of age, 26.87 per cent developed spontaneous hepatomas and 7.81 per cent developed spontaneous pulmonary tumors.

This subline of strain C3H mice is highly susceptible to spontaneous mammary gland tumors and spontaneous hepatomas but relatively resistant to spontaneous pulmonary tumors. Other types of spontaneous neoplasms are uncommon. This colony is regarded as a separate line of C3H mice, and results obtained with it are not directly comparable with those procured with other lines of strain C3H mice.—From author's summary.


Male and female mice, in which tumors produced by intradermal inoculation of sarcoma S 37 disappeared spontaneously, were reinoculated intradermally or intraperitoneally with the same tumor. Practically all females were resistant to reinoculation, whereas one-fourth of the males developed tumors.—Author's summary.


This valuable review covers the development of genetic research on cancer incidence in human and animal material. Certain types of animal tumors considered separately are mammary epithelial tumors, epithelial pulmonary tumors, nonepithelial tumors, and leukemias. The author points to the evidence that general factors such as coat color and hybridization may, in certain cases, influence the incidence of spontaneous tumors in mice. The bibliography includes 82 references.—L. L. W.


A group of 46 induced sarcomas obtained by subcutaneous injection of 20-methylcholanthrene and 9,10-dimethyl-1,2-benzanthracene were transplanted into rats of heterogeneous ancestry. Over half of the tumors were lost by failure to grow in the first passage, and the remainder showed marked variability in the number of takes and the rate of growth. One tumor, a fibrosarcoma (No. R-9), was selected on the basis of transplantability, since it had given about 55 per cent of takes in the first four passages. During subsequent passages of this tumor, susceptibility and resistance to it tended to be manifested in litters and was not a haphazard distribution in individual rats. Separation of the colony into two strains was made by inbreeding animals from susceptible or resistant litters. Of the 263 resistant-strain rats receiving transplants, 71 (27.0 per cent) grew tumors, and of the 1,038 implants made into these animals 187 (18.0 per cent) grew. However, of the 218 susceptible-strain rats into which transplants were made, 216 (98.8 per cent) grew tumors, and of the 892 transplants made, 823 (92.5 per cent) grew. The response observed was hereditary, but was obtained at once by selection of litters rather than by progressive selective breeding.—Authors' summary.


Tolerance to the lethal effects of salicylaldehyde parallels and may be used as a measure of the intrinsic susceptibility which partly determines carcinoma of the mammary gland in mice.

The genetic factor or factors may exert an influence on the individual prone to have carcinoma of the mammary gland at some future time apparently in two directions: (1) the development of the physiologic state of the organism of which tolerance to the lethal effects of salicylaldehyde may be an index and (2) the physiologic state of the mammary gland as influenced by hormones.—Author's summary.


In a series of 800 mice of the NH descent injected with 1 mgm. of methylcholanthrene dissolved in 0.1 cc. of sesame oil at 60 days of age, 45 showed definite evidence of carcinoma of the mammary glands. These carcinomas all occurred in female mice only. In addition to these neoplasms, hyperplasia of mammary tissue and squamous metaplasia of mammary tumors were also found in the treated animals. These types of mammary tissue response occurred separately and in combination with other types of tumor, such as (1) spindle cell sarcoma, (2) carcinoma of the skin, and (3) rhabdomyosarcoma. Mice of the NH descent are characterized by showing a high resistance to spontaneous tumors of mammary origin.—Authors' abstract.

PHYSICAL FACTORS


Mice bearing transplanted sarcoma 180 were exposed to high and low body temperatures with and without previous roentgen irradiation. Temperature changes alone had little effect on the number of takes or regressions although tumors grew more slowly in mice living in an environment at 4°C. The number of regressions was increased when the tumors were exposed to X-rays and further increased when this was followed by daily bouts of fever induced by ultra short radio waves. The number of regres-
sions was decreased when x-radiation was followed by exposure of the host to a cold environment.

Tumor fragments kept frozen in vitro at \(-70\, ^\circ\) C. for 7 days showed good growth capacity on subsequent transplantation.—C. E. D.

Radiation


The author produced multipolar cleavage in the eggs of Arbacia punctulata by roentgen irradiation of either the eggs or the sperm before fertilization. Excellent photomicrographs and drawings illustrate the distortion of spindle pattern and the unequal distribution of chromatin. Unequal distribution of genic material during mitosis generally leads to cell death but may result in viable daughter cells with altered characteristics. A somewhat similar change, generally described as a somatic mutation, is often invoked to explain the origin of spontaneous tumors. The evidence for and against this theory is discussed. The burden of evidence supports the view that the induction of cancer involves changes in the hereditary complex of the tumor cells. Environmental factors may affect the incidence of such changes as is shown by Bittner's work on the foster nursing of mice and the induction of tumors by chemical carcinogens. Multipolar cleavage is held to have possible relation to the production of cancer by radiation since it involves modification or loss of hereditary substances in the daughter cells.—C. E. D.

Biochemistry and Nutrition—Chemotherapy


Groups of rats and mice were fed with a variety of repeatedly heated fats in addition to an adequate basal diet. Within a year signs of avitaminosis A appeared among the rats, and cases of ulceration and papillomatosis of the forestomach were observed among those that died. Control animals fed with unheated fats and the same basal diet showed no gross pathological alterations. Extracts of the livers of rats fed with lard heated under various conditions in addition to an adequate basal diet contained reduced amounts of vitamin A as compared with controls. Multipolar cleavage was observed among those that died. Control animals fed with unheated fats and the same basal diet showed no gross pathological alterations. Extracts of the livers of rats fed with lard heated under various conditions in addition to an adequate basal diet contained reduced amounts of vitamin A as compared with controls. Additionally, the addition of raw carrot to the diet prevented the occurrence of gastric lesions, thus tending to confirm the association between these and the general condition of avitaminosis A.

The authors regard the results as correlating those of Cramer and of Passey with some of the observations made by Flibiger; and they suggest that the induction of papillomatosis in their experiments is related to the presence in repeatedly heated fats of a factor which interferes in some way with the absorption or metabolism of vitamin A.—A. H.


When normal and malignant homologous tissues from the identical animal host were studied, the malignant form exhibited much lower cytochrome system activity than the benign form. This was true of hepatoma, mammary cancer, rhabdomyosarcoma, and sarcoma 180 in mice. Sarcoma 180, which had been rendered slow-growing through "immunization" of the host, nonetheless reacted like other malignant tissues.

These findings suggested that the method might be used in clinical cases to supplement microscopic diagnosis of human tissue. A few examples were given, involving human skin, human breast, and human leukocytes. The white blood cells from human leukemia reacted like normal tissues.

Rabbit papilloma induced by the Shope virus behaved like normal skin for several weeks, and then lost much of its cytochrome system activity.—Authors' abstract.


The presence of an enzyme in certain tissues, and in the milk and sera of certain species, which catalyzes the depolymerization of sodium thymonucleate has been established. This was done by studying the effect upon the viscosity and streaming birefringence of the thymonucleate when mixed with various tissue extracts or body fluids. The rate of diminution of these specific properties served as an index of enzymatic activity. Tissues were studied in pairs: (1) transplanted hepatic tumor in rats and normal and regenerating rat livers; (2) spontaneous mammary tumors and lactating and hyperplastic mammary tissues (induced by stilbestrol). All of these tissues contained thymonucleodespolymerase. The hepatic tumor tissue showed less activity than its normal control, but the mammary tumor tissue was more active than either lactating or hyperplastic breast tissue. The enzyme was found to be present in the milk of the rat, mouse, rabbit, and guinea pig but absent in human milk and that of the cow, goat, and mare. It was found in the serum of the mouse, rabbit, dog, and guinea pig, but was absent in human and horse serum.—L. L. W.


Fleischmann baker's yeast (S. cerevisiae) was suspended in isotonic salt solution or water, sealed under a quartz coverslip on a quartz slide, and irradiated continuously on a microscope stage with the radiation (\(\lambda=2800\) A.) employed as the microscope illuminant. Photomicrographs were taken at 15-minute intervals. They showed a progressive increase in the ultraviolet absorption of the cells during irradiation.

Since the wave lengths employed were in the range highly absorbed by purines and pyrimidines, the results are interpreted as indicating the production by the injured cells of "nucleic-acid-like materials," which may function...
as growth promoting factors ("intercellular wound-hormones").

It is suggested that the release of such substances into a tumor mass and the surrounding tissues following irradiation may be a significant factor therapeutically.—R. J. L.

Cytology


The number of giant cells per unit area of sections of the spleen was estimated in normal mice and in mice bearing (1) subcutaneous grafts of a 1,2-benzanthracene-endo-succinate tumor, (2) subcutaneous grafts of Mal. sarcoma, (3) intraperitoneal grafts of Mal. sarcoma, (4) primary sarcomas from grafted lymph glands, (5) primary Mal. sarcomas, (6) primary Mal. filtrate tumors. Spleens of x-irradiated mice and of mice under treatment with the endo-succinate of 1,2,5,6-dibenzanthracene were also examined.

In mice bearing subcutaneous grafted sarcomas the splenic giant cells are increased to two or three times their normal number, in contrast to mice bearing intraperitoneal grafts, in which these cells are decreased to one-sixth of their normal number. Mice bearing primary induced sarcomas or receiving injections of a carcinogenic compound show about half the normal number of giant cells. X-irradiation greatly diminishes the number of giant cells in the spleen.

A significant degree of correlation was found between the blood leucocyte counts and the splenic giant cell counts in mice bearing subcutaneous grafted sarcomas or primary induced sarcomas.

Extramedullary myeloipoiesis, and changes in the lymphoid tissue of the spleens of mice bearing tumors and under other conditions are described.

The induction of 2 sarcomas by injection of the endo-succinate of the relatively noncarcinogenic hydrocarbon 1,2-benzanthracene with concurrent x-radiation of the mice is reported.—F. L. W.

Miscellaneous


This tumor strain was obtained by Gye from a spontaneous tumor. A full account is given of the changes in transplantiability, rate of growth and histological structure in successive generations. The original tumor was transplanted more readily in female (46%) than in male (28%) rats; when the sarcomatous transformation took place this sexual difference ceased. The acini showed secretory activity during pregnancy. Squamous metaplasia occurs, especially in male rats, and sebaceous metaplasia was observed in 3 female rats. There is a tendency for fibrous tissue to outgrow the epithelial cells of these adenomas, and this may continue until the epithelium has wholly disappeared and the tumor takes on the appearance of a fibroma or sarcoma, which is transplantable, but none of these tumors have shown invasion of normal tissues or formation of metastases.—E. L. K.

Clinical and Pathological Reports

Etiology


This is a synopsis of the available experimental data on the etiology of malignant disease, so designed as to be of interest to the general physician.—M. J. E.


Two cases illustrating a possible relationship of trauma to malignant tumors are recorded. A patient was struck on the thigh with a 100-pound container of white lead. Some swelling resulted after 2 weeks, but 9 months later the part was invaded by an extensive synovial sarcoma. A full account is given of the changes in the tumor mass and the surrounding tissues following irradiation may be a significant factor therapeutically.—R. J. L.


This paper is concerned with facts relating to consanguinity in marriage deduced from an examination into the parentage of in-patients of general hospitals and neurological and cancer hospitals in England and Wales. The consanguinity rate amongst the parents of sufferers from cancer, considered as a single disease, is no greater than has been noted amongst the total of parents of the general hospital population. When, however, the parental consanguinity rate is analyzed for different types of cancer, suggestive figures for cancer of the uterus are particularly striking. Thus the percentages of first cousin and consanguineous marriages amongst the parents of the general hospital population are 0.606 and 0.789, respectively, but for cases of uterine cancer, 1.609 and 1.724, respectively. These figures are suggestive of a recessive factor being operative.—R. J. L.


The incidence of twinning, whether measured by the percentage of families with twins or by the ratio of twin births to total births, is similar and consistently high in families of patients with ovarian dermoids, of patients with