Abstracts

Reports of Experimental Research

CARCINOGENIC COMPOUNDS


A solution of 3,4-benzpyrene in benzene and cyclohexane was irradiated in almost pure monochromatic ultraviolet light of 2,537 Å; the product, soluble in M/100 NaHCO₃, had produced at the time of writing squamous carcinoma of the skin in 4 out of 10 mice in 54 weeks. The active substance, which is unstable, has not yet been identified. Similar products were obtained from cholantherene, 1,3,5,6-dibenzanthracene, phenanthrene, anthracene, and naphthalene.—E. L. K.


Preliminary to the study of early carcinogenesis it was necessary to establish a technic by which cancer of the skin could be produced: (1) in a reasonably short time, (2) in a high percentage of animals, and (3) in the center of the skin area subjected to treatment. With the technic that was adopted as standard, cancer could be induced in 100% of the mice, with low mortality and a latent period of 6 to 24 weeks. More than two-thirds of the cancers developed between the 12th and the 20th weeks and all occurred in the area of the skin to which the methylcholanthrene had been applied. Variations in technic showed that the size of the skin area exposed to the carcinogen was a greater factor in determining the response than either the concentration of the carcinogen, the frequency of its application, or the total amount of carcinogen administered.

The gross changes induced were: (1) epilation of the skin usually within 3 days; (2) dilatation of subcutaneous blood vessels, due both to the carcinogen and to the benzene solvent; (3) the appearance of shallow ulcerations, which healed; (4) the most important gross change—the appearance of swellings of 4 types, (a) elongated, ridge-like elevations that always regressed, (b) small, semiglobular, wart-like swellings that may or may not have been preceded by swellings of the first type, (c) benign tumors that sometimes arose from type b, and (d) malignant tumors. Types a and b are not true tumors. The nature of such swellings can be established only by histologic examination.—F. L. H.


Studies were made of early changes taking place in the skin of 94 mice of the inbred Swiss strain, after cutaneous painting with a 0.6% solution of 20-methylcholanthrene in benzene according to a standard technic. The effects of a single application are compared with those resulting from repeated doses and are considered as proceeding in three phases: (1) traumatization and repair; (2) excessive repair leading to epithelial hyperplasia; (3) development of skin cancer in a hyperplastic area.

Mice in each series showed decided differences in susceptibility to the action of the carcinogen. Factors determining susceptibility and conditions determining carcinogenesis are discussed on the basis of the suggestion that the epithelial proliferation of the skin leading to the development of malignancy is elicited, not by the direct action of the carcinogen, which has a toxic inhibitory effect, but by a hypothetical substance formed in the skin in response to the carcinogen.

Four plates and 3 figures are shown.—F. L. H.


Experiments testing the response of tissue lining the vaginal tract to low doses of a known carcinogen were carried out in a preliminary investigation of the hypothesis that smegma may be carcinogenic.

From 7 to 9 doses of 0.35 cc. of a suspension containing 2.5 gm. of smegma in 25 cc. of lard filtered at 38° C. were injected subcutaneously at intervals of 4 to 8 weeks into the left axillary region of twelve 3 months old mice of the A strain. Abscess formation and ulceration occurred, and the mice died 9 to 15 months after the beginning of the experiment. No tumors were found except 1 adenocarcinoma, which was probably spontaneous.

In 20 mice trauma was induced by inserting a metal plunger into the vagina 130 to 140 times during a 15 to 17 month period. No tumors resulted. Five mgm. of smegma were introduced into the distended vagina of 20 other mice
2 or 3 times weekly for 12 months and less frequently thereafter. Five mice died during the 9th to 12th months after 72 to 115 applications. The rest died between the 14th and 16th months after 130 to 140 treatments. No gross evidence of tumor was found.

In a control experiment 5 mgm. of a 5% solution of 3,4-benzpyrene in cholesterol was introduced similarly into the vaginal canal of 10 mice 2 or 3 times a week. Two mice developed tumors during the 7th month, 6 more developed tumors by 10 months, and all mice had developed them by 14 months. The tumors appeared as papillary growths on the vaginal wall, and finally infiltrated the perivaginal tissues. Microscopically all were squamous cell carcinomas showing no involvement of the cervix. Each of 2 carcinomas was transplanted subcutaneously into 8 mice, but none of the transplants grew, possibly because of infection.

The authors believe that further investigation of the possible tumor-producing potency of smegma is warranted.—F. L. H.


Test papers are prepared in the dark by (1) soaking in aqueous solution of silver nitrate and methylene blue, (2) drying, and (3) soaking in aqueous solution of potassium bromide, and (4) drying. A drop of solution of hydrocarbon in toluene is applied and the paper is exposed to light of 4,200-4,400 Å for 5 minutes. Many carcinogenic hydrocarbons produce a blue-grey spot; non- or weakly carcinogenic hydrocarbons give a negative result.—E. L. K.


The intravenous injection of methylcholanthrene dispersions in which the particles were 101 to 200 in size induced at least 10 times as many pulmonary tumors in strain A mice as similar dispersions in which the particles measured 1.1 to 4.1. Apparently the neoplastic response depended upon the actual amount of the hydrocarbon that lodged in the lungs and not upon the amount injected into the organism. This indicates that the genesis of pulmonary tumors is the result of a local action of the hydrocarbon upon susceptible pulmonary tissue rather than a manifestation of a general systemic response to the chemical.

The lungs of young, small mice of strain A were more susceptible to the induction of pulmonary tumors with intravenous dibenzanthracene than the lungs of old, large mice of the same strain. The amount of hydrocarbon retained in the lungs was the same in both instances, but the smaller lungs of the young mice permitted a higher concentration of the hydrocarbon in the tissue.

The pulmonary tumor reaction was not influenced by foster nursing or by reticulo-endothelial blockade with trypan blue, and was the same whether the hydrocarbon was given in a single dose or in 5 repeated intravenous injections at 3 day intervals.—F. L. H.


Numerous extracts made from overfried and overbaked meats were tested for carcinogenicity in mice by subcutaneous injection and by feeding.

1. The fats from a mixture of beef, pork, and suet that had been overfried were tested by injection, by feeding, and by injection of the nonsaponifiable lipids.

2. The nonsaponifiable lipids from a second mixture of pork, beef, and suet prepared from the raw meat, after overfrying and after overbaking, were tested by injection.

3. The nonsaponifiable lipids from overfried beef were tested by injection.

4. The nonsaponifiable lipids from a mixture of beef, pork, and lamb were tested by injection.

Only one sarcoma was induced at the site of injection of these meat extracts. This occurred in a mouse injected with the benzene-soluble fats from overfried mixed meats.

Pure cholesterol heated to 200° C. and to 300° C. was not carcinogenic on subcutaneous injection into mice. Acrolein also was not carcinogenic. Sesame oil that had been heated to 350° C. was carcinogenic, inducing 3 sarcomas in 9 mice that lived for over 1 year.

These results, taken with those of other investigators, indicate that heated vegetable fats may be potentially more dangerous than the animal fats.—Authors' abstract.


This compound, and the 1'-keto derivative, were synthesized and were found by Haddow to have tumor inhibitory properties of a moderate order.—E. L. K.


Sixty young adult mice of the C strain received weekly 2.5 mgm. of e-aminocazotolueno dissolved in corn oil (mazola). Injections were made subcutaneously into the back, 0.1 cc. of oil being used for each dose. Forty animals were kept on Purina chow, while the remaining 20 received a supplement of dried fresh beef liver (20% by weight). After 11 months, 10 of the surviving mice were autopsied. All but 2 had developed subcutaneous sarcoma at the site of injection. Three of the 10 had been fed with liver and all 3 had sarcoma. While the feeding of liver gave protection against cirrhosis and cancer of the liver, it did not appear to influence the development of local sarcoma.—M. B.


Benzpyrene in the molecular state (i.e. in solution) exhibits a violet fluorescence. The fluorescence is green if the hydrocarbon is in the form of monoclinic crystals, blue for orthorhombic crystals, and yellow for colloidal suspensions. "The fluorescent spectrum of dissolved benzyrene is unchanged in various solvents except for a slight..."
displacement of the entire group of bands.” The absorption spectrum of the blue-fluorescing metastable orthorhombic modification of solid benzpyrene is entirely different from that of the dissolved hydrocarbon. (Cover slips upon which had been evaporated a benzene solution of benzpyrene provided thin crystal films of the hydrocarbon.)

The absorption spectra of the monocline crystal form and of the colloidal suspension can be studied only indirectly. A continuous spectrum is directed on a thin layer of the hydrocarbon so that there is little general absorption. Under these conditions the regions absorbing selectively show fluorescence and the absorption spectrum appears as fluorescent light (“fluorogram”). “The method permits of recording—at the present stage, qualitatively—the absorption spectra of systems which are not suitable for the usual spectroscopic methods.”—I. H.

Hormones


Interstitial cell testicular tumors appeared in 7 of 13 mice of the JK strain that had received weekly subcutaneous injections of 5 mgm. of triphenylethylene for 250 days or more.

Seven of 17 mice of the A strain similarly treated had interstitial cell tumors, one of which has been transplanted for 4 generations. Although it has grown progressively only in mice that have received estrogens for 10 weeks, it has grown for 3 months after the last injection and has continued to grow after hypophysectomy.

One of 14 male C3H strain mice that received triphenylethylene died with a testicular tumor that had metastasized to lymph nodes, spleen, and lungs. Tumors of the testes were not found in mice of the C3H, N, or CBA strains similarly treated. The interstitial tissue was present in increased amounts in only 7 mice of these strains, where it consisted of large, clear, Leydig cells and “brown cells.” Brownish cells and fibroblastic elements filled the small intercellular spaces in the other mice.

Hematopoietic foci occurred in the testicular tumors of all the JK mice and of 3 A mice. Some islands of hematopoietic tissues contained largely erythroblasts and normoblasts. Others showed developing granulocytes and megacaryocytes.—Author’s abstract.


Not only uterine but also extragenital subserous fibroids can be produced in the abdominal cavity of female guinea pigs by injection of estrogens. Fibroids were produced in the mesentery, spleen, pancreas, and abdominal wall. Under certain quantitative and timing conditions of treatment with estrogens, abdominal fibroids were almost always produced in the female guinea pig, seldom in the male, and not at all in the rat. All estrogens, natural and artificial, free and esterified, were shown to be tumor-inducing, even in very small doses. This tumor-inducing action is largely inhibited by the administration of estradiol benzoate or testosterone propionate, and especially by desoxycorticosterone. It cannot be predicted whether the same results will be obtained in women, because of the profound influence of species on the experimental results, but the method of inhibition awaits trial in the hands of the gynecologist.—H. G. W.

Radiation


Strain A mice were exposed to light from a G.E. model F ultraviolet lamp for varying periods. In the small numbers used, the cancer rate in the breeding females was less than in the controls (41% as compared with 80%).

(Note: References to Pierce in text and bibliography should read Pearce.)—A. H.


The paper presents the results of comparative studies on the penetration of ultraviolet radiation into human and mouse skin and the relation of these findings to carcinogenesis. The method consisted in illumination of the epidermis with a collimated beam of monochromatic light from a mercury arc source, and photoelectric estimation of the emerging radiation. Two methods of measuring the emerging radiation are given in detail.

Observations on human (cleared) and mouse epidermis led to the conclusion that the absorption spectra in both cases are of the same general character in the region of 3,000 Å, and that absorption in this region is principally due to protein. On the other hand, uncleared human epidermis transmitted wave lengths shorter than 3,200 Å (the region with tumor-inducing properties) less extensively than did mouse epidermis. The transmission of human epidermis was shown to vary considerably between individuals, and for different parts of the body.

Although mouse epidermis, repeatedly irradiated and thus comparable to well tanned human skin, showed greatly reduced transmission, both irradiated and normal mouse epidermis transmitted more radiation from the tumor-inducing region than any of the samples of human epidermis. These differences in penetration of radiation into mouse and human skin, respectively, are in harmony with the finding that tumors of dermal origin predominate in mice subjected to ultraviolet radiation, while virtually all human cutaneous tumors of exposed areas are epidermal in origin.—F. L. H.


The radiocurability of a transplantable mammary carcinoma in strain A mice was determined as 1% for doses
of 2,500 r and 48% for doses of 5,000 r. The same tumor could be transplanted readily into the F2 generation of a cross between strain A and strain C57. However, the tumors in these F2 mice were cured in 82% of the cases by 2,500 r and in 97% by 5,000 r. Even after allowance is made for the spontaneous tumor regressions, which were observed in 28% of these F2 mice, the radio-cureability of the tumors was much greater in the F2 hosts. Attempts to affect the radio-cureability of mouse tumors by altering the diet of the host or by foster nursing yielded inconclusive results. It is suggested that roentgen irradiation acts to augment natural host resistance to tumor growth.—C. E. D.

Biochemistry and Nutrition


In a previous paper (Cancer Research, 2: 567-570. 1942) it was demonstrated that esterase activity of the serum of two cancer susceptible strains (C3H and A) was higher than that of an insusceptible strain (C57). The esterase content of liver and the excretion of the enzyme were therefore studied. It was found that the livers of these strains were equally potent as regards esterase activity. Excretion of the enzyme, however, was very different in the two strains, being much greater in the insusceptible one. The values expressed in terms of cubic centimeters of decinormal NaOH per gram of the dry tissue were 52.96 cc. for the insusceptible strain, and 17.14 cc. and 17.49 cc. for the C3H and A strains, respectively. No difference appeared in kidney esterase activity. The findings suggest that the difference between the susceptible and insusceptible strains is not in the capacity of the liver to manufacture the enzyme but, probably, in its ability to maintain equivalent levels in the blood. A probable influence of liver metabolism on malignant growth is discussed.—Authors' abstract.


The metabolism of tumors of the liver and skin has been studied in relation to that of their tissue of origin. Two types of liver tumor have been used: (a) malignant liver tumors induced by feeding p-dimethylaminoazobenzene to rats; (b) highly differentiated spontaneous hepatomas of mice.

The skin tumors studied were carcinomas of the human vulva, and these were compared with the intermediate leukoplakia and normal skin of the vulva.

The respiration, glycolysis, and respiratory quotient of malignant liver tumors were similar to those found generally in tumor metabolism; viz., high anaerobic and moderately high aerobic glycolysis combined with an R.Q. below unity. In confirmation of Orr and Stick-land, it was found that in these tumors the glycogenolysis typical of normal adult liver is replaced by glucose breakdown. Precancerous states of the liver induced by p-dimethylaminoazobenzene, showed only a slight increase of aerobic glycolysis, and in some cases a slight anaerobic glycolysis also. The benign spontaneous mouse tumors had respiration, glycolysis, and R.Q. substantially the same as that of the adjacent liver tissue.

The following highly specialized functions were studied in both types of hepatic tumor, and were compared with the surrounding nontumorous liver tissue: formation of urea from ammonia, formation of urea from l(+)-alanine, formation of acetoacetic acid from caprylic acid, oxidation of uric acid, synthesis of fermentable carbohydrate from pyruvic acid.

These highly differentiated functions were entirely, or almost entirely, lost in the malignant tumors induced by p-dimethylaminoazobenzene, though urea synthesis usually persisted at a measurable but greatly reduced rate even after the other reactions were lacking. On the other hand, the spontaneous mouse hepatomas still retained almost intact all the foregoing functions of the normal liver cell.

The respiration and glycolysis of carcinoma of the vulva were typical of those of malignant growths in general, both aerobic and anaerobic glycolysis being high. On the other hand, the leukoplakia showed only a slight increase of both the latter reactions as compared with the normal skin of the vulva.

The interpretation of none of these results is affected by the use of the nucleic acid phosphorus content of the tissue, instead of its dry weight, as a basis for the calculation of the metabolic values.

The loss of special liver functions by the p-dimethylaminoazobenzene tumors is regarded as a reversion to a more primitive type of metabolism. It is suggested that the appearance of glycolysis, aerobically and anaerobically, in this as well as in other tumors, is a metabolic expression of the same primary process of dedifferentiation.—Authors' summary.


The carcinogenic azo dyes show a high degree of specificity in inducing tumors of the liver and hence might be expected to affect certain liver functions. Since carcinogenesis probably involves protein synthesis, the proteins that are produced in the liver and can be measured in the blood offer a means of detecting a precarcinogenic chemical change due to the influence of the dye. Liver cancer was produced in rats by feeding synthetic diets containing p-dimethylaminoazobenzene. Plasma albumin, fibrinogen, and total nitrogen were measured at intervals during the carcinogenic period, and hemoglobin was determined at 6 months. There were no changes in blood proteins that could be associated with the process of carcinogenesis. When the diet contained p-aminobenzoic acid, cirrhosis was greatly reduced without changing the incidence of liver cancer.—Author's abstract.

Benzpyrene was dissolved in aqueous solutions of caffeine and its effect on the activity of a urease system determined. Freshly prepared solutions were relatively nontoxic to the enzyme, but irradiation of the hydrocarbon solution with ultraviolet light produced at least two types of substances that definitely inhibited urease activity. One of these agents could be detected only after prolonged irradiation and presumably was derived from the benzpyrene, while the second inhibitory substance proved to be hydrogen peroxide. Apparently benzpyrene acted as a photosensitizing catalyst in the production of \( {H}_2{O}_2 \) during ultraviolet irradiation.---Authors' abstract.


Under the conditions described a deficiency of cystine, riboflavin, pyridoxin, or choline will induce hyperplasia and ulceration of the forestomach epithelium in rats. Sodium taurocholate or pepsin and hydrochloric acid increased the incidence of lesions in animals fed the poor diets but caused no macroscopic changes in rats fed the stock diet. Neither did limitation of intake of the stock diet cause any visible pathologic change in the forestomach, and the body weight remained constant in young rats. The action of the protective factors is interdependent so that a deficiency of one may prevent effective action by others. On the basis of these facts and the observation that squamous epithelial changes were occasionally observed in areas other than the stomach, it is postulated that the mechanism of formation of the gastric lesions is irritation of abnormally sensitive epithelium by hair, hard food particles, pepsin and hydrochloric acid, or bile.---Author's abstract.

VIRUSES


Domestic rabbits were inoculated with virus protein in scarified areas on the abdomen and sides. The resulting growths were sliced off when they were 1 to 2 mm. high, washed in 0.9% NaCl solution, and ground with sand particles, pepsin and hydrochloric acid, or bile.---Author's abstract.

Cytology


Attempts were made to determine in mice the influence of colchicine upon the mitoses in embryo hair follicles, in adult hair follicles made hyperplastic with methylcholanthrene, and in squamous cell carcinoma induced with methylcholanthrene. At suitable intervals after the colchicine injection, tissues were removed and subjected to ultracentrifugation to study displacement of chromosomal clumps. Other special technics were also employed: Feulgen reaction for thymonucleic acid, mitochondrial stains, micro-incineration for mineral constituents.

The authors concluded that slight differences existed in the "susceptibility to colchicine of mitoses" in the above three categories. Clumping of chromosomes after colchicine injection was most pronounced in cancer cells, less in hyperplastic hair follicles, and least in embryonic hair follicles. In the embryo hair follicles, displacement of chromosome clumps by ultracentrifugation was slightly greater after colchicine than in control tissue; no such effect of colchicine was observed in either hyperplastic hair follicles or carcinoma cells. No definitive findings by the other technics were reported.---H. B.

Miscellaneous


Epidermis can be loosened from the underlying dermis by treating the skin with isotonic ammonia for 35 minutes or by submitting it to 50°C for 2 minutes. The epidermis can then be removed as a complete sheet by blunt dissection. The epidermal cells thus treated adhere again to the dermis if not promptly separated. Epidermis isolated by either procedure continues to consume oxygen, as shown by manometric measurements.---A. C.


A description of an automatic milking apparatus by means of which 0.7 cc. of milk per mouse can be obtained.---R. C. R.