HORMONAL IMBALANCES AND TUMORS OF ENDOCRINE GLANDS. W. U. GARDNER. (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

Tumors of interstitial cells of the testes, pituitary glands, ovaries and adrenal glands have been induced experimentally in animals under conditions of hormonal imbalance produced by the addition of sex hormones, the removal of the sources of intrinsic sex hormones, or the production of excessive gonadotrophins. In most cases genetic factors are of importance in that the tumors are strain-limited. Testicular interstitial cell tumors appear in estrogen-treated mice of the A and JK strains and in their first generation hybrids. The pituitary is assumed to be involved in formation of these tumors. Chromophobe hyperplasias and adenomas occur in estrogen-treated mice of the C57 strain and in their hybrids but rarely in mice of other strains or hybrid groups. The simultaneous administration of androgen partially inhibits their appearance. Whether hormonal mechanisms prevent their appearance in the mice of the resistant strains is not known. Ovarian tumors occur in intrasplenic transplants of ovaries. Under such conditions the ovaries are exposed to excessive intrinsic gonadotrophin, presumably follicle-stimulating hormone, although sex differences exist. The ovarian tumors appearing in roentgen irradiated mice may be explained on a humoral imbalance basis. Adrenal cortical tumors also appear in mice (Woolley) subsequent to gonadectomy at birth or even when older (Gardner). These tumors as well as the testicular interstitial cell and ovarian tumors mentioned above produce physiologically active substances. These tumors will be discussed from some of their genetic and hormonal interrelationships.

COMPARISON OF THE CARCINOGENIC ACTIVITY IN EXTRACTS OF HUMAN LIVER AND OTHER HUMAN AND ANIMAL ORGANS. PAUL E. STEINER, D. WARREN STANGER, and MIRIAM BOLYARD. (Department of Pathology, University of Chicago, Chicago, Ill.)

Ethylene dichloride extracts after saponification were prepared from pooled human livers, kidneys, spleens, hearts and colons. The extracts were made in duplicate from cancer-bearing and noncancer-bearing patients. Similar extracts were made from pooled livers of stillborn infants, swine livers, bovine livers, and swine hearts. The extracts were tested for carcinogenic activity by subcutaneous injection into 1,044 mice of C57 black, A, or our albino strains. The percentage yield of sarcomas at the site of injection in C57 black mice surviving for 6 months was: Noncancerous livers, 58.7; cancerous livers, 14.8; cancerous spleens, 10.4; livers of stillborn infants, 8.1; swine livers tested in strain A mice, 7.5. The other extracts were essentially noncarcinogenic.

THE LOCALIZATION OF STEROIDS IN NORMAL AND CANCEROUS TISSUES BY THE USE OF RADIOACTIVE ISOTOPES AND HISTOCHEMICAL METHODS. S. ALBERT, J. COHEN, R. D. H. HEARD, and C. P. LeBLOND. (Departments of Anatomy and Biochemistry, McGill University, Montreal, Canada.)

By using fuchsin-sulphurous-acid and 2,4-dinitrophenylhydrazine, two reagents supposed specifically for ketosteroids, it can be shown that the histochemical reactions thus obtained in tissues are not suppressed by the removal of ketosteroid-producing organs. In normal and cancerous animals the most intense reactions are found in the ovary, testis, adrenal and accessory sex organs, with little or no reaction in cancerous tissue. It is concluded that these reactions reveal the presence of a non-ketosteroid substance, probably an acetal phosphatide of the plasmalogen family, which may be linked with steroid metabolism.

Using 3-estradiol iodinated with radioactive iodine, it was possible to follow the distribution of this compound in the tissues of cancerous mice by means of the Geiger counter. It was found that after 10 hours the largest concentration of this compound occurred in the gastrointestinal tract, feces and urine, while only minute amounts occurred in the genital organs, accessory sex organs and cancerous tissues.

A PHYSIOLOGICAL MEASURE OF HOST-TUMOR RELATIONSHIP AS SHOWN BY A TRANSPLANTABLE MOUSE RETICULOENDOTHELIOMA. ARTHUR M. CLOUDMAN. (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.)

Measurable physiological changes have been produced by parabiotic operations in mice normally refractory to the transplantable reticuloendothelioma, C198. This strain tumor always involves the liver of susceptible leaden mice that receive subcutaneous implants of tumor tissue. All other pure strains of mice are resistant to this tumor when the usual technics are employed in tumor tissue transfer.
The transfer of some substance or substances through the medium of body tissues and fluids not normally introduced frequently make refractory C57 black strain mice serve as a successful host for tumor C198. After the altered host has progressively grown the tumor for a certain time interval and the mass has become sizeable the tumor itself undergoes a physiological change. After this the tumor can be easily transferred to members of the C57 black strain. However, it will still grow in the leaden mice. Furthermore, whatever tumor change was induced by growth in a black mouse is weakened or lost by growth for one tissue transfer generation in a leaden mouse.

The data presented reveal that (a) parabiosis alters refractory C57 black strain mice, making many of them susceptible to implants of tumor C198; (b) the altered host can change the implanted tumor; (c) this changed tumor can be successfully transferred to other C57 black mice; and (d) C57 black mice growing the altered C198 tumor remain resistant to unaltered C198 taken directly from a leaden donor mouse.

THE NEOPLASTIC TRANSFORMATION OF GRANULOSA CELLS IN GRAFTS OF NORMAL OVARIUES INTO SPLEENS OF GONADECTOMIZED MICE. J. FURTH, and H. SOBEL. (Department of Pathology, Cornell University Medical College, New York, N. Y.)

Growth of granulosa cells were produced in 29 (67 per cent) of 43 mice by grafting fragments of normal ovaries into the spleens of gonadectomized mice as described by Biskind and Biskind. After intrasplenic subpassages into gonadectomized mice, the splenic growth in one of these mice became transformed into a neoplasm readily transplantable into the subcutaneous tissue of normal mice and occasionally metastasizing to the lung (Strain B1). A second transplantable strain (B2) was derived from another mouse that had a splenic growth of granulosa cells with secondary nodules in the liver. This growth proved readily transplantable in the spleen, from which it frequently metastasized to the liver, but not in the subcutaneous tissue. The secondary changes in mice bearing these 2 transplantable tumors indicate the discharge of estrogens by the tumor cells. The blood volume of mice bearing subcutaneous tumors of these strains is elevated and their livers show cavernous congestion characteristic of hypervolemia. These experiments serve to illustrate how hyperplasia of normal cells can lead to neoplasia and enable an analysis of the factors bringing about this transformation.

FURTHER STUDIES ON THE PATHOGENESIS OF THE OVARIAN TUMORS IN MICE. M. H. LI, and W. U. GARDNER. (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

Our previous experiments demonstrated that a pituitary-gonadal endocrine imbalance may be induced by the intrasplenic transplantation of ovaries in castrated male and female mice and that the imbalances may result in the formation of granulosa cell tumors, luteomas, or mixed cell tumors.

Most extensive studies have been made by using inbred mice of the A, C3H, and C57 strains, and several groups of hybrid mice (A X C3H and CBA X C57). Ovarian tumors have appeared in intrasplenic transplants in mice of the A, C3H and C57 strains and in hybrids; there is apparently no strain limitation in the development of ovarian tumors in intrasplenic auto- and homotransplants of ovaries in castrated mice. Ovarian tumors have not been observed in the intrasplenic ovarian transplant in unilaterally gonadectomized male and female mice. The formation of tumors in the intrasplenic ovarian transplants was prevented by weekly administration of small doses of a-estradiol benzoate or testosterone propionate. Similar treatment of progesterone, however, was not effective. Daily injection of a gonadotrophic hormone from pregnant mare serum for short periods exerts a stimulating effect on the growth of the transplants and on tumor formation. These observations are interpreted to substantiate further the assumption that overaction of gonadotrophic hormones is responsible for the genesis of the ovarian tumors in mice.

CORRELATION OF A BIOLOGICAL TEST WITH CLINICAL DIAGNOSIS IN HUMAN MALIGNANCY. HOWARD H. BEARD, SAMUEL L. LIBERT, and B. HALPERIN (by invitation). (Department of Physiological Chemistry, The Chicago Medical School, Chicago, Ill.)

Forty known malignant urines were extracted with an equal volume of alcohol and ether for 2 days in the small Koch extractor. The process was then repeated for another 2 days. Solvents were combined and evaporated under reduced pressure and the water residue diluted so that 2 cc. represented 100 cc. of the original urine. This amount was injected intraperitoneally into immature white rats and the animals were sacrificed from 1 to 4 days later. Litter mates of the same sex and approximate body weight were used as controls without injection. The gonads, spleen and body weight were made soon after death by an overdose of nembutal. The body weight/gonad and body weight/spleen ratios were then calculated for all the animals. In 39 of the 40 known malignant urines these ratios decreased from 20 to 80 per cent and this observation constituted the biological test of malignancy. Nonmalignant urines and those from normal individuals gave ratios that decreased from the control ratios by less than 15 per cent and were considered negative. The average degree of hypertrophy observed was as follows: spleen, 483 to 673 mgm. (39 per cent); male gonads, 1283 to 1991 mgm. (55 per cent), and female gonads, 219 to 365 mgm. (72 per cent). Histological studies showed an intense passive hyperemia of the spleen and intense spermatogenesis in the testes. The female gonads were not sectioned. These results are in agreement with those of Roffo, and Krebs and Gurchot. It is concluded that all malignant urines so far tested contain a cancer hormone (probably of sterol nature) which is the cause of the biological test described above. We believe
that this hormone acts through the pituitary to produce increased amounts of a gonadotrophic hormone which is the immediate cause of the hypertrophy of the spleen and gonads. We are not yet convinced that this is an Aschheim-Zondek test but further work may prove this to be so.

EFFECT OF DIET DEFICIENT IN CERTAIN AMINO ACIDS ON THE INDUCTION OF LEUKEMIA IN dba MICE. JULIUS WHITE, FLORENCE R. WHITE, and G. B. MIDER. (National Cancer Institute, Bethesda, Md., and Department of Surgery, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.)

A comparative study was made of the restriction of cystine, lysine and tryptophane, respectively, on methylcholanthrene-induced leukemia in strain dba mice. Each of the diets employed was so restricted in one of the foregoing amino acids that growth of young mice was prohibited, but indefinite maintenance was possible. The same diets, each supplemented by the amino acid in which it was deficient, permitted good growth. There was no significant decrease in the incidence of leukemia among the mice on diets restricted in either cystine. The data indicate that under the conditions of the experiment, cystine played a role in the development of leukemia not associated with its properties as an essential amino acid for growth but some other attribute not yet determined.

EFFECT OF VARYING THE PROTEIN (CASEIN) CONTENT OF THE DIET ON THE FORMATION OF TUMORS IN THE MOUSE. ALBERT TANNENBAUM and HERBERT SILVERSTONE. (Department of Cancer Research, Michael Reese Hospital, Chicago, Ill.)

Since it is generally believed that protein metabolism may play an important role in the formation of tumors, the effect of different levels of dietary protein (casein) was studied. "Synthetic" diets were utilized and protein levels of 9, 18, 27, 36 and 45 per cent were obtained by substituting casein for cornstarch. All other components of the diets were left unchanged. The modifying effect of the level of protein was evaluated with the carcinogen-induced skin tumor, the spontaneous mammary carcinoma, and the spontaneous hepatoma of the mouse.

No significant effect on either the incidence of induced skin tumors or their average time of appearance was observed. With the spontaneous mammary carcinoma, no difference in incidence was found but the tumors may have appeared somewhat earlier, on the average, in the group being fed 18 per cent casein. In the three groups of C3H male mice receiving diets containing 9, 18, and 45 per cent casein, the percentage of spontaneous hepatomas at 13 months were 11, 61, and 38 respectively, indicating that the "low" and "high" protein diets led to fewer tumors than the diet with moderate protein. It may be concluded that varying the protein (casein) content of the diets, within the limits as indicated, probably has little effect on the formation of many types of tumors, but may have a significant effect on certain special kinds.
DIFFERENCE IN ACTIVATION OF PROTEOLYTIC ENZYMES IN NORMAL LIVER AND HEPATOMA, AS DETERMINED BY MEANS OF A NEW MONOMETRIC METHOD FOR FOLLOWING PEPTIDE CLEAVAGES. PAUL C. ZAMECNIK and MARY L. STEPHENSON. (Medical Laboratories, Collis P. Huntington Memorial Hospital; and Tumor Clinic, Massachusetts General Hospital, Boston, Mass.)

A manometric method has been devised, which facilitates the study of reaction kinetics involved in the hydrolysis of tyrosine-containing peptides by catheptic enzymes. This method depends on the inclusion in the reaction mixture of a bacterial decarboxylase, which liberates carbon dioxide from L-tyrosine as the latter is split from peptide linkage. Since the decarboxylase is present in excess, the rate of carbon dioxide production from tyrosine reflects the rate of the peptide cleavage. This method makes it possible to follow in detail the activation mechanism of the catheptic enzyme.

Ultrafiltrates have been prepared from normal rat livers, and from primary hepatomas induced by butter yellow. The ultrafiltrates of normal livers and of the non-malignant portion of the hepatoma-containing livers activate a purified catheptic enzyme more than ultrafiltrates prepared from hepatoma nodules.

THE INHIBITING ACTION OF AMORPHOUS AND CRYSTALLINE PENICILLIN AND STREPTOMYCIN PREPARATIONS ON THE METABOLISM OF TUMORS AND OTHER TISSUES. DEAN BURK, MARIE L. HESSELBACH, and CLARA E. FISCHER. (National Cancer Institute, Bethesda, Md.)

Amorphous preparations of penicillin have been found to produce a marked inhibition of respiration of tumors and normal tissues (e.g., spontaneous breast adenocarcinoma, transplanted Barrett C3HBA adenocarcinoma, Earle L sarcoma, kidney, spleen, and liver of mice). The inhibition is immediate (detectable manometrically within a few minutes) and progressive, attaining practical completion (95 to 100 per cent) within one to several hours, depending upon the amorphous preparation and concentration employed (range, 0.1 to 10 mgm./cc.).

Penicillin G several-times recrystallized (1,660 Oxford units/mgm.) was approximately one-tenth as inhibitory on a weight basis as several amorphous preparations assaying 1,000 to 1,500 Oxford penicillin units/mgm. Whether this small activity is due to the crystalline penicillin itself, or to possible traces of the “amorphous factor” still extant as impurity, remains to be determined. Crystalline streptomycin salt was still less inhibitory on a weight basis.

Treatment of various amorphous preparations with B. subtilis penicillinase, to remove essentially all penicillin activity against microorganisms, reduced the respiration inhibiting activity per mgm. by 25 to 75 per cent, depending upon the ratio of the amorphous factor to penicillin in the preparation. The amorphous factor can thus act on respiration independently of the presence of penicillin. Synergistic action (as occurs in the case of the enhancement factor of Welch, Randall, and Price) has not been definitely indicated. In any event, metabolic analysis offers a rapid and comparatively sensitive method of assay. Tumor glycolysis was nearly as subject to inhibition by the amorphous factor as was respiration.

EFFECTS OF AN ASCORBIC ACID DEFICIENCY ON TUMORS. WILLIAM v. B. ROBERTSON, A. J. DALTON, and WALTER HESTON. (National Cancer Institute, Bethesda, Md.)

The effect of an ascorbic acid (vitamin C) deficiency on tumors was studied on transplants of a fibrosarcoma (N.C.I. C - 2663) in an inbred family of guinea pigs. Animals were maintained on an adequate diet until the tumor transplants became palpable, and then were placed on the scorbutic diet.

After the guinea pigs had been fed the vitamin C-free diet for 2 weeks, the tumors appeared to become attached to the skin and belly wall, whereas transplants in animals on an adequate diet were loose and easily movable. At necropsy, the scorbutic guinea pigs were found to have large amounts of hemorrhagic connective tissue connecting the tumor capsule with the deeper layers of epidermis and with the musculature of the body wall.

Transplants of this fibrosarcoma show a core of central necrosis surrounded by a margin of healthy tumor tissue, but the tumors in scorbutic hosts showed not only much larger areas of central necrosis but also many areas of focal necrosis scattered throughout the periphery.

The ascorbic acid concentration of the tumors in the scorbutic cavies was essentially zero; that of the non-neoplastic tissues, although considerably below normal, was still appreciable.

The collagen concentration of tumors in the scorbutic animals averaged 3.7 per cent, as compared with the concentration of 8.9 per cent found in the tumors from normally fed controls.

The rate of tumor growth as measured by external caliper was the same in the scorbutic and control groups for a fortnight, after which the tumors in scorbutic guinea pigs grew much more slowly. The average weight of tumors removed from 14 scorbutic and moribund animals was 7.6 gm., whereas tumors taken concurrently from 7 normal animals had an average weight of 29.6 gm. This difference was found to be statistically significant (p <0.01).

DESAMIDATION OF GLUTAMINE AND ASPARAGINE IN NORMAL AND NEOPLASTIC HEPATIC TISSUES. MAURICE ERRERA (by invitation) and JESSE P. GREENSTEIN. (National Cancer Institute, Bethesda, Md.)

Fetal rat liver possesses little or no asparaginase activity but does possess a high glutaminase activity. In adult rat liver, the relative activity of these enzymes is reversed, the glutaminase activity being extremely weak and the asparaginase activity very high. When the adult liver becomes neoplastic, the fetal pattern is noted in the hepatoma, i.e., a near-disappearance of asparaginase activity and concomitant rise in glutaminase activity. That a tumor may possess the metabolic characteristics...
of the corresponding embryonic form is not surprising by now.

The rate of desamidation of glutamine and asparagine in homogenates of all three kinds of hepatic tissues is greatly increased by added pyruvate. The pyruvate is not consumed in the reaction, but plays the role of a cosubstrate. This effect of pyruvate is almost exclusively a property of the liver, and is not observed to any great extent in other normal tissues. The fact that it is noted in hepatomas but not in any other tumors of different histogenesis shows that in this respect the hepatoma bears the imprint of its tissue of origin, and suggests a chemical method of distinguishing hepatomas from other kinds of tumors.

COBALT INHIBITION OF TUMOR RESPIRATION AND PROTECTION BY HISTIDINE. JOHN HEARON, ARTHUR L. SCHADE, HILTON LEVY, AND DEAN BURK. (National Cancer Institute, Bethesda, Md., and Overly Biochemical Research Foundation, New York, N. Y.)

Cobalt has been shown previously to inhibit tissue respiration at concentrations of approximately 5 to 50 p.p.m. The inhibition is progressive with time, and tumor tissue is particularly sensitive. It has been found that the inhibition of tumor respiration may be prevented by additions of histidine at a molar ratio of histidine to cobaltous ion of 2 to 1 or greater. This protection is explicable on the basis of the reversible formation of a 2:1 histidine-cobalt complex, cobaltodihistidine, whereby the equilibrium constant, $K = (\text{cobaltodihistidine}) (\text{H}^+)^2/\text{histidine}^2 = 7.5 \times 10^{-7}$ at $38^\circ$ C. The degree of protection afforded by a given concentration of histidine at any level of cobalt concentration may be correlated with the degree of completion of the reaction, and the observed inhibition in the presence of histidine is in accord with the concentration of free cobaltous ion calculated from the equilibrium expression. It may be concluded that the cobaltodihistidine is nontoxic. The rather slight protection given by other alpha amino acids and histamine parallels the observed lower coordination affinities of the compounds for cobaltous ion, except in the case of cystine which forms a 3 cystine: 1 cobaltous complex of high affinity that is rapidly oxidized to the cobaltic state and affords considerable protection. The progressive inhibition of tumor respiration by cobalt can only be halted but not reversed by additions of histidine. Thus, it appears that the combination of cobalt with the tissue component concerned is essentially irreversible, even in the presence of histidine. Further studies on the mechanism are in progress.

PURIFICATION AND PROPERTIES OF DEHYDROPEPTIDASES FROM NEOPLASTIC AND NORMAL TISSUES. JOSEPH SHACK. (National Cancer Institute, Bethesda, Md.)

Previous investigators have postulated from tissue distribution studies the existence of a dehydropeptidase I splitting gleykidehydroalanine with a uniformly high activity in tumors and of a dehydropeptidase II splitting chloracetyldehydroalanine and absent in tumors. A purification and study of these enzymes from rat tissues has been carried out. By differential centrifugation at 3,000 and 18,000 R.P.M., it was found that the bulk of dehydropeptidase I of kidney is firmly bound to particulates sedimentable only at high speeds. In contrast the dehydropeptidase I of liver and tumor and the dehydropeptidase II of liver and kidney remain in the supernatant. A hundred-fold concentration of dehydropeptidase I free of dehydropeptidase II has been achieved by differential centrifugation, enzymatic digestion and salt fractionation of kidney extract. The soluble enzymes have been purified by low temperature alcohol fractionation. The separation of activities made in the fractionation procedures confirm the existence of 2 distinct enzymes. They have been compared with respect to pH dependence, kinetics and specific inhibition. Neither is inhibited by azide, iodide or fluoride. Both are inhibited by cyanide and thioglycolate, inhibitions reversible on dialysis. Iodoacetate inhibits dehydropeptidase II (also reversed on dialysis) but has no effect on dehydropeptidase I.

These results indicate that the absence of dehydropeptidase II activity in hepatoma is due not to a change in specificity but to the disappearance of the enzyme as a result of malignancy. Comparative studies of purified dehydropeptidase I from liver and hepatoma have shown no differences in catalytic properties.

PHOSPHORYLATED INTERMEDIATES IN TUMOR GLYCOLYSIS. G. A. LePAGE. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

The rate of glycolysis has been reported to be high in tumors. There has been considerable controversy as to whether this was a phosphorylative or non-phosphorylative glycolysis. While data available can all be reasonably explained on the basis of a phosphorylative glycolysis and the enzymes necessary all appear to be present in tumors, the question of what type of glycolysis is operative had not been conclusively settled. In this investigation analyses were made, using methods established as adequate for other tissues, for the intermediates of the Meyerhof phosphorylative glycolysis system. Tissues were fixed in liquid air. Components analyzed for included inorganic phosphorus, the adenine nucleotides, phosphocreatine, glycogen, glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, hexosediphosphate, phosphoglyceric acid, phosphopyruvic acid and lactate. Analyses were confirmed in certain cases by isolation in high yield. Tumors so studied included several transplantable and certain primary rat and mouse tumors, and human carcinoma.

Glycogen was found to be low except in the human tumor samples. Lactic acid was elevated several fold above that of differentiated tissues in all cases. The levels of other intermediates conformed with those found in differentiated tissues. Modification of the physiological state of certain tumors by production of anoxia or hyperglycemia gave changes which were interpretable on the basis of a phosphorylative system.
THE DPN-CYTOCHROME REDUCTASE CONTENT OF CANCER TISSUE. M. RHIAN and VAN R. POTTER. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

The enzyme that has been referred to as DPN-cytochrome reductase catalyzes the reaction between diphenolphosphoryl nucleotide (DPN, coenzyme I, cozymase) and cytochrome c. When this enzyme is functioning, the substrate-hydrogen from the DPN-linked dehydrogenases is transported to oxygen via the cytochrome system.

Using the malic dehydrogenase system as a source of reduced DPN it has been possible to devise an assay system for the determination of DPN-cytochrome reductase. Assays have been carried out on normal rat liver, heart, kidney and brain tissue; and Walker carcinoma 256, Jensen sarcoma, Flexner-Jobling carcinoma and primary hepatomas, all from rats. It has been shown that the cancer tissues are extremely low in this enzyme as compared with the normal tissues studied thus far.

The results can be interpreted in terms of the balance between the glycolytic and the oxidative enzymes, since a relative deficiency of this enzyme would diminish the rate of oxidation of reduced DPN by oxygen, leaving it to be oxidized by pyruvate, which in turn would be converted to lactate. Thus a deficiency in cytochrome reductase could result in the glycolytic type of metabolism that is found in tumors.

SUCCINOXIDASE STUDIES OF THE LIVER CELLS OF MICE FED CARBON TETRACHLORIDE. KRETCHMER, N. (by invitation), TSUBOI, K. K. (by invitation), and BARNUM, C. P. (Department of Physiological Chemistry, University of Minnesota, Minneapolis, Minn.)

Alterations in the succinoxidase system that took place during the period of carbon tetrachloride feeding were presented from studies on cytoplasmic fractions of the mouse liver cell. Liver tumors were induced in C3H mice over a period of 200 days by feeding 0.1 cc. of 40 per cent carbon tetrachloride in olive oil every 4 days. Animals were sacrificed at intervals for the succinic oxidase assay. The assays were conducted on the cytoplasmic particulates obtained by methods of differential centrifugation.

During carbon tetrachloride poisoning of the mouse liver, there is initially a decrease in the cytoplasmic succinoxidase activity of the liver cell; as the induction proceeds, the enzyme activity reaches normal values and exceeds the normal in the 60, 75, and 90 day periods. After 90 days the enzyme activity follows a downward trend such that at 200 days and at tumor, the enzyme activity is somewhat below the normal.

THE INTERFACIAL DENATURATION OF PROTEINS IN THE PRESENCE OF AROMATIC DIAMIDINES AND NUCLEIC ACIDS. M. J. KOPAC. (Department of Biology, Washington Square College of Arts and Sciences, New York University, New York, N. Y.)

These experiments augment the work reported at the A. A. A. S.-Gibson Island Conference of 1946 (Cancer Research 7:44-46, 1947). The effects of stilbamidine, propamidine, and bis-amidinomethylbenzyl on the denaturation of bovine plasma albumin and of crystalline ribonuclease (Kunitz) at oil-water interfaces were measured.

The interfacial denaturation of albumin (2 mgm./ml.) was enhanced by stilbamidine (0.001M), less so by propamidine (0.001M), and completely inhibited by bis-amidinomethylbenzyl (0.001M). With an albumin concentration of 5 mgm./ml., or higher, only stilbamidine enhanced surface denaturation, whereas others depressed it.

On adding Na zymonucleate (1 mgm./ml.) to the albumin-diamidine preparations, the diamidines were nearly completely antagonized. The combination of stilbamidine + propamidine, each at 0.001M, produced a typical stilbamidine effect. On adding Na zymonucleate (1 mgm./ml.) to this preparation, the action of stilbamidine was abolished and a typical propamidine effect was elicited, indicating that stilbamidine was preferentially bound by the nucleic acid. Stilbamidine was partly neutralized by yeast adenylc acid (1 mgm./ml.).

The interfacial denaturation of crystalline ribonuclease (1 mgm./ml.) was strikingly enhanced by stilbamidine (0.001M) and by propamidine (0.001M) and completely prevented by bis-amidinomethylbenzyl (0.001M). All diamidine effects were abolished on addition of Na zymonucleate (1 mgm./ml.). Sodium thymonucleate (1 mgm./ml.) was less active than Na zymonucleate in abolishing the stilbamidine effect. Stilbamidine was not neutralized, however, if the Na zymonucleate was previously incubated with ribonuclease for 2 to 4 hours. Following incubation of Na thymonucleate with ribonuclease, stilbamidine was considerably neutralized.

These data indicate that certain diamidines enhance interfacial denaturation because they weaken side-chain linkages in protein molecules. No appreciable increase in interfacial denaturation was observed if these diamidines were removed before exposing the proteins to interfacial forces. The increased denaturation, therefore, results from the simultaneous action of surface forces with the diamidines.

Stilbamidine in the presence of other diamidines was preferentially bound by nucleic acids. These data may explain why stilbamidine produced the drastic action on the nucleoproteins tested to date. This compound, a denaturating adjuvant, is readily bound by nucleic acids.

LARGE SCALE PREPARATION OF THE TUMOR-NECROTIZING POLYSACCHARIDE FROM S. MARCESCENS. ADRIAN PERRAULT and M. J. SHEAR. (National Cancer Institute, Bethesda, Md.)

The cultivation of strain 724 of S. marcescens in a synthetic medium is being carried out in lots of up to 350 l. each. The organisms are separated, and the active agent in the filtrates concentrated to 1/200 of the original volume.
After further concentration and purification, bioassays for potency were carried out in mice bearing sarcoma 37. The potency of the final product is similar to that obtained in the small-scale preparations of the active polysaccharide previously obtained with culture filtrates from the "G. W." strain.

Active fractions have been obtained from the organisms themselves, and these fractions are being subjected to further purification. Yields are now measured in grams.

TUMOR NECROTIZING BACTERIAL POLYSACCHARIDE TAGGED WITH RADIOACTIVE IODINE. ARNOLD M. SELIGMAN, JOSEPH LEITER, BENJAMIN SWEET, and M. J. SHEAR. (Surgical Research Department, Beth Israel Hospital, and Department of Surgery, Harvard Medical School, Boston, Mass., and the National Cancer Institute, Bethesda, Md.)

The polysaccharide from Serratia marcescens, which produces necrosis in tumors, was tagged with radioactive iodine (I131). Unattached iodine was removed by dialysis.

Free iodine.—Iodination of 2.5 mgm. of polysaccharide with 0.25 mgm. of iodine resulted in the incorporation of 3 per cent of the iodine or 180 atoms of iodine per molecule of polysaccharide (approximate molecular weight 8,000,000). Ethylene linkages presumably were involved in the reaction. Some loss in tumor necrotizing potency was observed in mice bearing sarcoma 37.

Sodium hypoiodite.—Iodination of 2.5 mgm. of polysaccharide (P, R) with iodine in the presence of sodium carbonate (10 mgm.) resulted in incorporation of the iodine. When 1.25 mgm., 0.25 mgm., and 0.05 mgm. of iodine were used, 0.7 per cent, 3.5 per cent, and 14.4 per cent of the iodine respectively were attached; the number of atoms of iodine per molecule of polysaccharide attached was 226, 223, and 183 respectively. The polysaccharide molecule, therefore, was readily saturated within a wide range of iodine concentration. Hydrogen, alpha to a carbonyl group, presumably was replaced in this reaction. Some loss in tumor necrotizing potency of polysaccharide was observed in mice bearing sarcoma 37.

Mandler candle (1 in. long) filtration of a solution of tagged polysaccharide (25 mgm. per ml.) resulted in a 20 per cent loss of radioactivity.

Blood disappearance curves in mice, rabbits, and man showed 40 per cent loss of radioactivity in 10 minutes and 75 per cent loss in 30 to 60 minutes.

The ratios of the radioactivity of tissues to that of circulating blood, when normal mice were sacrificed 1 hour after the injection of 25 mgm. of iodo-polysaccharide were, liver 1.3; lung 0.44; kidney 0.30; and thyroid 0.30. The ratios of the radioactivity of liver and tumor to that of circulating blood, when mice bearing sarcoma 37 were sacrificed after injection of 12.5 mgm. of iodo-polysaccharide, were as follows:

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<td>At 1 hour</td>
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<td>At 24 hours</td>
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THE EFFECT OF SIMULTANEOUS ADMINISTRATION OF BACTERIAL POLYSACCHARIDE AND ADRENAL CORTEX EXTRACT ON CELLS OF MOUSE TUMORS AND ON THE ADRENAL GLANDS OF THE HOST. IRENE COREY DILLER. (Institute for Cancer Research, Lankenau Hospital, Philadelphia, Pa.)

In an attempt to alleviate some of the toxic effects of the tumor-necrotizing polysaccharide of Shear, Upjohn adrenal cortical extract and polysaccharide were administered simultaneously to mice (Beck and Diller, unpublished data). This did not inhibit the action of the polysaccharide on tumor tissue (sarcoma 37). The process of tumor cell degeneration, however, differed from that obtained following polysaccharide alone, and the responses occurred more slowly, reaching a maximum at about 24 hours instead of at 6. Marked fragmentation of tumor cells was apparent and unfragmented nuclei were condensed and crenulated. Acetic orcein stains these tumors very hazily and the cytoplasm is grayish and opaque, which suggests some chemical as well as structural...
change. Cellular changes occur also in adrenal glands of the host, particularly in the medulla, when polysaccharide only (0.01 mgm.) or Upjohn extract only (0.5 cc. in 5 single doses) are injected intraperitoneally. Damage to the adrenal cell appears to be largely overcome when the 2 are given simultaneously.

COMPARATIVE STUDIES OF THE IMMUNOLOGICAL, TOXIC, AND TUMOR-NECROTIZING PROPERTIES OF S. MARCESCENS POLYSACCHARIDES. HUGH J. CREECH, MARY ALICE HAMILTON, IRENE COREY DILLER, EDWIN T. NISHIMURA, and M. J. SHEAR. (Lankenau Hospital Research Institute and the Institute for Cancer Research, Philadelphia, Pa., and the National Cancer Institute, Bethesda, Md.)

Effective clinical utilization of the tumor-necrotizing property of S. marcescens polysaccharide has been hindered by the concomitant toxic and immunological properties. Although these 3 properties appeared to run parallel in the earlier preparations of this polysaccharide, fractionation of one preparation yielded products in which the toxic and antigenic properties were decreased considerably, whereas the tumor-necrotizing property was not altered significantly.

Studies have been made of the effects of passive immunization of mice against the toxic action of the polysaccharide from the “G.W.” strain using the γ-globulin fraction of rabbit antisera. These antibody-containing fractions upon injection into normal mice a few hours before the administration of a lethal dose of polysaccharide protected a high percentage of the animals. Injection of the γ-globulin fractions into mice bearing sarcoma 37 prior to the injection of relatively large tumor-necrotizing doses of polysaccharide afforded definite protection against the lethal action but did not seem to interfere significantly with the tumor-necrotizing action of the polysaccharide.

Two recent preparations of polysaccharide from a different strain (#724) of S. marcescens have been found to be less antigenic and less toxic that the preparations from the “G.W.” strain; in addition, they are not related antigenically to the latter. The influence of the γ-globulin fraction of antisera toward these preparations on the toxic and tumor-necrotizing actions of the polysaccharide is being investigated.

EFFECT ON SARCOMA 37 IN TISSUE CULTURE OF TWO TUMOR-NECROTIZING AGENTS. JANE R. McCONNELL, SUZANNE F. HALLETT and M.J. SHEAR. (Institute for Cancer Research, Philadelphia, Pa., and the National Cancer Institute, Bethesda, Md.)

The action in tissue culture of a preparation of the polysaccharide from S. marcescens and of emetine hydrochloride was studied. Each of these agents, when injected into mice bearing sarcoma 37, produces necrosis in the tumor.

Hanging drop cultures of sarcoma 37, grown for 18 hours, were treated directly with emetine hydrochloride (concentrations of 10 mgm./cc. to 0.00001 mgm./cc). The cells became rounded; blebs formed and were pinched off; nuclei shriveled and became pyknotic. The speed of action and the degree of destruction of the cells in culture were proportional to the concentration of the emetine hydrochloride. Control experiments showed that the damage was not attributable to the hydrochloric acid. The damage seemed to be correlated with the apparent surface reducing effect of emetine on the nucleus and cytoplasm.

When hanging drop cultures of sarcoma 37 were similarly treated with the polysaccharide (concentrations of 10 mgm./cc. to 0.001 mgm./cc.), however, no necrotizing effects were observed, even though these concentrations regularly produced hemorrhage and necrosis in vivo.

The tissue culture method is of value in studies on the mechanism of action of such chemical agents. However, it is clear that, while it may give evidence of a direct effect of some substances on normal and malignant cells in cultures, it may not be entirely dependable if employed as a screening procedure in a chemotherapy program in lieu of in vivo screening.

CHEMOTHERAPY OF CANCER. CLASSES OF COMPOUNDS UNDER INVESTIGATION AND ACTIVE COMPONENTS OF PODOPHYLLIN. JONATHAN L. HARTWELL (by invitation), and M. J. SHEAR. (National Cancer Institute, Bethesda, Md.)

More than 1,200 organic compounds have been assembled for screening in tumor-bearing animals. Among the classes of compounds that have been obtained are: alkaloids; isoquinolines; derivatives of phenylethylamine, phenylpropylamine and phenylisopropylamine; carbamates; azo compounds; derivatives of phenanthrene, acenaphthene, and fluorene; diphenylethenes; stilbenes; α, β-unsaturated ketones and quinones; acridines; quaternary ammonium salts; sulfonamides; mercurials; arsenicals; and substances known to affect intermediary metabolic processes.

About 300 of these compounds have been put through a preliminary first screening in mice bearing sarcoma 37. With a few compounds, more extensive biological work has been done. The exploratory screening indicated that some classes of compounds contain a higher percentage than others of members capable of inducing damage in tumor tissue under the conditions of these experiments. For example, of 20 isoquinolines screened, only 1 gave microscopic evidence of obvious damage as compared with: 10 of 38 acridines; 23 of 211 quaternary ammonium salts; 6 of 34 arsenicals; 8 of 53 alkaloids; 4 of 53 α,β-diphenylethylamines; 1 of 11 sulfonamides; 2 of 10 stilbenes; and 1 of 10 phenanthrene derivatives.

Podophyllin produced severe gross damage in the tumors. Fractionation yielded 2 white crystalline compounds, podophyllotoxin and a new substance designated provisionally as NCI-1074. Each of these 2 compounds possessed tumor-damaging properties in a single dose down to 3 µgm. per gm. body weight. Quercetin, another crystalline podophyllin component, yielded negative results at ten times this dose. The possible presence of
other active constituents is under investigation. Picro-
podophyllin, prepared from podophyllotoxin, gave nega-
tive results in doses up to 12 μg/mg, per gm, body weight.
NCI-1074 is isomeric with podophyllotoxin and picro-
podophyllin, has a melting point close to that of picro-
podophyllin, but is identical with neither.

HISTOLOGIC CRITERIA FOR EVALUATING THE
CAPACITY OF CHEMICAL AGENTS TO PRO-
DUCE DAMAGE RAPIDLY IN SARCOMA 37.
ROSS C. MacCARDLE and VIRGINIA DOWN-
ING. (National Cancer Institute, Bethesda, Md.)

The necrosis-producing capacity of chemical agents
injected in single doses subcutaneously into mice bearing
intramuscularly implanted sarcoma 37 was ascertained
histologically by observing the extent and speed of
changes in cells of tumor and intestinal epithelium fixed
in Zenker’s formol-bichromate fluid at 8, 20 and 48 hours
after administration. No regression experiments were
attempted in this preliminary screening of many com-
ounds. Control tumors showed resting and dividing
cells with varying amounts of spontaneous degeneration.
One feature of old necrosis is the presence of extracellular
blurish debris.

Tumors treated with some compounds showed exten-
sive degeneration and necrosis in which mitribund pro-
cesses seemed to be in approximately the same stage, sug-
gestting simultaneous induced injury. Compound 368,
N-acetyllycodocholin methyl ether, attacked tumors ap-
parently directly, arresting mitoses in metaphase
followed by necrosis. Compound 707, a quaternary
ammonium salt, attacked tumor cells evidently directly
and indirectly after vascular damage. Compound 497,
α-phenyl-α-(3,5-diiodo-4-hydroxyphenyl)-propionic acid,
induced necrosis in some tumors. Tumors, in treated
mice, showing necrosis sharply demarcated from healthy
tumor tissue were considered unaffected, since control
tumors occasionally presented this appearance tentative-
lly attributed to localized spontaneous vascular blockage.
Agent 851’, podophyllin, induced cell damage throughout
the tumor apparently directly and indirectly with
marked stasis and blood vessel damage. Intestinal and
some tumor cells were arrested in various stages of
mitosis. Mouse epidermis painted with podophyllin
showed many large clear cells and polymorphic nuclei in
atypical mitosis; while the intestine of the same animal
showed arrested mitoses. Rous sarcoma in chickens
treated with podophyllin showed induced necrosis; and
cerebellar Purkinje cells were also damaged. Cell death
is being studied by silver, orcin, Masson, microincinera-
tion and phase-contrast methods in these and other tumors.

THE EFFECT OF PODOPHYLLIN ON TUMOR
CELLS IN VITRO. RICHARD A. ORMSBEE
and IVOR CORNMAN. (Sloan-Kettering Institute
for Cancer Research, New York, N. Y.)

A sterile suspension of crude podophyllin, when
inoculated into the nutrient medium in roller tube tissue
culture preparations, exerts a toxic and repressive effect
against tumor cells from the in-strain transplantable
mouse tumors, sarcoma L946 A II and lung tumor MA
387. The effect on normal mouse embryonic skin growing
in the same tube is negligible at concentrations which
cause extensive tumor cell damage. This differential
toxic effect is more marked than that obtained with any
of the other known mitotic poisons which have been
tested so far. This material is now being tested in vivo
for repressive effect against a variety of tumors.

TRYPANOSOMA CRUZI IN THE TREATMENT OF
MOUSE TUMORS. THEODORE S. HAUSCHKA.
(Institute for Cancer Research, Lankenau Hospital,
Philadelphia, Pa.)

The work of Roskin and his collaborators on destruc-
tion of tumors in animals by Trypanosoma cruzi “endo-
toxins” has recently been extended to the clinical ap-
plication of Klyuva’s “cancerolytic” T. cruzi lysates and
has given impetus to related studies. Our experiments
were started in March 1945 under a joint institutional
program participated in by the Chemotherapy Section of
the National Cancer Institute and the Laboratory of
Zoology (National Institute of Health) and the Lankenau
Hospital Research Institute.

Infections of Trypanosoma cruzi (“B”-strain) signifi-
cantly retarded the growth of three transplantable tu-
mors: squamous epithelial carcinoma 119, mammary
adenocarcinoma, and sarcoma 37. Spontaneous breast
adenocarcinoma in C3H mice was slightly retarded in
growth.

The inhibitory effect was often accompanied by loss in
body weight and parasitemia of vital organs. Of the 4
tumor varieties studied, only carcinoma 119 was found
to be parasitized. Cancer cells proper were rarely invaded
by T. cruzi, but parasites were relatively abundant in the
stroma and in the encapsulating connective tissue. Re-
tardation of tumor development did not result in longer
survival.

Growth of carcinoma 119 was retarded or completely
inhibited by infection with (1) the lethal “R-strain of
T. cruzi (obtained from the same source as Roskin’s
strain); (2) a mixture of 5 avirulent strains (“A”-,”M”-
“P”, “T”- and “C”-strain); (3) the entirely avirulent
“C”-strain. Tumor-inhibition by “C”-strain was not
accompanied by loss in body weight or other symptoms of
Chagas’ disease, and infected tumor-bearing mice
lived longer than tumor controls.

Growth of spontaneous mammary adenocarcinoma
(C3H mice) was inhibited by infection with “R”-strain
T. cruzi. This otherwise lethal infection can be cured by
treatment with the quinoline derivative, Bayer 7602.

Heat-killed cultures (50° C.) and lysates of T. cruzi
(“B”-strain) were without effect against carcinoma 119
or mammary tumors. A lysate prepared from “R”-strain
of T. cruzi in the plasma of infected mice contained a
tumor-necrotizing “endotoxin” but also produced de-
generative symptoms in liver, spleen and kidney. Test
mice treated with this lysate died earlier than the controls.

THE EFFECT OF INHIBITORS OF INTER-
MEDIARY METABOLISM ON ADVANCED
HUMAN NEOPLASIA. MAURICE M. BLACK
and ISRAEL S. KLEINER. (New York Medical College, New York, N. Y., and Brooklyn Cancer Institute, Brooklyn, N. Y.)

Malignant tissues have long been known to exhibit greater aerobic and anaerobic glycolytic activity than homologous normal tissues. Attempts to inhibit the growth of such tissues by the use of inhibitors of glycolysis by other investigators have yielded indiscernible results. In view of the importance of the active phosphate bonds in energy-yielding reactions, we have attempted selective inhibition of such reactions in relation to these bonds.

The inhibitors used were sodium fluoride, iodoacetic acid, malonic acid and sodium azide. In the doses used, these substances, both singly and in combination, resulted in encouraging therapeutic effects without evidence of appreciable toxicity. The 31 cases studied, all far advanced, included acute leukemia and a diversified group of malignant tumors. Hematological and clinical remissions, for a period of 3 months, were observed in a significant number of leukemias studied. The beneficial results in patients with various types of malignant tumors included shrinkage of tumor mass, relief of pain, increase in weight and well-being, and degenerative changes in material obtained in repeated biopsies in 1 case of lymphosarcoma.

In this work, adaptation to such agents seems to be the limiting factor in continued therapeutic effect. Thus, after refractoriness to sodium fluoride and iodoacetic acid had developed, a therapeutic effect was obtained by the addition of malonic acid. Reversal of the refractory state with renewed sensitivity to the glycolytic inhibitors was also accomplished by the use of sodium azide.

The findings reported would appear to be consistent with the hypothesis of the importance of the active phosphate bond and of the possible role of accessory pathways in this phenomenon. This and other hypotheses are under investigation.

CHANGES IN THE REDUCING POWER OF PLASMA IN PATIENTS WITH MALIGNANT NEOPLASIA AND THERAPEUTIC IMPLICATIONS. MAURICE M. BLACK. (New York Medical College, New York, N. Y., and Brooklyn Cancer Institute, Brooklyn, N. Y.)

Determination of the reducing power of plasma (or serum) was made by the use of the redox dyes, brilliant cresyl blue and methylene blue. It was found that plasma of patients with malignant diseases tended to have a lowered reducing power and could usually be distinguished from normal plasma and from plasma of patients suffering from conditions other than malignancy. The decreased reducing power obtained with plasma from cancer patients tended to be grouped at different levels with individual sites of tumor origin. So far, in advanced pregnancy and in advanced hepatic cirrhosis, similar decreased reducing power has been observed. Adequate therapy (x-ray or surgery) increased the reducing power. Thus, this effect of therapy could be followed objectively.

The correlations between the results of this procedure and the diagnoses are illustrated by the following ratios.

The numerator represents the number of cases showing good correlation between the clinical pathological diagnoses and the alteration in reducing power; the denominator gives the total number of cases involved. Controls (normals) 50/50; nonmalignant diseases 111/120; active malignant disease 158/184.

These observations suggested the concept that some of the symptomatology associated with malignancy might be due to the altered enzyme activity as a result of diminished -SH potential. Accordingly, glutathione or cysteine was administered intravenously. This was followed rapidly by relief of pain and general symptomatic improvement. No effect was noted, however, on the growth rate of the tumor itself.

IN VIVO STAINING OF MALIGNANT TISSUE IN MICE. MARGARET REED LEWIS and PHILIP P. GOLAND. (The Wistar Institute of Anatomy and Biology; Department of Neurosurgery, Hospital of the University of Pennsylvania; and the Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania, Philadelphia, Pa.)

Certain dyestuffs, namely, 3 oxazine, one thiazine, 4 xanthene, one acridine and 1 anthraquinone dye added to the diet of tumor-bearing mice stained the tumors selectively. The in vivo staining was accompanied by retardation of their growth.

Twenty-five dyestuffs were pulverized with fox chow in amounts equivalent to 0.4 per cent of commercial and 0.15 per cent of other compounds. Thirty mice of an inbred strain were then implanted subcutaneously with a small graft of a sarcoma native in the strain used, placed in individual containers and given 10 grams of food each day; 25 of them receiving treated and 5 untreated fox chow. Samples of urine were examined, and the mice were sacrificed about 14 days later, by which time those receiving untreated food had large tumors. As each mouse was sacrificed records were made of its condition and of the size and color of its organs and tumor. If the mouse was normal and its tumor large and uncolored the test was not repeated. If, however, its tumor was small and stained, the test was repeated. Tests on mice that became ill were repeated using less dyestuff. Compounds that stained and retarded sarcomas were tested also on mice bearing spontaneous adenocarcinomas.

Our results show that compounds that stain and retard tumors have certain structural similarities. Further investigation should disclose the structural nature of compounds less toxic and more selective in their inhibiting action on malignant tissue so that they can be synthesized for use.

STUDIES ON PURIFICATION OF THE AGENT OF CHICKEN TUMOR I. W. RAY BRYAN and VERNON T. RILEY. (National Cancer Institute, Bethesda, Md.)

Progress has been made toward purification of the agent of chicken tumor I in experiments carried out with quantitative biological assays, quantitative nitrogen determinations, and electron microscopic observations. The results of investigations involving the principles of
ultracentrifugal fractionation and chromatography were described.

RELATIONSHIP BETWEEN THE LETHAL YELLOW (Ay) GENE OF THE MOUSE AND SUSCEPTIBILITY TO SPONTANEOUS PULMONARY TUMORS. MARGARET K. DERINGER and WALTER E. HESTON. (National Cancer Institute, Bethesda, Md.)

Susceptibility to induced pulmonary tumors has been shown to be associated with the lethal yellow gene (Ay) of the mouse. Yellow F1 hybrid mice from a cross between strains A and Y were more susceptible to pulmonary tumors induced by 20-methylcholanthrene than were their brown litter mates. In the present experiment the data indicated a relationship between the lethal yellow gene and spontaneous pulmonary tumors.

Eighty-two AYF1 hybrids were produced and were autopsied at 15 months of age. Sixteen of the 38 yellow mice and 9 of the 44 brown mice had pulmonary tumors. The results suggested a higher degree of susceptibility in the yellow mice but were not significant. A second group of 83 mice was therefore produced and was autopsied at 15 months of age. Fifteen of the 38 yellow mice and 10 of the 45 brown mice had pulmonary tumors. These results were not significant but the combined results for the 2 groups were highly significant, X² = 7.425; P < 0.01.

The previously demonstrated effect of the Ay gene on body size was shown by the weights of the second group at 6, 12, and 15 months of age. At 6 months the average weight for the yellow males was 14 gm. higher than that for the brown males; and that for the yellow females was 19.6 gm. higher than that for the brown females. This difference was approximately halved at 12 months and at 15 months the yellow and brown mice were of approximately equal weight.

MORPHOGENESIS AND EVOLUTION IN MALIGNANT TUMORS. SPONTANEOUS MATURATION AND REGRESSION OF TESTICULAR NEOPLASMS. NATHAN B. FRIEDMAN. (Army Institute of Pathology, Washington, D. C.)

Study of 1,000 tumors of the testis (Mil. Surgeon, 99: 573-593, 1946) has revealed that teratoid growths result from the maturation of originally undifferentiated neoplasms. It is suggested that primitive cells from such tumors may metastasize before differentiation takes place, a hypothesis which would explain why teratomas lacking histologically malignant components are sometimes associated with metastases.

Some trophoblastic tumors of the testis disappear completely despite progression of their metastases. The tendency toward vascular invasion, hemorrhage and necrosis and possibly the normally brief life span of trophoblastic tissue may account for such regression. The primary site of the neoplasm remains marked by a peculiar cicatrix, which, when overlooked, leads to the erroneous diagnosis of extragenital chorioepithelioma.

Regression is not restricted to trophoblastic tumors. The tuberculoid granulomas which are common secondary stromal components of germinalomas (seminomas) sometimes become more prominent than the seminomatous tissue. It is difficult or even impossible at times to identify residual neoplastic elements when the bulk of the “tumor” is made up of lymphocytes, epithelioid cells, fibroblasts and giant cells.

Teratoid tumors may be governed by oncologic principles which do not apply to other types of neoplasms. However, it might be worth investigating the new growths of other organs for evolutionary and regressive tendencies comparable to those of testicular tumors. The factors controlling neoplastic maturation and regression and the possibility of influencing them should be explored.

GENETIC FACTORS AFFECTING SYNERGISM OF LEUKEMOGENIC AGENTS. HARRY W. MIXER and ARTHUR KIRSCHBAUM. (Departments of Radiology and Anatomy, University of Minnesota Medical School, Minneapolis, Minn.)

Mice of the dba strain (subline 212) are susceptible to the induction of leukemia by either x-rays or methylcholanthrene administered independently. Although strain CBA is very susceptible to the leukemogenic action of x-rays, this stock has proved to be absolutely refractory to the induction of leukemia by methylcholanthrene.

When treatment with x-rays (1,000 r in divided doses) was combined with 18 skin paintings of methylcholanthrene dissolved in benzene (0.25 per cent solution), the incidence of induced leukemia was increased (59 per cent with combined treatment, 34 per cent with x-rays only, 33 per cent with methylcholanthrene only). When subthreshold doses of the 2 agents were combined, absolute synergism was obtained (no induced leukemia with either 6 skin paintings of methylcholanthrene, or 200 r of x-rays used alone, but 30 per cent induced leukemia when the 2 agents were combined).

Although synergism could be demonstrated in dba mice where susceptibility to each leukemogenic agent was manifest, neither synergistic nor additive effects could be obtained by combined administration of these agents to CBA mice. The incidence of leukemia was the same if 500 r were given in divided doses either alone or in combination with 18 skin paintings of methylcholanthrene.

These results suggest that leukemogenic agents may act synergistically only if the test animals are susceptible to each of these agents independently.

CHEMICAL FACTORS CONCERNED IN THE MUTUAL ADHESIVENESS OF EPITHELIAL CELLS. IRVING ZEIDMAN. (Department of Pathology, School of Medicine, University of Pennsylvania, Philadelphia, Pa.)

Experiments were designed to determine the chemical factors responsible for maintaining mutual adhesiveness of human squamous epithelial cells. The method used depended upon separation of pairs of cells by micromanipulation, the value of adhesiveness being determined by the bend produced in a calibrated microneedle when subjected to the strain of detaching the cells. Adhesiveness was decreased in the absence of calcium or magnesium, or both. Reduction in adhesiveness brought about in a calcium-free solution was not reversed by restoring
calcium to the medium. Excess of potassium in the solution did not alter adhesiveness. Decrease in adhesiveness was produced by methylcholanthrene, a substance reported to lower the calcium content of squamous epithelium. These results offer an explanation for changes in adhesiveness recently reported in cancer cells. In these malignant cells, adhesiveness was found decreased as compared with that of normal epithelium. Since the calcium content of cancer cells has been reported to be abnormally low, it is regarded as probable that lessened adhesiveness of cancer cells is explained by their deficiency in calcium.

CHANGES OF CARBOHYDRATE METABOLISM IN PATIENTS WITH GASTRIC CANCER AND IN MICE BEARING SARCOMA 180. J. C. ABELS, C. J. KENSLE, N. F. YOUNG, and F. HOMBURGER. (Sloan-Kettering Institute for Cancer Research, New York, N. Y.)

Studies of hepatic glycogen concentration in patients with gastric cancer and in controls undergoing laparotomies for benign abdominal disorders have shown that while the glycogen concentration is not different in the livers of patients with gastric cancer when the biopsy is taken after a 10 hour fast, it is considerably lower in the patients with cancer when both groups of patients receive glucose in the 10 hour period preceding the operation. This defect of glycogenosis can be corrected by the administration of adrenal cortical extract.

Studies on mice bearing transplanted sarcoma 180 revealed similar defects of hepatic glycogenosis. This metabolic defect is therefore independent of the type of tumor present and occurs, at least in the mouse, even when the tumor is located outside of the portal circulation.

RESPONSE OF MOUSE MYELOGENOUS LEUKEMIA TO URETHANE. ARTHUR KIRSCHBAUM and C. S. LU. (Department of Anatomy, University of Minnesota Medical School, Minneapolis, Minn.)

The administration of a single anesthetic dose of urethane resulted within 24 hours in a drop in the white blood cell count and the appearance of many mature cells in the bone marrow of mice with myeloid leukemia. The depression in white blood cell count continued until 72 hours after injection, following which the counts rose. However, they had not reached the initial level 6 days after the single injection in 8 of the 11 mice tested.

The ratio of segmented to mononuclear myeloid cells in the leukemic marrow ranged from 13.87 to 46.54, with an average of 26.74. This ratio was reversed within 24 hours following a single injection of urethane (1 mgm. per gm. of body weight in aqueous solution given IP).

The number of mitotic figures in the myeloid cells of leukemic marrow was decreased following the administration of urethane. Maturation may have been secondary to inhibition of mitosis in blast cells. However, in the treated mice there were fewer marrow cells capable of undergoing division, which may account for the reduced number of division figures.

It is suggested that the release of an increased percentage of mature cells into the circulating blood may be a factor in depression of white blood cell counts following the injection of urethane into mice with myeloid leukemia.

THE METABOLISM IN THE MOUSE OF 1, 2, 5-, 6-DIBENZANTHRACENE LABELED IN THE 9-POSITION With C14. CHARLES HEIDELBERGER and HARDIN B. JONES. (Radiation Laboratory, University of California, Berkeley, Calif.)

Previous investigations of the metabolism of dibenzanthracene, using ultraviolet absorption spectroscopy as the analytical tool, has led to the isolation and characterization of 4',8'-dihydroxydibenzanthracene and some preliminary information as to the distribution of this carcinogen in the animal body. This work was summarized by R. Norman Jones (Cancer Research, 2:237, 1942) who made a considerable contribution to this field, and who pointed out the difficulties inherent in this method of analysis.

Dibenzanthracene labeled in the 9 position with C14 has been synthesized by Heidelberger, Brewer, and Dauben (J. Am. Chem. Soc. In press) and this material which has a specific activity 0.385 μc./mgm. is being used in an investigation of the metabolism and mechanism of carcinogenic action of this compound. Small doses of known radioactivity are administered in various ways to mice, which are kept in metabolism cages to recover the carbon dioxide of respiration as well as the urine and feces. The animals are dissected, the organs to be assayed are burned with oxygen in a combustion furnace, and the carbon dioxide is precipitated and counted in the form of barium carbonate with thin-window Geiger-Mueller counters. Dibenzanthracene has been administered to mice as a colloid in isotonic glucose, and in fat solutions.

The most striking fact observed in the metabolism of dibenzanthracene injected intravenously as an aqueous colloid, is the rapid elimination of large quantities in the feces. A bile-fistula was performed on a mouse and after injection of the colloid, all detectable activity was present in the bile. Since there was no activity observed in the intestines or the intestinal contents, the excretion must be entirely through the bile. Chemical investigation of this bile reveals that the activity is due almost entirely to unaltered dibenzanthracene. In some cases there has been a small amount of radioactivity in the carbon dioxide of respiration, indicating the complete oxidation of at least one carbon in the molecule. This point is undergoing further investigation.

In general, it can be stated that the activity is not highly concentrated in any specific tissue of the body, but seems to be distributed fairly evenly throughout the internal organs. The mode of administration affects the amount absorbed in the body, exclusive of the gastrointestinal contents. At the end of 24 hours, 25 per cent of the activity was absorbed from intraperitoneal injection, whereas 5 per cent was absorbed from stomach tube and from intravenous injection. When the substance is given to animals bearing highly developed mammary
carcinoma, there is no appreciable concentration in the neoplasm. When the compound is administered intra-peritoneally, either in oil or as an aqueous colloid, there is a higher concentration of activity in the intestines than in the intestinal contents. This is not observed with other methods of administration, and indicates the path of absorption to be across the exposed peritoneum of the gut. Subcutaneous administration in oil indicates that at the end of six weeks, 52 per cent of the dibenzanthracene is retained near the site of injection. However, a small tumor which appeared at the site in that time interval, showed that an appreciable part of the radioactive carbon in the tumor was no longer present in the form of dibenzanthracene, but 14 per cent has been converted into an acidic form, and 21 per cent to a phenolic form. Thus 65 per cent of the dibenzanthracene is unaltered.

Aliquots of an extract of this tumor were assayed by both possible methods. The amount of dibenzanthracene calculated from the spectrophotographic data was twice the quantity obtained by direct radioactive assay, and this justifies previous observations that spectrophotographically interfering substances other than the carcinogen tend to give unreliable results.

Work is now in progress in these laboratories on the mechanism of tumor production and regression with radioactive dibenzanthracene. Distribution studies over longer periods of time, using various modes of administration, are being continued with the ultimate aim of establishing, if possible, the site and mechanism of dibenzanthracene metabolism, degradation, and elimination in the mouse.

This paper is based on work performed under Contract #W-7405-Eng-48 with the Atomic Energy Commission in connection with the Radiation Laboratory, University of California, Berkeley, Calif.

Carcinoma of the Colon in Rats Following the Feeding of Radioactive Yttrium. Herman Lisco, Austin M. Brues, Miriam P. Finkel, and Walter Grundhauser. (Metallurgical Laboratory, University of Chicago, and Argonne National Laboratory, Chicago, Ill.)

Yttrium, one of the common radioactive fission products obtained in a chain-reacting pile, is a pure beta-emitter with an energy of 1.5 mev and a half-life of 57 days. It is essentially not absorbed and, since the material remains longer in the colon than in any other portion of the intestinal tract, most of the damage occurred in this region.

One group of rats received a single feeding by stomach tube of from 1.0 to 6.0 mc. of Y91. Of the 33 animals in this group, 4 died with adenocarcinoma of the colon. The earliest tumor was seen at 135 days and the latest at 506 days. Additional animals died with acute and chronic ulceration of the colon accompanied by benign and atypical hyperplasia of the mucosa.

A second group of rats was given 78 feedings of 0.46, 0.20, or 0.06 millieuries of Y91 per feeding over a period of 3 months. The total accumulated doses were 31.20, 15.60, and 4.68 mc., respectively. Clinically all animals appeared well during the feeding period and growth was not impaired. Six of the 8 animals at the 2 higher levels died with carcinoma of the colon from 304 to 548 days after the first feeding. No malignancies were observed at the lowest level. However, many of these animals died with superficial ulcerative lesions of the colon.

The Influence of Cosmic Radiation on the Induction of Cancer. Frank H. J. Figge. (University of Maryland Medical School, Baltimore, Md.)

The hypothesis that the action of carcinogenic substances may be related to their efficient conversion of some form of penetrating radiation such as cosmic radiation to a form of energy capable of inducing intracellular malignant transformations was suggested by previous work. To test this hypothesis, 182 mice of inbred strains were injected with 0.25 mgm. of methylcholanthrene. They were divided into two groups and subjected to different intensities of cosmic radiation. One group consisting of 69 mice in 3 aluminum cages, used as controls, received normal, unmodified sea-level cosmic radiation. The remaining 113 mice, in 5 aluminum cages with lead plate covers, were subjected to the normal sea-level cosmic radiation plus the showers of radiation resulting from passing cosmic radiation through 1 or 2 lead plates 1 cm. thick. The average latent period for carcinogenesis in the controls was 80 days. The average latent period for induction of sarcomas in the 113 experimental mice receiving the intensified cosmic radiation was only 60 days, or three-fourths that of the controls. A repetition of this experiment gave the same results. While these results are significant, experiments to test this hypothesis in a more conclusive manner are desirable, and are contemplated.


A number of early human carcinomas in various tissues were studied in order to determine the kind of immediate environment out of which cancer arises. These observations pointed to morphologic evidence that certain neoplasms have a multicellular origin. It was concluded that the majority of neoplasms originate in tissues that are in an involutionary phase and that have widespread atrophy of the parenchyma. Furthermore, such tissues may, in some areas, show hyperplasia. The degree of atrophy was usually severe. Also, a number of cases were studied that illustrate grossly and microscopically the qualitatively different type of organization manifested by neoplasms. Cases are given of (1) osteogenic sarcoma, (2) adenocarcinoma of the colon, and (3) melanocarcinoma. It is suggested that the degree of organization is dependent upon the level at which the tissue control is destroyed at the time the neoplasm is established. Another aspect, that of morphologic evidence that certain neoplasms have multicellular origin, was studied in carcinoma in situ of the breast, carcinoma in situ of the stomach, epidermoid carcinoma
of the skin and cervix uteri. In all of these it was possible to show in a focal area that the cells were approximately of the same age of neoplastic development. Additional data was submitted from tissue culture studies and induced carcinomas in lower species. In summary, it may be stated that this evidence points to the origin of cancer from tissues that have widespread atrophy. The type of cancer that is established morphologically depends upon the level at which the tissue organization breaks down.

GENETIC AND ENDOCRINE FACTORS IN ADRENAL CORtical Tumor Formation. GEORGE W. WOOLLEY and MARGARET M. DICKIE. (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine)

There are important genetic factors behind the occurrence of adrenal cortical tumors. This is indicated by pronounced strain differences in response to gonadectomy. The adrenal cortex of strain JAX C57 brown, for example, undergoes only slight change in size or structure following gonadectomy. Strain JAX dba develops nodular hyperplasia of the adrenal cortex and strain JAX ce, adrenal cortical carcinoma. Hybridization experiments now in progress also point to the importance of the genetic factors.

Endocrine factors are also of great influence. In our series, adrenal cortical tumors of the types described in this report did not occur without gonadectomy. Following gonadectomy certain endocrine preparations will prevent their occurrence in a genetically susceptible strain. It is known that there is an intimate relationship between the adrenal cortex and the pituitary gland. In these experiments the relationship is evidenced by occasional abnormalities of the anterior lobe of the pituitary in the experimental and not in the control series.

It seems evident that there is a relationship between the adrenal cortex and the gonad, and probably between gonadotropic and adrenocorticotrophic factors. An hypothesis is that with the gonad absent there is increased need of activating materials which the adrenal cortical cells may be at least partially equipped to supply. The extent to which these are needed and/or the ability of the cells to fulfill the need is undoubtedly under genetic control.

THE EFFECT OF CALORIC RESTRICTION ON THE INCIDENCE OF MAMMARY TUMORS IN CASTRATE HORMONIZED C3H MICE. CARMEN B. CASAS, JOSEPH T. KING, and M. B. VISSCHER. (Department of Physiology, University of Minnesota Medical School, Minneapolis, Minn.)

Thirty C3H mice were ovariectomized at 21 to 23 days of age. They were divided into two groups of 15 each. One group was fed ad libitum; the other was restricted 33 per cent in caloric intake. Both groups were fed 0.5 gamma of diethylstilbestrol daily, and both received the same absolute amounts of protein, minerals and vitamins.

Vaginal smears made by the lavage method showed a constant dense, mixed-cell picture with predominance of cornified cells in both control and experimental groups, with no recognizable difference between the two.

At the time the first tumor appeared in the control group, accidents and sacrifice of animals for tissue study had reduced the control group to 13 animals and the restricted to 10.

In the control series 3 tumors appeared in the 24th week after ovariectomy; 2 in the 27th; 1 in the 28th; 2 in the 31st and 3 in the 33rd week. Only 2 controls are tumor-free at the end of the 39th week.

Only 1 tumor has been found in the restricted animals; it appeared in the 38th week. Evidently caloric restriction did not influence the vaginal response to estrogen in these animals but did significantly alter the tumor age.

THE MILK AGENT. SAMUEL GRAFF, DAN H. MOORE, WENDELL M. STANLEY, HENRY T. RANDALL, and CUSHMAN D. HAAGENSEN. (Columbia University, and Rockefeller Institute for Medical Research, New York, N. Y.)

This communication reports our progress toward isolation and characterization of the mouse mammary carcinoma agent transmitted by and present in the milk of the high-cancer strain. Milk, a fluid of fairly constant composition, offers obvious advantages for this work. Although ordinarily a colloidal suspension of casein and fat in a solution of proteins of lower molecular weight, the milk of the high-cancer strain also contains another protein, the virus.

Fractionation by a variety of physical and chemical methods is under way. Electrophoretic, ultracentrifugal, and electron microscopic evidence on the elimination of some components, and the isolation and concentration of other components was demonstrated.

EXCRETION OF STEROIDS IN THE FECES OF MICE OF VARIOUS STRAINS WITH AND WITHOUT THE MAMMARY TUMOR MILK AGENT. LEO T. SAMUELS (by invitation), JOHN J. BITTNER, and BARBARA K. SAMUELS (by invitation). (Department of Biochemistry, University of Utah Medical School, Salt Lake City, Utah, and Division of Cancer Biology, University of Minnesota, Minneapolis, Minn.)

The excretion of ketosteroids has been studied in various strains of mice with and without the mammary tumor milk agent. It appears that the absence of the milk agent is associated with increased fecal excretion of ketosteroids. Most of the ketosteroids excreted have been found in the unconjugated form. The possible significance of the difference was discussed.

COMPARATIVE STUDIES OF THE ESTROUS CYCLES IN RELATION TO THE MAMMARY TUMOR MILK AGENT. ROBERT A. HUSEBY and JOHN J. BITTNER. (Division of Cancer Biology, University of Minnesota, Minneapolis, Minn.)

Chemical analysis of the excreta of mice has shown that mice possessing the milk agent virus have a much lower 17-ketosteroid excretion than genetically similar
mice lacking the virus (Samuels and Bittner). As androgens inhibit the action of estrogens upon the vaginal mucosa as well as upon other organs, if the altered 17-ketosteroid excretion noted is due at least in part to a change in the production and/or metabolism of androgenically active compounds, the estrous cycles of mice should vary according to the presence or absence of the agent. To test this the estrous cycles of groups of mice differing only in this one respect were compared. Strain A and C3H mice and their F1 hybrids and hybrids between the dba and C3H strains were studied. It was found, generally, that mice possessing the agent showed vaginal cornification a greater percentage of the time than did those mice lacking the virus. Also the percentage of vaginal cornification of mice lacking the agent could be increased by foster-nursing such mice to females that possessed the agent. Mice of the C3H strain differed from the other groups studied, for in this strain there was no difference in the estrous cycles whether the animals possessed or lacked the agent. The reason for this is obscure at the present time.

EXPERIMENTAL ALTERATION OF THE CELLS OF A TRANSPLANTED TUMOR. C. W. HOOKER, C. A. PFEIFFER, and L. C. STRONG. (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

A testicular tumor composed of primitive Leydig cells that arose spontaneously in a mouse of the Strong C strain has been carried through 24 serial subcutaneous transfers in untreated mice of the same strain. No change in the cytology of the tumor has been encountered. Occasionally the grafted tumor has invaded the muscle of the body wall, and in a few instances metastases to the kidneys, liver, and lungs have been recorded. In the last 20 transfers the graft has consistently attained a size of 2.5 by 1.5 cms, in 3 weeks. When grown in castrated males the cytology of the tumor remained unchanged and the condition of the genital system of the hosts indicated no production of androgen. When equine gonadotrophin was injected daily into castrated mice carrying the tumor, the tumor cells were transformed into morphologically mature Leydig cells, and the condition of the genital system indicated the secretion of levels of androgen approximating that of the normal male mouse. Thus an agent that will provoke cellular differentiation in the normal testis has also brought about full morphological differentiation and physiological activity in the cells of an apparently malignant tumor.

EFFECT OF ORCHIECTOMY ON ADVANCED CANCER OF THE BREAST IN MALES. IRA T. NATHANSON. (From the Medical Laboratories of the Huntington Memorial Hospital of Harvard University and the Tumor Clinic at the Massachusetts General Hospital, Boston, Mass., and Pondville Hospital, Massachusetts Department of Public Health, Walpole, Mass.)

Five males with advanced, primary, recurrent or metastatic cancer of the breast were subjected to bilateral orchietomy and exhibited some form of beneficial response therefrom. These effects were seen primarily in the original lesion, lymph node and pulmonary metastases. Changes in osseous metastases were less well defined. All the patients exhibited a general improvement in their physical status. These effects are akin to those seen in patients with advanced cancer of the prostate gland following orchietomy or estrogen therapy. In 2 patients estrogen therapy was instituted after recrudescence of the disease. No definitive effect was seen. The response is apparently only temporary, however, as 2 patients have succumbed, and the remaining patients have shown no evidence of reactivation of their disease 9 to 15 months following orchietomy. These findings suggests further a hormonal control of certain type of neoplasms.

METABOLIC EFFECTS OF TREATMENT OF CARCINOMA OF THE PROSTATE. JOSEPH C. AUB, DOROTHY M. TIBBETTS, and IRA T. NATHANSON. (Massachusetts General Hospital, Boston, Mass.)

Over a period of several years, we have studied metabolic effects of castration and of stilbestrol in a few patients with carcinoma of the prostate. Changes in the excretion of calcium, phosphorus, nitrogen, and citrate were not dramatic as were the variations in the blood phosphatase levels. We are trying to analyze the influence of treatment upon the viability or function of tumor cells by determining the acid phosphatase in multiple biopsies of metastatic lymph nodes.

URINARY SEX STEROID BALANCE IN PROSTATIC DISEASE. WILLIAM T. SALTER, FRANCES D. HUMM, and JOHN B. GOETSCH. (Laboratories of Pharmacology and Toxicology and the Department of Surgery, Section on Urology, Yale University School of Medicine, New Haven, Conn.)

That hormones can influence the progress of prostatic cancer is well supported by clinical and laboratory evidence. Such work has led, in general, to the theory that the affected organism presents an environment in which androgenic elements are predominant. This has led logically to methods of therapy in current practice which are aimed at upsetting the prevailing hormone balance either by (a) removal of the testis or (b) supplying exogenous estrogen, or by using a combination of both methods.

By actual test, however, the urinary excretion in such cases indicates an imbalance in the opposite direction from that which has been assumed to exist. The ratio of estrogen (in μgm.) to "androgen" (17-ketosteroids in μgm.) as determined microchemically, is strongly in favor of estrogens in a high percentage of cases of prostatic disease. The E/A ratio is under 1.0 in healthy young adult males, while ranging from 2.0 to 10 in ovulating adult females. In contrast, males with prostatic hypertrophy or prostatic carcinoma frequently show ratios in the female range, and occasionally above 10. This paradoxical trend of the steroid ratio bears no relationship to the degree of malignancy involved. It does fur-
nish evidence which indicates that the relation of androgens and estrogens to prostatic disease must be re-evaluated.

THE RELATIONSHIP OF THE NUCLEOLUS TO CYTOPLASMIC NUCLEIC ACIDS AND PROTEINS IN DIFFERENT CONDITIONS OF GROWTH IN RAT LIVER. ROBERT E. STОWELL. (Department of Pathology, Washington University School of Medicine, St. Louis, Mo., and Institute for Cell Research, Karolinska Institute, Stockholm, Sweden)

Rats were kept on a protein-free diet for periods up to 3 months to deplete the protein of the body. A few protein-depleted animals were then placed on a high protein diet for intervals up to 8 days. The liver of numerous normal controls, of 6 protein-depleted and 3 partially protein-repleted rats were frozen-dried or fixed in Stieve or Carnoy fluid. Some sections were stained with hematoxylin and eosin and others by the Feulgen reactions for thymonucleic acid. Fixed and unfixed sections were photographed with ultraviolet light of 2,570 A.

The nucleoli of the hepatic cells of rats on a protein-free diet increased to twice their normal size and the nuclei and cytoplasm decreased in volume. After a few days on a high protein diet the size of the nucleoli decreased and their number per nuclear section increased. The changes in the cytoplasmic absorption at 2,570 A were suggestive of an increased nucleotide content. The results of these preliminary experiments, when compared with similar experiments on liver cells in regeneration and in neoplastic transformation, show that there are large morphologic variations in the nucleolus of hepatic cells under different conditions of growth.

GROWTH RATE OF TRANSPLANTED TUMORS IN RELATION TO LATENT PERIOD AND HOST VASCULAR REACTION. GLENN H. ALGIRE, and FRANCES LEGALLAIS. (National Cancer Institute, Bethesda, Md.)

Transplanted tumors that have been studied in transparent chambers inserted into mice fall into two general classes in respect to their growth rate and vascular development. Among the rapidly growing group studied so far are included sarcomas, mammary gland carcinomas, and a malignant epithelial tumor of the skin. These elicited new capillary sprouts from the host as early as 2 to 3 days, the surrounding host vessels became hyperemic and numerous leukocytes accumulated about the implants. The percentage of the vascular tissue rose to approximately 50 per cent then stabilized at that level. The capillaries of the tumors mentioned above had an average diameter 5 times greater than those in a normal tissue (striated muscle), appearing as enormous sinusoid-like vessels which showed little tendency to differentiate into arterioles and venules.

In striking contrast to the rapidly growing tumors that killed the host in from 3 to 6 weeks, were the slowly growing tumors which killed the host in from 3 to 6 months. These included the Harding-Passay and Cloudman S91 pigmented melanomas, and an amelanotic melanoma derived from the S91. The slow growth rate of these tumors was correlated with a prolonged latent period prior to capillary proliferation, usually 8 days or more. In addition, vascular levels in these tumors rarely exceeded that of the vessels in the surrounding subcutaneous connective tissue, and were less than one half that of the rapidly growing tumors. There was very little leukocytic accumulation about the implants and vascular hyperemia in the surrounding tissues was lacking. The capillaries formed were small in diameter, like those of normal striated muscle, and showed considerable differentiation into arterioles and venules.

METABOLIC CHARACTERIZATION OF TRANSPLANTED MOUSE MELANOMAS BY HIGH OXIDATIVE RESPONSE TO PARAPHENYLENEDIAMINE. MARIE L. HESSELBACH, DEAN BURK, GLENN H. ALGIRE, CLARA FISCHER, and FRANCES Y. LEGALLAIS. (National Cancer Institute, Bethesda, Md.)

Tissue slices of the 3 transplantable mouse melanomas, the Harding-Passay, the Cloudman S91 pigmented melanomas and the S91A amelanotic melanoma, showed a metabolism consistent with that of malignant tumors generally, in regard to aerobic and anaerobic glycolysis, oxygen consumption, respiratory quotient, and other related derived quotients.

On the other hand, all 3 melanomas showed a much greater percentage stimulation (400 to 1,000) of oxygen consumption by paraphenylenediamine than any other tumors tested to date (0 to 150 per cent), and the stimulation was in all cases essentially eliminated by cyanide. This greatly enhanced stimulation of oxygen consumption by paraphenylenediamine offers the possibility of a biochemically new characterization and mode of diagnosis of melanomas, amelanotic as well as pigmented, that is readily subject to further testing with a variety of other melanomas.

The marked stimulation of oxygen consumption caused by paraphenylenediamine in cyanide-free tissue slices of the melanomas, as compared with other tumors tested, may be interpreted as indicating that the ratio oxidized/reduced cytochrome c is considerably higher in these melanomas than in other tumors, and, in fact, in the range in normal and embryonic tissues generally. This is indicative of a relatively high level of oxidation-reduction potential within the melanoma cells, either intracellularly throughout or locally in certain cell areas.

THE EFFECT OF AGE ON REGENERATION OF RAT LIVER FOLLOWING PARTIAL HEPATECTOMY. NANCY L. R. BUCHER, ANDRÉ GLINOS, and JOSEPH C. AUB. (Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University at the Massachusetts General Hospital, Boston, Mass.)

It seems possible that the frequent association between cancer and old age may express some significant aspect of the genesis of cancer. Accordingly it seems important to formulate the response of ageing tissues to growth stimuli.
Regenerating rat liver has been chosen for such a study because its restorative capacity can be accurately quantitated. Previous investigators have found that age delays mitosis in this tissue, and that it retards restoration of liver.

In the present experiment rats of accurately known ages were divided into young (4 to 6 weeks), adult (6 to 8 months) and old (1½ to 2½ years) animals. Many of the latter group showed the characteristics of senility. The main lobes of the liver, constituting approximately 68.4% of the total, were removed, and the method of Brues, Drury and Brues (Arch. Path., 22:658, 1936) was followed in determining the percentage of restoration in terms of (1) mass and (2) number of cells. Rats were autopsied at intervals of 16 and 30 hours, and 3, 7 and 14 days after operation.

The young rats, in whom regeneration was superimposed on active growth, were not strictly comparable to the other two groups. In general the regenerative capacity decreased with age. Restoration of liver mass was retarded in the adult and old rats as compared with the young rats, and restoration of hepatic cells to their original number was retarded in the old rats as compared with the other two groups.

THE CITRIC ACID CONTENT OF TUMOR TISSUE AND OF TUMOR-BEARING ANIMALS. FRANCES L. HAVEN, and CHALLISS RANDALL. (Department of Biochemistry, The University of Rochester School of Medicine and Dentistry, Rochester, N. Y.)

The citric acid content of Walker 256 and of liver, blood, and kidneys of rats bearing this tumor has been determined. The necrotic portion of 21 tumors contained 4 to 20 times more citric acid than the non-necrotic portion. The blood of tumor-bearing rats is normal in citric acid. The kidneys and, to a lesser extent, the livers of rats bearing this tumor were higher in citric acid than similar organs of rats without tumors.

ON DEFECTIVE PLASMA PROTEIN FORMATION IN PATIENTS WITH GASTRIC CANCER. F. HOMBURGER, AURELIA POTOR, and N. F. YOUNG. (Sloan-Kettering Institute for Cancer Research, New York, N. Y.)

Intractable hypoproteinemia is part of the systemic disease found in patients with gastric cancer. This study of nitrogen balance and plasma protein regeneration was made on 13 patients with gastric ulcers, operable and inoperable cancer of the stomach. It was found that on high protein intakes, sufficient to produce plasma protein regeneration in patients with gastrectomies for ulcers, plasma protein levels of patients with gastric cancer remained low or continued to fall. The increase of circulating plasma protein in 2 cases was due to globulins. This finding, together with the fact that even in the presence of positive nitrogen balance for as long as 80 days no increase of circulating plasma protein occurred, suggests an anomaly of protein metabolism in these patients.

HISTOCHEMICAL PHOSPHATASE REACTION IN MOUSE SARCOMAS CR 180 AND 37 FOLLOWING ADMINISTRATION OF BACTERIAL POLYSACCHARIDE. MORRIS BELKIN, and ELMER D. BUEKER (by invitation). (Departments of Pharmacology and of Anatomy, Medical College of South Carolina, Charleston, S. C.)

Swiss albino mice carrying 2 weeks old implants of sarcoma CR 180, and dba mice carrying similar implants of sarcoma 37 were each given 0.1 mgm. of bacterial polysaccharide intraperitoneally. They were then sacriﬁed, as were control animals, in groups of 2 or 3, at half-hourly or hourly intervals for the first 4 hours, and at 8, 12 and 24 hours after injection. The tumor tissue was fixed in chilled acetone for acid phosphatase, and in 80 per cent alcohol for alkaline phosphatase preparations. Gomori’s histochemical method was used for both acid and alkaline phosphatase reaction, with minor modifications.

For both acid and alkaline phosphatases, 5 different substrates were used, with varying incubation periods as follows:

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Acid pH 5, hours</th>
<th>Alkaline pH 9.4, hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerophosphate</td>
<td>6½</td>
<td>5</td>
</tr>
<tr>
<td>Adenyllic acid</td>
<td>72</td>
<td>3</td>
</tr>
<tr>
<td>Fructose diphosphate</td>
<td>72</td>
<td>2</td>
</tr>
<tr>
<td>Lecithin</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>Yeast nucleic acid</td>
<td>48</td>
<td>3</td>
</tr>
</tbody>
</table>

No striking effects were obtained, for any of the substrates, for either acid or alkaline phosphatase reaction. The tumors incubated with glycerophosphate and yeast nucleic acid on the acid side, and all the substrates on the alkaline side, showed a mild increase in staining properties 2 to 3 hours after polysaccharide administration.

Microscopically, at this interval, the nuclear wall and nucleoli were somewhat more darkly stained. But, as the cytotoxic action of the polysaccharide continued, with progressive dissolution of nuclear contents into granular fragments, and their dispersal into the cytoplasm, the intensity of the staining diminished.

It is concluded that the cytotoxic effect of bacterial polysaccharide is not mediated through attenuation or destruction of the phosphatase enzymes in so far as it has been studied by this particular histochemical technic.

REDUCTION IN TOXICITY OF SERRATIA MARCESENS POLYSACCHARIDE TO TUMORBEARING MICE PRODUCED BY UPJOHN CO. BEEF ADRENAL EXTRACT. LYLE BECK, IRENE DILLER, BERTINA BLAUCH, and MARY FISHER. (Lankenau Research Institute, Philadelphia, Pa.)

The Serratia marcescens tumor-necrotizing polysaccharide isolated by Shear and his co-workers (J. Nat. Cancer Inst., 4) produces toxic effects which may culminate in death when the mice bear large tumors or when a dose many times that required to produce extensive necrosis is given to mice bearing small tumors.

Five hundred micrograms of S. marcescens polysaccharide preparation P, of the National Cancer Institute
AN EXPERIMENTAL STUDY OF SINGLE PARTURITION INTERVAL.

agent tended to vary inversely with the injection-response of pulmonary tissue to poly saccharide. Another group of mice, bearing 7 day tumors, were injected with a single narcotizing dose of poly saccharide and another 0.25 cc. of adrenaline extract at the end of the working day. Of these mice, 22 survived 48 hours or longer. The probability of this difference in survival being due to chance was calculated using Chi Square and was found to be about 1 in 10,000.

On the other hand, no evidence was secured that Upjohn Co. concentrated hog adrenal extract in oil is effective in counteracting the lethal effects of 500 mgm. of poly saccharide preparation P, given to mice bearing 7 day tumors.

STUDIES OF PULMONARY TUMOR INDUCTION IN MICE BY DERIVATIVES OF CARBAMIC ACID. C. D. LARSEN. (National Cancer Institute, Bethesda, Md.)

Examination of the phenomenon of pulmonary tumor induction in mice by ethyl carbamate and other derivatives of carbamic acid have been extended. In strain A mice, single injections of a narcotizing dose of ethyl carbamate initiated increases in incidence and frequency of lung tumors. Although an initial response was noted after 1 month, maximum effects were not observed until 5 months had elapsed.

Ethyl carbamate (urethane) was relatively specific in its capacity to induce lung tumors. Studies of ester homologues of urethane, other than those previously reported, substantiate the specificity of urethane. α-chloro-ethyl carbamate and trichloroethyl carbamate were inactive; propyl carbamate and isopropyl carbamate exhibited about 1 and 5 per cent, respectively, of the activity of the ethyl ester. N-alkylated ethyl carbamates, with one exception, tended to decline in activity as the extent of alkylation was increased. Mono-N- and di-N-methyl ethyl carbamates exhibited about 10 and 5 per cent, respectively, of the activity of urethane. Mono-N-isopropyl ethyl carbamate, however, elicited a striking increase in lung tumors; an activity approaching 50 per cent of that of urethane was noted.

Embryonic lung tissue was found susceptible to the oncogenic action of urethane. Litters from pregnant mice that had been injected with a single narcotizing dose of urethane prior to parturition were kept until 6 months of age. striking increases in the incidence and multiplicity of lung tumors in the offspring were observed. Essentially identical results followed either intraperitoneal or intravenous injection of the pregnant mice. The response of pulmonary tissue to in utero exposure to the agent tended to vary inversely with the injection-parturition interval.

AN EXPERIMENTAL STUDY OF SINGLE TRAUMA MALIGNANCY. WILLIAM L. SIMPSON. (The Barnard Free Skin and Cancer Hospital, and Washington University School of Medicine, St. Louis, Mo.)

The skin of the Swiss strain mouse is rendered unusually susceptible to the action of carcinogenic agents by the prolonged application to it of carcinogenically inactive solutions of methylcholanthrene in anhydrous lanolin. Unless actively carcinogenic compounds are applied to the skins of these "sensitized" mice, they usually remain free from cancer until death, and neither structurally nor chemically do their skins resemble those in which precancerous changes have been initiated by "active" solutions of methylcholanthrene in benzene.

Experiments have been conducted on such hypersusceptible skin, as well as on normal mouse skin, to test the cancer-evoking potentialities of a single severe trauma. Three types of injury were inflicted: (1) burning with a hot glass rod, (2) crushing of the skin with pliers, and (3) exposure to a massive localized dose of roentgen irradiation.

Only rarely does the normal mouse respond to a solitary trauma by the development of a malignant tumor, a result in agreement with most earlier observations. In sharp contrast are the results on "sensitized" mice. Malignant tumors did not follow injuries by crushing or by x-ray "burns," but in two groups of mice subjected to trauma by burning with a hot glass rod malignant tumors appeared in 42 per cent and 65 per cent respectively. Carcinomas, sarcomas and carcinosarcomas were produced. In both groups approximately 80 per cent of the tumors arose at the site of the preceding injury. The average period of induction, dated from the time of injury, was 7 months.

The bearing of these experiments on the much discussed problem of single trauma cancer in man was considered.

DIFFUSIBLE AND NON-DIFFUSIBLE CALCIUM IN NORMAL AND METHYLCHOLANTHRENE-TREATED MOUSE EPIDERMIS. A. I. LANSING and M. H. AU. (Department of Anatomy, Washington University School of Medicine, and Barnard Free Skin and Cancer Hospital, St. Louis, Mo.)

Carruthers and Suntzeff in 1943 established that epidermal calcium is significantly decreased in methylcholanthrene-induced hyperplasia and carcinoma. The present investigation was designed to explore further this pronounced shift in total calcium and to determine whether the ratio between free and bound calcium in early and late hyperplasia and carcinoma is altered.

The method employed for separation of free and bound calcium (measured as diffusible and non-diffusible calcium) was based upon the ultrafiltration technic of Mazia in 1937, and calcium was determined by the method of Lindner and Kirk, the same year.

The ratio of diffusible to non-diffusible calcium in normal 3 month old Swiss mice was 1:1.6; early (20 days) hyperplastic epidermis revealed no significant alteration of this ratio but confirmed the 50 per cent drop in total calcium reported by Carruthers and Suntzeff. Study is being made of the diffusible and non-diffusible calcium ratio in late hyperplasia (60 days) and carcinoma.
HYALURONIDASE AND THE GROWTH OF MALIGNANT EPITHELIAL TUMORS. A. R. GOPAL-AVENGER, and WILLIAM L. SIMPSON. (The Barnard Free Skin and Cancer Hospital, and Washington University School of Medicine, St. Louis, Mo.)

Although an association between “spreading factors” and the growth of malignant tumors has been recognized for some years, the nature of the relationship has never been elucidated. We have now tested by direct experiments the hypotheses formulated in 1943 by Cramer and Simpson on a possible mechanism for this association. Included in the investigations were: (1) the effects of a spreading factor from testis (hyaluronidase) on the growth and invasive capacities of a mouse-transplantable squamous cell carcinoma, (2) the relation of hyaluronidase and anti-hyaluronidase antibodies to the development of the transplantable tumor, and (3) the effect of the enzyme on carcinogenesis in response to methylcholanthrene.

Local injection of the hyaluronidase about the base of established cancer transplants resulted in the enhancement of invasive growth with a striking destruction of muscle and bone by line tumors. In a few instances the local injection was followed promptly by the appearance of distant metastatic lesions.

Results of the other experiments, which were not then quite completed, were described at the meeting.

THE ROLE OF SEBACEOUS GLANDS AND HAIR FOLLICLES IN EPIDERMAL CARCINOGENESIS IN MICE. V. SUNTZEFF, C. CARRUTHERS, and E. V. COWDRY. (From the Barnard Free Skin and Cancer Hospital, and the Department of Anatomy, Washington University School of Medicine, St. Louis, Mo.)

Previous studies in this laboratory revealed that young New Buffalo mice developed squamous cell carcinoma more rapidly and in a higher percentage than did old mice of the same strain after the topical application of methylcholanthrene. This difference led to an investigation of the response of the skin of very young mice (2 to 10 hours after birth) to a single application of the same carcinogen. Thirty mice were treated in this fashion, and 19 months after the application of the carcinogen, 23 mice were alive without evidence of tumor formation. A possible morphological basis for this lack of responsiveness was found in a detailed study of the development of the skin and its associated structures from the time of birth until the skin was completely developed. The hair follicles and sebaceous glands were found to be rudimentary at the time the carcinogen was applied, and the epidermis was well differentiated and covered with a thick layer of keratin. The failure of very young mice to develop cancer may be due to the following factors: Inability of the carcinogen to penetrate through the thick epidermis or to reach the few rudimentary sebaceous glands via the hair follicles, only a few of which have hair reaching the exterior. That the hair follicles and sebaceous glands play an important role in epidermal carcinogenesis in mice is quite apparent from this study.

STUDIES ON THE TRANSMISSION OF AVIAN VISCERAL Lymphomatosis. I. VARIATION IN TRANSMISSIBILITY OF NATURALLY OCCURRING CASES. BURMESTER, B. R., and DENINGTON, E. S. (U.S. Regional Poultry Research Laboratory, East Lansing, Mich.)

The transmissibility of tumors from 10 cases of naturally occurring visceral lymphomatosis was tested by inoculation of cellular and cell-free preparations into groups of 14 to 21 chicks 1 day of age. The recipient chicks were relatively free from prior infection since none of 41 non-inoculated controls developed tumors during an experimental period of 183 days.

Lymphomatous tumors of the viscera were reproduced (an incidence of 14 to 85 per cent in 93 to 183 days) in recipients of cell-containing preparations from 8 of the original tumors. Similar tumors were produced (an incidence of 39 to 94 per cent in 183 days) by cell-free preparations from 5 of the original tumors. In addition to the visceral tumors, preparations from 1 tumor also produced a high incidence of osteoporosis.

Of the 10 donors that supplied visceral tumors, 7 also had gross or microscopic evidence of neurolymphomatosis. Gross neural lesions appeared in 1 to 4 chickens of several groups; however, there appeared to be no direct relation between the presence of this lesion in the donor and the number of recipients that developed neural or visceral lymphomatosis.

Tumors of some, but not all, cases of visceral lymphomatosis are transplantable, and part of these tumors may be transmitted to chicks by inoculation with filtrates. The active agent or agents are of a size which will allow them to pass readily through bacteria-retaining filters.

TRANSPLANTATION OF THE ROUS CHICKEN SARCOMA INTO THE ANTERIOR CHAMBER OF THE MOUSE EYE. EDWARD W. SHIRLEY. (From the Department of Bacteriology and Immunology, Yale University School of Medicine, New Haven, Connecticut)

The Rous chicken sarcoma placed into the eye of the mouse grows to fill the chamber and frequently herniates to the exterior through the cornea. The growth behavior of the sarcoma in the mouse eye is similar to that in the eye of the guinea pig. However, in the former the tissue persists longer before undergoing regression. Transplants capable of producing growths in chicks have not been obtained from mice after 15 days of residence. Chicks injected directly with this mouse growth may show, in addition to the local tumor, hemorrhagic disease and periosteal sarcomas. Subsequent passages in chicks indicate that unlike the guinea pig passage agent, the virus has not undergone alteration in specificities nor has it increased in potency. On the contrary, data suggest that the mouse passage virus has lost some of its virulence while its tissue specificities are no different from those of the stock Rous agent.

THE MORPHOLOGIC STABILITY OF SIX STRAINS OF MALIGNANT MOUSE FIBRO-
BLASTS GROWING IN VITRO. WILTON R. EARLE. (National Cancer Institute, Bethesda, Md.)

The production of six strains of sarcoma cells from one parent strain of mouse fibroblast growing in an entirely heterologous medium in vitro has been previously reported. Of these six, strains D, H, J, L, N, and O had been treated with a concentration of 1 μg/ml. of 20-methylcholanthrene per ml. of culture media for 6, 32, 111, 184, and 406 days respectively. The degree of morphologic alteration in these cell strains was apparently directly associated with the time the cell strains had been subjected to the carcinogen. Strain D, the presumably untreated control strain, also underwent a very limited morphologic alteration, but never showed as great a change as the cells of strain H, which were subjected to the carcinogen for 6 days.

The last of these cell strains was removed from 20-methylcholanthrene on September 16, 1942, and since that time all strains have been grown in the same heterologous culture medium of chicken plasma, horse serum, and chick embryo extract, and under the same experimental culture conditions.

Periodic photographs of these living cultures from December 17, 1942, through December 19, 1946, showed that strains J, N, and O have undergone certain limited secondary alterations within this interval. Strains D, H, and L, however, have shown no recognizable change in their respective characteristic induced morphologies since December 17, 1942. Allowing generously 5 days for each mitotic interval in these three cell strains, it seems that the respective characteristic induced morphologies of these three cell strains have been stable for over 290 consecutive cell generations.

THE USE OF PURIFIED FIBRINOGEN WITH CERTAIN STRAINS OF NORMAL AND MALIGNANT FIBROBLASTS IN TISSUE CULTURES. VIRGINIA J. EVANS, HELEN M. DYER, and MARGARET G. KELLY. (National Cancer Institute, Bethesda, Md.)

An attempt was made to obtain a more chemically reproducible solid culture medium for tissue culture metabolic studies than has been possible by the use of plasma. A study has been made of bovine fibrinogen prepared by a number of different procedures. Test cell strains used have all been subcutaneous mouse fibroblasts and have included 3 strains of presumably normal mouse fibroblasts, one freshly explanted in vitro and two grown in vitro for more than 3 years. Earle's sarcoma strains D, H, J, L, N, and O were also used. All cultures were grown in Carrel D3.5 flasks and the supernatant culture medium has been 40 per cent saline, 40 per cent horse serum and 20 per cent chick embryo extract.

Results to date indicate that different cell strains vary substantially in their tendency to lyse this solid substrate. Of the cell strains tried, strain L alone was unable to lyse the clot to any perceptible degree. All three strains of normal cells showed rapid lysis of the clot as did the sarcoma strains D, H, J, N, and O.

HEREDITARY EOSINOPHILE LEVELS IN THE ACQUIRED RESISTANCE OF THE RABBIT TO THE BROWN-PEARCE TUMOR. ALBERT E. CASEY and GEORGE R. DRYSDALE. (Department of Pathology, The Baptist Hospital, Birmingham, and the Holy Name of Jesus and Baptist Memorial Hospitals, Gadsden, Ala.)

Previous studies by our group demonstrated hereditary variations in the blood eosinophile levels of normal rabbits but none for the neutrophiles or monocytes. High pretransplantation eosinophile levels were associated with a lower incidence and number of metastases, and a lower mortality in animals receiving successful transplants than low pretransplantation levels. No such relation for the neutrophile or monocyte levels could be demonstrated.

Because the eosinophile effect did not seem to become manifest until the seventh week after inoculation 98 additional animals were studied, giving a cumulative total of 283 young adult male rabbits received from breeders. Of these, 159 had the blood level of each of nine blood cell factors within normal limits for the species, and were seemingly free from intercurrent disease. These 159 normal animals were inoculated intrathecally with the Brown-Pearce tumor, and surviving animals were sacrificed two months thereafter.

The cumulative data indicate that the pretransplantation eosinophile level bears no apparent relationship to the course of the Brown-Pearce tumor during the first six weeks after inoculation. Its effect appears in the seventh week and persists with the characteristic and statistically significant pattern described above. It especially seems to affect the incidence of hematogenous metastases.

The seventh week corresponds to the beginning of regression or the turning point of this neoplastic disease as first described by Brown and Pearce and later by Malluche. Thus a relationship between the hereditary eosinophile level and the acquired resistance of the rabbit to the tumor is indicated.

RETARDATION OF GROWTH AND METABOLISM OF NORMAL AND MALIGNANT CELLS DURING CONTINUOUS CULTURE. JOHN H. HANKS (by invitation), GEORGE O. GEY, and RACHEL BARRETT (by invitation). (Division of Cell Physiology, Department of Surgery, Johns Hopkins Hospital and Medical School, Baltimore, Md., and Leonard Wood Memorial Department of Bacteriology, Harvard Medical School, Boston, Mass.)

Throughout the life of multicellular organisms, most of the tissues and organs are capable of carrying on their specific function with a fairly stable cell population and, therefore, at a low maintenance rate of growth. When cells are released from the organization and control of the host and are explanted in tissue cultures, conditions are usually provided which cause them to migrate and divide rapidly. Since a major portion of biological and medical interest in the results of tissue cultivation depend
on interpretation in terms of post-embryonic or adult physiology and pathology, it is obvious that the art and science of tissue cultivation need some reorientation in the direction of maintaining more stable populations of cells without rapid multiplication. Maintaining cells at metabolic levels approximating those of postpartum physiology is of value in studying problems concerned with cytology, nutrition, endocrine secretion, antibody formation, the interrelations of cells and infectious agents, and the riddle of differentiation and malignancy. By lowering cell metabolism through reduction in temperatures of maintenance and by decrease in concentration of nutrients, it has been possible to perpetuate strains of normal and malignant cells over long periods of time with minimal effort. Reduced temperature levels thus far investigated include 28°, 31°, and 34° C. The results reported include studies on normal human and rat fibroblasts and several strains of rat sarcoma. The effects of lowering temperature and nutrient supply upon rate of growth, duration of mitosis, cultural behavior, and cytology were discussed.

**FURTHER OBSERVATIONS ON THE CONVERSION OF NORMAL INTO MALIGNANT CELLS IN VITRO.** GEORGE O. GEY, and MARGARET K. GEY (by invitation). (Division of Cell Physiology, Department of Surgery, Johns Hopkins Hospital and Medical School, Baltimore, Md.)

This study is concerned with a series of permanent alterations occurring in continuous cultures of normal rat mesenchyme cells and leading to the production of malignant cells. The strains studied include normal, altered normal, and malignant cell strains of autologous origin which have been under cultivation for eight and one-half years. It has been possible to make direct comparison between a normal and a malignant strain derived from it. The data to the present time implicate factors contributed by a culture medium totally heterologous to the strains studied. No known extraneous carcinogenic agents have been found to play a part in these conversions which occurred in stocks of normal cell strains. Differences between normal and malignant autologous strains were discussed.

**IS AEROBIC GLYCOLYSIS OF AN INTENSITY CHARACTERISTIC OF CANCER TISSUE? A NORMAL METABOLIC FEATURE OF THE MUCOSA OF THE SMALL INTESTINE? OTTO ROSENTHAL.** (Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania, Philadelphia, Pa.)

In 1941 Dickens and Weil-Malherbe reported that the rate of aerobic glycolysis of normal duodenal or jejunal mucosa of rat and mouse equals that generally obtained with cancer tissue. These authors believed they had eliminated the possibility of an artefact in spite of the exceptional observation that the aerobic glycolysis was as high as the anaerobic glycolysis. Such complete absence of the Pasteur effect has never before been observed in undamaged tissues whether normal or malignant.

Since the mucous membranes of murine species are extremely fragile the metabolism of the more stable duodenal mucosa of the rabbit was studied manometrically by means of Warburg's indirect method. In addition, lactic acid was determined colorimetrically with the method of Barker and Summerson.

The rates of respiration and of anaerobic glycolysis of the rabbit mucosa approximated those obtained with duodenal mucosa of the rat by Dickens and Weil-Malherbe. $Q_o$ and $Q_e$ averaged 9.5 and 7.9 respectively (initial dry weight basis, 60 minutes). The aerobic glycolysis, however, amounted to but 10 per cent of the anaerobic glycolysis. The Pasteur effect was thus evident. Persistence of a small aerobic glycolysis is commonly found with normal tissues in vitro.

While these results do not eliminate the possibility that the high aerobic glycolysis of murine mucosa of the small intestine is a peculiarity of the species, the known absence of species differences in the metabolism of colonic mucosa does not favor this interpretation, but rather suggests an artefact.

**MICROMETRIC INVESTIGATIONS ON MYELOMA CELLS AND NORMAL BONE MARROW PLASMA CELLS.** HARALD GORMSEN. (Department of Pathology, University Institute of Forensic Medicine, Copenhagen, Denmark)

Micrometric investigations by ocular micrometer have been carried out on normal bone marrow plasma cells (smears of sternal punctures and sections of bone marrow from 15 normal adult persons) and on myeloma cells (smears of sternal punctures and sections of myeloma tissue from 29 patients). In each preparation 50 cells and their nuclei have been measured in longitudinal and transverse direction. The average values of the 50 measurements have been subjected to statistical analysis.

In 18 of the 29 cases of myeloma both the nuclei and total cell size were significantly larger than normal plasma cells in bone marrow. In 8 cases, only the nuclei of the myeloma cells were significantly larger than nuclei of normal plasma cells in the bone marrow, whereas 3 myeloma cases showed cell- and nuclei-sizes that did not differ from normal bone marrow plasma cells.

Consequently, in the majority of myeloma cases (in the present material 26 out of 29) the myeloma cells differ unmistakably from normal bone marrow cells. In a few cases (in the present material 3 out of 29) myeloma cells in all aspects (cell size, nucleus size, nuclear structure, etc.) are morphologically identical with normal bone marrow plasma cells.

This observation is of practical importance in the use of sternal punctures for the differential diagnosis between myelomatosis and conditions with reactive plasma cell proliferation in the bone marrow (infections, etc.).

No significant relation could be demonstrated between the degree of morphological abnormality of myeloma cells and the clinical symptoms or the course of the myeloma cases.
THE EFFECT OF SOME CARCINOGENIC AMINOazo DYES ON THE AUTOXIDATION OF LINOLEIC ACID. H. P. RUSCH, and J. A. MILLER. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

Previous publications from this laboratory have demonstrated that carcinogens including certain azo dyes inhibit the autoxidation of unsaturated lipids. The present paper describes in more detail the effect of p-dimethylaminoazobenzene and its demethylated derivatives on the autoxidation of purified linoleic acid. The aminoazo dyes were purified by chromatographic adsorption. Known quantities of linoleic acid and the azo dyes were placed in Warburg flasks and the rate of autoxidation was followed manometrically at 36.5°C. The flasks contained linoleic acid alone or with varying levels of p-dimethylaminoazobenzene (DAB), p-monomethylaminoazobenzene (MAB), or p-aminooazobenzene (AB).

DAB and MAB both increased the latent period of oxidation of linoleic acid, the former being more effective as an antioxidant than the latter, and the antioxidant effect of each dye was proportional to the concentration employed. Thus, when DAB was used at concentrations of M/200, M/100, and M/50 the oxidation of the linoleic acid at the end of the first 24 hour period had progressed only 56, 29, and 0 per cent respectively as compared to the acid alone. With the same levels of MAB, the amount of oxidation was 73, 45, and 35 per cent respectively. Contrary to the inhibiting effect of the methylated dyes, AB shortened the latent period slightly.

As the autoxidation proceeded, the azo dyes disappeared from the flasks and DAB and MAB were found to be demethylated. At the end of 30 hours 90 per cent of the DAB initially added had disappeared from the flask but MAB appeared in amounts equal to 85 per cent of the starting level of DAB. Small amounts of AB were also found throughout the run. MAB disappeared more slowly than DAB during the course of the oxidation and it was found to be demethylated to AB. AB disappeared very rapidly in oxidizing linoleic acid and no other basic dye was detected in the mixture.

THE INHIBITION OF THE GROWTH OF LACTOBACILLUS CASEI BY p-MONOMETHYLAMINOazoBENZENE AND ITS REVERSAL BY RIBOFLAVIN. E. C. MILLER, H. N. KINGSLEY, and J. A. MILLER. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

The activity of the hepatic carcinogens p-dimethylaminoazobenzene and p-monooethylaminoazobenzene can be greatly modified by the character of the diet in which they are fed. In particular, feeding diets high in riboflavin to rats greatly delays tumor development due to these compounds. This antagonism has now been studied using the growth of L. casei as the end point; the growth of this organism is proportional to the riboflavin content of the medium. The bacteria were grown in the medium of Roberts and Snell except that in certain cases amino acids were substituted for a part of the hydrolyzed casein. Growth was measured turbidimetrically 24 hours after inoculation. p-Monomethylaminoazobenzene was generally used because it is 10 times as soluble as p-dimethylaminoazobenzene in aqueous media.

When 1 to 3 mgm. of p-monooethylaminoazobenzene were added per ml. of medium, growth was inhibited by 60 to 90 per cent at riboflavin levels of 0.01 to 0.15 mgm. per ml. Increasing the level of riboflavin to 1.25 mgm. per ml. decreased the inhibition to 0 to 40 per cent; higher levels of riboflavin were impractical because of the poor solubility of the vitamin. The inhibition due to the dye could also be reversed by an unidentified constituent present in fresh pancreatic digests of casein; the activity of this factor decreased on storage in the cold for 4 to 8 weeks. When Saccharomyces cerevisiae was grown anaerobically in the same medium, its growth was inhibited 20 to 40 per cent at levels up to 0.2 mgm. of riboflavin per ml. Larger amounts of riboflavin usually reduced the inhibition to 10 per cent or less. S. cerevisiae destroyed 80 to 90 per cent of the p-monooethylaminoazobenzene in the medium at the high levels of riboflavin while L. casei destroyed only 10 to 20 per cent.

SUSCEPTIBILITY OF STRAIN C MICE TO p-AMINOazoTOLUENE. H. B. ANDERVONT, and THELMA B. DUNN. (National Cancer Institute, Bethesda, Md.)

Female mice of strain C are much more susceptible than males to hepatic lesions induced by p-aminoazotoluene. Castration of males considerably increases their susceptibility while castration of females lowers their susceptibility. Administration of testosterone propionate to castrated males or females lowers their susceptibility to that of intact males. The compound induces hemangioendotheliomas in both sexes. The site of origin of these tumors is influenced by the site of administration of the compound.

TUMORS PRODUCED IN RATS AFTER INGESTION OR PAINTING OF 2-NITRO, 2-AMINO, N-ACETYL-2-AMINO, AND N-DIACETYL-2-AMINO FLUORENE. H. P. MORRIS, C. S. DUBNIK, T. B. DUNN, and J. M. JOHNSON. (National Cancer Institute, Bethesda, Md.)

The carcinogenic effect on the rat of 4 derivatives of fluorene were studied after both ingestion and painting. In the feeding experiments each derivative was fed to rats at a level of 0.05 per cent in a low-fat synthetic diet for 160 days. The average daily ingestion of carcinogen ranged from 4.0 to 4.7 mgm. In the painting experiments a 2 per cent acetone solution of each compound was applied thrice weekly to the scapular region. The estimated amount of carcinogen given with each application was 0.5 mgm. during the first 6 months and 1.00 mgm. thereafter. The painted rats were fed a stock diet. Autopsies were made after the appearance of tumors or...
when death appeared imminent. Sixty-four tumors were identified histologically in 104 treated animals. No tumors were found in control animals.

Distant tumors were produced by all 4 derivatives after either ingestion or painting. 2-Aminofluorene was the only compound producing skin tumors. The majority of liver tumors were observed in rats after ingesting either the mono or diacetyl derivative. The 2-nitro derivative induced no liver tumors. The results of these experiments suggest for both types of administration an increasing order of carcinogenicity from the nitro to the amino to the mono or diacetyl derivative. The type of distant tumors produced, while not dependent on the route of administration, seems to be influenced by it.

PARALLEL EFFECTS OF CERTAIN DIETS UPON THE RETENTION OF RIBOFLAVIN AND THE FORMATION OF HEPATIC TUMORS IN THE LIVERS OF RATS. A. C. GRIFFIN, and C. A. BAUMANN. (Department of Biochemistry, University of Wisconsin, Madison, Wis.)

When p-dimethylaminoazobenzene was fed to rats, the amount of riboflavin in the liver varied with the concentration of this vitamin in the diet: liver storage was lower on synthetic or semi-synthetic diets containing 0.7% of riboflavin per gm. of diet than on similar diets containing 2.0% of riboflavin per gm. The rate of tumor formation was faster on the lower level of riboflavin intake, and on any one level it was essentially the same whether the other B vitamins were supplied as a synthetic mixture or as a crude rice concentrate. In the presence of m'-methyl-p-dimethylaminoazobenzene the hepatic storage of riboflavin was low on both dietary levels of the vitamin; and previous studies have indicated that tumors due to m'-methyl-p-dimethylaminoazobenzene form at essentially the same rate on either diet.

In the presence of p-dimethylaminoazobenzene more riboflavin was retained in the liver when the fat of the diet was hydrogenated coconut oil than when it was corn oil. Hepatic tumors are known to form more rapidly when the latter oil is fed. In the presence of m'-methyl-p-dimethylaminoazobenzene, however, essentially the same amounts of riboflavin were found in the liver whether corn oil or hydrogenated coconut oil were fed, and on the basal diets used, the nature of the oil does not appear to affect the rate at which liver tumors develop when the m'-methyl dye is the carcinogen. These results, and the quantitative relationship between the carcinogenicity of the many azo dyes and their effects on hepatic riboflavin, suggest that riboflavin retention parallels the ability of the liver to resist the formation of tumors due to azo dyes.

THE LEVELS OF LIPIDS AND CARCINOGENIC AZO-DYES IN THE LIVERS OF RATS FED VARIOUS DIETS CONTAINING p-DIMETHYLaMINOAZOBENZENE. RELATIONSHIP TO THE FORMATION OF HEATOMAS. HERBERT SILVERSTONE, and ALBERT TANNERBAUM. (Department of Cancer Research, Michael Reese Hospital, Chicago, Ill.)

The hypothesis that diets affect the formation of azo-dye-induced hepatomas in rats through modifying the level of carcinogenic azo dyes in the liver has been studied. The possibility that carcinogenicity might be influenced by the liver lipid level was also considered. Groups of 24 rats were fed the following diets: (a) brown rice; (b) brown rice plus 15 per cent brewers' yeast; (c) a "synthetic" diet high in protein and fat; (d) a "synthetic" diet low in protein and high in fat; (e) a similar "synthetic" diet low in both fat and protein. Six hundredths per cent p-dimethylaminoazobenzene was incorporated into each of the diets for 4 months; the dye was then omitted and the diets continued until death of the animal or the termination of the experiment 2 months later. The same diets with azo dye were also fed to groups of 5 rats for 7 weeks, following which the animals were sacrificed and their livers analyzed for total lipids, free and total cholesterol, lipid phosphorus, and carcinogenic azo dyes (p-dimethylaminoazobenzene plus p-monomethylaminoazobenzene). The levels of azo dyes appeared to be positively associated with the formation of hepatomas. There was no evidence that either hepatoma formation or the concentration of carcinogenic azo dye in the liver are dependent on the level of liver lipids.

INFLUENCE OF THIOURACIL UPON THE CARCINOGENIC ACTION OF ACETYLAMINOFLUORENE. K. E. PASCHKIS, A. CANTAROW, and J. STASNEY. (Jefferson Medical College and Hospital, Philadelphia, Pa.)

Rats fed 2-acetylaminofluorene develop a variety of malignant tumors. We have reported previously that treatment with certain sex hormones hastens and intensifies the development of cancer of the liver by this carcinogen. We have now found that administration of thiouracil protects the liver against the carcinogenic and other effects of acetylaminofluorene and also against the "potentiated" carcinogenicity of combined acetylaminofluorene and testosterone treatment. At the same time there are indications that the androgenic effect of testosterone is more pronounced in animals receiving thiouracil than in those receiving the hormone alone. These findings suggest that the protective action of thiouracil may consist, in part at least, in preventing the transformation of testosterone to a compound of carcinogenic (or co-carcinogenic) and at the same time of lessened androgenic potency. Thiouracil treatment failed to protect the liver against the carcinogenic effect of dimethylaminoazobenzene.

The thyroid glands of animals treated with acetylaminofluorene and thiouracil show essentially the same changes (hyperplasia, adenoma) as those observed in rats treated over long periods of time with thiouracil alone. Malignancy of the thyroid developed in a few animals treated with acetylaminofluorene and thiouracil. Inasmuch as this has been reported by others in rats treated with thiouracil alone, the carcinogen appears merely to hasten and intensify the development of thyroid malignancy without being essential to it.
THE CARCINOGENICITY OF CERTAIN COMPOUNDS RELATED TO p-DIMETHYLAMINOAZOBENZENE. KANEMATSU SUGIURA. (Memorial Hospital, New York, N. Y.)

Many aminoazobenzene derivatives have been tested for carcinogenic activity in the rat. \(N,N\)-dimethyl-p-aminoazobenzene and N-methyl-p-aminoazobenzene have been found to be equally carcinogenic. They produced cholangiomas and hepatomas in all animals tested in approximately the same period of time. \(N,N\)-dimethyl-3'-methyl-p-aminoazobenzene was more carcinogenic than the parent compound \(N,N\)-dimethyl-p-aminoazobenzene; but the \(N,N\)-dimethyl-2'-methyl-p-aminoazobenzene and \(N,N\)-dimethyl-4'-methyl-p-aminoazobenzene were very much less active. \(N,N\)-diethyl-p-aminoazobenzene and all other higher alkyl homologues of \(N,N\)-dimethyl-p-aminoazobenzene tested failed to produce cirrhosis or neoplastic changes in the liver of the rat when fed in equimolecular amounts.

The investigation has been extended to several compounds of this series which have not been previously tested. Rats were fed a rice diet to which 0.06 per cent of \(N,N\)-dimethyl-p-aminoazobenzene dissolved in cottonseed oil or molar equivalent amounts of the other compounds were added. The diet was supplemented with a slice of fresh carrot and water daily. Feeding was continued until the animals either succumbed or were sacrificed at the end of the experimental period of 250 days. The results showed the N-methyl-3'-methyl-p-aminoazobenzene was at least as carcinogenic as the N-methyl or \(N,N\)-dimethyl compound. However, the N-methyl-2'-methyl-p-aminoazobenzene and the N-methyl-4'-methyl-p-aminoazobenzene were very much less carcinogenic. Although \(N,N\)-diethyl-p-aminoazobenzene was noncarcinogenic, N-methyl-N-ethyl-p-aminoazobenzene was definitely carcinogenic, an indication of the importance of the methyl radical for carcinogenesis. \(N,N\)-diethanol-p-aminoazobenzene was also noncarcinogenic. The livers of rats fed \(N,N\)-dimethyl-4'-hydroxy-p-aminoazobenzene had smooth surfaces and histological examination showed no evidence of tumors, bile duct changes, or abnormal regeneration of the ducts and liver cells, or any abnormal nuclear alteration.
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