A.A.A.S. Gibson Island Research Conference on Cancer, 1946

Abstracts of Papers Presented

On August 12 to 16, 1946, sessions of the summer meeting of the Section on Chemistry of the American Association for the Advancement of Science, at Gibson Island, Maryland, were devoted to a research conference on cancer. Dr. Harold P. Rusch, Professor of Oncology and Director of the Department of Cancer Research of the University of Wisconsin was Chairman, and Dr. Austin M. Brues, of the Argonne National Laboratory, University of Chicago was Vice-Chairman. Although there was no single topic of the conference many of the papers dealt with problems of carcinogenesis. Among the papers there were reports on the biological effects of radioactive compounds and nitrogen mustards. Abstracts of the papers presented follow.

STEROID HORMONES IN THE INDUCTION OF CANCER.* W. U. GARDNER. (Department of Anatomy, Yale University School of Medicine, New Haven II, Connecticut.)

Estrogens are the only normally occurring steroid hormones, if not the only known chemically identified substances, produced within the body, the application of which results in cancer under specific conditions. Because non-steroid, synthetic estrogens have carcinogenic effects similar to the normally occurring estrogens, the neoplasia-inducing action is associated with the biological responses rather than with an independent, specific chemical structure. Therefore the collective term, estrogen, is used in the following abstract although nine different estrogenic chemicals have been used in most of the experiments enumerated below.

In mice tumors of the pituitary gland, lymphoid tissue, testis, mammary gland and uterine cervix have appeared subsequent to estrogen-treatment, and in animals in which they would not have appeared unless given estrogens. With few exceptions, however, inherited factors or influences transmitted from parent or parents to offspring are of great significance. Estrogen-treated mice of one strain give rise to tumors of one or more tissues or glands but not of others. In this respect the estrogens are not unique; the carcinogenic hydrocarbons also produce different responses in mice with different genetic backgrounds and certainly differ greatly in effectiveness in different species. It is not improbable that two human beings differ more from one another than mice from one strain differ from mice of another strain. That a stimulus capable of eliciting neoplasia in one individual is inadequate in another is not improbable.

* The investigations of the author referred to here were supported by grants from The Jane Coffin Childs Fund for Medical Research and The Anna Fuller Fund. Several of the experiments mentioned have been undertaken in collaboration with other investigators in the laboratory, namely, Dr. T. F. Dougherty, J. H. Dougherty, Dr. C. W. Hooker, Dr. C. A. Pfeiffer and Dr. L. C. Strong.

Pituitary tumors.—Almost all mice of the C57 strain, but very few mice of other strains, acquire chromophobe adenomas and/or extensive hypertrophies (12 to 200 mgm., whereas the mouse's pituitary gland normally weighs about 2 mgm.) of their pituitary glands when given estrogens for long periods of time. Although the males are more susceptible to the tumors than females both male and female mice of the C57 strain transmitted the tendency for pituitary tumors to their estrogen-treated hybrid young (C57 strain × CBA strain or C3H strain). Young obtained by backcrossing the hybrids to the parent stocks showed that the tendency for pituitary tumors among estrogen-treated mice is genetically transmitted, probably by multiple factors. Pituitary tumors have not appeared in the untreated mice of these strains.

Androgens inhibit but do not entirely prevent the development of pituitary tumors in estrogen-treated mice.

Several pituitary tumors have been transplanted. They have grown only in related and estrogen-treated hosts. The incidence of grafts that grow is low, growth is slow and delayed and no transplants could be carried for over 3 generations. The original tumors appear to continue growth after discontinuance of estrogen treatment because mice removed 138 days after cessation of injection still had large tumors; as large as those of mice continuously treated.

Lymphoid tumors.—Among 7 strains of mice with low incidences of lymphoid tumors in control animals, the estrogen-treated mice of 3 strains showed an augmented incidence of such tumors (15 per cent); of two strains a slight increase (5 per cent); and of two strains no effect. Large doses of estrogens were more lymphomagenic in the susceptible strains than small doses. The discontinuance of estrogen-treatment after 30 weeks did not reduce the incidence of lymphoid tumors although the tumors did not appear for several months after cessation of treatment. The leukemogenic effect of estrogens was inhibited by the simultaneous administration of testosterone.

The hybrid offspring of mice of 2 strains susceptible to estrogen-induced leukemia acquired a very high incidence of such tumors (40 to 45 per cent). The incidences of leukemia were intermediate in hybrid offspring of mice of strains susceptible to and not susceptible to estrogen-induced leukemia, and were equivocal when hybrids with parents of two non-responsive strains were studied. All lymphoid tumors were transplantable into untreated and related mice.

Carcinogenic hydrocarbons do not induce increased incidences of leukemia in mice of the strains used in which estrogens proved leukemogenic.

Testis tumors.—Tumors of the interstitial cells develop in the testes of estrogen-treated mice of the A and JK strains although such tumors occur rarely in untreated mice. The tumors metastasize to the lumbar and renal nodes, and occasionally to the lungs. They are transplantable into estrogen-treated hosts of the same strain for many transfer generations. In this laboratory they
have not grown in untreated mice. Fragments of transplanted tumor tissue may remain dormant for several months in untreated hosts and start growth when estrogens are administered.

Both male and female mice of susceptible strains transmit the tendency for testicular tumors to their hybrid young. Three untreated hybrid male mice (A × C3H and A × C57) have also acquired testicular tumors at advanced ages.

Mammary tumors.—Estrogen-treated male mice from genetically susceptible strains and with the maternally transmitted mammary tumor inciter acquire mammary adenocarcinomas (Table I). The incidence is usually lower than among repeatedly bred females. Among the mice with genetic susceptibility but without the mammary tumor inciter the tumors appear at advanced age, grow slowly, and many are of the squamous cell type. Estrogen-treated male and female mice of the tumor-susceptible C3H strain acquired few mammary tumors when large doses of testosterone propionate were administered simultaneously. Not only was the incidence of mammary tumors reduced but localized overgrowths of the mammary glands seldom appeared and even mammary duct growth was subnormal.

Table I: Influence of Several Factors on Mammary Carcinogenesis

<table>
<thead>
<tr>
<th>Strain of origin</th>
<th>Average chromatin of susceptible strain, (%)</th>
<th>Mammary tumor (％)</th>
<th>Estrogen-treated</th>
<th>Forced bred controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57 ♂ × CBA ♀</td>
<td>50</td>
<td>—</td>
<td>0</td>
<td>43</td>
</tr>
<tr>
<td>CBA ♂ × C57 ♀</td>
<td>50</td>
<td>—</td>
<td>59</td>
<td>85</td>
</tr>
<tr>
<td>CC3 ♂ × CBA ♀</td>
<td>75</td>
<td>—</td>
<td>54</td>
<td>79</td>
</tr>
<tr>
<td>CC3 ♂ × C57 ♀</td>
<td>75</td>
<td>—</td>
<td>14</td>
<td>30</td>
</tr>
<tr>
<td>C57 ♂ × C57 ♀</td>
<td>25</td>
<td>—</td>
<td>1</td>
<td>7</td>
</tr>
</tbody>
</table>

In order to determine how the mammary tumor inciter might get into the milk and hence be transmitted to the offspring, studies were made on the milk which revealed that the formed elements including macrophages. Counts of cells in the milk obtained from the tail showed that during active lactation the white blood cells were almost 50 per cent below that of the non-nursing mouse (Table II). Lactating mice given large doses of estrogen had high white blood cell counts on the 4th day of treatment, at which time the weights of their nursing young began to decline.

Table II: The Average Total White Blood Cell Counts of Lactating and Control Mice under Different Conditions of Lactation

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of mice</th>
<th>First count</th>
<th>24 hours</th>
<th>72-78 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Lactating control-suckling interrupted and not resumed</td>
<td>10</td>
<td>10,740(66)</td>
<td>15,610(62)</td>
<td>16,185(62)</td>
</tr>
<tr>
<td>2. Lactating-suckling interrupted and resumed</td>
<td>18</td>
<td>7,480(63)</td>
<td>12,970(72)</td>
<td>6,850(66)</td>
</tr>
<tr>
<td>3. Control-non-lactating females or males</td>
<td>13</td>
<td>13,653(76)</td>
<td>12,597(71)</td>
<td>12,177(68)</td>
</tr>
</tbody>
</table>

* Percentage lymphocytes.

Uterine cervical tumors.—Carcinomas appear in the uterine cervices or upper vaginas of estrogen-treated mice of all strains that have been studied, but have not been observed among untreated mice except for one strain. The tumors usually appear, as judged by the detection of small infiltrative epithelial lesions, in the lower cervical canal, external cervix or vaginal fornix. Many stages of development, ranging from small tumors to large, and metastasizing cancers have also been observed. Several untreated mice of one stock, which shows a high incidence of imperforate vagina, have acquired cervical carcinoma; estrogen treatment augments the incidence in mice of this stock.

Simultaneous administration of testosterone propionate and estrogen did not prevent uterine cervical tumors. The tumors, however, showed less tendency to show cornified cells or pearls.

Uterine leiomyosarcomas have been noted in the uterus of several very old mice but not among estrogen-treated animals. Some overgrowth of uterine glands, with extension to the serosa (adenomyosis), is commonly observed but primarily malignant growths of the uterus proper have not been found.

By proper hybridization it is possible to combine transmitted tendencies so that any two or more types of tumors may appear in animals of the same group or even in the same animal within limitations of sex (Table III). Pituitary, lymphoid and mammary; or testicular, pituitary and mammary tumors have been found in single estrogen-treated hybrid mice. Other than for mammary tumors, there is no evidence of any tendency for tumors that is transmitted specifically by mice of one sex or the other.

Table III: The Relative Tendency for Estrogen-Treated Mice of Several Strains to Acquire Tumors

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mammary Carcinoma</th>
<th>Lymphoid Cell</th>
<th>Testicular Cell</th>
<th>Pituitary Tumor</th>
<th>Uterine Cervix</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3H</td>
<td>+++++</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>CBA</td>
<td>+++++</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>PM</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>A</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C57</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>C121</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>JK</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>?</td>
</tr>
</tbody>
</table>

Testosterone is antagonistic to tumorigenesis of the mammary, pituitary and lymphoid tissues but not to tumors of the uterine cervix, or probably, of the testicular interstitial cells. No evidence of changes in the adrenal glands has been noted that could be associated with tumors of organs other than the pituitary gland.

Tumors in Intrasplenic Ovarian Transplants in Castrated Mice. M. H. Li and W. U. Gardner. (Department of Anatomy, Yale University School of Medicine, New Haven 11, Connecticut.)

It has been shown that many steroid gonadal hormones are inactivated when circulated through the hepatic portal system, and that the intrinsic production of gonadotrophins is increased subsequent to castration. This investigation demonstrated the role of the endogenous gonadotrophic hormones of the pituitary gland in ovarian tumor formation by the transplantation of ovary into spleens of castrated mice.

Inbred mice of the Strong A and C3H strains, and
hybrid mice (AC3, AC5) were used. Six groups of experiments were set up: (a) Twenty-one castrated male mice with homotransplantation of an ovary into the spleen; (b) 52 castrated female mice with autotransplantation of an ovary into the spleen; (c) 25 castrated female mice with subcutaneous homotransplantation of an ovary into the right axillary region; (d) 12 intact male mice with subcutaneous homotransplantation of an ovary into the right axillary region; (e) castrated male mice with subcutaneous homotransplantation of an ovary either into the right axillary region or into the abdominal region; and (f) 33 intact male mice with homotransplantation or heterotransplantation of an ovary into the right testis. Castration and grafting were done when the mice were 1 to 3 months old, except in the group (f) in which a few mice were operated upon at 130 days of age. The donors of the ovaries for homotransplantation or heterotransplantations were usually younger than the recipients.

Five granulosa-cell tumors, 2 probably tumors of the same type and 1 mixed tumor consisting of both granulosa and luteomatous cells were found among the 21 castrated male mice with intrasplenic transplants. Four luteomas and 7 mixed tumors were found among the 33 castrated female mice that showed no adhesion of the intrasplenic ovarian graft to the left uterine horn or to the adjacent peritoneum. Among the 19 castrated female mice with vascularized adhesions of the ovarian transplant 1 luteoma was observed in a mouse that had irregular estrous cycles during the experimental period. Mice of the A strain were used in these 2 groups of experiments and most of the ovarian tumors developed in castrated mice carrying the intrasplenic ovarian transplants for more than 7 months. No ovarian tumors were found in other sites of transplantation except for one small granulosa cell tumor-like growth observed in a subcutaneously transplanted ovary in a castrated female mouse. The mouse was the only one that was operated upon during pregnancy.

THE METABOLISM AND CARCINOGENICITY OF p-DIMETHYLAMINOAZOBENZENE AND RELATED COMPOUNDS IN THE RAT. J. A. MILLER and E. C. MILLER. (McArdle Memorial Laboratory, Medical School, University of Wisconsin, Madison 6, Wisconsin.)

These studies cover some of our efforts to define the carcinogetic operative in carcinogenesis by the aminoazo dyes through investigations on their metabolism and the effect of structure on their activity.

Using a sensitive quantitative method for the determination of basic aminoazo dyes in tissue we have demonstrated that of the initial pathways in the metabolism of p-dimethylaminoazobenzene (DAB) is its reversible demethylation to p-monomethylaminoazobenzene (MAB) followed by an irreversible demethylation to p-aminophenyl benzene (AB) (3, 4). These basic aminoazo dyes were initially characterized by their absorption spectra and adsorption characteristics. We have further characterized the dyes by reduction to their component amines which were then determined by an independent method described below. DAB and MAB have been found to be metabolically and carcinogenically interchangeable (6). In animals fed these compounds the liver is the only organ that contains appreciable amounts of all 3 dyes. The tumors arising in livers containing the basic dyes contain similar amounts of these compounds. AB is temporarily stored in the red blood cells and is also excreted in the bile. The same amount of AB appears in the blood after any of the 3 dyes are fed; this seems to indicate that the metabolism of DAB and MAB proceeds largely through an initial demethylation to AB. The amount of AB in the blood varies directly with the concentration of dye in the diet. The dye levels in the tissues reach a maximum in about a week of feeding. When the dye is withheld from the diet the dye contents of the tissues drop about 50 to 90 per cent in 3 days and in a week very little is left. The basic aminoazo dyes found in the body at any one time could account for about 8 per cent of the dye ingested within the previous day.

None of the various diets known either to increase or decrease the rate of hepatic tumor formation with DAB have been found to alter greatly the levels of dye in the liver and blood.

A study of the over-all metabolism of DAB, particularly the dyes and their constituent amines that may be found in the urine has also been made. In general our observations on the over-all metabolism of DAB agree with those of Stevenson and her group (7) in that p-phenylene diamine and p-aminophenol appear to be quantitatively the major final metabolites of the dye. The application of a sensitive and relatively specific method for the determination of six monophenyl amines in urine has disclosed the presence of minor quantities of other metabolites. In addition a new class of aminoazo dyes appears to be present. The urine of a rat fed DAB contains much less basic aminoazo dye than can account for the red color given with acid. The urine becomes orange when made alkaline but the color cannot be extracted with petroleum ether or benzene. These properties indicated that one or more amphoteric aminoazo dyes were present. No hydrolysis to simplerazo dyes has been found possible and a means has not been found for their removal from urine uncontaminated with monophenylamine. Instead, we have had to rely on an approximate means for their estimation. This consists in reducing the aza dyes in the urine in the cold at an acid pH with Na₂S₂O₅ and then determining the free amines formed. This procedure determines only free aminotera dyes and the free portions of conjugated dyes. It was unnecessary to release the conjugated monophenyl amines present in the urine for analysis by hot acid hydrolysis under reducing conditions. The 3 basic aminoazo dyes and their p-hydroxy derivatives, which are representative amphoteric aminoazo dyes, were found to be reduced quantitatively to their constituent amines by refluxing in dilute HCl in the presence of tin. Hence the monophenylamines were determined by the difference between the total amines found after reductive hydrolysis in acid and the amines found after reduction in the cold.

Four aromatic amines could arise in the reduction of the basic aminoazo dyes, viz., dimethyl-p-phenylene diamine (DMPD), monomethyl-p-phenylene diamine (MMPD), p-phenylene diamine (PD), and aniline (A). p-Aminophenol (p-AP) was already known as a metabolite of DAB. After a method had been devised for the estimation of these amines, it became evident that still another aminophenol, o-aminophenol (o-AP), was present in the urine. The method employed allows the separation and estimation of these 6 amines in microgram quantities as colored dyes in the presence of tin in the determination of the Böniger reaction. Primary aromatic amines were found to react instantly in the cold at pH 7 with excess sodium-p-naphthoquinone-4-sulfonate to form highly col-
ored phenolic Schiff bases. Benzene will extract the Schiff bases formed with DMPD, MMPD, o-AP, and A. The first two amines form blue and purple solutions respectively and the MMPD base may be selectively destroyed with acetic anhydride. o-AP and A form orange and yellow solutions respectively. Hence these four Schiff bases in the benzene extract can be separately determined by measurements of light absorption at different wave lengths before and after the addition of acetic anhydride. PD forms a highly insoluble Schiff base which can be quantitatively filtered on asbestos and then dissolved therefrom by dilute base to form an orange-red solution. The extracted and filtered aqueous solution still contains the p-AP base, which may then be extracted with amyl acetate in which it forms an orange solution. The interference by normal urine constituents is low and satisfactory recoveries of added amines were obtained.

Each of the 3 basic aminoazo dyes, the three p'-hydroxy aminoazo dyes, and the six monophenyl amines have been fed to rats and the urines examined for these compounds and their derivatives. When free amines were excreted the urines were collected through funnels coated with hydroquinone and caught in dilute HCl in the presence of either tin or hydroquinone and benzene. Approximately 50 per cent of each of the azo dyes could be accounted for in the urine in terms of the azo dyes and amines found. While the major metabolites in each case were PD and p-AP, small amounts of MMPD, A, and o-AP were noted. Very small quantities of DMPD, close to the limit of the method, appeared to be present in the urines of rats fed the methyl- and monomethylaminoazo dyes but were not noted in the urines of rats fed either of the completely demethylated dyes. Basic azo dyes were found only in the urines of rats fed the basic dyes. Amphoteric azo dyes were found in the urines of rats fed any of the 6 azo dyes. The probable composition of the amphoteric azo dyes in the urine is indicated by the observation that from the urines of rats fed DAB and MAB the amines MMPD, PD, p-AP, and o-AP were formed after cold acid reduction. The urine of rats fed AB yielded PD, p-AP, and o-AP under both conditions. After reduction, the urine of rats fed the p'-hydroxy derivatives contained similar compounds except that no o-AP was noted. The amphoteric aminoazo dyes appear to account for only a few per cent of the azo dye fed. No free monophenyl amines were found in the fresh urines of rats fed any of the 6 azo dyes. When the urine of azo dye-fed rats was allowed to stand at room temperature without a preservative the amphoteric aminoazo dyes disappeared and free PD and p-AP appeared. A different situation was found when the monophenyl amines were fed since considerable free amine was excreted in each case although the majority was in the conjugated form. Seventy-three or more per cent of each of the dianimes was recovered in the urine. Although most of the DMPD and MMPD fed was demethylated to PD appreciable amounts of DMPD and MMPD were found in the urine after feeding DMPD. MMPD yielded only MMPD and PD and only PD was found in the urines of rats fed PD. When A was fed it was metabolized to p-AP although some o-AP and A were excreted. Each aminophenol when fed yielded only that aminophenol in the urine.

The above data indicate that a considerable portion of the metabolism of DAB probably proceeds through the basic aminoazo dyes to the amphoteric aminoazo dyes and thence to the monophenyl amines. A minor pathway from AB direct to A and PD probably exists also. There is as yet no evidence for deamination at any step, rearrangement of hydroazo compounds, or for the direct reduction of the methylated basic aminoazo dyes since the MMPD and the small amount of apparent DMPD in the urine could also come from the reduction of hydroxy aminoazo dyes. Preliminary data indicate that the over-all metabolism of DAB is not greatly altered by diets which significantly alter its carcinogenicity.

Our studies on the effect of structure on the carcinogenicity of the aminoazo dyes have been designed to define more clearly the structural features necessary for strong carcinogenic activity and to test the activity of possible metabolites of DAB. Each compound was fed to at least 10 rats at a molar level equivalent to 0.06 per cent of DAB, the reference compound, for 4 to 8 months in a diet which allows a high incidence of tumors with DAB. The compounds were rated on a rough scale of activity in which DAB is assigned a value of 6. Approximately 40 azo dyes and amines were tested and only a few of the azo dyes were found to possess strong activity. Alterations in the azo linkage, the ring substituents, and the substituents of the amino groups have been tested. When the azo linkage in DAB was replaced by =N =CH~ or by CH~CO~N~ (benzamide of DMPD) the activity was abolished. When the methyl groups in DAB were replaced by 2 H, =CH~ or CH~CH~CH~CH~, or 2 CH~CH~CH~CH~ compounds of zero activity resulted. On the other hand if the amino group carried H and CH~ or CH~ and CH~CH~, the activities were equal to that of DAB. In contrast to the latter case the azo dye with CH~ and HOCH~CH~ on the amino group was inactive. Inactivity was also produced when the amino group substituents were CH~ and phenyl=CH~ or H and CHO=, Ring substituents produced great variations in activity. When the ring hydrogens were substituted one at a time with methyl groups the activities of the compounds ranged from 0 to 10-12 (1, 5), in contrast to the prediction of Kensler and his co-workers (2). Although --NO~ groups in the p'--NO~ compound, like the p'=CH dye, was more active than the corresponding o'--derivative. None of the possible metabolites of DAB other than MAB have shown any activity. The p'-hydroxy derivatives of DAB, MAB, and A were inactive. DMPD and A led together were inactive even at 3 x the equivalent to 0.06 per cent DAB. MMPD, PD, A, p-AP, and o-AP were likewise inactive. 2,4'-Diamino-5-dimethylaminodiphenyl, 3-dimethylaminocarbazole, and 4-hydroxyazobenzene also proved to be inactive.

The conclusion drawn from the above studies is that the real carcinogenic operative in carcinogenesis by DAB is either one or both of the two dyes, DAB and MAB, or some azo dye which is very close in its structure to these compounds.

Recently, we have found a tight combination, presumably chemical, between an unidentified aminoazo dye and a cellular constituent, probably protein, in the livers of rats fed DAB. The dye could not be extracted from isolated crude liver protein by boiling organic solvents, but it could be extracted with ethyl ether-toluen after the protein had been degraded with either hot alkali or trypsin. For an approximate estimation of the "bound dye," tissue protein was digested with boiling alcoholic KOH and the digest was extracted with ethyl ether; after removing the solvent the residue was dissolved in alco-
holic HCl and the color intensity was read at 520 ms.
In rats fed DAB the bound dye was found only in the liver and not in the blood, lungs, spleen, heart, skeletal muscle, intestine, or kidneys. The livers of rabbits, guinea pigs, and cotton rats did not contain the bound dye while mouse livers had only about a fourth as much as rat livers. The livers of rats fed DAB and MAI appeared to contain similar quantities of the same bound dye(s) while the livers of rats fed AB had very little of this dye. These findings correlate well with published data on the specificity of DAB in producing tumors of the liver, on the inability of other workers to induce tumors in rabbits or guinea pigs, the lesser susceptibility of mice as compared with rats, and the extremely low carcinogenicity of AB. About a month was required for the level of bound dye to reach a maximum after feeding with DAB was initiated, but the bound dye content dropped approximately to zero within a few days after the rats were red dye-free diets. The tumors arising in livers containing the bound dye contained none of this compound. The data suggest that the bound dye may have an important place in carcinogenesis by the aminoazo dyes.

REFERENCES

NUCLEAR DIFFERENTIATION AND THE ORIGIN OF TUMORS. JACK SCHULTZ. (The Institute for Cancer Research, The Lankenau Hospital, Philadelphia 30, Pennsylvania.)

This is a review in which the basis for a concept of the differentiation of cell nuclei during development is discussed. It is shown that a new theory of the origin of tumors, based on this concept, can be developed without any additional ad hoc assumptions.

Examples of the correlative differentiation of the nucleus and cytoplasm are given under four categories. (a) Losses of parts of chromosomes during early development (Ascariis). (b) Increase in chromosome number without nuclear division: "endomitosis." (c) Differential reproduction of genes, during endomitosis. (d) Changes in state of chromosomes; increase in nucleoprotein content in pycnotic nuclei, relatively high protein content of "dissolve" nuclei. This last category of nuclear differentiation involves visible changes in the chromosomes, hence in the immediate vicinity of the genes, and possibly within the genes themselves. The differences between the same chromosome region in the two types of nuclei discussed are analogous to those found between the heterochromatic regions (rich in deoxyribosenucleic acid) and the euchromatic regions (relatively poor in this substance) within a single nucleus. The analysis of the genetic function of the two types of chromosome region provides a basis for a working hypothesis regarding nuclear differentiation. In particular, the combination of cytological and genetical studies in Drosophila gives evidence for the connection of the heterochromatic regions with the overall nucleoprotein metabolism of the cell: ribonucleicprotein in the cytoplasm, deoxyribonucleic in the chromosome. The euchromatic regions seem by this at present, an oversimplified picture, to be concerned with the diversified synthetases that are diagnostic of different genes. In Drosophila there are many cases in which euchromatic regions placed next to heterochromatin change their properties; individuals carrying such chromosome rearrangements show somatic "mutation" for the genes in the abnormally placed euchromatin. These changes are effectively losses of the specific activities of the genes, and are correlated cytologically with the assumption of the properties of heterochromatin by the regions of chromosome involved, in the differentiation of the nuclei of the different tissues. There is thus a basis for the postulate that the changes in "pycnosis" of the different types of nuclei are reflections of changes in gene activity, and in extreme cases, of the genes themselves.

The extension of this postulate leads to the point of view that during development a process of "adaptation" takes place. This "adaptation" occurs concurrent with the establishment of a semi-autonomous system of elements in the cytoplasm, associated with protoplasmic ribo-nucleoproteins. The genes whose specific activities are essential for the maintenance of the cytoplasmic system differ for the different cell types, and probably relatively few remain in the "active" (euchromatic) state in any one nucleus. The much larger remaining number of genes is adapted to a state of activity comparable to that of the heterochromatic regions, a state of generalized function concerned with gene reproduction and the mitotic process. The further the process of nuclear differentiation has proceeded, the more these adaptations are irreversible; and in the germ cell all genes are in the "adaptable" state almost by definition. It should be stated that this discussion is intended especially for the last of the four categories of nuclear differentiation; for the other types, where differentiation is obvious, its consequences for the discussion to follow are the same in principle.

On the assumption that an irreversible adaptation of genes for generalized and for specific function occurs during development, the nuclei of differentiated cells have lost an adaptability present in their embryonic forbears. Of the total number of genes present, the majority are conceived to be adapted for the generalized function; but since in the ordinary life of a differentiated cell, the stimuli for mitosis are either rare or highly specific and mediated by an already functioning cytoplasmic system.
the reactions in which they participate are conceived rarely to occur. Should, however, the established cytoplasmic system be broken down, and a new system be regenerated, this regeneration of the cytoplasmic system takes place under the influence of the differentiated nucleus, with the preponderance of genes adapted for generalized function ("heterochromatin-adapted"). Such genes are maximally functional in embryonic tissue; consequently the type of cytoplasmic system regenerated will resemble that of the embryonic cell. Mitosis will occur; but since the nucleus is a differentiated one, without the "adaptable" genes of an embryonic cell, the daughter cells, instead of being responsive to "organizing" influences about them, will continue to produce the embryonic type of cytoplasm. Thus a cell type, committed under most circumstances to mitosis, and relatively unresponsive to the needs of differentiation about it, will arise. Its characteristics are those of the malignant cell.

Comparison of this hypothetical picture with the actual data strengthens a belief in its validity. (a) The multiplicity of stimuli evocative of tumors calls for an effect on some constituent of the cell. Recent cytological work (see especially Oppel) shows a stage in tumor development to be the breakdown of the nucleoprotein granules of the cytoplasm. Viruses, on this view, act by the cytoplasmic damage they cause, and propagate in the tumor cell at the expense of its embryonic cytoplasm. The precancerous lesions have the characteristics of embryonic tissue. (b) The nuclei of tumor cells resemble most the more differentiated of the nuclei in the tissue of origin (Biesele); their high nucleoprotein content (Stowell) indicates possible direct evidence for heterochromatization. (c) The direct chemical analyses of the constitution, the enzymatic activity, and the metabolism of tumor cells accord more closely with those of embryonic cells than with any other group. It seems likely, and in some of these cases it is already clear, that these measurements reflect similarities in the cytoplasts. (d) Other groups of data (antigenic properties, behavior on transplantation, relation of malignancy to tissue type of origin, differentiation in the tumor itself), insofar as available, are consistent with the view. Obviously many kinds of experiment are necessary, to test the basic assumptions, and to make precise the properties of the tumor cell in usable terms. The working hypothesis is tenable, that the malignant cell is the result of the combination of an "old" nucleus with "young" cytoplasm.

CARCINOGENESIS AND THE MECHANISM OF GENE ACTION. S. SPIEGELMAN. (Department of Bacteriology, Washington University School of Medicine, St. Louis 10, Missouri.)

The basic problem of carcinogenesis involves explaining the appearance of a sudden heritable change in somatic cells which implies the involvement of the hereditary mechanism at some stage in the process. On this assumption it becomes imperative to examine the question from the point of view of what is known about the mechanism of gene action. Here one must emphasize the distinction between two quite different aspects of the gene problem. One involves simply their transmission from one generation to the next and the other the mechanism whereby genes effect their control over cellular physiology. It is with the latter problem that the present paper is primarily concerned.

Recent work employing micro-organisms as genetic materials has led to some rather well defined concepts on the nature of gene action. The basic assumption underlying much of this work is that since the enzymes of a cell determine its physiological capabilities, genes must control the phenotype of a cell by virtue of their ability to control enzymatic constitution. Adaptive enzyme formation and its inheritance in yeast was studied in an attempt to learn more about the nature and extent of this control.

We may summarize the important findings and conclusions relating to genes and enzyme formation by the following statements.

1. Usually, the transmission of characteristic enzymes and the products of their activities follows the classical Mendelian laws derived from the assumption that the controlling units are self-duplicating entities, genes, located on chromosomes in the nucleus.

2. The existence of a particular gene in the nucleus of a cell does not guarantee that the corresponding enzyme will be found in the cytoplasm, as evidenced by such phenomena as cellular differentiation and enzymatic adaptation. Genes, therefore, have as their primary function the indefinite retention for the cell of the potentiality for enzyme formation.

3. The actual formation of an enzyme in the cytoplasm is mediated directly by a cytoplasmic unit (plasmagene) which possesses the capacity for self-duplication in the presence or absence of the corresponding gene.

4. The presence of the homologous substrates accenuates the capacity of these self-duplicating plasmagenes to produce enzyme.

5. Competitive interactions exist amongst the cytoplasmic enzyme-forming units.

6. Nucleoproteins are involved in the synthesis of enzymes.

These findings and conclusions have been synthesized into a theory of gene action which assumes that genes continually produce at various rates more or less complete replicas of themselves which enter the cytoplasm. These replicas or plasmagenes are presumed to be nucleoprotein in nature in view of their origin and to possess to varying degrees the capacity of self-duplication. Their presence in the cytoplasm controls the types and amounts of proteins and enzymes synthesized. These plasmagenes, like all self-duplicating entities, would compete with each other for protein and energy, and the outcome of such competitive interactions would then determine the enzymatic constitution of the cytoplasm. Inherent in this concept is the possibility of changing the ultimate result of this competition by varying the conditions (e.g., substrates available) under which it takes place. The various reactions and the role of substrate in the process are detailed in Fig. 1 in terms of one gene G and its corresponding enzyme E.

All the double arrows denote self-duplicating reactions. Gene G continuously produces its plasmagene (Pl) at a rate denoted by k. The plasmagene by its very nature must possess heterocatalytic potentialities, i.e., in addition to being autosynthetic it must possess the capacity of catalyzing the synthesis of units (enzymes) other than itself. Consequently, once in the cytoplasm, several things may happen to plasmagene (Pl). If it is successful in obtaining the proper material (M) in the cytoplasm it will duplicate itself. It may, on the other hand, combine with precursor protein (Pr) and convert it to E, resulting in the formation of the PLE complex.

Since little enzyme is found experimentally in the absence of substrate, one must assume that this complex is
highly unstable and quickly breaks up into its two components. A plasmagene once formed cannot of course exist indefinitely, particularly in a population of other such units actively competing for the material of which it is composed. The reaction leading from Pl to IP (inactive protein) in Fig. 1 describes this fact. Enzyme may also break down to inactive protein as is indicated by loss of activity or removal of substrate. By IP or inactive protein we mean merely that the plasmagene has broken down to a protein unit which has lost the capacities for self-duplication and enzyme formation, and in the case of enzyme, into a protein which no longer possesses enzyme activity. Thus far we have described the reactions which take place in the absence of substrate. It is clear that little enzyme would be found in the cell, unless the rate of Pl production by G, were extremely high or the stability of the enzymes or enzyme-plasmagene complex very great. Neither condition is apparently satisfied in the cases of the enzymes reported on here. When substrate S is added, however, it will combine with E, and two things may result. It is well known that the addition of substrate to enzyme stabilizes it against inactivation. Hence the presence of substrate would decrease the rate at which the active enzyme is converted to inactive protein. More critical however is the possibility that S would combine with E, while the latter is still united with Pl, thus resulting in a Pl:E:S complex. This substrate would now not only stabilize the enzyme, but could also stabilize the unstable plasmagene-enzyme (Pl:E,) combination. Such stabilizations of unstable complexes by the addition of a third component are quite common in organic chemistry.

Inherent in the very definition of a self-duplicating entity is the concept that should such a unit undergo a modification at any given moment, all subsequent replicas would bear this modification. Thus when Pl exists alone it duplicates only Pl. However, when substrate is added and the stable Pl:E:S combination results this is now duplicated, which is indicated in the diagram by the double arrow. This scheme provides therefore a concrete mechanism whereby substrate can modify competitive interactions between plasmagenes. What is essentially accomplished by substrate is the creation of a new self-duplicating unit which duplicates not only the plasmagene but also the enzyme corresponding to the substrate added.

From a general point of view, the unique feature of the theory outlined above of gene action is that while supplying a link between the gene and the enzyme, it at the same time predicts that cells with identical genomes need not possess identical enzymatic constitutions. Whether a particular enzyme will be transmitted from one cell generation to the next in a Mendelian fashion will thus depend on the relative rates of duplication of the controlling cytoplasmic units as compared with their rate of production from the genome. If the latter is quantitatively determining, Mendelian inheritance will be observed. If the former is determining, the Mendelian picture will be obscured to varying degrees, depending upon the self-duplicating capacity of the plasmagenes. It is clear also how substrate could so intensify cytoplasmic inheritance of a particular enzyme as to completely obscure the segregation of the corresponding gene.

As a tentative working hypothesis this theory has the advantage of furnishing a unified point of view from which such diverse and apparently contradictory phenomena as classical Mendelian genetics, cytoplasmic inheritance, cellular differentiation, and enzymatic adaptation may be analyzed. Its implications for the cancer problem are evident. From the viewpoint of the plasmagene mechanism of gene action, heritable changes such as carcinogenesis need not necessarily be referred back to the genes. The plasmagene by being self-duplicating provides us with another level at which a mutation can take place and be subsequently transmitted via the cytoplasm from one cell generation to the next. This concept may also furnish a basis for reconciling the somatic mutation versus the virus theories of cancer, since, if the mutation does occur at the plasmagene level, the difference between these two opposing views becomes tenuous indeed.

PATHS OF GENE ACTION IN MAMMARY TUMOR DEVELOPMENT IN MICE. W. E. HESTON. (National Cancer Research Institute, National Institute of Health, United States Public Health Service, Bethesda 14, Maryland.)

Now that genetic differences in the development of mammary tumors in mice have been demonstrated, the present problem is that of determining the physiologic mechanisms through which these variations in genic complex become manifest. From recent work on gene chemistry such genic differences can be expected to affect enzymatic differences controlling specific metabolic reactions related to the subsequent development of the tumor.

At present three paths through which differences in gene action affect the probability that a mammary tumor will appear have been identified. There is evidence suggesting that genic differences can influence the response of the mammary tissue cell to the hormonal stimulation and to the milk agent stimulation. In either case the gene action must occur in the mammary tissue cell. There is evidence that genic differences can influence the development of mammary tumors through the hormonal mechanism, possibly in controlling the production of the hormonal stimulation. In this path the gene action can be expected to be in the physiology of the endocrine system. Genic differences can influence the propagation and
transmission of the milk agent. Since it has not been demonstrated that the milk agent is limited to any particular tissue, one cannot predict just where the gene action occurs in this case. Through the effect on the transmission of the agent, gene action in one female may influence the probability that a tumor will appear in her daughter or in a foster-nursed female.

Possible bearings that these findings in the mouse may have on the investigations of breast cancer in the human being were discussed.

THE INDUCTION OF GERMINAL MUTATIONS
BY A CARCINOGENIC CHEMICAL (METHYLCHOLANTHRENE). LEONELL C. STRONG.
(Department of Anatomy, Yale University School of Medicine, New Haven 11, Connecticut.)

Fifty-two germinal mutations affecting intensity and distribution of pigment and spotting patterns of the hair of mice have been obtained in the descendants of mice, whose ancestry has been injected with methylcholanthrene over a number of generations. None of these mutants have occurred in mice of the untreated control series. In the series treated earlier (4 generations), no germinal mutations were obtained and the mice, as a rule, died early following the injection of methylcholanthrene, with the progressive growth of local tumors appearing at the site of injection. However, selection toward resistance to tumors induced by methylcholanthrene has resulted in the suppression of local tumors, as well as other types of internal tumors whose latent periods are considerably longer than the ones obtained with local tumors at the site of injection. When the effect of selection toward resistance to tumors had been great enough to permit the appearance of abdominal tumors (with long latent periods) such as primary carcinoma of the liver, adenocarcinoma of the gastric mucosa, leiomyosarcoma of the uterus, and granulosa cell carcinoma of the ovary, the descendants of these selected mice began to show many germinal mutations.

At present, it is intended to discuss only three groups of these mutants. (A) There has apparently appeared a series of alleles at the brown-black locus. Not all genetic tests have been made on all these mutants but apparently each type breeds true and segregates following an outcross. Any mouse may have one or two of these genes but no more. A cross between two of these new mutants gives one or the other of the new mutants, not the wild type. This series of alleles (new mutations) classified according to intensity of pigmentation by visual inspection is (1) black (established dominant = BB), (2) graphite, (3) dark gunmetal, (4) gunmetal, (5) dark bronze, (6) bronze, (7) darker sepia, (8) dark sepia, (9) sepia, (10) chestnut, (11) brown (established recessive = bb), (12) light brown, (13) lighter brown, (14) velvét, and (15) buff. (B) Another series of mutations involving pigmentation produces a lighter ventral area than occurs on the dorsum. This series has occurred in mice whose ancestry had shown nothing for many generations but non-agouti, where there is no differential pigmentation on the dorsum and ventrum. Thus there has been obtained a series of mutants classified according to pigmentation on the ventrum of (1) black or non-agouti, (2) light black, (3) rusty, (4) red, (5) chestnut, (6) tan, (7) sandy, (8) derris and (9) buff. (C) The third series of new mutants involves the formation of different colored spots on either the forehead or ventrum. The colors of spots have been, (1) white, (2) gray, and (3) yellow.

In addition to these clear-cut germinal mutations there has been obtained many somatic mosaics between any two of the above color mutants; such as between buff and brown, chestnut and brown and rusty and graphite, etc. Many of the new color mutations are permanent from the growth of the hair until old age, while others shift more and more toward the recessive condition with advancing age—reminiscent of the shift of black toward brown with advancing age particularly when a black mouse is heterozygous for brown.

In several cases, the appearance of a germinal mutation affecting color of the hair has also suddenly brought about physiological changes that alter susceptibility or resistance to tumors induced by methylcholanthrene.

It is concluded that there must be some correlation between the induction of germinal mutations by methylcholanthrene affecting body characteristics and the unknown changes in somatic tissue which leads to cancer induced by the same carcinogen.

THE ACTION OF DIAMIDINES AND RELATED COMPOUNDS ON NUCLEOPROTEINS. M. J. KOPAC. (Department of Biology, Washington Square College of Arts and Sciences, New York University, New York 3, N. Y.)

Cellular structures, in particular those containing nucleoproteins, can be modified by a number of chemical agents. Various oncoytic agents (bacterial polysaccharides, quaternary ammonium halides, diphenylethylamines, stilbamine, and x-radiation) may initiate abnormalities ranging from minor disturbances in nuclear configurations to the complete destruction of the nucleus.

The changes induced in cells by the action of nitrogen mustards frequently cannot be recognized except by careful cytological study or by genetic analysis. These substances, at sub-lethal concentrations, may increase the rate of incidence, in Drosophila, of sex-linked lethals—an effect previously obtained only by the application of high frequency radiation.

The so-called mitotic poisons cause still different effects. Colchicine, in most instances, produces a metaphase block. Some of the diphenylethylamine derivatives are also mitotic poisons of the colchicine type. Several nitrophenols induce a prophase block in cleaving sea-urchin eggs. Nitrogen mustards have been reported to disturb the interkinetic phase in a variety of cells.

The virus nucleoproteins are also susceptible to chemical agents, especially to those compounds capable of denaturating proteins. Accordingly, detergents, pyridines, picolines, guanidines, and urea have been frequently employed either as destroyers of virus activity, or as tools for the elucidation of virus structure.

The experiments described in this report were designed for two purposes: (1) to measure the effect of selected organic compounds on the structure of various types of nucleoproteins, and (2) to develop and test methods that might be employed later for determining the action of such compounds on living cells.

Experimental.—Changes in protein structure can be followed by measuring denaturation rates. Unfortunately, classical methods were not sufficiently sensitive to measure the denaturation of proteins induced by compounds at low concentrations. A procedure was developed, however, so that protein molecules could be exposed to the simultaneous
action of interfacial forces and one of the compounds maintained at physiologically effective concentrations. In this way, the denaturing effect of one agent could be used to augment a similar action initiated by another agent. For example, with these surface chemical methods, it was previously shown that stilbamidine, at physiologic concentrations, enhanced the interfacial denaturation of various protein molecules. Several other diamidines, however, depressed interfacial denaturation.

In general, three-dimensional protein molecules when exposed to appropriate surface or interfacial forces will unfold and become two-dimensional platelets. Such changes in architecture require the splitting of several critical side-chain linkages. Apparently, some of these linkages can be split by surface forces alone, while others may be split only by the action of chemical agents. When both forces are in operation, the effect can be demonstrated by an increase in surface denaturation of the proteins tested.

On the other hand, other compounds which reinforce side-chain linkages will indicate the effect by a decreased surface denaturation. Several compounds, including fatty acids and certain amino acids, when added to protein solutions can protect such proteins against heat and other denaturing agents. These protecting compounds also appreciably reduce the surface denaturation of protein molecules.

The nucleoproteins (liver nucleoproteins, tobacco mosaic virus nucleoprotein, protamine = nuclear complexes, and a cytoplasmic fraction, rich in mitochondria) were exposed to the simultaneous action of interfacial forces (oil-water interfaces) and one of the chemical agents (at concentrations of 0.001 M, or lower). The interfacial denaturation of the nucleoproteins was measured with the semi-automatic, drop-retraction apparatus. The method is fundamentally a micro-adaptation of the procedure developed by Devaux for measuring protein monolayers at oil-water interfaces. The same procedures and apparatus may be used to study oil-cytoplasm interfaces. In this way, the action of chemical agents on cytoplasmic proteins can be determined.

Liver nucleoproteins.—The action of the various compounds already investigated on liver nucleoproteins may be classified into 4 groups:

I. Compounds that augment surface forces by weakening certain side-chain linkages in the protein molecule. Increased interfacial denaturation (stilbamidine, methylbis-, ethyl-bis-, and tris-2-chloroethylamine, benzylisothiourea).

II. Compounds that counteract surface forces by strengthening critical side chain linkages. Decreased interfacial denaturation (propanidide, nentamidine, phenamiline, 1,2-di-ethylisothiourea, phenethylypyridinium bromide, bis-amidinomethylbenzyl).

III. Compounds that neither augment nor counteract surface forces. Unchanged interfacial denaturation (1,2-diethylisothiourea, benzylisothiourea).

IV. Compounds that simultaneously augment and counteract surface forces by weakening some linkages and strengthening others within the molecule. The net effect depends on the predominance of one action over that of the other. A balance between the two would produce no change in interfacial denaturation. Under such conditions, groups III and IV can be differentiated by testing such compounds in combination with stilbamidine (colchicine, phenethylypyridinium bromide, 2-p-dimethylaminostyryl-4-methylpyridine-methobromide, 2-b-dimethylaminostyrylpyridine-methobromide, benzylamine).

Tobacco mosaic virus nucleoprotein.—The surface denaturation of molecules of the virus type requires: (1) dissociation of the large molecules into smaller molecules, and (2) unfolding of the smaller molecules at the interface. Interfacial forces are sufficiently large in some instances to produce both dissociation and unfolding. Agents that only dissociate the large molecules will enhance interfacial denaturation. If these agents also facilitate unfolding, interfacial denaturation would be enhanced to an even higher degree.

Stilbamidine, propamidine, or pentamidine increased the interfacial denaturation of the virus nucleoprotein. The increased denaturation by these compounds was, in all probability, the result of dissociating the giant virus molecule. The experimental data indicate that the subsequent unfolding of the small molecules was induced primarily by surface forces. In no instance has propamidine or pentamidine enhanced interfacial unfolding of protein molecules to any appreciable extent. On the other hand, phenamidine or bis-amidinomethyl-dibenzyl appeared to strengthen the virus molecule so that interfacial forces were unable to dissociate the large molecule. Accordingly, interfacial denaturation of the virus nucleoprotein was depressed by these two compounds. Neither bis-amidinomethyl-dibenzyl nor phenamidine were able to antagonize the action of stilbamidine.

Cytoplasmic proteins.—Stilbamidine enhanced the interfacial denaturation of cytoplasmic proteins, buffered at pH 7.5, while the other diamidines depressed. Propamidine depressed considerably more than phenamidine or bis-amidinomethyl-dibenzyl. Thus, the latter two diamidines produced similar effects with both virus nucleoproteins and cytoplasmic proteins.

Either stilbamidine or propamidine were equally effective in enhancing the interfacial denaturation of cytoplasmic proteins, buffered at pH 7.9. Colchicine depressed interfacial denaturation, thus producing effects on cytoplasmic proteins similar to those previously described for liver nucleoproteins. Tris-2-chloroethylamine depressed interfacial denaturation of the cytoplasmic proteins, in contrast to its action on liver nucleoproteins.

Combination of chemical agents with stilbamidine.—The striking denaturing action by stilbamidine was not obtained by adding this agent to liver nucleoproteins previously treated with propamidine, pentamidine, or phena-

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Combination of chemical agents with stilbamidine.—The striking denaturing action by stilbamidine was not obtained by adding this agent to liver nucleoproteins previously treated with propamidine, pentamidine, or phenamidine. The stilbamidine effect was thereby antagonized and in proportion to the depressing action of the other diamidines tested alone, e.g. pentamidine > propamidine > phenamidine. The interfacial denaturation of liver nucleoprotein treated with phenethylypyridinium Br + stilbamidine was nearly identical to that measured with phenamidine + stilbamidine. These data indicate that phenamidine and phenethylypyridinium Br have a similar action.

The interfacial denaturation of liver nucleoproteins in the presence of colchicine + stilbamidine was higher than in the presence of stilbamidine alone. Colchicine, at the same concentration, depressed interfacial denaturation. Phenethylypyridinium Br antagonized stilbamidine slightly, however, subsequent analyses of the denaturation curves suggest that this compound is similar to colchicine. Some linkages are weakened while others are strengthened in the liver nucleoprotein molecules by either colchicine or phenethylypyridinium Br. If stilbamidine was added with these compounds, interfacial denaturation was enhanced since stilbamidine tends to weaken side-chain linkages. Phenethylypyridinium Br is a stronger depressor than colchicine and accordingly this agent antagonized stilbamidine to a greater degree.

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Significance of chemical structures.—Relatively slight changes in structure of an agent may produce considerably different effects on the interfacial denaturation of nucleoproteins. The striking differences between phenethylpyridinium Br and phenethyl-4-piclonium Br show that a single p-methyl group can modify the action of a compound on liver nucleoproteins.

All aromatic diaminodiones, with the exception of stilbamidine, depressed interfacial denaturation of liver nucleoproteins. Liver nucleoprotein molecules were fortified against interfacial forces by bis-amidinomethyldibenzyl and weakened by stilbamidine. When the two agents were present simultaneously, the weakening action of stilbamidine was almost entirely antagonized. The other diamidines also antagonized the action of stilbamidine and in proportion to their ability to antagonize interfacial forces.

Many onecyclic compounds induce morphological or functional changes in tumor cells especially in those structures that contain nucleoproteins. These agents also produced clear-cut effects on the interfacial denaturation of liver nucleoproteins, virus nucleoprotein, protamine—nucleate complexes, or of cytoplasmic proteins.

Any change in interfacial denaturation implies some functional alteration in the structure of such nucleoproteins and these alterations may, in many instances, be sufficient to affect the functional activity of those cells in which corresponding changes in nucleoprotein structure might occur. The ability of certain compounds to bind side-chain linkages and at the same time weaken others may explain, in part, the action of mitotic poisons.

Nucleoproteins may even be dissociated by a chemical agent, providing the protein moiety is, at least partially denatured. In complex nucleoproteins, denaturation of the protein moiety is a prerequisite for the separation of nucleic acid from the protein molecule.

Thus, nucleoproteins can be inactivated in two ways: (1) the nucleic acid may be irreversibly dissociated from the protein moiety or (2) critical intramolecular structure of the nucleoprotein may be blocked by strengthening side-chain linkages. In either event, some molecules must become less capable of performing essential functions.

There is surface chemical evidence to indicate that specificity of action might be further enhanced by employing a combination of agents, in which the individual properties are mutually modified. For example, the combination of stilbamidine + colchicine was different in its action on nucleoproteins than that obtained by the individual action of either agent. Similarly, the effects produced by stilbamidine + phenethyl-4-piclonium Br or by stilbamidine + diphenylethyamine differed strikingly from those produced by any one of the agents tested individually. Similar combinations of chemical agents should, therefore, be tested on living cells.

Some Chemical Changes Induced by Methylcholanthrene in the Transformation of Mouse Epidermis to Squamous Cell Carcinoma. C. Car ruthers and V. Sunzteff. (Research Department, The Barnard Free Skin and Cancer Hospital, St. Louis 3, and the Department of Anatomy, Washington University Medical School, St. Louis 10, Missouri.)

The research program at this hospital on some of the chemical, histological, and physical changes induced in the epidermis of mice undergoing carcinogenesis by methylcholanthrene has led to the accumulation of considerable data on the chemical composition of normal, methylcholanthrene-treated (hyperplastic) epidermis, and transplantable squamous cell carcinoma of mice, and also some information on the mineral composition of human epidermis.

For the various analyses, the epidermis was separated from the dermis at 50°C. on a warm plate by the procedure of Baumberger, Sunzteff, and Cowdry (1), or at room temperature by a recently devised method. Since only small amounts of epidermis were obtained from normal mice, it was necessary to devise microchemical methods for the quantitative determination of the minerals, vitamins, and lipids.

Using nucleoprotein phosphorus as a basis of reference, the potassium, sodium, magnesium, iron, copper, and zinc contents of normal epidermis, hyperplastic epidermis, and of a transplantable squamous cell carcinoma were determined. While significant decreases in the calcium, iron, copper, and zinc nucleoprotein phosphorus ratios were observed as a result of the application of 1 to 3 paintings of the carcinogen, the potassium, sodium, magnesium, and ascorbic acid ratios showed no perceptible change. Moreover, the decrease in the heavy metal and calcium ratios so manifestly evident throughout hyperplasia was even more spectacular with the onset of carcinoma. On the other hand, the ascorbic acid nucleoprotein phosphorus ratio remained unchanged throughout the hyperplastic and carcinomatous stages, while the sodium, magnesium, and potassium ratios decreased slightly in the tissues of squamous cell carcinoma.

In this connection, analyses were also made of the mineral compositions of human epidermis (for purposes of comparison) and it was discovered that decreases occurred in the calcium, copper, and zinc contents in squamous cell carcinoma strikingly similar to those detected in the comparable stage of the mouse. These experiments also revealed that normal human and hyperplastic epidermis of the mouse agree well with respect to their mineral composition, which is in conformity with their somewhat similar morphology.

Determination of the water content of the epidermis undergoing carcinogenesis established the fact that the amount of water increased from 60 per cent in the normal to an average value of 66 per cent in the hyperplastic stage, and rose to nearly 82 per cent in the transplantable carcinoma.

Investigations on the epidermal content of choline, biotin, p-aminobenzoic acid, inositol, and pyridoxine by Tatum and his group (4) using mutant strains of Neurospora demonstrated that only biotin was significantly altered during carcinogenesis. This vitamin decreased to 64 per cent of the normal.

Wicks and Sunzteff (5, 6) also showed that the total lipid- and total cholesterol-protein-nitrogen ratios were about 50 per cent less than normal in hyperplastic epidermis. The decrease in total lipid was found to synchronize with the disappearance of the sebaceous glands. The relationship of the latter structures to carcinogenesis has been investigated by Simpson and Cramer (3), and is briefly discussed.

Experiments were also conducted with a view to elicit information concerning the influence of age on the response of carcinogen in relation to epidermal calcium and total lipid content. Young and old mice of the Swiss and New buffalo strains were used for the purpose. The results showed conclusively the absence of any correlation between the decrease in the content of these substances and the response to the carcinogen.
The activities of succinic dehydrogenase and cytochrome oxidase in epidermal carcinogenesis were followed by employing the procedure of Schneider and Potter (2). Our study showed that the activity of succinic dehydrogenase remained the same both in the normal untreated epidermis and in the hyperplastic epidermis, but increased significantly in the carcinoma. On the other hand, the activity of cytochrome oxidase in the late hyperplastic epidermis was twice that of the normal, but the value for its activity in the carcinoma was less than that of hyperplastic epidermis, but greater than that of the normal.

Finally, an attempt was made to correlate the chemical changes induced by the carcinogen with some of the morphological alterations.

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THE SIGNIFICANCE OF INDUSTRIAL CANCER IN THE CANCER PROBLEM. W. C. HUEPER.

(Warner Institute for Therapeutic Research, New York, N. Y.)

In view of the known differences existing in the susceptibility and reactivity of various species to the numerous recognized physical and chemical carcinogenic agents, industrial cancers possess the great advantage over experimental cancers in animals in that they permit the direct study of many of the most important and fundamental aspects of cancer in the human organism. The considerable rise in the absolute number and in different types of occupational cancers paralleling the enormous growth of modern industrial developments reflects an important biological maladjustment of civilized man to his new artificial industrial environment and represents a striking illustration of the momentous rôle which external environmental agents apparently play in the causation of cancer in general. There are several reasons for assuming that the majority of occupational carcinogenic agents is still unknown, and this applies especially to those occupational carcinogens which have a low potency and therefore produce a low incidence of cancer among the individuals exposed. This conclusion is supported by evidence obtained during recent years which makes it likely that new industrial carcinogenic agents and heretofore unknown types of occupational cancers will be discovered in the next few years in American industries. When these facts and their implications are properly and fully appreciated, occupational cancers are bound to become a public health problem of the first order, which deserves serious and extensive investigation.

The existence of wide differences in the physical and chemical properties of the various established or suspected occupational carcinogens suggests that these agents do not exert their specific pathogenic action through the same mechanism. Some of them seem to cause a cancerous cellular transformation by acting directly either in their original form or as metabolites or conjugates upon the cellular substrate. Others may elicit such an effect by changing some normal chemical constituent of the cells or tissue fluids in such a way that it becomes endowed with carcinogenic properties. A third group of occupational carcinogenic agents may display such an action by producing functional disturbances in certain organs, such as the liver, adrenal glands, pituitary gland and gonads leading to the endogenous generation of carcinogenic substances.

Inasmuch as the great prevalence of industrial cancers in males is the result of an exposure factor and is evidently not sex-conditioned, it is suggested that environmentally carcinogenic factors may be responsible for the greater frequency of several cryptogenetic cancers (lung, larynx, gastrointestinal tract, skin) among males than among females.

Observations made in connection with occupational cancers show clearly that a hereditary factor is not involved in the production of these tumours, which have been observed in individuals between the ages of 10 to 80 years. The age at onset of exposure together with its duration and intensity controls the manifestation age of occupational cancers.

There is no concrete and valid evidence indicating that a genetic hereditary predisposition plays an important rôle in the production and incidence of occupational cancers. The intensity, time and duration of exposure to and the potency of the carcinogenic agents involved appear to be the main factors in determining the susceptibility of the individual and the length of the latent period.

The rôle of the various procarcinogenic, anticarcinogenic, procarcinotic and antircarcinotic factors is evidently not a fixed one, but varies with the type and contact of the carcinogenic agent involved and the intensity of its action.

Although the United States has been for several decades the leading industrialized country, only very incomplete data as to the actual number and variety of occupational cancers, their causes, and the agents and operations responsible for their development are available, because these industrial diseases are not reportable in most of the states and because American industries have been reluctant to file their cases of occupationally induced cancers on record. By the end of 1945 only 32 states had passed some form of compensation law covering industrial diseases in general. Since the majority of such laws either contain time limitation clauses as to the filing of compensation claims or apply only to a limited and well defined number of hazardous agents or operations, they provide inadequate or defective coverage against industrial cancer hazards. No competent and extensive investigations exist in regard to the possible rôle that industrial or related exogenous agents might play in the causation of some of the large numbers of cancers of the cryptogenetic type. A comprehensive and thorough survey of all industries producing or handling known or suspected carcinogenic agents, including...
those occurring in the form of contaminants, by-products or waste products, would furnish a sound basis for the development of effective measures controlling the industrial cancer hazards of this country.

CARCINOGENIC ACTION OF SOME SUBSTANCES WHICH MAY BE A PROBLEM IN CERTAIN FUTURE INDUSTRIES. AUSTIN M. BRUES, HERMANN LISCO, and MIRIAM P. FINKEL. (Argonne National Laboratory, University of Chicago, Chicago 90, Illinois.)

In the course of manufacturing plutonium in a chain-reacting pile, products of uranium fission are evolved. These atomic split products are largely radioactive and must be separated from the pile uranium as part of the plutonium separation process.

We have investigated the late or chronic effects of exposure to certain fission products and plutonium. The fission products, since they occupy the middle of the periodic table, include most of the rare earths, also iodine, barium and strontium. Experiments were set up to observe the effects of radioactive Sr$^{+}$, Ca$^{++}$, Ba$^{++}$, and plutonium. Radium was also studied for comparison with earlier human and animal work. We used Sprague-Dawley rats, Carworth CF-1 and ABC hybrid mice and a few CS8 mice, as well as rabbits and dogs. All animals received complete autopsy, terminal blood study, and roentgenograms. This is a progress report.

Sr$^{+}$, with a 55-day half-life and a tendency to concentrate in bone, has no visible effect on mammary tumor incidence and a slight positive effect on lymphoma incidence. It is a producer par excellence of bone tumors. Analysis of bone tumor incidence in a series of over 3,000 mice, 400 to 500 days after treatment, shows that expectation of tumor development is approximately proportional to dose and to time after the onset of the "latent period," but that the latent period itself is a function of dose which increases only gradually (perhaps logarithmically) with decreasing dose. Such a scheme is shown to fit, as well as can be determined, the existing human radium data. It appears that age of the animal at the time treatment is administered has little effect on latent period or tumor expectancy, even when the isotope is absorbed by the growing fetus.

Radium, in addition to bone tumor induction, produces heavy calcification in the media of the larger arteries and in certain other sites.

Radioactive cerium—presumably, which gives an initial high liver concentration, causes atrophy of this organ except in the marginal areas, where ionization is less and regeneration can proceed. Bone sarcoma is also produced.

Yttrium$^{+}$, which was fed by stomach tube, is virtually unabsorbed. One-tenth of the acute lethal dose can be fed daily for 3 months with no measurable detriment. Animals surviving this treatment, as well as those surviving a single acute dose of 20 to 30 mc. per kgm., show after several months a variety of intestinal lesions with obstruction.

Plutonium (Pu$^{+}+$), when injected intravenously, has its highest immediate concentration in liver and spleen, and is later translocated to bone. Acute plutonism is similar to acute total body radiation sickness, with the additions of splenic atrophy and gross liver changes. Chronic plutonism involves greying of hair, progressive liver damage, and bone sarcoma. Following subcutaneous injection of 1 mgm. of plutonium, local fibrosarcomas may appear within a year. Local epilations, ulceration, keratosis, and spontaneous amputation are also seen.

THE CARCINOGENIC EFFECT OF PILE RADATIONS. P. S. HENSHAW, E. F. RILEY and G. E. STAPLETON. (Clinton Laboratories, Oak Ridge, Tennessee.)

A variety of high energy radiations (fast and slow neutrons, gamma rays, beta rays, etc.) are associated with the uranium chain reaction in a pile reactor. All of these radiations are known to cause deleterious biological effects and hence constitute health hazards.

In an attempt to understand more fully the types of injury induced, and at the same time obtain information on threshold and tolerance levels, numerous animal experiments were carried out. Four strains of mice (CF, ABC, A, CS8) and one strain of rats (Sprague-Dawley) were the main types of test material. The different radiations were obtained by means of selective filtration, secondary emitters and radioactive isotopes. Single massive dose treatments as well as small daily treatments were administered. The radiations used, with the exception of beta rays, were of the penetrating type, and for the most part were distributed throughout the animal bodies.

Results revealed that the length of life varied inversely with the amount of exposure, and further that the amount of effect varied directly with the concentration of ions in space and time. The terminal effects obtained when penetrating radiations were used were mainly of two types: generalized atrophy, and neoplasias of the hematopoietic organs. The atrophy was attended by lowered leukocyte level, emaciation, loss of weight, and premature death. The neoplasias seen consisted mainly of medullary lymphomatoses. The incidence of the latter was raised from less than 15 per cent in the controls to more than 60 per cent in certain of the experimental groups.

When non-penetrating radiations were used the changes were limited mainly to the skin. Several months after single massive doses skin abnormalities of every type were present. In animals that rarely show skin lesions of any type, the incidence of carcinoma was raised to 100 per cent and loci per animal were as great as 50 to 100.

The relative effectiveness of fast neutrons compared with gamma rays appeared to be definitely greater when late modifications were considered rather than acute. Threshold levels of daily exposure, so far as shortening of life is concerned, were found to be in the range of 1 r of gamma rays, and something well less than 0.1 r.

ETIologic STUDIES IN Hodgkin's DISEASE. HERMAN A. HOSMER. (The Division of Cancer Research, The Ohio State University, Columbus, Ohio.)

An introductory reference was made to the distribution of Hodgkin's disease: the significance of tubercle bacilli and Brucella in Hodgkin's tissue and body fluids; the age distribution of patients with Hodgkin's disease; the prognostic importance of the sedimentation rate, lymphopenia, and anemia; the absence of pain in early Hodgkin's disease; and the selective preference of the disease for specific tissue systems.

Observations following inoculation of tissue culture cells with cell-free material from Hodgkin's disease, lymphomatoses, and control tissue extracts and body fluids were described. Embryo spleen cells in tissue culture, stained with Sellier's stain, were used routinely to demonstrate the presence or absence of fuchsinophilic cytoplasmic inclu-
Sources of embryo spleen were the chicken, duck, and guinea pig. All preparations of fresh, frozen, or lyophilized Hodgkin's tissue, lymphomatosis, and control tissues were minced and centrifuged for 30 minutes at 4,000 r.p.m. The supernatant fluid obtained was used as the source of inoculum before and after differential low gravity and ultracentrifugation, ultrafiltration, and dilution to one part in 2 billion. Cyto-stimulating and cytolytic changes were noted following inoculation of tissue cultures with cell-free material of Hodgkin's disease and lymphomatosis. These changes were not observed in cultures inoculated with control tissues and body fluids. Inocula composed of the re suspended sediment following high gravity centrifugation produced in tissue cultures an increase in cyto-stimulation and the number of inclusion bodies.

Fuchsinophilic intracytoplasmic inclusions were found not only in tissue cultures inoculated with material from Hodgkin's disease and lymphomatosis, but also were noted in some control preparations containing either chicken or duck constituents only. Investigation of this phenomenon resulted in the finding that chickens and ducks, from which the plasma and embryos had been obtained, were infected with lymphomatosis. Because of this potential source of contamination, adult and embryo chicken and duck materials were considered unsuitable for further use in tissue cultures. Therefore homologous guinea pig substrate and nutrient medium were adopted.

Inactivation of the agents of Hodgkin's disease and lymphomatosis by ultraviolet light was described.

The third phase of the work deals with clinical observations made by the author and his associates. Under the direction of Dr. Perk Lee Davis of the Women's Medical College of Pennsylvania, two patients with Hodgkin's disease received intramuscular injections of 10 cc. of pleural fluid obtained from a terminal case of Hodgkin's disease of the thoracic type. The donor also received an identical injection of his own fluid. At the end of 48 to 72 hours all 3 recipients developed a generalized maculopapular eruption with severe itching. Most of the lesions soon disappeared, but a few still remained; these enlarged, and some of the central necrosis became visible between the fourth and seventh days following the injections. These lesions varied in size from 0.2 to 2.0 cm. and did not respond to treatment with chemotherapeutic agents applied locally.

Examination of biopsy material obtained from the skin lesions revealed the typical Sternberg-Reed cells (Hodgkin's cells) in all cases. Following therapeutic doses of x-rays, these lesions disappeared leaving brown pigmented areas, but the downhill course of the patients appeared to be accelerated following the injections.

Lesions similar to, but less acute than, those just described have been observed by the author in several patients with Hodgkin's disease:

(a) Multiple skin lesions occurring immediately after a series of five daily x-ray treatments to a greatly enlarged spleen were seen in case of H.O., a 37 year old white male with acute Hodgkin's disease.

(b) M. H., a 34 year old white female, developed multiple skin lesions of the back 48 hours following the subcutaneous inoculation of an extract of Hodgkin's tissue that had been inactivated by ultraviolet irradiation.

(c) The author has observed 4 cases of Hodgkin's disease that exhibited skin lesions of spontaneous origin similar to those described above by Dr. Davis. One of the author's associates, Dr. Anthony Rottino, of St. Vincent's Hospital in New York City, has investigated and made biopsies of 2 similar cases.
time of the exposure (about 2 days) all mitotic activity is abolished. The affected mitotic cells exhibit clearly two distinct reactions. They either break down rather rapidly into chromophilic fragments or increase strikingly in size. The nuclei of these large cells may fragment in later development, while some of them seem to recover. In organs where the processes of proliferation and differentiation occur in different cells, as in the nervous system for example, enlargement and early breakdown of the mitotic cells occur side by side. In the embryonic gut, however, where differentiation and proliferation occur within the same cell, only cell enlargement occurs, while at the same time cellular differentiation continues unhindered. This results in the formation of giant cells which apparently function normally. It was found that the cells showing the enlargement reaction are characteristically cells which in later development make up proliferating regions of the embryo, while the cells exhibiting the early breakdown reaction without enlargement are those whose mitotic activity is limited to early embryonic development. Consequently fewer and fewer broken cells of this type are found as the age of the animals at exposure increases, because more and more of these mitotic cells have entered their differentiation phase and are hence not affected.

By its selective action in showing up regions of proliferation the agent is an exceedingly useful tool for the study of growth patterns. The agent's specific ability to arrest mitotic activity without interfering with differentiation allows us to control experimentally the size of organs and gives us a new approach to the problems of growth control.

**EXPERIMENTAL OBSERVATIONS ON THE EFFECTS OF THE NITROGEN MUSTARDS ON NEOPLASTIC TISSUES**


The effects of the nitrogen mustards \(N\left(CH\_2\_Cl\right)\_2\) (called \(HN3\)), \(CH\_N\left(CH\_2\_Cl\right)\_2\) (called \(HN2\)), \(CH\_N\left(C\_2H\_Cl\right)\_2\) (called \(HN1\)) and \(CH\_\_\_\_CH\_N\left(CH\_2\_Cl\right)\_2\) (called \(HN4\)) used in all cases as hydrochloride salts, have been studied on normal animals and tissues, and then assayed for chemotherapeutic activity against various types of neoplastic tissues under several conditions: Leukemia in mice, mouse sarcoma 180 growing on the chick chorioallantoic membrane and sarcoma 180, adenocarcinoma 1025 and lung carcinoma MA 387 in roller-tube cultures.

In normal animals it is found that certain tissues, including the hematopoietic and lymphatic tissues and the intestinal epithelium, are unusually sensitive to the action of these compounds. It may be significant that the sensitive tissues are those which possess relatively high rates of cell division. \(HN2\) and \(HN3\), the only ones tested, on intravenous injection into animals have a very rapid and presumably direct action on the affected tissues. In many respects the sulfur and nitrogen mustards are found to resemble x-rays in their action; for instance, in their toxicological effects on animals, their inhibiting action on cell division, and in their ability to produce mutations in Drosophila (2) and Neurospora (3).

The additive lethal effects in mice of \(HN2\) and x-rays vary with the sequence of administration. If 80 per cent of a lethal dose of x-ray is followed an hour later by 80 per cent of a lethal dose of \(HN2\), the lethal action of the two agents is only slightly additive. If the sequence is reversed, and the same dose of \(HN2\) is given 1 hour before the x-rays, an additive, much greater lethal effect results.

Four compounds, the methyl-bis, ethyl-bis, isopropyl-bis and the tris-(β-chloroethyl)amine were tested against 1 myeloid and 3 lymphoid leukemias in mice. The mice used were of a highly inbred stock Afb in which transmitted leukemias of the 4 strains used take in approximately 100 per cent. The experimental procedure was as follows: a leukemic mouse with enlarged spleen and nodes was weighed and injected intravenously with a given mgm./kgm. dose of the compound to be tested dissolved in saline. One hour later (less in the higher doses where toxic effects often killed the mouse in from 5 to 50 minutes) the mouse was killed, the spleen removed, and the splenic brei injected subcutaneously into 4 young mice of the Afb stock as a bioassay. Sections of liver, spleen, and kidney of the donor mouse were made. All mice used for bioassay were then watched for development of leukemia, and in all questionable cases sections were made. It has been our experience that larger doses of the nitrogen mustards, even when not completely cytocidal to leukemic cells, so affect them that the incubation period in the bioassay animal may be much prolonged over the normal time.

The problem of how long the nitrogen mustard should stay in the live animal in order to have a maximal effect on the leukemic cells of the spleen is unsolved. Frequently the animal died rapidly and we arbitrarily set 7 minutes survival time for the donor animal as necessary for a valid bioassay.

From the preliminary data, it would appear that for the 4 strains of leukemia tested the cytocidal dose of tris-(β-chloroethyl)-amine varies from 7 to 22 times the LDS0 of the \(HN3\) or 10 to 33 mgm./kgm. The methyl-bis compound inactivates the leukemic cells at 8 to 12 times the LDS0 or 33 to 75 mgm./kgm. With the ethyl-bis compound, only in the A9417 leukemia were there any consistent results and there the cytocidal effect was manifest at 22 times the LDS0 or 33 mgm./kgm. With the isopropyl-bis(β-chloroethyl)-amine, a dosage of 37 times LDS0 or 50 mgm./kgm. inactivated the cells of 3 strains of leukemia in some experiments.

It appears from this experiment that with the tris- and the methyl-bis compounds there is a definite cytocidal dose for leukemic cells. With the ethyl and isopropyl analogs, the evidence of cytocidal activity is not clear even at much higher multiples of the LDS0. If this is borne out in the finished experiments, it would seem to indicate that the former compounds are more likely to be of value clinically than are the latter.

The lethal dose of \(HN3\) in fertile chick eggs is almost directly proportional to the mass of the embryo; the LDS50 by injection into the yolk sac is about 0.015 mgm./egg in the 3-day chick and about 0.5 mgm./egg in the 12-day chick. Anomalies of the beak and legs have been produced in 3-day-old chicks surviving LDS50 doses of \(HN3\), but this has not occurred in the older eggs. A similar effect has been produced with the sulfonamides (1). In 5-day eggs with actively growing sarcoma 180 tumors on their chorioallantoic membrane, a dose of \(HN3\) which is not lethal to the egg will stop the growth of the sarcoma 180, render it incapable of growth when transplanted into mice and produce, as shown by histological examination, dissolution of a majority of the sarcoma cells and the appearance of remarkable giant cells containing large ab-
normal nuclei, occasionally multilobed or fragmented. The appearance and location of these giant cells strongly suggest that they stem from the sarcoma cells. No significant change in the chick membranes or vascular tissues was observed. The LD50 of HN3 for the tumor in the egg is in the range of 0.05 to 0.10 mgm./egg, when injected at 12 days, about 1/5 to 1/10 of the LD50 for the chick embryo. Preliminary experiments designed to show the speed of the lethal action of HN3 in such egg-tumor preparations indicate that the tumor can be fatally damaged within less than 30 minutes (the shortest period studied) by 0.2 mgm./egg of HN3.

Using the roller tube technic, cultures of sarcoma 180, lung carcinoma Ma387, and normal mouse epithelium were exposed to various concentrations of N(C2H5Cl)2, dissolved in the nutrient medium. The results of these experiments up to the present time indicate that certain tumors appear to be more susceptible than others to the action of this compound. Sarcoma 180 cells have consistently been killed at concentrations of 20 mgm./l after 24 hours exposure as indicated by their failure to cause tumors when transplanted into susceptible mice. Ma387, however, has in some cases survived concentrations as high as 80 mgm./l.

There was suggestive evidence that the tumor cells were somewhat more sensitive to concentrations of HN3 than were the corresponding normal cells. At concentrations of up to 80 mgm./l, the deleterious effects on normal cells appeared less distinct than on tumor cells. At 70 mgm./l, Ma387 and sarcoma 180 cells were visibly affected although not disintegrated, whereas normal cells appeared unaffected. At 20 mgm./l, both normal and tumor cells looked normal cytologically, but those of sarcoma 180 were apparently rendered nonviable as evidenced by negative bioassay results.

While these lethal concentrations will have to be more precisely defined by further experiments, it is interesting to note the difference in susceptibility of the tumors employed and that tumor cells could be consistently rendered nonviable by concentrations which caused little visible damage to the cells involved.

The clinical evaluation of the methyl-bis and tris-(β-chloroethyl)amine hydrochlorides is in progress. In addition to varying the dosage level, the effect of these drugs on various neoplasms not connected with the blood-forming organs is being evaluated. Neuroblastoma, Ewing's tumor, glioblastoma, and malignant melanoma are under treatment at the present time, but it is still too early to state any definite results.

It is to be re-emphasized here that this is in the nature of a preliminary report and that much work remains to be done before these data can be considered definitive.

REFERENCES


THE CLINICAL APPLICATION OF A NITROGEN MUSTARD COMPOUND METHYL BIS (β CHLOROETHYL) AMINE TO THE TREATMENT OF NEOPLASTIC DISORDERS OF

THE HEMOPOIETIC SYSTEM.* CHARLES L. SPURR, LEON O. JACOBSON, TAYLOR R. SMITH, and E. S. GUZMAN BARRON. (Dept. of Medicine, University of Chicago, Chicago, Illinois.)

Gilman, Goodman and their co-workers (1) were the first to study the effects of a nitrogen mustard compound on human neoplasia. They studied the pharmacological and clinical effects of tris-(β-chloroethyl) amine administered intravenously in 7 terminal cases. Independently in March, 1943, Jacobson and his group (2, 3) undertook a similar clinical study in an attempt to evaluate another nitrogen mustard; namely, methyl-bis (β-chloroethyl) amine as a therapeutic agent. An unselected group of 59 cases of neoplastic and allied disorders of the hemopoietic system have been studied.

Methyl-bis (β-chloroethyl) amine which is highly cytotoxic is administered intravenously in a dose of 0.1 mgm. per kgm. of body weight diluted in approximately 100 cc. of normal saline on consecutive days in series of 2 to 6 injections. The toxic reactions observed after the administration of this compound have been nausea and vomiting, phlebothrombosis, varying degrees of destruction of hemopoietic tissues and consequent depression of the hematological constituents of the peripheral blood. Nausea and vomiting which are partially central and local in origin usually follow each dose within 3 hours and persist for a similar period. Phlebothrombosis occurs only if the drug is not sufficiently diluted when injected or delayed by obstructing masses in the veins used for the administration.

Evaluation of the reaction of the hemopoietic system to the therapeutic application of the methyl-bis (β-chloroethyl) amine demonstrated that, following each series, a characteristic lymphopenia became apparent within 48 hours. Recovery of the lymphocytes usually begins in the second week following treatment.

A neutropenia develops in the third week and is usually of about one week's duration. There is also a prompt but variable thrombocytopenia during the first 3 weeks. This is occasionally sufficient to produce an increase in the bleeding time but only on 3 occasions have petechiae appeared.

Series of tri-weekly sternal marrow aspirations were made on 5 cases over a period of 6 weeks after treatment. These show a depression of the total nucleated cell count from an average of 100,000 per cu. mm. to 6,400 per cu. mm. on the 21st day and a sharp recovery to 65,000 per cu. mm. in the fourth week. There is a slight depression in the blast forms and promyelocytes during the first week and a moderate increase in the second week. The myelocytes and metamyelocytes decline through the first 2 weeks and regenerate to a peak in the third and fourth weeks respectively. The percentage of polymorphonuclear cells increases in the first 2 weeks and declines sharply in the third week, regenerating to a peak in the fifth week. There is also a depression in the erythroid series for 2 weeks with orderly regenerative peaks of proerythroblast and basophilic erythroblasts in the third week and of the orthochromatric erythroblast in the sixth week. Many giant forms of the granular series are seen in the peripheral blood especially during the first week after administration of the drug. Infections and other clinical manifestations usually attributed to severe leukopenias were conspicuously rare. In the one instance in which an infec-
tion occurred it was successfully controlled by penicillin therapy.

The reaction of the peripheral blood and the clinical response served as the principal guides in evaluating the most efficacious total dose per course of treatment. In a series of 79 courses the severity of the depression of the peripheral blood increased with the number of consecutive injections per course. There was a fall of hemoglobin and erythrocyte values from 0.7 gm. per cent and 140,000 per cu. mm. respectively (average total dose 12 mgm.) to 1.6 gm. per cent and 460,000 per cu. mm. respectively after 6 injections (average dose 36 mgm.). An increasingly severe decline in leukocytes was also noted. The average percentage of fall in the total leukocyte count increased from 35 per cent to a plateau at 65 per cent and 67 per cent as the courses were increased from 2 to 4 and 6 injections. An irregular increase in the incidence of leukopenias of less than 3,000 cells per cu. mm. developed as the courses were prolonged; however, there was a sharp increase in the severity and duration of leukopenias with total doses above a 25 to 30 mgm. level or with 4 or more injections.

The possibility that this hemotoxic material may have a cumulative effect with repeated doses and eventually lead to a permanent hypoplastic reaction in the bone marrow has been assessed by grouping 58 courses according to the total dose administered in 50 mgm. steps from 50 to 250 mgm. The reaction of the peripheral blood in each group as judged by the average pretreatment and post-treatment leukocytic counts shows no remarkable trend. However, the percentage incidence of leukopenias increases from 40 per cent in the 0 to 50 mgm. group to 80 per cent in the group with a cumulated dose of 200 to 250 mgm. This is the only indication that the hemopoietic system may become more sensitive to the compound as the courses of treatment are repeated. Two factors tend to contradict this: (a) the leukocytes return to normal range following each course, and (b) the cases in this group are of longer duration and several had leukopenia prior to initiating treatment. It is suggested that a cumulated dose of 600 mgm. or more will be necessary before an unequivocal answer may be made to this question.

The clinical response to methyl-bis (β-chloroethyl) amine hydrochloride in 29 cases of Hodgkin’s disease observed over a period of 3 to 36 months shows instances in which the various symptoms of the disease have been controlled. In this group of cases 94 per cent of 120 courses of treatment produced clinically significant remissions. Among the 8 cases that failed to respond to treatment, 5 were in a terminal phase of their disease, 2 had advanced disease and were no longer responsive to roentgen therapy and 1 had a remission from fever of only 3 weeks. Of particular interest are 4 cases which had been considered “ray resistant.” This group has responded repeatedly to courses of nitrogen mustard. The remissions induced in this group of patients with Hodgkin’s disease vary from 0 to 10 months, although the length of remission increases with the total dose per course. A course of four injections of 25 to 30 mgm. was selected as optimum because of the plateau in the severity of leukopenia at this level. There is a slight decrease in the average remission with repeated courses; however, the number of cases cumulating doses above 150 mgm. is too small to evaluate this question. Two cases have appeared to develop a tolerance or resistance to the material.

A group of 9 cases of chronic lymphatic leukemia has shown remissions of 2 to 24 months. This variation depends largely upon the activity of the disease. Although each case responded to the initial course, the group survival is poorer than in Hodgkin’s disease. Five patients have died within 18 months after the initial treatment from advancing disease or its complications.

Six cases of lymphosarcoma have been treated with methyl-bis (β-chloroethyl) amine. Two cases, both afflicted with an extremely undifferentiated type of tumor, failed to respond to the initial course of treatment. The remaining cases had remissions of 2 to 6 months and have been followed for 2 years. A case of giant follicular lymphoma responded in a manner similar to those of lymphosarcoma.

Seven cases of chronic myeloid leukemia have responded to treatment with methyl-bis (β-chloroethyl) amine with transitory symptomatic and hematological improvement. We do not believe that the remissions induced were clinically significant.

Two cases of acute leukemia, 1 lymphatic and 1 myelogenous respectively have been treated without symptomatic relief, severe leukopenia developed in the latter case.

Seven cases of polycythemia rubra vera have responded favorably following courses of methyl-bis (β-chloroethyl) amine with remissions of from 3 to 17 months. Six continue in their initial remission. The hematological picture in each reverted to normal values within 1 month and remain within normal values. One case although free of symptoms had a moderate increase in the hemoglobin and erythrocyte levels and was treated 6 months after his first course.

The remissions produced in Hodgkin’s disease and lymphosarcoma and in some cases of chronic lymphatic leukemia are sufficient to warrant extended study with a view to using methyl-bis (β-chloroethyl) amine or a related compound as an adjunct to roentgen therapy. The remissions produced in cases of polycythemia rubra vera are comparable to those following treatment with radioactive phosphorus and possibly superior to roentgen therapy. The nitrogen mustards have not been proven superior to roentgen therapy in the treatment of any of the lymphomas.

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These authors have another article with similar title in press for publication in the Journal of Clinical Investigation.


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