Of the many aspects of cancer research, three
which are perhaps of particular interest to chem-
ists are the problems of cell metabolism, of chemo-
therapy, and of carcinogenesis. All these aspects
are closely related, and at the present stage of de-
velopment of the subject chemotherapy and car-
cinogenesis are intimately connected, as most of
the physical and chemical agents used in therapy
of cancer are themselves carcinogenic. The correla-
tion between these two properties has been so close
in the past few years that, when a new agent is in-
troduced for therapy, one is immediately sus-
picious that it may be carcinogenic.

From the human point of view, the two aspects
of the cancer problem which are most impor-
tant are prevention and cure. Experiments on chemo-
therapy are likely to lead to control of the disease
and a study of carcinogenesis to prevention or re-
duction of the incidence. The investigation of car-
cinogenesis might reduce the incidence of the dis-
ease in at least two ways. One of these is the identi-
fication of carcinogens which might be acting as
extrinsic carcinogenic agents in the external en-
vironment. It is obvious that any known car-
cinogens should be avoided, but they may be dif-
ficult to recognize. One approach to carcinogenesis
is through the examination of the incidence of can-
cer in sections of the population. In the case of
chimney-sweeps and the early operators of x-ray
apparatus the incidence was so great as to be ob-
vious, but careful statistical analysis might lead to
the recognition of many, as yet unsuspected, ex-
trinsic carcinogenic factors.

This method has until now been mainly instru-
mental in demonstrating carcinogenic factors con-
cerned with occupations such as the bone cancer
of girls who painted the luminous dials of watches,
the bladder cancer of men working with β-naph-
thylamine, and the scrotal cancer caused by the
lubricating oils used in the spinning mills in the
Lancashire cotton industry. The incidence of all
these forms of cancer has been greatly reduced by
the introduction of precautions based on our
knowledge of carcinogenesis. A great deal of can-
cer not due to special occupational risks could be
reduced by application of similar principles. The
increase in the incidence of cancer of the lung of
man which has occurred during the present cen-
tury is probably due to increased contact with car-
cinogenic stimuli. We have reason to be suspicious
of the air we breathe, the food we eat, and the
medicaments we use, and such suspicions should
be allayed or justified by research.

Fundamental knowledge of carcinogenesis
might lead to prevention of the disease in another
way. The effect of carcinogens may be merely to
increase the incidence of the change of normal
cells to cancer cells, a change which is almost cer-
tainly analogous to a mutation or sport, and may
occur to a small extent without any external agent.
Understanding of the nature of this change might
even lead to its prevention, and so reduce that
part of cancer incidence in which no external stim-
ulus was involved. It is of course possible that
there would be no cancer without external stimuli,
but that would seem to be unlikely.

The recognition of the carcinogenic nature of
chimney soot and coal tar was followed by the isolation of the pure carcinogenic hydrocarbon 3,4-benzpyrene. This led to the synthesis and testing of a large number of carcinogenic polycyclic hydrocarbons by Cook and Kennaway (18, 19) in England and Fieser and Shear in the United States. Progress in this field was rapid in the thirties, partly because of the effectiveness of the biological tests for substances of this type. The application of the substances by painting on the skin of mice or injection into mice or rats produced easily visible papillomas, epitheliomas, or sarcomas. During the last 10 years consideration of the essential nature of the carcinogenic hydrocarbons had indicated that activity was usually associated with the presence in the molecule of a region of high electron density. Such consideration was made easier by the publication of the survey of compounds tested for carcinogenic activity by Hartwell (30). Sir Robert Robinson (45) pointed out that carcinogenic hydrocarbons usually contained a phenanthrene double bond, and the French workers, Daudel (21) and Pullman (44), made many theoretical calculations showing that the carcinogenic activity was dependent on a high electron density of a part of the molecule which Schmidt (46) had termed the K region—the K standing for Krebs or Cancer. The conclusions of the theoretical chemists have in general been supported by the determinations of the rate of oxidation by osmium tetroxide made by Badger (1), following the study of this reaction made by Criegee (20). That all carcinogenic hydrocarbons contain a region of high electron density or, in other words, a bond with considerable double bond character in Pauling's sense (43) seems to be established. It has never been claimed that all substances which contain an active double bond or a region of chemical unsaturation are carcinogenic, so that the presence of an active K region is only one requisite of a carcinogenic molecule. On the other hand, there are a number of carcinogenic substances outside the groups of hydrocarbons which have no typical K region.

The activated phenanthrene double bond is only one type of a number of chemical groups which appear to confer carcinogenic activity on molecular structures. The term "carcinogenophore" analogous to chromophore has been suggested (9, 10) as a general term for such groups in carcinogenic molecules and the term auxocarcinogen for groups which enhance the chemical activity and biological activity of such structures. Although many carcinogenic hydrocarbons are known, they form only one group of carcinogens. As long ago as 1938, Haddow (26) drew attention to "the striking multiplicity of tumour-producing agents including the hydrocarbons and radioactive agents." His own work had shown that the carcinogenic hydrocarbons, like the carcinogenic radiations, inhibited the growth of tumors and animals. Haddow suggested that "the carcinogenic agents operate by producing interference with certain normal functions of the cell in such a way as to induce variation." The total number and types of known carcinogens has increased enormously since 1938, and our knowledge of their biological action has also increased; but the similarity of action between the carcinogenic chemicals and the ionizing radiations is known with more certainty and is even more striking.

In describing a substance as carcinogenic, I propose, for the purpose of this discussion, not to differentiate between sites and animal species in which carcinogens sometimes show remarkable specificity. Thus, if an agent produces tumors in any site of any species it is considered a carcinogen. An interesting example of this specificity is sodium arsenite, which seems to be carcinogenic for man (40) but not in any laboratory animals in which it has been tested.

The known carcinogenic agents can be conveniently divided into four groups: physical, inorganic, aliphatic, and aromatic. The physical agents include the application of localized cold in the form of carbon dioxide snow (4), implanted cellophane (41), and the ionizing radiations. Although the ionizing radiations are physical agents, they are thought to produce their biological effects by liberation of free hydroxyl radicals and so have a chemical mechanism. Tumors appear to have been induced by a number of inorganic agents including metallic nickel (38) and by salts of zinc (9) and beryllium, but the difficulties of testing inorganic substances are possibly greater than with the aromatic compounds.

Investigation of aliphatic carcinogens has been largely a post-war development, but a number (cf. Table 1) are now known. During the war it was recognized that the vesicants mustard gas and the nitrogen mustards resembled the ionizing radiations in several biological actions. Compounds which produce some or all the effects of radiations and the effects themselves have been described as radiomimetic (22). Many effects of this type are known, but for the purpose of the present discussion perhaps the most interesting are the inhibition of cell division and of growth, the induction of mutations and of specific chromosome damage. If treatment with an agent produces these effects, then there appears to be some chance that the treatment will also produce cancer, because these
effects are produced by known carcinogens. Because of this correlation, Horning and I (13) tested the nitrogen mustards in mice and found them to produce tumors, although rather slowly. Heston (32) and Haddow have since induced tumors with sulfur mustard or mustard gas. Experiments on the effect of substances of this type on chromosomes of the nuclei of tumors growing in rats and on the inhibition of growth of tumors indicated that only substances with at least two active chemical centers were effective. Thus, methylbis(β-chloroethyl)amine, the usual nitrogen mustard, is active, but dimethyl-β-chloroethylamine is inert (12). Following such observations, some of Haddow's co-workers (25) suggested that these substances acted as cross-linking agents. With this hypothesis in mind, diepoxides including butadiene diepoxide were investigated and found to be radiomimetic agents, and some of these have induced tumors in animals. Other substances of this type which have been found to be carcinogenic are trimethylol melamine (31), which has also been used in the clinical treatment of leukemia (42), and the dimesyl a-a-glycols, which have been investigated by Haddow and Timmis (29) and of which 1,4-dimethyl sulfonoxbytane may be a useful drug for treatment of myeloid leukemia (24). All these compounds are able to esterify acid groups, possibly through the intermediate formation of carbonium ions, and it is probable that these substances produce their effects in the body by reactions of this type.

Induction of tumors is one of the radiomimetic effects which is more difficult to produce than induction of chromosome breakage. Now, although chromosome breaks can be induced with mono-functional compounds such as ethyleneimine (6), bifunctional compounds are almost a hundred times more active in this respect. Interesting mono-functional carcinogenic compounds of this type, however, are the stearoyl ethyleneimine and caproyl ethyleneimine (31).

Urethan, or ethyl carbamate, induces lung tumors in mice and is also used in the chemotherapy of leukemia. The specificity of ethyl carbamate is remarkable, as methyl carbamate, propyl, and butyl carbamates are inactive.

The aromatic carcinogens can be divided rather arbitrarily into three classes: the hydrocarbons, the aromatic heterocycles, and the aromatic amines.

Most of the carcinogenic hydrocarbons are derivatives of phenanthrene or anthracene in which the chemical activity is increased by substituent groups such as methyl or condensed benzene rings which do not destroy the planarity of the molecule (Table 2). Thus, the simplest carcinogenic hydrocarbons are 1,2,3,4-tetramethylphenanthrene, 9,10-dimethyl anthracene and 1,2-benzanthracene, but the parent hydrocarbons, phenanthrene and anthracene, are inactive. In each of these three cases, as in most carcinogenic aromatic hydrocarbons, there are probably at least two centers of specific chemical reactivity — thus, the 9,10 double bond, and the 1,4 positions of the tetramethylphenanthrene, the 9,10 meso positions, and probably the α-β (1,2) bonds of 9,10-dimethylanthracene and the 3,4 bond and 9,10 meso positions of 1,2-benzanthracene are all active centers. The ability to partake in 1,4 addition reactions is possibly important in carcinogenic hydrocarbons; the meso positions which can partake in such reactions may be carcinogenophores. Examined in this light, many carcinogenic hydrocarbons are found to have two carcinogenophores. The carcinogenic

<table>
<thead>
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<th>TABLE 1</th>
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<tbody>
<tr>
<td>ALIPHATIC AND HETEROCYCLIC CARCINOGENS.</td>
</tr>
<tr>
<td>MUSTARD GAS.</td>
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<tr>
<td>NITROGEN MUSTARDS.</td>
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<tr>
<td>BUTADIENE DIEPOXIDE.</td>
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<tr>
<td>DIMESYL GLYCOLS.</td>
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<tr>
<td>TRIMETHYLOL MELAMINE.</td>
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<tr>
<td>CAPROYL ETHYLENEIMINE.</td>
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<tr>
<td>URETHANE.</td>
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1,2,5,6-dibenzanthracene, 3,4-benzphenanthrene, and 3,4-benzpyrene each have two phenanthrene double bonds or K regions. With the dibenzfluorenes it is possible that bonds in each of the naphthalene nuclei are active. Thus, in the case of most carcinogenic hydrocarbons, two carcinogenophores can be indicated, if one accepts the meso positions of anthracene or analogous positions as such.

A large number of aromatic heterocyclic car-

TABLE 2
CARCINOGENIC HYDROCARBONS.

There are a large number of carcinogenic amines with two benzene groups. The first group of compounds of this type to be discovered were the azo compounds—the aminoazotoluenes and the N-methylaminoazobenzences. An interesting point about these aminoazobenzences is that at least one methyl substituent group is necessary for carcinogenic activity. Aminoazobenzene is inactive, but N-methylaminoazobenzene and butter yellow (4-N,N-dimethylaminoazobenzene) are active, as is o-aminoazotoluene. Thus, the methyl group can be attached to carbon or nitrogen. These essential methyl groups, like those of carcinogenic hydrocarbons, may be merely repelling electrons and so increasing the activity of some other part of the molecule, or they may react directly with some cell constituent.

The carcinogenic aminostilbenes which were discovered by Haddow, Harris, Kon, and Roe (28) have a formal similarity to the azo compounds with the ethylene group replacing the azo group. These tumor-producing aminostilbenes were discovered through their high activity in inhibiting the growth of tumors. In the stilbenes and azo compounds unsaturated groups occur as the amino group, the stilbene double bond and the azo group.

TABLE 3
AROMATIC HETEROCYCLIC CARCINOGENS.

In the aminoazobenzene and aminostilbene series the introduction of a methyl or chloro group into the 4' position of the molecule appeared to destroy the carcinogenic or growth-inhibiting effect. If the group introduced is a fluoro group, the activity is increased, and 4'-fluoro-4-dimethylaminoazobenzene (38) appears to be the most potent of the aminoazo compounds.

In experiments on the structural requirements of carcinogens related to acetylaminofluorene,
Miller, Miller, Sandin, and Brown (37) found that dimethylxenylamine (p-dimethylaminodiphenyl) was carcinogenic. This molecule is like the carcinogenic azo compounds or stilbenes without the azo or ethylene group. Unless one writes this structure in a quinonoid form, for which there seems to be no real reason, it appears to have only one reactive center.

The aminodiphenyl compound is a link between aminoazobenzene and aminostilbenes and the aminofluorene derivatives. Aminofluorene and acetylaminofluorene are carcinogenic, and Miller and his colleagues (37) have found that a number of analogs of acetylaminofluorene are carcinogenic. These include acetyaminobenzathiophene, acetyaminodibenzoithiophene oxide, and acetyaminodibenzfurane and dimethylaminodiphenyl. In this series consideration of the structures of active compounds suggests that the shape of the part of the molecule attached to the amino group is of great importance but that the atoms forming that shape can vary considerably.

The aromatic nitrogen mustards which were developed by Haddow, Kon, and Ross (see 27) form another group of carcinogenic amines. These are compounds in which the aliphatic residue of the nitrogen mustard molecule is replaced by aromatic residues, such as substituted or unsubstituted phenyl or naphthyl residues. Such compounds are much less toxic than the aliphatic nitrogen mustards, and one of them, the β-naphthyl-bis(β-chloroethyl)amine has been used in the clinical treatment of Hodgkin’s disease and has induced tumors in mice and rats. Although it is an aromatic compound, its chemical and biological properties are closer to the aliphatic carcinogens than to the other aromatic amines. Thus, the active chloroethyl groups have a direct and obvious chemical reactivity, since such groups can esterify acid residues or react with amino groups.

The carcinogenophoric groups in typical aromatic compounds include the phenanthrene and stilbene double bonds, the meso positions of anthracene or tetramethyl phenanthrene, the azo group, the heterocyclic nitrogen, the aromatic amino group, and the sulfide link. Now all these groups are capable of reacting with oxidizing agents such as perbenzoic acid and are considered unsaturated groups. With the outstanding exception of the dimethylxenylamine, most of the aromatic carcinogens appear to have two such centers of unsaturation. Whether two such groups are really essential and, if so, what part they play in the biological actions are still matters for conjecture and experiment.

This brief survey of the different types of carcinogenic agents indicates the wide variety of causes of cancer, although the effects are, to some extent, specific for each type. This diversity of structure probably means that the same end can be brought about by a variety of means. The author made the suggestion (8) that the carcinogens produced their effects by inhibition of enzymes such as the phosphokinases. Investigation of this hypothesis has not been very fruitful, thus the reduction in glycolysis of tumor tissue of animals treated with nitrogen mustard occurred after the chromosome damage (12). This is therefore more likely

<table>
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<td>CARCINOGENIC AROMATIC AMINES.</td>
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| I | \[
\begin{align*}
\text{R}^1 \text{R}^2 \text{N}^3 \text{X}^4 \\
\text{ACTIVE WHEN R}^1 \text{R}^2 = \text{CH}_2 \\
\text{B-ΝΑΡΦΗΤΥΛΑΜΙΝΕ}
\end{align*}
\] |
| II | \[
\begin{align*}
\text{R}^1 \text{R}^2 \text{N}^3 \text{X}^4 \\
\text{ACTIVE WHEN R}^1 \text{R}^2 = \text{CH}_2 \\
\text{AMINOAZOBENZENE}
\end{align*}
\] |
| III | \[
\begin{align*}
\text{R}^1 \text{R}^2 \text{N}^3 \text{X}^4 \\
\text{ACTIVE WHEN R}^1 \text{R}^2 = \text{CH}_2 \\
\text{AMINOFLUORENE}
\end{align*}
\] |
| IV | \[
\begin{align*}
\text{AROMATIC} \text{R}^1 \text{R}^2 \text{N}^3 \text{X}^4 \\
\text{ACTIVE WHEN R}^1 \text{R}^2 = \text{CH}_2 \\
\text{AMINOFLUORENE}
\end{align*}
\] |

to be an effect of cell damage rather than the cause of chromosome changes. The reduction of glycolysis of tissues receiving moderate doses of x-rays is also negligible. Although some carcinogenic compounds may produce their specific effects by enzyme inhibition, the hypothesis that the results are due to the more direct action on chromosome nucleic acid seems more likely in some cases. Because the carcinogens produce specific damage to chromosomes and chromosomes contain deoxyribosenucleic acid, it is tempting to assume that the carcinogens act by producing abnormalities in the nucleoprotein. Such abnormalities might be induced in different ways.

The most direct way in which damage in the nucleic acid might produce the observed effect in the chromosomes is by cleavage of the nucleic acid chains. Such fission occurs when solutions of deoxyribosenucleic acid are exposed to x-rays (47) or treated with mustards (17). This has been fol-
Purines are possibly anti-carcinogenic agents.

...have been pursued for many years. The noncarcinogenic and noncarcinogenic aromatic compounds has now been investigated further and have found aqueous solutions of nucleic acid and may be functioning in this way. Such precipitation may be a form of cross-linkage by salt formation with a divalent ion. It has been suggested that the effects of beryllium are due to inhibition of cellular phosphatase, although the data on such inhibition (34) would not indicate that this is a likely mechanism.

The biochemical mechanism underlying the action of aromatic carcinogens is still not known, but two lines of investigation may throw light on the subject. The first investigations are concerned with reaction of aromatic compounds with purines and pyrimidines. Weil-Malherbe (48) and Neish (39) had shown that aromatic carcinogens dissolve in solutions of purines such as caffeine and tetramethyluric acid to form complexes. Dr. J. Booth and I have investigated this reaction further and have found that aqueous solutions of nucleic acid as well as simpler purines dissolve polycyclic hydrocarbons and aromatic amines. The amount of hydrocarbon which dissolves increases with increase in concentration of purine and depends upon the nature of the purine. Although these complexes are easily broken down, the results show that the aromatic compounds including carcinogens do combine—if only loosely—with nucleic acid and that this association may change the nucleic acid sufficiently for the chromosome aberrations to result. The fact that caffeine combines more readily with benzpyrene than does nucleic acid means that the caffeine would compete with the nucleic acid for the hydrocarbon and so reduce the effect on the chromosomes. This would account for the observation that the carcinogenicity of benzpyrene was reduced when it was injected in tetramethyluric acid solution (49). Thus, simple purines are possibly anti-carcinogenic agents.

The study of the metabolism of carcinogenic and noncarcinogenic aromatic compounds has now been pursued for many years. The noncarcinogenic hydrocarbons, naphthalene (50, 7), anthracene (14), and phenanthrene (16) are converted to phenols and dihydroxy dihydro derivatives or "diols" in the body. As the diols are easily dehydrated to phenols by treatment with acid, it was thought that the diols were the biological precursors of the phenols. During the last year, however, we have found (15) that the processes of phenol formation and diol formation are separate and independent. If rats are injected with naphthalene for the first time, they convert some of the hydrocarbon into α-naphthol, but after repeated injection they convert increasing amounts of the hydrocarbon into the diol. At the same time that the animals are trained by injection of the hydrocarbon the lethal dose increases, giving clear evidence of adaptation or drug resistance. There are two independent metabolic pathways for naphthalene, and the same alternatives may be possible for more complex carcinogenic compounds. As the metabolism of the hydrocarbons seems to be associated with carcinogenesis, one of these processes, or both, may be the cause of chromosome damage and cancer development. If the oxidative process occurs while the hydrocarbon is held in the nucleic acid of the cell, then the nucleic acid might also be modified at the same time. If the oxidation were of the type leading directly to a phenol, it is likely to involve free radicals, and the free radicals produced in situ would be expected to disrupt the nucleic acid. If the oxidation were analogous to the production of the dihydroxydihydro derivative, then an epoxide is a very probable intermediate product. Such an epoxy derivative might then act like the aliphatic carcinogenic epoxides.

For the carcinogenic action of urethan, still another mechanism must be postulated. Urethan is relatively inert in so far as very large doses must be given to produce any biological action and the action is produced only slowly. In order to produce the same degree of chromosome damage, 10,000 times as much urethan must be used as nitrogen mustard, and the effect takes 24 hours longer to be produced. McKinney (36) showed that urethan is much more effective than methyl carbamate as an inhibitor of the transmethylation of glycocoyamine or nicotinamide by methionine in tissue homogenates. An analogous inhibition of methylation of uracil might lead to deficiency of thymine, an essential constituent of the desoxyriboenucleic acid. In experiments carried out with Dr. Koller, partial neutralization of the chromosome-damaging action of urethan was observed when thymine was administered with the urethan.

There are thus at least five hypothetical processes (see Table 5) by which the changes in nucleic
acid which might lead to cancer can be brought about. Of these the process involving fission of the nucleic acid by free radicals produced by radiation or other means or by mustards seems to be the least uncertain. In this a change resembling that seen in the cell can be seen to take place in the nucleic acid treated in vitro. Of the other suggested three processes, precipitation by salt formation, complex formation with hydrocarbons may be related in that the nucleic acid reacts with a divalent reagent. The mechanism suggested for the action of urethan would be similar to the effect of choline deficiency in causing cancer in animals (23). By choline deficiency a shortage of methyl donors would have a similar final effect as inhibition of the means of using such methyl donors as choline and methionine.

It is clear that there is much work to be done by chemists, biochemists, pharmacologists, and pathologists before we know enough about the processes involved to be able to prevent the occurrence of cancer even from external carcinogens. It should eventually be possible to reduce the incidence of human cancer by application of our knowledge of the nature of carcinogens and the processes by which they operate.

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