Abstracts

Reports of Experimental Research

Carcinogenic Compounds


Five dimethylandanthrenes (1,2, 1,3, 1,4, 2,3, 9,10-) were tested for carcinogenic activity by applying to the skin, by subcutaneous injection and implantation, in mice. The 9,10 compound alone produced epitheloma of the skin, and adenomas of the lung were most abundant in mice receiving this compound. None of the 5 compounds produced tumors of the subcutaneous tissue. The results indicate: 1. the importance of substitution in the 9,10-positions as a source of carcinogenic activity, and 2. the inadequacy of subcutaneous injection as a sole test for carcinogenic power.—Authors’ summary.


Of 586 mice which developed tumors as a result of the application of methylcholanthrene for 2 months, 92% did not develop them until after the carcinogenic treatment had been discontinued.

Methylcholanthrene was applied to the skin of mice in amounts insufficient to produce tumors in the majority of the animals, and methylcholanthrene, benzpyrene, dibenzanthracene, or benzanthracene were then applied for relatively short periods of time. Benzanthracene failed to increase the incidence of tumors, but the other hydrocarbons exerted additive effects which were equivalent to the known carcinogenic potencies of the compounds for mouse skin.

When methylcholanthrene was applied to the skin of mice for 2 months, and intervals up to 4 months allowed before the application of hydrocarbon was resumed for 1 month more, many tumors developed as a result of the repainting procedure.

These observations suggest that the initial application of hydrocarbon had produced subcarcinogenic alterations in the tissue. An attempt has been made to correlate the results with an hypothesis that carcinogenesis is essentially an accumulation of abnormal protein within the cell.—Authors’ abstract.


A cod liver oil concentrate prepared by saponification and extraction with ethylene dichloride has not been carcinogenic when injected into mice.—Author’s summary.


Three series of female Swiss mice, totaling 123 animals, were painted on a large area of the skin of the back with methylcholanthrene dissolved in acetone and in benzene, respectively. The carcinogenic response to a 0.3% solution of methylcholanthrene was better in acetone than in benzene solvent. Mice painted with a 0.3% solution of methylcholanthrene in acetone 3 times a week and with pure benzene on alternate days developed cancer even more rapidly.

The application of solutions to the ears of a rabbit showed that the hyperemia after the application of methylcholanthrene in benzene was a combined effect of the carcinogen and of its solvent. The less pronounced hyperemia after the application of methylcholanthrene in acetone was caused by the carcinogen alone, since pure acetone did not produce appreciable hyperemia.—Authors’ abstract.

Hormones


Estradiol in sesame oil was administered in a total dose of 3.5 mgm. per mouse intermittently over a 12-month period to groups of castrated and intact male Marsh-Buffalo mice. In the castrated mice the incidence of lymphosarcoma was increased significantly above that occurring in both estrin-treated and control intact mice. No mammary gland tumors appeared in the series of intact males and only 6 per cent (2 tumors) developed in the castrated group, which had received estrin. To date carcinoma of the breast has not been produced in intact male Marsh-Buffalo mice by the administration of estrogens over a long period of time either in toxic or nontoxic doses.—Authors' abstract.


When tablets or pellets of estradiol and synthetic desoxycorticosterone acetate are implanted simultaneously under the skin of the female guinea pig, the fibromatogenic action of the estrogen can be inhibited by equimolecular quantities of the adrenal cortical steroid. This holds only for sufficiently high levels of the fibromatogenic estrogen; at lower levels the equimolecular ratio often loses its inhibitory action and the quantity of the antifibromatogenic steroid necessary to prevent fibroids is then a multiple of the tumorigenic one. This may be explained by the assumption that the inhibitory effect depends also
upon a threshold dosage of the antitumorigenic steroid. Ineffectiveness of very small pellets may depend also on a prematurity more or less complete absorption of the source of the antitumorigenic steroid.—Authors’ summary.


The antifibromatogenic threshold, or the minimum quantity which must be released per day by a subcutaneous tablet of the steroid so as to inhibit fibroids, was lowest with progesterone; desoxycorticosterone (acetate) was next in order; testosterone (propionate) was highly active but less than progesterone and desoxycorticosterone. Androstenediol (propionate) had no antifibromatogenic action.

The diminution of the average tumorigenic effect and other quantitative criteria of antifibromatogenic action were in accord with the statements referring to the antifibromatogenic threshold.

The descending order of antifibromatogenic faculty of different steroids was coincident with their antiestrogenic activity in the rabbit. Species differences prevent generalization of these results.

Antifibromatogenic activity of steroids is apparently reinforced by the ketonic oxygen in position 3 or by the double bond at position 4-5 in ring I, or by both of these, and by the carbons attached in position 17 (the short side chain of carbons 20 and 21).—From authors’ summary by S. B-J.

GENETICS


Strain A mice were crossed with strain W mice containing the shaker-2, waved-2, and flexed-tail genes. The F1 generation of this mating were backcrossed with the parental strain W mice. The resulting generation was classified as to whether the mice were shaker or normal, waved-coated or normal-coated, or flexed-tailed or normal-tailed. These animals were all inoculated with 20-methylcholanthrene and killed for observation 16 weeks later. The mice bearing the normal alleles of shaker-2 and waved-2 genes displayed a higher incidence of induced pulmonary tumors than the shaker-waved group. A similar but more striking difference was observed between the shaker animals and the nonshaker animals irrespective of the waved-2 gene. The nonshakers bearing the normal allele from the A strain showed higher susceptibility than the shakers. On the other hand, if they were classified as to the waved-2 gene, the normals and waved-2 cross showed about the same susceptibility. There was a marked decrease in susceptibility of the flexed-tail animals over the normal-tailed animals bearing the dominant character from the susceptible strain A mice. This is evidence that the shaker-2 and flexed-tail genes are associated with chromosomes which in turn are associated with the susceptibility factor. Similar differences were noted when these crosses were compared as to the number of pulmonary nodules produced.

A similar experiment was made using waltzer and waved-1 genes. No significant evidence was found to associate these genes with susceptibility to induced pulmonary tumors.—R.C.R.


The degree of resistance against progressive growth of inoculated malignant cells characteristic of certain refractory strains of mice has been reduced by foster nursing of the refractory mice by mothers of the susceptible strain. The influence present in the milk of Dba subline 2 mice has been shown to affect the growth of two transplantable lymphoid leukemias, line L449 and line P1534. On the other hand, foster nursing was shown to be ineffective in influencing the growth of a myeloid leukemia, line L493.

The “susceptibility” influence apparently is present in the milk of lactating females during the first 6 or 7 days of the lactation period. Data are not as yet available to determine the presence or effectiveness of the susceptibility influence during the latter part of the lactation period. Total deprivation of susceptible milk in young of the susceptible strain followed by foster nursing by females of the refractory strain does not affect the response of these mice to inoculations of leukemic cells.

A change in the growth capacity of the malignant cells of the two lymphoid leukemias, line L449 and line P1534, and of the myeloid leukemia, line L493, whereby leukemic cells can be grown by serial transfer in refractory mice has been observed. This occurs following initial growth of the malignant cells in refractory foster-nursed mice.—Author’s summary.


Three myeloid tumors produced by subcutaneously transplanting the buffy coat, particles of lymph nodes, or spleen of dilute brown mice with induced leukoses were found to be 100% transplantable in Bagg albino mice. In the dilute brown mice, as the tumor grew, a greenish fluid collected around the tumor and bilateral edema and ascites developed. The area of injection was characterized grossly by a flat, wide, hemorrhagic tumor with edema and ascites developed. A similar picture except for absence of ascites was seen in the Bagg albino strain with transplants.

When the tumors remained in the dilute brown mice longer than 8 days the animal died. In the Bagg albino mice, however, the smaller tumors began to regress on the 8th day and had entirely disappeared in a few days thereafter. The larger tumors in this series of mice continued until the 10th day and then regressed. The
The occurrence of malignancy late in life was dominant over longevity was a dominant; it was also found that the strain which still showed a high incidence of carcinoma, the extremes. Crossing a sarcoma strain with one in which carcinoma occurred resulted in hybrids that demonstrated a medium incidence of both carcinoma and sarcoma. The readiness with which the mammary glands pass from the first phase, that of resistance to growth, to a phase of preparatory growth and that the difference in the readiness with which the cancerous phase is reached depends not only on the readiness with which the mice pass from the stage of preparatory growth by way of a precancerous stage to the fully developed carcinoma but also on the readiness with which mice pass from the first phase, that of resistance to growth, to the preparatory growth phase and on the rapidity with which they pass through the stage of preparatory growth. The readiness with which the mammary glands of various strains respond with the progressive growth that is characteristic of the preparatory growth phase to long-continued stimulation by endogenous or exogenous estrogenic substances is at least one of the factors determining the difference in the hereditary tendency of various strains of mice toward the development of mammary gland carcinoma.—Authors' summary.

A statistical study of 756 mice, in which a microscopic examination of sex organs and certain other organs was made, confirmed the conclusion that the various strains of mice that reach the stage of cancerous growth of the mammary gland pass first from the phase of resistance to growth to a phase of preparatory growth and that the difference in the readiness with which the cancerous phase is reached depends not only on the readiness with which the mice pass from the stage of preparatory growth by way of a precancerous stage to the fully developed carcinoma but also on the readiness with which mice pass from the first phase, that of resistance to growth, to the preparatory growth phase and on the rapidity with which they pass through the stage of preparatory growth. The readiness with which the mammary glands of various strains respond with the progressive growth that is characteristic of the preparatory growth phase to long-continued stimulation by endogenous or exogenous estrogenic substances is at least one of the factors determining the difference in the hereditary tendency of various strains of mice toward the development of mammary gland carcinoma.—Authors' summary.

The author feels that this is evidence for the existence of mammary cancer could be eliminated in a certain strain which still showed a high incidence of carcinoma. The author's belief that radioactive phosphorus is a useful therapeutic agent in leukemia and lymphosarcoma, but that its value in other forms of cancer cannot be determined until more time has elapsed.—Author's abstract.

Inasmuch as mammary cancer could be eliminated in a certain strain which still showed a high incidence of carcinoma, the author feels that this is evidence for the existence of separate factors for the occurrence of malignancy and for its site of origin.

In studying hybrids between short and long-lived strains, longevity was a dominant; it was also found that the occurrence of malignancy late in life was dominant over the tendency to have malignancy early.—H. B.
Small doses of different carcinogenic agents, each given during separate periods, were administered to mice or rabbits in order to determine whether their carcinogenic activities were additive. The carcinogenic process initiated by the application of benzpyrene, methylcholanthrene, or 9,10-dimethyl-1,2-benzanthracene to the backs of mice could be completed by either of the other two hydrocarbons. Ultraviolet light did not increase the number of tumors produced in mice rendered precarcinogenic with methylcholanthrene or 9,10-dimethyl-1,2-benzanthracene, and the hydrocarbons failed to increase the number of tumors in animals which were precarcinogenic with respect to irradiation tumors. Certain hormones, vital dyes, and other substances likewise failed to increase the number of tumors induced by ultraviolet irradiation. Ultraviolet irradiation and x-rays both failed to increase the carcinogenicity of the Shope papilloma virus.—Authors' abstract.

Biochemistry and Nutrition—Chemotherapy


By means of the Carr-Price reaction (development of blue color produced by the reaction of the vitamin and antimony trichloride) the levels of vitamin A in the plasma of patients with gastrointestinal cancer were found to be below the normal range in 86% of the patients examined.

The plasma vitamin A of 62 normal males was 170 ± 38 U.S.P. units per 100 cc. and the plasma carotene was 0.12 ± 0.13 mgm. per 100 cc. For 62 normal females these values were 149 ± 46 U.S.P. units per 100 cc. for plasma vitamin A and 0.18 ± 0.10 mgm. per 100 cc. for carotene. As long as the individuals in this study, both normal and those with cancer, remained untreated and on the same diet, their plasma levels of vitamin A never varied more than ±10 U.S.P. units from day to day.

In contrast to the above were the levels in the plasma of 38 males with cancer of the gastrointestinal tract: vitamin A ranged from 32 to 186 U.S.P. units per 100 cc. and averaged 84 U.S.P. units, and carotene ranged from 0.04 to 0.50 mgm. per 100 cc. and averaged 0.14 mgm. Thirteen female patients with cancer of the gastrointestinal tract provided similar findings: vitamin A ranged from 50 to 148 U.S.P. units per 100 cc. and averaged 78.5 U.S.P. units, and carotene ranged from 0.5 to 0.27 mgm. per 100 cc. and averaged 0.135 mgm.

The explanation for the low plasma levels of vitamin A is thought to be an inadequate ingestion and absorption of the vitamin, or an hepatic dysfunction in connection with the storage of vitamin A or its formation from carotene. A dietary deficiency or malabsorption could not account for the low plasma vitamin A values.

Patients who have had successful resection of gastro-intestinal cancer have a lower incidence of reduced plasma levels of vitamin A than do patients in whom gastro-intestinal cancer is still present. The administration of yeast, lipocar or choline chloride, themselves free of carotenoids, raised the reduced plasma vitamin A levels of the patients with gastrointestinal cancer.

Patients with other malignant diseases (lymphomas, cancer of the head of the pancreas, and bone sarcoma) also showed a high incidence of low plasma vitamin A levels.—J. L. M.


Chemical assay of glycogen in 92 Walker 256 tumors in intact and hypophysectomized rats yields values that are inversely correlated with tumor size and rate of growth. Elimination of the effect of hypophysectomy did not alter the correlation between glycogen content and rate of growth. The visible periphery of the tumor and the tumor as a whole, including the necrotic center, yield glycogen values that are almost identical and which show a high degree of positive correlation. Chemical methods appear to demonstrate the presence of glycogen in amounts greater than would be inferred from histological preparation where particulate (undissolved) glycogen seems to be the only form shown.—Authors' abstract.


The influence of repeated biweekly intraperitoneal injections of methylcholanthrene and phenanthrene for 18 weeks was without effect upon the hepatic vitamin A stores in mice, although tumors and ascites were induced by methylcholanthrene. Large doses of phenanthrene, 3,4-benzpyrene, methylcholanthrene, and 1,2,5,6-dibenzanthracene were also without effect upon the hepatic vitamin A stores when the latter were maintained at a high level by the oral administration of vitamin A to mice.

The intraperitoneal administration of large doses of 3,4-benzpyrene and methylcholanthrene to rats induced a marked lowering of the hepatic vitamin A content which was much greater than the inhibition of growth. The inhibition of growth by phenanthrene somewhat paralleled the slight decrease in the hepatic vitamin A content.—Author's abstract.


The citric acid content of numerous tissues was found to be as follows (mgm./100 gm.): Rabbit tissues: Brown-Pearce carcinoma 136; (? same rabbit) liver 2.8, skeletal muscle 2.5, kidney 6.6, whole brain 4.6. Rat tissues: Walker No. 256 carcinoma 16.6, 18.4; Guérin tumor 16.8, liver (same animal) 3.0. Mouse tissues: Crocker No. 180 sarcoma 14.2, 14.3; mainly necrotic part of a Crocker tumor 12.3, mainly necrotic part (same animal) 7.7; normal tissues (probably different animals from tumorbearers) skin 12.2, fur 133, seminal vesicles 128, skeleton 46. Guinea pig tissues: liver 1.6, kidney 3.9, whole brain.
The fate of dibenzanthracene was investigated in mice, rats, rabbits, dogs, and monkeys by chemical and spectroscopic methods. In the excreta of mice, rats, rabbits, dogs, and monkeys by chemical and spectroscopic methods.

If these pure aromatic substances are mixed and subjected to the fractionation procedures, the resulting absorption bands in the different fractions are in similar positions to the absorption bands from the same fraction obtained from the urine.

Thus far, we have had occasion to study some 150 different urines from 48 normal and diseased individuals, and have not encountered appreciable variations from the findings presented. Possibly the same sort of separation and classification will enable a clearer picture of the absorption spectra of other body fluids to be made. — Authors' summary.

The fate of dibenzanthracene was investigated in mice, rats, rabbits, dogs, and monkeys by chemical and spectroscopic methods. In the excreta of mice, rats, and rabbits, evidence was obtained spectroscopically for the presence of phenolic derivatives of dibenzanthracene. In all species investigated, no suggestion of a conjugated derivative of dibenzanthracene has been found. The presence of dibenzanthracene and derivatives was investigated spectroscopically in tissues.

The phenolic derivatives of dibenzanthracene excreted by mice, rats, and rabbits were isolated and the physical and chemical properties showed that the compounds excreted by mice and rats are identical. Evidence is presented for the identity of the dibenzanthracene metabolite excreted by mice and rats with 4',8'-dihydroxydibenzanthracene. Rabbits excrete a dihydroxydibenzanthracene which is not identical with that excreted by mice and rats. The derivative excreted by rabbits is without carcinogenic activity when injected into mice. The biological significance of the conversion of dibenzanthracene to phenolic metabolites is discussed.

Authors' summary.


A method for the treatment and preliminary separation of the ether-soluble substances normally present in urine into groups suitable for the study of absorption spectra is described. Following such a separation, absorption spectra do not present the confused picture of overlapping bands which has been found in the past with whole urine. While no claim is made for complete identification of any one substance, its probable nature may be inferred from comparison with the absorption spectra of certain aromatic substances which are known from previous chemical study to be present in normal urine.

If these pure aromatic substances are mixed and subjected to the fractionation procedures, the resulting absorption bands in the different fractions are in similar positions to the absorption bands from the same fraction obtained from the urine.

Thus far, we have had occasion to study some 150 different urines from 48 normal and diseased individuals, and have not encountered appreciable variations from the findings presented. Possibly the same sort of separation and classification will enable a clearer picture of the absorption spectra of other body fluids to be made. — Authors' abstract.


Fowl-pox lesion or epithelioma, liver, and, in some experiments, skin were removed from chicks from 7 to 16 days after inoculation with fowl-pox virus. Thin sections of each tissue were placed in Ringer-phosphate, Ringer-bicarbonate, or Ringer-acetate solution to which dextrose and sulfanilamide were then added in 0.2% and 0.02% concentration respectively. After shaking for 5 hours at 39°C in the Warburg apparatus the fluid in each flask was analyzed for free and total sulfanilamide. The conjugating capacity (mgm. of sulfanilamide conjugated per mgm. dry tissue) of the fowl-pox lesion was significantly greater than that for normal skin and of the same magnitude as that for liver. Since the fowl-pox lesion has been reported to possess the ability to split glucose both anaerobically and aerobically and lactic acid is the probable precursor of the acetic acid shown to be necessary for conjugation of sulfanilamide, such conjugating capacity should be considered in connection with malignant tumors. — F. L. H.


A study has been made of the nature and incidence of crystalline material found within or associated with the phagocytic cells of the lungs of mice of the Swiss strain. The crystal substance proved to be a protein.

A few crystals are found in stock mice 3 weeks old. The number increases with age, giving a correlation coefficient, r_{cr}, of 0.64. There is a still closer correlation between the quantity of crystals and the volume of spontaneous pulmonary tumor (r_{cr}=0.76). In mice injected subcutaneously with 1,2,5,6-dibenzanthracene, the incidence of both crystals and tumors is increased. However, the rise in crystals does not occur until several months after the development of multiple tumors.

Crystals were found in the lungs of mice of strains other than the Swiss. The incidence in these strains does not show a positive correlation with the incidence of lung tumors. Mice of the Bagg albino and A strains, both relatively susceptible to lung tumors, show crystals in a rather small percentage of individuals. There is some evidence that the quantity of crystals is increased in Bagg mice by injection with 1,2,5,6-dibenzanthracene.

Attempts to influence the occurrence of crystals by intranasal inoculation of extracts of lung tissue have so far been without effect, and the tumor incidence in inocu-
lated and uninoculated mice was approximately the same. Further experiments along this line are in progress.

The occurrence of consolidation of portions of one or more lobes of the lung in inoculated mice suggests the presence of an agent which might account for the condition of chronic inflammation with which the crystals are associated. The agent did not become lethal on repeated passage.

The possible relations of the crystals to inflammation and tumors are discussed. From the evidence as it stands no positive correlation between any of these factors can be assumed.—Author's abstract.


Analyses of serum proteins by means of the salting-out technic (Howe method) and electrophoresis (Tiselius method) were carried out in normal adults, in patients with hyperproteinemia due to various chronic infections or to cirrhosis, and in patients with multiple myeloma.

The following results were obtained when the serum proteins of 38 cases of multiple myeloma were partitioned by the Howe method. In some cases hyperglobulinemia resulted from an increase in both eglobulin and pseudo-globulin I fractions. These increases were of the same type found in the hyperproteinemia occurring in patients with lymphogranuloma venereum, cirrhosis, sarcoid, and miscellaneous infections. In other cases anomalous results were obtained: the increase involved only the pseudo-globulin I, without any accompanying rise in eglobulin; or the pseudo-globulin II or albumin fractions exceeded normal limits. Electrophoretic analyses in 10 cases of multiple myeloma also gave varied results: an increase in components moving with the mobility of gamma-globulin, of beta-globulin, or of a globulin with an intermediate mobility. These anomalous Howe and electrophoretic patterns were found to be of value in diagnosis.

The significance of these peculiarities is brought out in a study of the analogous salting-out and electrophoretic properties of urinary Bence-Jones proteins obtained from patients with multiple myeloma. The variations found correspond with the anomalies observed in the salting-out and electrophoretic behavior of myelomatous serums.

The addition of urinary Bence-Jones proteins from different patients to normal serum produced the essential characteristics of some anomalous Howe patterns and of all electrophoretic patterns observed in myelomatous serums.

Myelomatous serums fall into 3 groups: (1) Serums with hyperglobulinemia due to an increase in the eglobulin and pseudoglobulin I fractions; electrophoretically, to an increase in the gamma-globulin. The globulin increment in these cases include little or no Bence-Jones protein, and is comparable to that observed in chronic infections. (2) Serums with or without hyperglobulinemia giving a variety of patterns by the salting-out or electrophoretic methods, for the most part due to Bence-Jones proteinemia. (3) Serums of apparently normal composition with respect to serum proteins.—J. L. M.
those which had received the linseed oil. In 2 rats receiving linseed oil, small nodules formed but failed to develop further. The 5 “linseed oil” rats in which tumors grew well showed a lower average proportion of lipoid (skin 44.6%, carcass 33.4% of fat-free dry weight) than that in 2 rats in which only small nodules developed (skin 90.4%, carcass 34.5%). In the fat-starved rats, the percentage of lipoid was lower in those bearing tumors (skin 28.6 to 42.5%, carcass 20.3 to 26.7%) than that in those in which no implantation had been made (skin 65.8 to 98.3%, carcass 30.8 to 46.4%). A considerable lowering of highly unsaturated acid in the skin, but not in the carcass, accompanied the development of tumors.—E. L. K.


The epidermis of rats on fat-free diets is thickened and the stratum granulosum is very distinct; if such a diet were supplemented with unsaturated fatty acids the epidermis was more nearly, or quite, normal.—E. L. K.


A study was made of the effect of the following conditions on the blood hemoglobin level: i. growth of tumor transplants in mice, ii. methylcholanthrene deposits in mice, 3. ingestion of butter yellow by rats in conjunction with diets of Purina dog chow and cooked whole rice and raw carrots.

The results obtained led to the conclusion that tumor tissue has an antagonistic action on the hemoglobin of the host. The hemoglobin level was depressed by the time the implant had grown to measurable size and thereafter became progressively lower until death intervened.

The precancerous condition as induced by methylcholanthrene was associated with a gradual fall in blood hemoglobin concentration in both cancer-susceptible and cancer-resistant mice indicating that precancerous tissue was also antagonistic to hemoglobin in the degree to which it approached the cancerous state.

Ingestion of butter yellow by rats induced a sudden drop in the hemoglobin to a new level in the first few weeks and thereafter it remained constant over a total period of 162 days until hepatoma developed. The hemoglobin of butter yellow-fed rats on a rice-carrot diet dropped to a much lower level than was true for butter-yellow-fed rats on a diet of Purina dog chow.—Authors’ abstract.

Clinical and Pathological Reports

URINARY SYSTEM—MALE AND FEMALE


At the Pondville Hospital, which is devoted to the care of malignant disease, there were but 1 case of carcinoma of the male urethra and 10 cases in the female in almost 19,000 admissions. The treatment of these cases is discussed in detail, and the conclusion drawn that radium treatment is better than surgery in early cases. Metastases to the regional nodes occur relatively late.—H. G. W.


Neoplastic growth in the renal pelvis extends rather to the ureter than to the renal cortex, so complete nephroureterectomy is the procedure of choice. Case report.—H. G. W.


A study based on 60 cases, the oldest patient being but 9 years, the youngest 2 months. This is a marked contrast to hypernephroma, for in this hospital no case has been...