A review of the progress made in the study of the carcinogenic properties of chemical compounds, incorporating literature published during the triennial period between the First and Second International Cancer Congresses, was presented to the Second International Congress in Brussels, in September 1936, and was subsequently reprinted with an addendum covering most of the literature published up to the end of 1936 (231). The year 1937 has again witnessed a large output of new work in this field and this is summarised in the present report. For convenience of reference the general classification adopted in the original report has been retained and the references are numbered continuously.

Chemical Relationships

Group I. Cholanthrene Derivatives

An interesting review of the carcinogenic properties of a number of compounds structurally related to cholanthrene was published early in the year by Fieser, Fieser, Hershberg, Newman, Seligman, and Shear (250). In the case of many of the compounds discussed the results of biological testing were necessarily of a preliminary character, and the points of view expressed may eventually require slight modification. Reference is made to some of these compounds under Group III (below). The synthesis of 4:10-dimethylene-1:2-benzanthracene, an isomeride of cholanthrene, has now been described by Fieser and Seligman (256) and the same compound was prepared by Dansi (239, 240) by a different method. This compound, when injected into rats, produces tumours of connective tissue (308); so the interesting fact emerges that of the four known isomeric "ace-1:2-benzanthracenes," the three in which substitution occurs at a meso position of the benzanthracene molecule are all carcinogenic (cholanthrene, 8:9-dimethylene-1:2-benzanthracene, 4:10-dimethylene-1:2-benzanthracene), whereas the fourth, 3:4'-dimethylene-1:2-benzanthracene (228), in which this condition is not fulfilled, is inactive.

Cholanthrene is related to 5:10-dimethylene-1:2-cyclopentenoanthracene, which has been synthesised by Fieser and Hershberg (251), in the same way that 1:2:5:6-dibenzanthracene is related to the slightly more carcinogenic 5:6-cyclopenteno-1:2-benzanthracene. Fieser and Hershberg (253) have recently reported that Shear obtained tumours at the site of injection in 3 of 20 mice within ten months after injection of a lard solution of 5:10-dimethylene-1:2-cyclopentenoanthracene.

The loss of carcinogenic activity following the introduction of a methyl
group into the five-membered ring of methylcholanthrene was mentioned in our previous report (p. 245). For comparison, the isomeric 15:20-dimethylcholanthrene, in which the added methyl group occupies the alternative position in the five-membered ring, has been synthesised (albeit in poor yield) by Bruce and Fieser (221).

A new method of synthesis of methylcholanthrene has been described by Bergmann and Blum-Bergmann (215). The claim of these authors that their method is a non-pyrolytic process is difficult to sustain in view of the fact that the final stage of the synthesis involves heating at 330° for a period of forty-eight hours. The carcinogenic activity of methylcholanthrene is destroyed by conversion into a dinitro derivative, a sulphonic acid derivative, and a compound which is probably an amino derivative (333). Another observation (333), which might have considerable significance, is that methylcholanthrene arises from the selenium dehydrogenation (at 360°) of the complex carbazole derivatives which are formed by condensation of cholestanone and cholestenone with phenylhydrazine. Hitherto the correlation between the carcinogenic compounds and the sterol group of natural products has been dependent upon the chemical transformation of the bile acids, with a suitably disposed hydroxyl group for cyclisation of the bile acid side chain, into the carcinogenic hydrocarbon methylcholanthrene. The new observation that suitable cholesterol derivatives can be converted into methylcholanthrene in a single operation suggests an inherent tendency for the sterol molecule to pass into the cholanthenne ring system, for in this case there are no polar groups or unsaturated centres to facilitate cyclisation, either in the side chain or in the appropriate ring of the nucleus.

Of interest in this connection are the experiments of Polettini (325), who obtained tumours of adenomatous type by repeated application of an oily solution of an irradiated ergosterol preparation to the sacral region of white
mice. The importance of this result is such that independent confirmation is desirable. Roffo's work on the experimental production of epithelial tumours in rats by exposure to ultra-violet light has received additional confirmation by the work of Beard, Boggess and von Haam (211). It is an attractive hypothesis that the action of the light is an indirect one involving chemical changes in some of the constituents of the skin, possibly cholesterol, as postulated by Roffo, who attributes importance to a photoactivation of cholesterol by ultraviolet light. This phenomenon has been examined by Stavely and Bergmann (342) and independently by Mayneord and Roe (302), who point out that the effect is due merely to liberation of hydrogen peroxide, or a similar product, from an unstable peroxide resulting from the photo-oxidation of cholesterol.

The experimental production of tumours in the forestomach of mice by feeding the animals with diets containing (a) 3:4-benzpyrene and (b) cholesterol oleate has been recorded by Waterman (359, 360, 361). (See also p. 70.) The production of cancer in such an organ with a recognised carcinogenic agent such as benzpyrene is interesting, but not surprising. If cholesterol oleate has also a demonstrable carcinogenic action, then important new fields of enquiry will be opened up in which attention must be devoted, inter alia, to the question of preparing cholesterol oleate in a state of chemical purity and to the nature of the impurities likely to be present in such a preparation as that used by Waterman. It yet remains, however, to be established beyond doubt that the tumours obtained with cholesterol oleate were true malignant neoplasms.

As wheat-germ oil is rich in sterols, this is perhaps an appropriate place to refer to the remarkable experiments of Rowntree, Lansbury, and Steinberg (334; see also Dorrance and Ciccone, 247), of which a fuller account has since been published (335). These workers obtained transplantable sarcomas in the abdominal cavity in 70 Wistar rats following oral administration of a crude wheat-germ oil. Similar tumours were also produced in Buffalo and Yale albino rats. These results were all obtained with one particular batch of wheat-germ oil and completely negative results were observed when many other samples of wheat-germ oil were fed to rats. It cannot, therefore, be accepted at present that a potent carcinogenic material can be extracted from purely vegetable sources, and until confirmation of this work is forthcoming with samples of oil of which the manufacture has been carefully supervised it would seem that the most likely interpretation of these results is that extraneous carcinogenic material (possibly mineral oil) may have been accidentally introduced in the process of extraction of the oil.

It is likewise difficult until the experiment has been repeated on a much larger scale to assess the significance of the findings of Burrows and Mayneord (223), who observed spindle-celled sarcomas in 2 out of 20 mice receiving injections of a solution in lard of cholesterol which had been submitted to roentgen irradiation.

**Group II: Benzpyrene**

New variations in Cook and Hewett's synthesis of 3:4-benzpyrene (232), with improvement of yield in the less satisfactory stages, have been described
by Vollmann, Becker, Corell, and Streeck (358), who have also made a study of the quinones formed by oxidation of 3:4-benzyrene, and have thereby cleared up a small discrepancy between the results of Cook and Hewett, and those of Winterstein and Vetter (364). Winterstein, in a review article (363), mentions that he was able, by chromatographic separation, to isolate 2.5 gm. of almost pure 3:4-benzyrene from 3 kg. of a coal-tar fraction boiling above 450°. This yield (about 0.1 per cent) is an indication of the relatively high concentration of benzyrene in such high-boiling tar fractions. In this connection Almasy (197) has described a technique whereby the concentration of the benzyrene in the appropriate zones of adsorption may be readily followed by fluorescence-spectroscopic methods. The fluorescence spectrum of 3:4-benzyrene is also the subject of a publication by Hieger (275), who has described in some detail his original fractionation of coal-tar pitch, leading to the isolation of 3:4-benzyrene.

Several substitution products of 3:4-benzyrene (tribromo-, mononitro-, dinitro-, monoamino-, sulphonato-) have been prepared by Windaus and Rennhak (362) and have been found to be devoid of carcinogenic activity. In view of the fact that carcinogenic activity is often readily destroyed by slight modification of molecular structure, and this has proved to be especially the case with the molecule of 3:4-benzyrene, it is of interest that a high order of carcinogenic activity is shown by three hexacyclic derivatives of 3:4-benzyrene, namely, 1:2:3:4-dibenzpyrene, 7-methyl-1:2:3:4-dibenzpyrene, and 3:4:8:9-dibenzpyrene (208). These were synthesised by Dr. E. Clar; the synthesis of 1:2:3:4-dibenzpyrene was described some years ago (226), and 3:4:8:9-dibenzpyrene was obtained from the corresponding quinone by an elegant new method of zinc dust distillation (E. Clar, Czechoslovakian Patent application, N.P. 3276-36/2). Another hexacyclic derivative of 3:4-benzyrene, anthanthrene, has been reported as inactive by Domagk (244), and our own experiments with this compound have so far given negative results. The preparation of 3:4:8:9-dibenzpyrene has been described by Silverman (341) and also by Vollmann et al. (358).
New relationships of considerable theoretical importance have come to light during the year under review, as the result of further studies of simple homologues of 1:2-benzanthracene. Fieser and his collaborators undertook the preparation of simplified forms of the cholanthrene molecule, with the object of determining the essential structural features required for carcinogenic activity among compounds of this type. In our own laboratory we have been concerned with the completion of a scheme begun several years ago: namely, a comparison of the biological properties of all of the twelve theoretically possible monomethyl derivatives of 1:2-benzanthracene. As a result, two independent investigations, conceived from somewhat different angles, have converged, and, incidentally, two important key compounds, 9-methyl-1:2-benzanthracene and 10-methyl-1:2-benzanthracene were both synthesised independently in both laboratories (255, 318, 233). New 5-alkyl (233) and 10-alkyl (252) derivatives of 1:2-benzanthracene have also been synthesised. Moreover, the synthesis of all twelve monomethyl derivatives of 1:2-benzanthracene has now been completed, new compounds still under test being the 1'- and 8-methyl compounds (J. W. Cook and A. M. Robinson unpublished). One outcome of this work has been to confirm the importance of substitution in position 5 as a factor favourable for the development of carcinogenic properties in benzanthracene derivatives (208). But the striking new fact also emerges that substitution at position 9 or 10 is equally effective and indeed probably more so in the latter case. The Harvard school claim that 10-methyl-1:2-benzanthracene is a carcinogenic agent of the same degree of potency as the cholanthrene group, but this is perhaps an over-statement, for although these workers observed the very rapid development of sarcomas following the injection of 10-methyl-benzanthracene into mice, our own experiments on the application of the hydrocarbon to the skin of mice show that the
CHEMICAL COMPOUNDS AS CARCINOGENIC AGENTS

average latent period before the development of tumours is greater than in the case of the cholanthrene hydrocarbons and approaches that found with hydrocarbons of the dibenzoanthracene type. The synthesis of di-, tetra, and hexahydrides of 10-methyl-1:2-benzanthracene has recently been recorded by Fieser and Hershberg (253).

9-Methyl-1:2-benzanthracene, when injected subcutaneously by Shear, gave one tumour in 20 mice after three and a half months (318). Our specimen of this hydrocarbon has been applied to the skin of 20 mice and has given 3 tumours after eleven and a half months (2 epitheliomas; 1 papilloma; 2 mice still living). The first tumour appeared after 269 days. This represents an order of activity approximately equal to that of 5-methyl-1:2-benzanthracene.

In 5:10-dimethyl-1:2-benzanthracene we have a hydrocarbon which contains all of the carbon atoms of the cholanthrene skeleton, and this hydrocarbon is said to resemble cholanthrene in being a very potent carcinogenic compound (255). Moreover, a similar high order of potency appears to obtain in the case of 5:9-dimethyl-1:2-benzanthracene (318).

It is evident that our earlier generalisation regarding the positions of substitution favourable for carcinogenic activity among the simple derivatives of 1:2-benzanthracene requires extension. The conclusion to be drawn from the facts now available is that positions 10, 5, 9 and 6 are all favourable positions of substitution for the development of carcinogenic properties, and from the examples which have been studied up to the present it seems that the order given is roughly the order of decreasing efficiency. Furthermore, it is evident that if suitable simple substituents are introduced into two of these favourable positions they then reinforce each other and a highly carcinogenic compound results. This applies to the combinations 5:6, 5:9, and 5:10, but whether the same is true for the combination 9:10 (substituents in both meso positions) we cannot say, as suitable examples of this type of compound have not yet been examined. The introduction of two meso alkyl groups into the molecule of 1:2:5:6-dibenzoanthracene has a depressant effect on carcinogenic activity (29, 7), but it is already clear that it would be unwise to place much weight on this in considering the simpler compounds of the 1:2-benzanthracene series. From the standpoint of the above considerations the pre-eminent position of methylcholanthrene as a carcinogenic hydrocarbon is readily appreciated, for it is a derivative of 1:2-benzanthracene containing simple substituents in no less than 3 favourable positions.

A point of major interest which emerges is that the relationships outlined establish a correlation between the two important classes of carcinogenic hydrocarbons dealt with under the headings of groups I and II, namely, the cholanthrenes and the benzpyrenes. For in the former class we have substitution at one of the meso positions (position 10) of the benzanthracene system, and in the latter class we have substitution at the other meso position (position 9). In this connection it would be of interest to synthesise and test 1':9-dimethyl-1:2-benzanthracene, which contains all the carbon atoms of the molecular framework of 3:4-benzpyrene. It is a little doubtful, however, whether such a hydrocarbon could be obtained, as from stereochemical con-
siderations it would appear that it would be impossible to introduce two methyl
groups at the positions shown without distorting at least one of the valency-
directions of an aromatic ring out of the plane of the ring, and such deviation
has rarely, if ever, been observed. R. B. Akin and M. T. Bogert ² (196)
have synthesised by a new method the 9:10-dimethyl-1:2:5:6-dibenzanthracene
formerly obtained by Cook (229), and found to have little, if any, carcino-
genic activity (29), and have extended their method to the synthesis of a
bis-dimethylene ether of a tetrahydroxy derivative of this dimethyl-diben-
zantracene.

Dihydro-compounds of several carcinogenic hydrocarbons, including 5-
methyl-1:2-benzanthracene, 1:2:5:6-dibenzanthracene, and methyl-cholan-
threne, have been prepared by Bachmann (206) and Bachmann and Pence
(209). The melting point of 9:10-dihydro-1:2:5:6-dibenzanthracene is dif-
ferent from that originally attributed to this compound (230), confirming the
spectroscopic evidence (89) that the substance made by Cook’s method con-
tained 1:2:5:6-dibenzanthracene. The absorption spectrum of a specimen of
the dihydro-compound prepared by Bachmann’s method has been examined by
Miss E. Roe (unpublished), who finds that it is in agreement with the struc-
ture suggested. The results of tests for carcinogenic activity with these new
dihydro-compounds have not yet been reported. In these cases negative re-
sults will be of greater significance than positive results, for the production of
tumours might well be due to preliminary dehydrogenation in vivo to the
known carcinogenic hydrocarbons, a process which can be effected by chemi-
cal means with great facility.

Bachmann and Bradbury (207) give a preliminary report of the produc-
tion of sarcomas after six to seven months in four out of five rats which had
received four subcutaneous injections of a solution in sesame oil of the potent
oestrogenic compound, 9:10-dihydroxy-9:10-di-n-propyl-9:10-dihydro-1:2:5:
6-dibenzanthracene. This would thus appear to be a compound having well
marked sarcoma-producing capacity as well as oestrogenic activity. Tumours
were not obtained in an experiment carried out in this Institute, in which this
oestrogenic diol was applied to the skin of 40 mice (208).

Substances in which 1:2:5:6-dibenzanthracene is coupled, through a
carbamido group, with amino acids and proteins have been prepared by
Creech and Franks (237), and used by them in immunological studies. When
linked in this way to glycine, 1:2:5:6-dibenzanthracene was found to retain
its carcinogenic activity.

Lonsdale (299) has examined the magnetic anisotropy of a number of
aromatic polycyclic compounds, including anthracene, phenanthrene, pyrene,
chrysene, and 1:2:5:6-dibenzanthracene. The crystal structure of three car-
cinogenic hydrocarbons (3:4-benzpyrene, methylcholanthrene, and 5:6-cyclo-
penteno-1:2-benzanthracene) (278) and of three isomeric carcinogenic di-
benzcarbazoles (279) has been determined by x-ray methods by Iball.

² It is a misstatement on the part of these authors to say that “this hydrocarbon has been re-
ported by Cook, who . . . found that his product invariably was contaminated with chrysogenic
substances removable only by sulphuric acid.” Although I have shown that 1:2:5:6-dibenzanthra-
cene is colourless when pure I have never at any time suggested that this applies to its 9:10-dimethyl
derivative. On the contrary, I have every reason to believe that a pale yellow colour is inherently
associated with meso-methyl anthracene derivatives.—J. W. Cook.
CHEMICAL COMPOUNDS AS CARCINOGENIC AGENTS

Mayneord and Roe (303) have recorded the ultra-violet absorption spectra of a further series of polycyclic compounds (9:9:10:10-tetramethyl-9:10-dihydro-1:2:5:6-dibenzanthracene, 6-isopropyl-1:2-benzanthracene, cholanthrene, fluorene, 1:2-benzfluorene, 3:4-benzfluorene, 1:2:5:6-dibenzfluorene, 3:4-benzphenanthrene, and 1':2'-naphtho-2:3-fluorene), and have made some preliminary experiments at low temperatures.

Group IV: Water-soluble Carcinogenic Compounds

Increasing interest is being shown in the question of procuring water-soluble compounds having potent cancer-producing properties. New methods of preparing colloidal solutions of carcinogenic hydrocarbons have been described, although this is not, of course, a solution to the problem. Thus, Windaus and Rennhak (362) obtained a 1 per cent aqueous dispersion of 3:4-benzpyrene in sodium cholestenonesulphonate, and O'Hara and Pollia (321) have described the preparation of 0.05 per cent colloidal solutions of dibenzanthracene and methylcholanthrene by a modification of the process of Berenblum (9) whereby a pyridine solution is diluted with water, and the pyridine dialysed out.

The angles from which the major problems are being attacked are mainly (a) the investigation of salts of carboxy derivatives of carcinogenic hydrocarbons, and (b) the search for carcinogenic hydroxy compounds which might be linked to solubilising groups. In the former category reference may be made to several compounds, although results of biological tests are not yet available. Bachmann and Pence (209) have prepared dicarboxylic acids derived from the meso dihydro compounds of methylcholanthrene and 1:2:5:6-dibenzanthracene; Windaus and Rennhak (362) have described a carboxy derivative of 3:4-benzpyrene of unknown orientation, while Fieser, Fieser, et al. (250) remark on liver changes following subcutaneous injection of 3:4-benzpyrene-3':4'-dicarboxylic anhydride (249). It is stated that the disodium and dipotassium salts corresponding with this anhydride appeared to injure the vascular system. Dansi (240) prepared 1:2-benzanthracene-10-carboxylic acid and Cook and de Worms (unpublished) have obtained the corresponding 5-carboxylic acid. It will be noted that methyl groups in corresponding positions to the carboxyl groups in the two last named compounds give rise to carcinogenic substances. Dr. W. E. Bachmann has informed us privately that he has succeeded in effecting a considerable improvement in the yield of the carcinogenic water-soluble sodium 1:2:5:6-dibenzanthracene-9:10-endo-β-succinate, and has extended his technique to the preparation of similar water-soluble derivatives of other carcinogenic hydrocarbons including methylcholanthrene.

Two hydroxy derivatives of potent carcinogenic hydrocarbons were mentioned in our previous report (231). These were the 4'-hydroxy-3:4-benzpyrene of Fieser, Hershberg, and Newman (47) and 3-hydroxy-20-methylcholanthrene. The latter compound was selected for study because the position in which the hydroxyl group has been introduced is the position corresponding with the hydroxyl group of the sterol molecule. The synthesis of this hydroxymethylcholanthrene has now been recorded (234, 257). Pre-
liminary attempts to introduce groups conferring water-solubility were made by Cook and de Worms (234), but it has been found in the meantime that neither 4'-hydroxy-3:4-benzpyrene, nor 3-hydroxy-20-methylcholanthrene, nor their respective methyl ethers produced tumours when applied to the skin of mice (208, see also 254). In these circumstances the conversion of the hydroxy compounds into water-soluble derivatives loses much of its interest. The complete loss of carcinogenic power resulting from the introduction of hydroxyl groups into the molecules of two very potent carcinogenic hydrocarbons is, however, of considerable interest, and in order to determine to what extent this is due to the position of the hydroxyl group rather than its nature, it is desirable to make a comprehensive study of a range of hydroxy derivatives of the benzanthracene hydrocarbons. In this connection a valuable lead has been given by the finding that 3-hydroxy-1:2-benzanthracene has weak carcinogenic properties (preliminary report of experiments of Shear, quoted by Fieser, Hershberg, Long and Newman, 254, who have also prepared some esters of this hydroxy-compound). Fieser and Hershberg (252) have also synthesised 3-hydroxy-10-methyl-1:2-benzanthracene, but have not yet reported on its biological testing.

Group V: Hydrocarbons Not Related to Benzanthracene

If any workers have obtained tumours with s-triphenylbenzene or tetraphenylmethane it is desirable that they should publish their results. In our hands these two compounds have given no tumours after exhaustive tests (208), and we are acquainted with other unsuccessful attempts to confirm the carcinogenic activity claimed for these compounds by Morton, Branch, and Clapp (91).

On the other hand, there are strong indications that a new group of potent carcinogenic hydrocarbons, not related to 1:2-benzanthracene may arise. The parent compound of this group is 3:4-benzphenanthrene, which was shown some years ago to have feeble carcinogenic activity (7). This hydrocarbon has something in common with 1:2-benzanthracene, of course, for both are tetracyclic aromatic hydrocarbons related to phenanthrene. 2-Methyl-3:4-benzphenanthrene, a compound synthesised by C. L. Hewett (273) by a new synthetic method, has proved to be a carcinogenic hydrocarbon of considerable potency, giving tumours after a latent period which is not very much longer than in the case of 3:4-benzpyrene (208). 1:2:3:4-Dibenzphenanthrene, a new pentacyclic hydrocarbon derived from 3:4-benzphenanthrene, has also
been synthesised by Hewett (274). 6,7-Dimethyl-3:4-benzphenanthrene has been synthesised, in poor yield, by Fieser, Fieser and Hershberg (249), and 8-methyl-2-isopropyl-3:4-benzphenanthrene has been synthesised by Adelson and Bogert (195), by a simple process which, however, cannot have general application.

![Chemical structures](image)

Reference should be made in this section to a recent paper by Schiller (338), who found that subcutaneous injections, once or twice weekly for a year, of an aqueous solution (2–3 per cent) of "light green FS" produced in 4 out of 7 rats "fibrosarcomas, chiefly of the spindle-cell type" at the site of injection. Apparently one of these tumours has been carried on by grafting for six years; it seems that during this period the experiment has not been repeated. Tumours were not obtained in mice. Dyes of this class have, of course, no chemical relationship to the other groups of known carcinogenic agents.

Tumours of the stomach were found by Kinosita (280) in rats which had received tetramethyldiaminobenzophenone (Michler's ketone) with their food. This ketone also caused changes in the livers of the animals.

**Group VI: Azo Compounds**

An interesting historical review of carcinogenesis by azo compounds has been published by Shear (340), who also supplemented the work of Yoshida (370) and found that subcutaneous injection of o-aminoazotoluene in pure strain mice gave transplantable liver-cell carcinomas, but no tumours at the site of injection. Nagao (311) found that oral administration of 4'-hydroxy-2:3-azotoluene to rats gave no liver tumours, but gave, to a less extent than 2:3'-azotoluene, tumours of the urinary bladder. The hydroxy compound thus resembles in its action the parent azo compound rather than the corresponding aminoazo compound.

3 From the description given this is probably the dye classified as No. 670 in the Colour Index, having the formula:
<table>
<thead>
<tr>
<th>Compound</th>
<th>Species</th>
<th>Method of Administration</th>
<th>Tumours</th>
<th>Site of Application</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Chemical Structure]</td>
<td>Rat</td>
<td>With food</td>
<td>Liver-cell carcinoma and bile-duct carcinoma</td>
<td>Liver</td>
<td>Sasaki and Yoshida (118) Zylbersac (374); Yoshida (133)</td>
</tr>
<tr>
<td>4'-Amino-2 : 3'-azotoluene (o-Aminoazotoluene)</td>
<td>Rat</td>
<td>Subcutan. With food + glucose</td>
<td>Hepatoma (one in 6 rats)</td>
<td>Urinary Bladder</td>
<td>Yoshida (131) Nishiyama (93)</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>Subcutan. With food</td>
<td>Hepatoma; no cholangioma</td>
<td></td>
<td>Nishiyama (94)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Injection into embryomata</td>
<td>Nil</td>
<td></td>
<td>Nil</td>
</tr>
<tr>
<td>4'-Acetylamino-2 : 3'-azotoluene</td>
<td>Rat</td>
<td>With food</td>
<td>Carcinoma</td>
<td>Carcinoma</td>
<td>Maruya, Harada, quoted by Kinosita (280)</td>
</tr>
<tr>
<td>4'-Diacetylamino-2 : 3'-azotoluene</td>
<td>Rat</td>
<td>With food</td>
<td>Carcinoma</td>
<td>Carcinoma</td>
<td></td>
</tr>
</tbody>
</table>

1 Adenocarcinoma of large intestine in one mouse.
The carcinogenic action of azo compounds has also been reviewed (in English) by Kinosita (280), who contributes in addition a number of new observations made by himself and his collaborators. Numerous derivatives of azobenzene containing substituent amino and methyl groups were tested, but none of these produced liver cancer except o-aminoazotoluene and its mono-acetyl and diacetyl derivatives, and p-dimethylaminoazobenzene. One of the chrysoidines gave considerable proliferation of the bile ducts as seen in the earlier stages of treatment with o-aminoazotoluene or p-dimethylaminoazobenzene. Kinosita has made an extensive investigation of the action of p-di-

\[
\begin{align*}
&\text{o-Aminoazotoluene} \\
&\text{2:3'-Azotoluene} \\
&\text{4'-Hydroxy-2:3'-azotoluene} \\
&p\text{-Dimethylaminoazobenzene (Butter yellow)}
\end{align*}
\]

methy laminoazobenzene (a dyestuff known as "butter-yellow" and formerly used as a food colouring matter). This is isomeric with o-aminoazotoluene, differing from it in the positions occupied by the two methyl groups. This compound was found to produce liver tumours when fed to rats as a 3 per cent solution in olive oil (280). These were hepatomas or cholangiomas, which metastasised and were capable of transplantation. Similar tumours were obtained in mice after a more prolonged latent period, and pathological changes, but not hepatomas, were produced in rabbits fed with the compound. Chickens showed no changes. Hepatic cancers were also observed by Mariya and Tanaka (quoted by Kinosita) in 5 rats out of 16 which lived more than one hundred and fifty days, which had received weekly injections of 0.5 c.c. of a 3 per cent solution of p-dimethylaminoazobenzene in olive oil. Many stomach tumours were found by Kagawa (unpublished experiments cited by Kinosita) in rats receiving p-dimethylaminoazobenzene, and the percentage of these tumours was raised from 6 to 22 when cholic acid was administered simultaneously. Some of these results are summarised in Table I. See also pp. 72-73.

The production of tumours of the bladder by these azo dyes is of interest particularly in connection with the abnormally high incidence of cancer of the bladder among workmen engaged in the manufacture of dyestuffs and their intermediates. On statistical evidence this has been attributed to the naphthylamines, but hitherto no reliable experimental evidence has been adduced in support of this view. Considerable importance, therefore, attaches to the observations of Hueper and Wolfe (276), who in a preliminary note record daily subcutaneous injections of 12 to 15 mg. of commercial β-naphthylamine (98 per cent pure, containing almost 1 per cent of β-dinaphthylamine) in 16
<table>
<thead>
<tr>
<th>Compound</th>
<th>Species</th>
<th>Method of Administration</th>
<th>Liver</th>
<th>Urinary Bladder</th>
<th>Stomach</th>
<th>Site of Application</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:3'-Azotoluene</td>
<td>Rat</td>
<td>With food</td>
<td>Nil</td>
<td></td>
<td></td>
<td></td>
<td>Otsuka and Nagao (178)</td>
</tr>
<tr>
<td>4'-Hydroxy-2:3'-azotoluene</td>
<td>Rat</td>
<td>With food</td>
<td>Nil</td>
<td></td>
<td></td>
<td></td>
<td>Nagao (311)</td>
</tr>
<tr>
<td>Diazoaminobenzene</td>
<td>Mouse</td>
<td>With food</td>
<td></td>
<td></td>
<td>Papilloma of forestomach</td>
<td>Otsuka (97)</td>
<td></td>
</tr>
<tr>
<td>p-Aminoazobenzene</td>
<td>Rat</td>
<td>With food</td>
<td>Nil</td>
<td></td>
<td></td>
<td></td>
<td>Sasaki and Yoshida (118)</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>With food</td>
<td>Nil</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound</td>
<td>Species</td>
<td>Method of Administration</td>
<td>Liver</td>
<td>Urinary Bladder</td>
<td>Stomach</td>
<td>Site of Application</td>
<td>Tumours, especially when cholic acid was also given</td>
</tr>
<tr>
<td>-------------------------------------------------------------------------</td>
<td>---------</td>
<td>--------------------------</td>
<td>---------------------------------------</td>
<td>-----------------</td>
<td>---------</td>
<td>---------------------</td>
<td>---------------------------------------------------</td>
</tr>
<tr>
<td>( \text{p-Dimethylaminoazobenzene (Butter yellow)} )</td>
<td>Rat</td>
<td>With food</td>
<td>Liver-cell carcinoma, cholangioma and sarcoma</td>
<td>Nil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>Subcuta.</td>
<td>Liver-cell carcinoma</td>
<td>Nil</td>
<td></td>
<td></td>
<td>Sarcoma (1 rat)</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>With food</td>
<td>Liver-cell carcinoma</td>
<td>Nil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rabbit</td>
<td>With food</td>
<td>Adenomatous proliferation of bile ducts, no hepatoma</td>
<td>Nil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fowl</td>
<td>With food</td>
<td>Nil</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Dimethyl-(p)-phenylenediamine \textsuperscript{a}} )</td>
<td>Rat</td>
<td>With food</td>
<td>Nil</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{4-Amino-2:2'-azotoluene ((p)-Aminoazotoluene)} )</td>
<td>Rat</td>
<td>With food</td>
<td>Nil</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{4:4'-Tetramethyldiaminobenzophenone \textsuperscript{a}} )</td>
<td>Rat</td>
<td>With food</td>
<td>Tumour</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Form in which "butter yellow" is excreted in the urine. \textsuperscript{b} Not an azo-compound but with somewhat similar structure to azo compounds in the table.
female dogs; after one year 10 of these received also 150 to 450 mg. by mouth. After a period of a year and a half to two years 4 dogs showed "papillary lesions of benign and malignant character" in the urinary bladder. One dog showed cirrhotic and proliferative changes in the liver, and 7 of the 16 showed lactating mammae.

*Group VII: Inorganic Compounds*

Occasional epitheliomas have been observed by Guzman (266) among workmen engaged in the Chile salpetre industry, and he states that he is carrying out experiments on the application of salpetre to the ears of rabbits. So far it has not been established that the tumours described by Guzman are due to any component of the salpetre, which contains 98.9 per cent of sodium nitrate, and in such a climate as that of Chile the possibility that sunlight is an aetiological factor requires consideration.

**BIOLOGICAL CONSIDERATIONS**

1. **Mechanism of Cancer Production by Chemical Compounds**

J. W. Orr (322) applied tar, 1:2:5:6-dibenzanthracene, or 3:4-benzpyrene weekly to the skin of mice subjected to staining *intra vitam* with phenol red. After seven to sixteen weeks, about the time when tumours began to develop, yellow areas appeared after the injection in the pink-stained skin of the painted region. A large proportion of the tumours were situated within or at the edges of these yellow areas. The change from pink to yellow would indicate a more acid reaction, due perhaps to deficient circulation. Rapid growth of the tumours was generally associated with disappearance of the yellow colour, while its persistence was often accompanied by retarded growth or even retrogression of warts.

Morelli (306) observed the histologic changes in the subcutaneous tissues of rats injected with 3:4-benzpyrene or methylcholanthrene in lard, or with lard alone, and killed after thirty to one hundred and twenty days. The first result of the action of these substances is the appearance of minute circumscribed foci of necrosis which produce a characteristic reaction. The tumours are multicentric in origin.

Wolbach (365, 366) studied the histologic changes accompanying the action of 1:2:5:6-dibenzanthracene on connective tissue, of 3:4-benzpyrene on the skin, and of 4'-amino-2:3'-azotoluene on the liver, and concluded that all three were destructive agents, and that their carcinogenic property did not depend upon direct stimulation of cell growth (cf. Haddow, 61).

Rondoni (330) injected a non-carcinogenic (pyrene) and carcinogenic (3:4-benzpyrene) hydrocarbon subcutaneously in rats and considers that the action of the latter is characterised by the appearance at different points, and after varying times, of areas of an atypical granulation tissue from which sarcoma develops.

Hval (277) injected 1:2:5:6-dibenzanthracene in lard subcutaneously in mice and observed the same changes (leucocytic infiltration, dilatation of capillaries) found by Kreyberg in skin after application of tar. Injections of
CHEMICAL COMPOUNDS AS CARCINOGENIC AGENTS

Salvarsan, and of lard alone, were given to controls. Hval's monograph is illustrated by 69 photographs.

Margaret Reed Lewis (293) found that the presence of carcinogenic hydrocarbons (1:2:5:6-dibenzanthracene, 3:4-benzpyrene, and methylcholanthrene) up to 0.1 per cent in cultures of chick-embryo cells did not cause any distinct interference with the growth of the tissue; mitosis continued normally under dull illumination, but on exposure to bright light was inhibited within two to ten minutes. Spindle material disappeared more or less completely, chromosomes spread from the equatorial plate, and the mitosis did not continue; if the spindle did not disappear entirely the mitosis might proceed in some abnormal form. Cultures which had been growing in the presence of these hydrocarbons were much more photosensitive than those to which the compound had just been added. Most of the cultures which continued to proliferate after exposure to light showed many abnormal mitoses (described) similar to those seen after exposure to radium or in malignant tissue, except that increased or tetraploid numbers of chromosomes on one spindle were not observed. No control experiments with non-carcinogenic hydrocarbons are described.

Doniach and Mottram (246) found that the area of skin of white mice to which 3:4-benzpyrene in benzene is applied becomes sensitive to the blue-violet rays of the visible spectrum, the skin becoming red and oedematous, while the mouse becomes ill at ease, scratches the painted region and tries to hide it from the sun. The same authors (310) found that Paramecium can be used as a test object for this photodynamic effect; the organisms placed in a suspension of 3:4-benzpyrene or 1:2:5:6-dibenzanthracene in tap water (not more than 0.01 per cent) are killed and lysed in a few minutes on exposure to light (3500 Å to 4100 Å); neither the hydrocarbon nor the light alone is harmful, but Paramecia kept in the suspension overnight and then washed in tap water remain photosensitive.

Bisceglie and di Grazia (217) observed that colloidal 3:4-benzpyrene in Tyrode solution (0.02 to 0.01 per cent) has no effect upon cultures of chick embryo tissues if these are not exposed to strong light. No tumours were obtained when such cultures, after 8 subcultures in the presence of the hydrocarbon, were inoculated into young fowls (cf. Des Ligneris, 80, 81). Exposure to light (Philips Argenta lamp, 150 to 160 W; wave-lengths not given) causes more or less destruction of the achromatic spindle with consequent derangement of mitosis.

Oesterlin (320) has published some preliminary observations upon trypanocidal agents of the styryl quinoline group, described by Browning, Cohen et al. (21, 22), which are vital stains for the parasites and cause the blepharoplast to fluoresce in ultraviolet light. One member of this group (No. 430) produces sarcoma. Oesterlin infers that there is a close association between three properties of certain chemical compounds, namely (1) carcinogenicity, (2) blue fluorescence, (3) chemotherapeutic action against trypanosomes. (But any such generalisations about trypanocidal action are rendered difficult by the fact that the styryl compounds, while acting on T. brucei, do not act on T. congolense infections.) He supposes, also, that a substance which produces Browning and Gulbransen's phenomenon, "chemotherapeutic interfer-
ence” in the action of a trypanocidal drug in vivo, will also prevent tumour production. He has then found experimentally that a red-fluorescing substance which he prepared, which proved suitable owing to its forming a depot in the tissues like No. 430, viz. 2\((p\text-acetylaminostyryl)\)-6\((p\text-acetylamino-benzoylamino)\) quinoline methochloride, had such annulling effects on both activities of styryl quinoline compounds. Further, the carcinogenic action of 3:4-benzpyrene is likewise neutralised by Oesterlin’s compound.

Dannmeyer and Treplin (238) give a graph showing the residual potential, as measured by their method, of an extract of human serum to which 1:2:5:6-dibenzanthracene was added.

Goldstein (264) states that cultures of a bacillus (Escherichia communior) containing colloidal 1:2:5:6-dibenzanthracene or methylcholanthrene show in the eighth to ninth hour of growth about 50 per cent more organisms than do control cultures or those containing the non-carcinogenic compound phenanthrene.

Pourbaix (327) used styryl 430, on account of its solubility in water, to test the action of a carcinogenic compound upon the metabolism of yeast. This substance in a concentration of 0.01 per cent lessened respiration by 90 per cent (tests by subculture showed that this was not due to death of cells); lessened aerobic and anaerobic glycolysis by similar amounts; and delayed very considerably the induction of fermentation by zymase (this delay can be lessened by the addition of hexose diphosphate). The suggestion is made that the styryl compound has a destructive action on cozymase. De Gaetani (260) found that a watery suspension of 3:4-benzpyrene increases after twenty-four hours the aerobic glycolysis in cultures of chick-embryo heart.

Rondoni and Beltrami (214, 331, 332) applied 3:4-benzpyrene (0.5 per cent in benzene) to the skin of rabbits and found after 8 to 18 applications (1) an increase of lipase (tributyrin esterase) and later of autolytic changes, in the skin treated, and (2) an increase of lipase and of amylase in the blood. The tissues of rabbits painted with benzene only were used as controls.

Hayashi and Tomita (271) have studied the metabolism of liver tissue of the rat during administration of 4′-amino-2:3′-azotoluene with the food. Nakahara and his fellow workers have continued their comparative studies of the chemical composition of induced hepatoma and normal liver tissue (281, 259, 315, 282).

II. Factors Affecting Carcinogenesis and the Growth of Transplanted Tumours

Maisin, Pourbaix and Jonard (300) have continued the earlier work of Maisin (173) upon the inhibitory effect of unsaturated compounds upon the development of cancer. The crude product of the action of a mixture of concentrated and fuming sulphuric acids upon ether has an activating effect; this crude mixture was therefore distilled and the fractions were tested for inhibitory action. Details are given of an experiment with the fraction distilling between 50° and 85°; this was neutralised with KOH, and a single subcutaneous injection (0.1 mg.) given to one of two batches of 80 mice, all of which re-
ceived applications three times weekly of 3:4-benzpyrene, until 20 applications had been made (Table II).

**Table II: Inhibitory Effect of Unsaturated Compounds on Tumour Development**
*(Maisin, Pourbaix and Jonard)*

<table>
<thead>
<tr>
<th>Days</th>
<th>Control mice</th>
<th>Injected mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number alive</td>
<td>Tumours</td>
</tr>
<tr>
<td>80</td>
<td>72</td>
<td>50%</td>
</tr>
<tr>
<td>100</td>
<td>71</td>
<td>65%</td>
</tr>
<tr>
<td>120</td>
<td>66</td>
<td>67%</td>
</tr>
</tbody>
</table>

Thus the effect of a single very small dose persists for some months. The same fraction injected into persons subject to asthma prevents attacks for several months.4

Taschner, Gottlieb and Spritzer (346) applied methylcholanthrene (0.5 per cent in acetone) to the skin of three series of mice: series A and B irradiated one month previously with x-rays; series C, controls. In series A and B the area of skin to be painted was screened with lead during irradiation. Some of the differences observed are shown in Table III.

**Table III: Influence of Irradiation on Carcinogenic Action of Methylcholanthrene**
*(Taschner, Gottlieb, and Spritzer)*

<table>
<thead>
<tr>
<th>Time after first painting</th>
<th>Series A x-rays: 650 r</th>
<th>Series B x-rays: 150 r (? *)</th>
<th>Series C controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 weeks</td>
<td>Cancers 40%</td>
<td>Hyperkeratosis with papillomas 40%; no cancers</td>
<td>Cancers 30%</td>
</tr>
<tr>
<td>18 weeks</td>
<td>Cancers 50%</td>
<td></td>
<td>Cancers 10%</td>
</tr>
<tr>
<td>22 weeks</td>
<td>Cancers 80%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* This dose is given in the text as 15 r and in the table as 150 r.

The larger dose of x-rays thus appears to accelerate the development of tumours, while the lower dose had if anything the opposite effect. (On the action of x-rays in promoting carcinogenesis see Mayneord and Parsons, under "Systemic Effects of Carcinogenic Compounds and of Tumours," below.)

Mottram (309) applied 3:4-benzpyrene in benzene (0.5 per cent) to the skin of mice twice weekly for seventy-five days; this treatment had been found to produce very few warts. About the 60th day gamma radiation was applied to the painted skin at the rate of 4 r per minute in amounts of 1440, 480, or 160 r. In comparison with controls the incidence of tumours was increased by the radiation and roughly in proportion to its amount.

Boyland and Warren (220) gave one or more subcutaneous injections of 1 mg. methylcholanthrene in lard to mice of the Simpson (high-cancer) and CBA (low-cancer) strains; the former produced more tumours in a shorter time (Table IV).

4 With reference to the shock-like action of these preparations, see also Maisin: Compt. rend. Soc. de biol. 126: 89, 1937.
No significant difference was found in the percentage of tumours among normal and castrated or among male and female mice of either strain. A comparison of the results of one and two injections gave no suggestion that the action of this carcinogenic agent depends on sensitisation. Similarly, repeated injections in guinea-pigs of serum of this species incubated with carcinogenic hydrocarbons, or exposure of the uterus to such serum, did not reveal any anaphylactic effects (cf. Landsteiner and Jacobs, 76).

Reimann and Hall (329) found that when \( 1:2:5:6 \)-dibenzanthracene and \( p \)-thiocresol are applied to the skin of mice in various combinations the incidence of cutaneous tumours is reduced by the action of the latter compound.

Shear (339) found that injections of skatole and of hexamethylene tetramine were without effect upon grafted sarcomas of the mouse produced originally by \( 1:2:5:6 \)-dibenzanthracene.

Dobrovolskaia-Zavad skaia and Raynaud (242) record negative results from the treatment by injection of a compound of rhenium (atomic weight 186.3) of mice bearing spindle-cell and polymorph subcutaneous sarcomas induced by \( 1:2:5:6 \)-dibenzanthracene.

Haddow and Russell (268) found that the addition of wheat germ oil to the diet did not affect the development of cancers induced by the application of \( 3:4 \)-benzpyrene to the skin of mice; two graphs give data on the rate of development of 52 such tumours. Cameron and Meltzer (224) found no evidence that a diet very rich in vitamin \( E \) affected the production of epithelioma of the skin of mice by \( 1:2:5:6 \)-dibenzanthracene.

Tomita (350) concludes that the production of liver cancer by \( 4'- \)amino-2:3'-azotoluene in rats takes place more readily in males, and is promoted by injections of lecithin, but the number of rats used in these experiments was very small.

### III. Action of Carcinogenic Compounds in Different Species and Tissues

Moore and Melchionna (305) injected \( 3:4 \)-benzpyrene (5 per cent in lard) into the prostates of white rats, of which 20 were castrated at the time of the injection. Rats over 500 days old at the beginning of the experiment are designated "senile."

The earliest tumour in an intact rat was observed on the 117th day, and in a castrated rat on the 83d day. Six of the intact series were castrated after the establishment by biopsy of the presence of a tumour; thirty-five days later these rats were killed and the tumour showed no difference in structure from the biopsy specimen. Ten intact and 6 castrated rats which received injec-
Intact | 210 | 18 | 13 | 1  
Castrated | 210 | 20 | 13 | 1  
Senile | 352 | 12 | 11 | 2  

<table>
<thead>
<tr>
<th>Duration of experiment in days</th>
<th>Number of rats</th>
<th>Carcinoma of prostate</th>
<th>Sarcoma of prostate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>210</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>Castrated</td>
<td>210</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>Senile</td>
<td>352</td>
<td>12</td>
<td>11</td>
</tr>
</tbody>
</table>

The carcinomas were of squamous-cell type and were formed by metaplasia of columnar epithelium. The sarcomas, which were pleomorphic, originated in the stroma adjacent to benzpyrene cysts. All the sarcomas occurred in glands which also contained carcinoma, but the two forms of growth might not be in the same area. Sarcomas developed later than carcinomas.

Four castrated rats received injections of 3:4-benzpyrene and, after the 86th day, daily injections of "male sex hormone" (oreton, Schering). All showed well developed carcinomas, and three of them sarcomas also. This high incidence of sarcoma, in comparison with the data in Table V, suggests the need for further experiments.

Valade (355, 356) injected methylcholanthrene in various concentrations in arachis (peanut) oil into the lumen of the trachea of 50 rats, and obtained peritracheal sarcomas in 8, and rhabdomyosarcomas of the oesophagus in 5, of these animals. The tumours appeared after periods of from twenty-five days to five months, the average being about three months. No epithelial tumours and no tumours of the lung were observed.

Nakahara and Fujiwara (313) made biweekly intraperitoneal injections of 3:4-benzpyrene (0.2 c.c. of 0.5 per cent in olive oil) in mice, and between the 103d and 157th days obtained polymorphous and spindle-cell sarcomas in 19 out of 27 animals alive at the beginning of that period. Seven of these tumours invaded the liver, and in 15 of the mice there were tumours involving the diaphragm. There was remarkable atrophy of the spleen. Metastasis to the lung was found in one case. One tumour was carried on successfully by transplantation. The same authors (314) injected methylcholanthrene (0.1 c.c. of 0.5 per cent in olive oil) intraperitoneally in 40 mice, and between the 77th and 114th day obtained spindle-cell sarcomas identical in appearance with the 3:4-benzpyrene tumours of the peritoneum in 9 out of 17 mice alive on the 77th day. Ascites was conspicuous in all of these 17 mice.

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5 This appears to be the shortest period recorded in the literature for the induction of a sarcoma. Dr. L. D. Parsons in this Institute obtained a sarcoma in a mouse twenty-two days after eleven subcutaneous injections of 0.25 c.c. of 0.4 per cent sodium-1:2:5:6-dibenzanthracene-9:10-endo-αβ-succinate (unpublished observations).
Domagk (245) gives some data for tumour production by 3:4-benzpyrene and records metastases of epitheliomata in the lungs and kidneys of mice, and peculiar endothelial proliferations in the peribronchial glands of sarcoma-bearing rats.

Kinosita (280) describes and figures proliferation of bile-ducks around a pellet of cholesterol containing 3:4-benzpyrene inserted into the liver of a rat.

Schabad (337) records experiments in which numerous papillomas and carcinomas of the skin, and sebaceous adenomata, appeared in parts remote from the site of application of 1:2:5:6-dibenzanthracene (Table VI). In this experiment, and in another (Table VII) in which the hydrocarbon was injected subcutaneously and intraperitoneally, there was a high incidence of adenomas of the lung, which were found in 3 out of 47 control mice painted with benzene only. The number of mice showing lung tumours increased with the amount of hydrocarbon injected (cf. Lettinga, under "Tumours of the Lung," p. 91, below). The figures for mammary carcinoma are difficult to assess in the absence of more data as to the normal incidence.

**Table VI: Production of Tumours by Application of 1:2:5:6-Dibenzanthracene in Benzene to Skin (Schabad)**

<table>
<thead>
<tr>
<th>Initial number of mice</th>
<th>Alive after 6 months</th>
<th>Local papilloma or carcinoma of skin</th>
<th>Distant papilloma or carcinoma of skin</th>
<th>Multiple sebaceous adenoma</th>
<th>Mammary carcinoma</th>
<th>Primary adenoma of lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>103</td>
<td>58</td>
<td>32</td>
<td>21</td>
<td>15</td>
<td>2</td>
<td>21</td>
</tr>
</tbody>
</table>

**Table VII: Production of Tumours by Injection of 1:2:5:6-Dibenzanthracene in Sunflower Oil (Schabad)**

<table>
<thead>
<tr>
<th>Injections: number, amount, and site</th>
<th>Initial number of mice</th>
<th>Alive after 6 months</th>
<th>Local sarcoma</th>
<th>Sebaceous adenoma</th>
<th>Distant mammary carcinoma</th>
<th>Primary adenoma of lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>One: 2 mg. subcut.</td>
<td>22</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Two: 4 mg. subcut.</td>
<td>36</td>
<td>8</td>
<td>5</td>
<td>2</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>Two: 10 mg. subcut.</td>
<td>51</td>
<td>5</td>
<td>14</td>
<td>2</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Two: 4 mg. intraper.</td>
<td>17</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>126</td>
<td>26</td>
<td>22</td>
<td>4</td>
<td>5</td>
<td>34</td>
</tr>
</tbody>
</table>

* "De nombreux animaux furent sacrifiés à dates précoces aux fins de recherches histologiques."

Waterman (359, 360) gave 3:4-benzpyrene (0.4 per cent in lard) daily to 6 mice by mouth, the animals licking the material off a glass rod. Between the 112th and 336th days 5 of the mice showed squamous-cell carcinoma of the forestomach, and in 4 metastases were present in two or more of the following situations (portal gland, peritoneum, liver, spleen, lung). One of the 5 mice developed a papilloma on the lower lip. The sixth mouse died early in the experiment. A watery colloidal solution (1 in 6,000) of the same hydrocarbon, administered by a pipette to 12 mice, produced no tumours.

Oberling, Guérin and Guérin (319) introduced a crystal of 3:4-benzpyrene
CHEMICAL COMPOUNDS AS CARCINOGENIC AGENTS

under the pia mater, in contact with or within the brain tissue, in 10 rats, and in another rat a drop of solution of the hydrocarbon in “oil” (0.1 per cent) was injected into the brain. Of the 10 rats, 3 lived more than ten months; all of these showed tumours of the hypophysis. At autopsy the crystal was found on the surface of the brain, without reaction around it. The three hypophyses showed adenomata, in one composed of chromophobe cells, in another of spongiocytes, in the third of castration cells. The rats showed also hyperplasia, sometimes adenomatous, of the adrenal medulla, degeneration of the seminal tubules, adenomatous proliferation of the prostate and vesiculae seminales, hyperplasia of the thyroid and parathyroid, and pigmentation of the liver, spleen, and adrenals. The rat which received the oily solution died in the eighth month; the anterior lobe of the pituitary was invaded by a tumour of an epitheliomatous type. The method by which these changes are produced is not at all clear; no hypophyseal tumours were found in rats after application to the skin of 3:4-benzpyrene in benzene for more than a year and a half.

Thomas (349) found that methylcholanthrene injected into rats (a) was more active in producing sarcoma subcutaneously when dissolved in olive oil than when made into a paste with the same, and (b) gave negative results in the anterior chamber of the eye.

The response of an animal to two different carcinogenic agents is described by Bullock, Curtis and Dunning (222), who obtained in a rat a Cysticercus tumour composed almost entirely of plasma cells (the only one of this kind found in more than 6,000 Cysticercus tumours), and four fibrosarcomata at the sites of subcutaneous injection of 2 mg. of 3:4-benzpyrene in paraffin wax. An interesting discussion of various aspects of carcinogenesis has been given by Dunning (248).

Athias (205) records a polymorphous-cell sarcoma in the auricular portion of the heart in one of 33 guinea-pigs which had received intracerebral injections of methylcholanthrene in arachis oil.

Claude (227) gave weekly subcutaneous injections of commercial 1:2:5:6-dibenzanthracene (0.4 per cent in lard) to 18 rabbits, of which 12 died with signs either of acute liver necrosis or of chronic liver degeneration and cirrhosis. No local tumours were produced, although one rabbit received in two and a half years 275 mg. of the compound, which was shown to be active by the production of sarcoma in 70 to 80 per cent of mice (cf. Pourbaix and Denisoff, 184). These results were confirmed with a purified sample of the hydrocarbon. None of the compound could be detected in the liver, bile, or ascitic fluid (cf. Chalmers and Peacock, 147). The livers showed acute necrosis of the central zone of the lobules when injections were frequent, and cirrhosis with interstitial fibrosis and proliferation of bile-ducts when time was allowed between injections for recovery. Degenerative changes and haemorrhage in the spleen and lymph nodes are described.

Lambret, Driessens and Cornillot (288) injected 3:4-benzpyrene in lard into 15 rabbits subcutaneously, twice monthly, in some cases for more than two years, and obtained no tumours, although the same preparation produced sarcomas in rats.

Berthelot and Amouex (216) describe cellular proliferations produced in
sunflower seedlings by application of a suspension of 3:4-benzpyrene in vaseline to the stem.

Boyland and Brues (219 and unpublished observations) have found that 3:4:5:6-dibenzcarbazole produces (a) epithelioma of the skin in mice; (b) sarcoma of the subcutaneous tissue in mice and in rats; (c) hypertrophic changes in the bile-ducts in 80 per cent of the mice in which the compound was applied to the skin, ranging from a simple localised increase in the number of ducts in the portal areas to a diffuse growth of metaplastic biliary tissue throughout the lobules. Most of the mice which lived more than 200 days showed nodules of altered liver cells resembling hepatoma. No metastases from the liver lesions were found. These changes in the liver, and an epithelioma, can be produced in the same mouse. The authors estimated that these changes could be brought about by amounts of 0.8 mg. (bile-duct hypertrophy) or 6 mg. (hepatoma-like growth), quantities considerably smaller than those of 4'-amino-2:3'-azotoluene which appear to be necessary to produce similar changes. In two female rats in which sarcoma was produced by subcutaneous injection, the adjacent portions of the mammary gland showed a condition which was, in so far as can be judged from microscopic appearances, undoubtedly carcinomatous. No growth of carcinoma, however, was obtained in grafts. Spontaneous mammary cancer is extremely rare in the rat.

The 1:2:5:6-dibenzcarbazole is less carcinogenic to the skin of the mouse than is the 3:4:5:6-compound, and the 1:2:7:8- is much less active again. No tumours of the skin or liver were produced by αα- or ββ-dinaphthylamine.

Shear (340) injected 4'-amino-2:3'-azotoluene (m.p. 99–101°) subcutaneously in the solid state (10 mg. given six times at intervals of two months) into 23 mice. No macroscopic changes in the liver were found in 7 mice dying in the first nine months. The first liver tumour was obtained in the eleventh month. During the fourteenth month all the remaining mice, numbering 7, were killed, and all showed multiple tumours of the liver. Altogether liver-cell carcinomas, almost always multiple, were obtained in 13 mice; one of these had an adenocarcinoma of the large intestine also. Two of the liver tumours were successfully transplanted subcutaneously, and one was growing with increasing vigour in the tenth generation. The characteristic appearance of liver cells was maintained in these grafts. Similar grafts of normal liver ("this was done because the great regenerative capacity of mouse liver tissue might possibly have been responsible for the successful growth of the implants of the liver tumours") were negative. No tumours appeared at the site of injection "although the compound was present in large amount for over a year in the subcutaneous tissue and was found in appreciable amount at the site of injection at autopsy."

(This paper by Shear contains also valuable summaries of the literature of three subjects: (a) the numerous investigations which followed upon Bernard Fischer's discovery in 1906 of the effects of scarlet red; (b) the results of Japanese workers with azo-compounds subsequent upon Yoshida's first paper on o-aminoazotoluene in 1931; (c) the geographical distribution of primary cancer of the liver in man; to this last bibliography the papers of Bonne (Am. J. Cancer 25: 811, 1935, and 30: 435, 1937) require to be added.)

Zylberszac (374) has confirmed Yoshida's results by obtaining carcinomas
of the liver, with metastases in the lung, in rats receiving 4'-amino-2:3'-azo-toluene in arachis oil with the food.

Nagao (311) gave 4'-hydroxy-2:3'-azotoluene by the mouth (2 mg. and later 3 mg. in 1 gm. food) to 20 rats, and in 2 of these, after 182 and 208 days, found a localised papilloma of the bladder much smaller than those produced by 2:3'-azotoluene, and showing no penetration into the submucosa. The bladders of some of the other rats showed thickening of the epithelium. The livers showed no changes.

With reference to carcinogenic azo compounds see also p. 59 and Table I.

In section VIII, on Tumours of the Lung, below, reference is made to the remarkable observation of Andervont and Lorenz (202) that tumours of the liver appeared in C₃H mice receiving 1:2:5:6-dibenzanthracene subcutaneously or intravenously. In describing one of these experiments, in which the hydrocarbon (0.6 mg.) was injected subcutaneously in two series of 20 mice each, in one series in dog-serum and in the other in lard, the authors say that when the surviving mice were killed to end the experiment "none had developed a tumor at the site of injection. . . . Of the 14 serum-injected mice, 2 had multiple liver growths and 4 had large single growths; and of the 3 lard-injected mice, 2 had huge masses within their livers. The liver growths ranged in size from 6 mm. in diameter to large pedunculated and lobulated growths measuring 22 by 15 by 15 mm. . . . Stained preparations showed that all were hepatomas. Histological studies of the hepatomas are still in progress." (Cf. Ilfeld (66), who produced tumours of the liver in 2 mice of the same strain, C₃H, by direct introduction of the same hydrocarbon into the liver.)

IV. Systemic Effects of Carcinogenic Compounds and of Tumours

Mayneord and Parsons (301) subjected 4 series of mice to treatment as follows: Series 1, subcutaneous injection of sodium 1:2:5:6-dibenzanthracene-9:10-endo-αβ-succinate; Series 2, the same, with x-radiation (usually 550 r) of the whole body; Series 3, subcutaneous injection of normal saline; Series 4, the same, with x-radiation as in Series 2. The results were as follows:

1. All x-irradiated mice showed a fall in the absolute numbers of lymphocytes immediately after irradiation.

2. Of the mice receiving the carcinogenic compound, those irradiated (Series 2) developed sarcoma at an earlier date and in greater numbers than the non-irradiated (Series 1).

In view of Murphy's work upon the lymphocyte as a defensive agent against malignant growth, it is possible that the diminution of lymphocytes following irradiation facilitates some stage in the development of the tumour.

3. In Series 1 and 2 the mice which produced sarcomas showed a series of changes in the blood, namely: (a) an increase in eosinophil cells, generally in the last half of the latent period before tumour formation; (b) about the time of appearance of the tumour, a rapid increase in the total number of leucocytes. This last is due to an increase in the number of polymorphs, for the lymphocytes at this time show an absolute decrease. At the same time many immature forms of leucocytes appear in the blood, and the average area of the polymorph cells increases by about 75 per cent.
These changes were not found in those mice of Series 1 and 2 which did not develop tumours, nor in the mice in Series 3 and 4, which received saline only and produced no tumours. Hence these changes in the numbers and types of leucocytes appear to be associated in some way with the processes which give rise to these tumours, and the same changes have now been observed in stock mice and in CBA mice bearing grafts of this tumour; in stock mice bearing grafts of sarcoma L.M. 4 produced by 1:2:5:6-dibenzanthracene itself; in dilute brown and in CBA mice bearing sarcoma 37, and in a Bagg albino mouse, sent for examination by Dr. Gorer, bearing a grafted mammary tumour of this strain which had undergone a sarcomatous change.

De Gaetani and Lanza (261, 262) studied the cells of the blood and of various organs in more than 100 rats and mice receiving 3:4-benzpyrene, either (a) in solution in benzene on the skin, or (b) in oil subcutaneously or intraperitoneally. Before appearance of the tumour there was anaemia, more marked following the subcutaneous or intraperitoneal injections, often with an increase of lymphocytes up to 75 per cent; the spleen generally was of normal size. In some animals, especially rats, the development of a tumour does not cause any further changes in the blood; in others, most frequently rats, anaemia progresses with the appearance of the tumour (e.g. to 2,500,000 red cells, with erythroblasts), and the spleen shows considerable enlargement with myeloid areas containing megakaryocytes.

The following changes are nearly always present in mice, but often absent in rats, even when the tumour is very large. When the tumour appears there is a sudden fall in lymphocytes (to 5 to 10 per cent) with a sudden rise in polymorphs (up to 90 per cent), while the leucocyte count rises to 35,000–40,000. Young granulocytic cells, metamyelocytes, myelocytes, and Türck cells and plasma cells may appear. The spleen is not enlarged, or only slightly so, and shows myeloid metaplasia with megakaryocytes. Two mice which are thought to have developed a definite myeloid leukaemia are described in detail.

Myeloid changes in the spleen were observed by Kinosita (280) and by Mizuta-Maruya (quoted by Kinosita) in mice treated with carcinogenic hydrocarbons, and in rats, and more abundantly in mice, receiving p-dimethylaminobenzene with the food.

Picard (324) found the spleen to be reduced to as little as one-tenth of the normal weight in mice receiving injections (subcutaneous or intraperitoneal) of colloidal 3:4-benzpyrene in water. There was atrophy also of lymph nodes, the thymus, and the bone marrow. Microscopically the spleen showed diminution of all elements of the pulp, and sclerosis, as in the senile spleen of man. Intravenous injections of 3:4-benzpyrene in rabbits, though causing no noteworthy changes in the blood even when continued over a year, often produced a similar condition of the spleen.

Margaret Reed Lewis (294) has published a continuation of her study with Lichtenstein (165, 166) which showed that the resistance of mice of a pure strain to the transplantation of sarcomas induced in mice of another strain by 1:2:5:6-dibenzanthracene could be broken down by repeated inoculation of the tumour. The varying resistance to such transplants suggested an examination of the blood cells and spleen of these and other mice.

"Eighty tumors that were induced in mice of pure inbred strains by means
of a single injection of 0.2 mg. of 1:2:5:6-dibenzanthracene [elsewhere in the paper the amount is given as 0.8 mg.] were 100 per cent transplantable into mice of the same strain but, with two exceptions, were not transplantable in mice of a different pure inbred strain."

Normal young mice of the strains used (A, BA, \(C_1\), \(C_2\)) were found to have a leucocyte count of 8,000 to 16,000 with 20 to 40 per cent polymorphs. Mice inoculated with straight-line tumours (i.e. tumours induced and carried on in the same strain) showed a degree of neutrophil leucocytosis which varied with, and was characteristic of, the particular tumour used, but was never as great as that seen in repeatedly inoculated mice that developed tumours from mice of a different strain, or in those bearing one of the tumours that were transplantable into more than one strain. Mice bearing tumours from another strain developed a myeloid hyperplasia with enlargement of the spleen to 0.4 to 0.8 gm. (normal 0.09 to 0.13 gm.). The blood of mice grafted with these tumours induced by 1:2:5:6-dibenzanzanthracene showed a neutrophil leucocytosis "concurrent with the growth of the graft, but remained unchanged if it failed to grow." If "the graft grew and later regressed, the blood became altered in proportion to the amount of tumor growth, then returned to normal after the tumor regressed."

The chief change in the blood of mice which developed a myeloid hyperplasia consisted in the presence of many large polymorphs "containing more than the usual amount of chromatin distributed into many lobes; in a few instances some myelocytes, myeloblasts, and even some cells undergoing mitotic division were present." These changes did not follow the single injection of 1:2:5:6-dibenzanthracene used to induce a tumour, although a considerable accumulation of polymorphs and mononuclears occurred at the site of injection; the blood showed little change until after a tumour had formed. The blood picture of mice bearing a primary tumour varied considerably, "but on the whole it was similar to the blood picture that became characteristic of that particular tumor after its serial transplantation in healthy young mice. The alteration of the blood was shown most clearly after the tumor had reached somewhat more than medium size; it became obscured by the ill-health of the host when the tumor became open or necrotic." This last observation is of especial value in showing that the polymorph leucocytosis is not due to infection of these tumours or of grafts from them. M. R. Lewis remarks that "it was necessary to keep all tumors free from bacteria for tissue culture purposes."

In an elaborate experiment, which is described in detail, it was shown that those primary tumours which produce the greatest degree of neutrophilia and myeloid change in the spleen in the original host are those which prove to be most capable of growth on repeated inoculation into mice of other strains.

Dr. J. S. Potter, who examined some of the material, also observed a polymorph leucocytosis which might reach 96 per cent, and great numbers of 6 to 8 lobed polymorphs. "The many lobed polymorphonuclears dominating the blood picture are atypical in that there are a tremendous number of them." Dr. Potter reported further that in tissue taken from mice whose resistance to the growth of an alien strain had been broken down, there was a distinct myeloid change in the spleens.
This study of the 80 tumours mentioned above was extended (M. R. Lewis, 295) to include the blood and adrenals of (a) 35 mice of pure strains (BA and C 57) bearing primary dibenzanthracene tumours, and of (b) the hosts of each of these tumours as it was transplanted through several serial passages. The neutrophilia was less in (a) than in (b), but the blood pictures were on the whole characteristic of the individual tumours. The spleens of mice bearing the primary tumours were small or only moderately enlarged, while those of mice bearing grafts enlarged as the tumours grew, some attaining a weight of 0.4 to 0.8 gm.

Eight of these 35 tumours were of the myeloid-stimulating type, and myeloid infiltration of the adrenals (myeloblasts, of which some were in mitosis, myelocytes, and polymorphs) was found both in the mice bearing primary tumours and those bearing grafts from these. Similar changes in the adrenals were found in the mice described above, bearing tumours causing considerable neutrophilia and myeloid change in the spleen, marrow and lymph nodes, and in mice bearing any one of the 10 tumours which had become adapted to grow in other strains. Injection of dead tumour cells or extracts of tumour did not bring about neutrophilia or myeloid change.

Takizawa (345) describes enlargement of the spleen, up to four times the weight of controls, in rats bearing transplants of a hepatoma produced by 4'-amino-2:3'-azotoluene which has reached the 40th grafted generation. The spleen enlarges more or less in proportion to the growth of the tumour, and diminishes in size if the tumour regresses. The spleen shows numerous areas of myeloblasts, myelocytes, polymorphs, and giant cells, and the development of these areas is proportional to that of the tumour. The giant cells appear to develop from the megakaryocytes of the normal spleen. Myeloid areas appear also in the liver and lymph nodes. In the blood the polymorphs become more numerous than the lymphocytes. The changes described above were not found in rats with primary hepatoma nor in those bearing grafted sarcoma.

Taschner, Spritzer, Gottlieb and Lazar (347) applied 3:4-benzpyrene (0.5 per cent in acetone) to the skin of mice and observed a diminution of leucocytes (e.g. from 11,600 to 4,600), and especially of lymphocytes, in the blood within a few days, while acetone alone caused an increase of lymphocytes. The guinea-pig, which is cancer resistant, reacts in the same way. 1:2:5:6-Dibenzanthracene and methylcholanthrene had a similar action when applied to the skin; the latter compound sometimes caused a leucopenia when given by mouth, but not when injected intraperitoneally in olive oil. 3:4-Benzpyrene when given orally to mice produced less leucopenia than when applied to the skin; when give by mouth to man it produced a fall in the leucocyte count, e.g. from 12,000 to 7,500, and in a case of leucaemia from 13,000 to 7,800.

J. M. Twort and C. C. Twort (354) give some data (Table VIII) for female mice after application of 1:2:5:6-dibenzanthracene (? in what solvent) to the skin. "All the animals surviving to the end of the experiment were killed, i.e. at the fortieth week . . . The differences in the benign tumour and hyperplastic groups are insignificant." The growth of malignant tumours was accompanied by enlargement of the spleen.

Nakano (quoted by Kinosita 280) obtained spindle-cell sarcoma, fibrosarcoma, myxosarcoma, and reticulosarcoma in mice after subcutaneous injec-
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Table VIII: Comparison of the Organs of Tumour and Tumourless Animals Painted with Dibenzanthracene (Twort and Twort)

<table>
<thead>
<tr>
<th>Skin</th>
<th>Number of mice</th>
<th>Body weight (gm.)</th>
<th>Pituitary area (sq. mm.)</th>
<th>Spleen (mg.)</th>
<th>Brain (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant tumour</td>
<td>101</td>
<td>22.9</td>
<td>1.77</td>
<td>407</td>
<td>438</td>
</tr>
<tr>
<td>Benign tumour</td>
<td>134</td>
<td>24.5</td>
<td>1.83</td>
<td>250</td>
<td>439</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>76</td>
<td>24.7</td>
<td>1.82</td>
<td>248</td>
<td>431</td>
</tr>
</tbody>
</table>

Tuchmann and Demay (353; cf. 192, 193) injected 1 mg. 3:4-benzpyrene on two occasions into one testis of rats; ninety to one hundred days later they found atrophy of both testicles; reduction of the vesiculae seminales; some enlargement of the prostate, and active growth of the mamma. Spermatogenesis is entirely arrested, while the interstitial tissue appears unaltered, in the injected testicle. In the other testis, there is very little spermatogenesis. The prostate shows epithelial desquamation and leucocytic infiltration. The pituitary is enlarged and shows an increase of eosinophils, diminution of chromatophores, and congestion.

Gottlieb, Plonskier, Spritzer, and Taschner (265) describe experiments of which the indications appear to be as follows: If a sarcoma be inoculated into a mouse A, to the skin of which a carcinogenic hydrocarbon has been applied in acetone in the ordinary way, the growth of the sarcoma in A is more or less inhibited (cf. Lees, 291), but if the tissues (blood, liver, or spleen) from A are then inoculated into a mouse B, a sarcoma (resembling the tumour in A) is much more likely to develop in B at the site of this inoculation than would be the case if A had not been treated with a carcinogenic compound. The same authors (348) describe experiments which suggest that the Jensen rat sarcoma persists longer and grows larger in mice if these have been treated with methylcholanthrene (to the skin or peritoneum).

Halberstaedter and Back (270) found that oestrus ceased in 21 out of 23 rats inoculated with a sarcoma produced by 3:4-benzpyrene, which had been maintained by grafts for two years. Oestrus reappeared (a) in a rat in which the tumour regressed and disappeared, and (b) in 4 rats from which the grafts were excised; and in one of these latter 4, oestrus ceased again after reinoculation with the tumour. The authors refer to other agents (e.g. thallium acetate) which have a similar action.

Tuchmann (351) states that the enlargement (to threefold) of the pituitary of the guinea-pig after castration depends largely upon increase of the eosinophils. Injections of oestrone, or of testosterone in larger doses, lessen this eosinophilia. "Le benzopyrene diminue également l'eosinophilie du castrat, bien que d'une façon moins nette et se distingue en outre des hormones citées par son action irritante sur les capillaires." For control observations on injections of cholesterol, and of olive oil, into the testes see Tuchmann and Demay (352).
Natoli (316) injected intravenously in rabbits suspensions of sarcomata of the rat induced by 3:4-benzpyrene. Sera were obtained which reacted with alcoholic extracts of these tumours, and of other rat tumours (Jensen, Walker) but not with those of human tumours, and they reacted to a variable extent with similar extracts of normal organs of the rat.

Vlès and Ugo (357) describe blue-violet fluorescence of the whole skin of the mouse after introduction into the peritoneal cavity of 3:4-benzpyrene in a glass tube sealed with coagulated albumen.

Supniewski and Hano (343) injected cats and rabbits intravenously with a watery lecithin emulsion of 3:4-benzpyrene (0.25 per cent), and observed very little action upon the arterial pressure, the heart, the volume of organs, respiration, metabolism, blood sugar and blood lipoids, and temperature. There was a considerable diminution in the secretion of bile, but no change in the amount of urine. The white corpuscles and percentage of neutrophils were diminished, while the red corpuscles and haemoglobin were not altered. Similar experiments with methylcholanthrene (344) gave results of the same kind.

V. Action of Carcinogenic Compounds on Tumours

Haddow and Robinson (267), continuing the earlier work of Haddow (61), tested the effect of a large number of hydrocarbons, given intraperitoneally, upon sarcomas of the rat, and chiefly upon the Walker and Jensen tumours. A strongly inhibitory effect was shown by all the definitely carcinogenic compounds used (1:2:5:6-dibenzanthracene, sodium-1:2:5:6-dibenzanthracene-9:10-endo-2β-succinate, 5:6-cyclopenteno-1:2-benzanthracene, 3:4-benzpyrene, and 3:4:5:6-dibenzacridine). The action is not strictly proportional to the carcinogenic power; thus 1:2:5:6-dibenzanthracene is more inhibitory than 3:4-benzpyrene. This inhibitory power was shown also to a variable but sometimes very considerable extent by some compounds which are either feebly carcinogenic or non-carcinogenic (chrysene, 1:2-benzanthracene, 4-6- and 7-methyl-1:2-benzanthracene). Any very close coincidence of inhibitory and cancer-producing power is hardly to be expected, since there is no single conclusive proof of carcinogenicity; the production of epithelioma of the skin in the mouse is an arbitrary test depending upon the response of a single tissue in a single species, and this may not give a complete picture of the property in question.

On the other hand, a considerable series of related non-carcinogenic compounds when tested under the same conditions gave no inhibition of tumour growth (anthracene, phenanthrene, dodecahydro-1:2-benzanthracene, pyrene, fluoranthene, triphenylene, dehydronorcholene, perylene, 1:9-benzanthrone and diphenylene oxide).

During these experiments it was observed that treated animals grew less rapidly than the controls, and this suggested that the reduction in rate of tumour growth was probably not a tumour-specific effect but was the result of an inhibition of growth affecting the body as a whole. Further investigations, by Haddow, Scott and Scott (269), showed that a single intraperitoneal injection of a carcinogenic compound (1:2:5:6-dibenzanthracene, 3:4-benzpyrene, 1:2:5:6-dibenzacridine) in sesame oil produces in young rats an immediate
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retardation of growth which is extremely prolonged if not permanent, as no recovery was observed in any of the experiments. Examination by Dr. Hieger of the fluorescence spectra of extracts of the bodies of these rats indicated that the hydrocarbon might no longer be detectable at a time when the inhibitory effect was still maintained. This slowing of growth (in the rat) is not exactly parallel to the carcinogenic power (on the skin of the mouse) since the order of influence upon growth is $1:2:5:6$-dibenzacridine $> 1:2:5:6$-dibenzanthracene $> 3:4$-benzpyrene, which is the reverse of the carcinogenic order. The non-carcinogenic compounds pyrene, fluoranthene and dodecahydro-$1:2$-benzanthracene when tested in the same way did not affect growth. Chrysene, which is of doubtful carcinogenic power, in a dose of $30$ mg. retards growth rather less than does $3$ mg. of $1:2:5:6$-dibenzanthracene. Several agents (x-rays, lead nitrate, colchicine) produced a temporary interference with growth followed by recovery to the normal rate with or without a compensatory rise above this level, but they did not, in the doses employed, have any long persistent inhibitory effect like that of the carcinogenic hydrocarbons. Phenobarbitone (luminal) showed no inhibitory action.

Lees (289, 290, 291) carried out a series of investigations suggested by those of Haddow and his fellow workers, and similar in technique. Lees found that single injections of $1:2:5:6$-dibenzanthracene ($5$, $10$, or $20$ mg.) produced a persistent interference with body growth of young rats of a type not seen with other toxic agents, as thallium acetate and bismuth salicylate, which cause an immediate but only transient inhibition even when given in doses up to $80$ per cent of the mean lethal dose. Mercury in oil produced an interference with growth for at least thirty days, which was perhaps due to the persistence of the metal in the tissues, as shown by x-rays. Twenty-one out of $48$ of the animals injected intraperitoneally with $1:2:5:6$-dibenzanthracene died within sixty-four days. Hydrocarbons which are non-carcinogenic or feebly carcinogenic (anthracene, phenanthrene, $1:2$-benzanthracene) produced an immediate but transient inhibition of growth. Amounts of $1:2:5:6$-dibenzanthracene which produce a very considerable reduction of growth rate do not lower the absorption of oxygen nor the consumption of food; hence the effect on growth cannot be attributed to loss of appetite or to diminution of metabolism. The inhibitory effect on growth was not altered by the administration of thyroid extract or of insulin, and rats under the influence of dibenzanthracene responded normally to the growth hormone of the anterior pituitary. Amounts of $1:2:5:6$-dibenzanthracene ($20$ mg.) which almost completely arrest the growth of young rats cause no lasting alteration in the oestrus cycle of adults.

Lees (291) confirmed the observations of Haddow as to the effect of $1:2:5:6$-dibenzanthracene upon the growth of the Jensen sarcoma and compared the effects of the hydrocarbon (intraperitoneal) upon the growth of the body and of the tumour in the same animal, in $4$ series of $10$ rats each (Table IX). One might say that, during the period of the experiment, the production of tumour was about double that of other tissues ($7.4:3.9$) under the influence of the hydrocarbon.

Rats whose growth had been considerably retarded by injection of dibenzanthracene five months earlier (e.g. body weight $132$ gm. as against $158$ gm. in
controls) were found to have the normal susceptibility to inoculation with Jensen sarcoma. The tumour-inhibiting action of dibenzanthracene was not affected by injection of oestradiol nor by castration in either sex.

Morelli and Dansi (307) injected (apparently subcutaneously and intraperitoneally) into rats bearing grafted tumours a series of carcinogenic and other compounds. The property of inhibiting the growth of grafts was strongest in 1:2:5:6-dibenzanthracene and 3:4-benzpyrene and more or less marked in 1:2-benzanthracene, while pyrene, naphthalene and cholesterol had no such power, and anthracene gave varying, but usually negative, results. The inhibition was greatest if the injection was made simultaneously with the graft and diminished in proportion to the time elapsing between the grafting and injection.

Pybus and Miller (328) injected 1:2:5:6-dibenzanthracene in water, gum saline, or olive oil intraperitoneally in mice, chiefly of the Simpson strain, bearing spontaneous mammary tumours. Among 60 mice repeated injections (total dose 10 mg.) were followed by partial or complete regression in 8 instances, and in 3 of these the tumour did not reappear before death. Single doses of 10 or 15 mg., or two or more doses of 7.5 mg. or 5 mg., caused a temporary regression having its maximum about the third or fourth week in 26 out of 61 animals, but growth was resumed later; there was a high mortality from peritoneal lesions. Treatment in cases of sarcoma and leukaemia, and administration of the hydrocarbon by mouth, were without effect.

K. H. Bauer (210) treated 22 human cases of cancer by injecting into the tumour 0.5 to 1 c.c. of a 0.5 per cent solution in ether of 3:4-benzpyrene, or in the case of quite superficial growths by allowing this solution to drip upon the surface; both these forms of treatment may be given repeatedly. Seven of these cases have remained healed for over two years; coloured figures of the successive stages of healing in five of these are given.

Pollia (326) made intraperitoneal injections of lard, acacia, and sesame oil, and of retene and 1:2:5:6-dibenzanthracene in sesame oil or in acacia, in rats bearing a grafted sarcoma. The growth of the tumour was inhibited in every instance by 1:2:5:6-dibenzanthracene, and to a less extent by retene also. “All animals treated intraperitoneally showed considerable damage to the viscera. . . . When distilled sesame oil caused definite peritoneal irritation, it inhibited tumour growth. . . .” Pollia emphasises the occurrence of “pathologic effects in the abdomen, deposits of the hydrocarbon on the abdominal organs, death of embryo, fluid in the peritoneal cavity, adhesions and swelling of the liver,” and evidently considers that these effects may influence the growth of tumours. The 1 per cent solution of 1:2:5:6-dibenzanthracene, used in many of the experiments, deposited a large part of the hydrocarbon as

<table>
<thead>
<tr>
<th>Tumour + dibenzanthracene</th>
<th>Animals</th>
<th>Tumours</th>
<th>Animals less tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3</td>
<td></td>
<td>7.4</td>
<td>− 3.1</td>
</tr>
<tr>
<td>46.8</td>
<td></td>
<td>25.5</td>
<td>+21.3</td>
</tr>
<tr>
<td>3.9</td>
<td></td>
<td>−</td>
<td>+ 3.9</td>
</tr>
<tr>
<td>37.5</td>
<td></td>
<td>−</td>
<td>+37.5</td>
</tr>
</tbody>
</table>
crystals on cooling to body temperature, and might thus cause irritation. [Injury to the peritoneum cannot account for the results described by Bauer (see above) nor for those of Morelli (306), who used subcutaneous injections. The evidence available at present indicates that retene is not carcinogenic (Kennaway: Biochem. J. 24: 497, 1930.)]

VI. Oestrogens in Relation to Tumours

Bonser, Stickland and Connal (218) gave (a) subcutaneous injections of oestrone benzoate (500 international units weekly in olive oil) or (b) applications of oestrone benzoate (0.01 per cent in benzene) twice weekly to the skin, together with fortnightly injections of prolactin, to nulliparous female mice of a strain derived from Little's black agoutis (CBA), which "has been under observation in the laboratory for four years, during which time no spontaneous breast tumours have been observed in either sex." Mammary carcinomas appeared in 3 out of 22 mice treated subcutaneously and in 2 of 10 mice given applications to the skin, after from fifty-five to one hundred and three weeks. There was no relation between the size of the pituitary and the development of tumours, and the suprarenals, examined in about one-half of the mice, were normal. Thus 5 tumours were obtained from 32 nulliparous mice, while 41 untreated females which had borne one or more litters and reached tumour age gave no tumours; hence it seems clear that the treatment determined the development of the tumours. "In male mice of the CBA strain it was found that prolonged treatment with oestrone benzoate caused great growth and development of breast tissue but that this was not associated with the development of malignant disease." Strong obtained 4 spontaneous mammary cancers in females in a line of CBA selected for longevity.

Zuckerman (373) found that squamous metaplasia occurs in the cylindrical epithelium of the cervix uteri of Rhesus monkeys receiving daily injections of oestrone for several months. The squamous cells form masses which invade the myometrium, but they are contained within a distinct basal membrane, and no malignant growths have been obtained. The down-growths regress when oestrone is withheld.

Dessau (241) observed in the cervix of guinea-pigs, after injections of oestrone for ninety days, down-growth of epithelium which did not penetrate the muscular coat.

Champy (225) describes and figures tubular adenomata of the ovary in rats and guinea-pigs receiving injections of oestrone.

Warren O. Nelson (317) injected oestrone benzoate or oestradiol benzoate in "oil" for from two to ten months into 32 female guinea-pigs and obtained fibromyomata of the uterus in 6 of these. "Associated with these experimental fibroids, particularly the larger ones, there has been always a marked adenomatous hyperplasia of the endometrium with areas of hemorrhage into the lumina of glands and widespread metaplasia of the tips and crypts of the glands. In the cervical region extensive metaplastic downgrowths from the surface epithelium, with keratinization and pearl formation, are regularly present."

Lacassagne (285) injected oestrone and progesterone daily in various pro-
portions into R3 mice (high-cancer strain); 2 females died without cancers, the remaining 8 (3 males and 5 females) developed mammary cancer between the 123d and 194th day. Two R3 males receiving injections of oestrone and testosterone acetate showed mammary cancer at the 108th and 126th day, and testosterone in the dose given did not prevent the appearance of mammary cancer in the females of this strain.

Lacassagne (283) obtained 5 sarcomas in mice injected with oestrogens; of these tumours, 4 (Table X) were at the site of injection (r. axilla) and one

| Table X: Tumours at Site of Injection of Oestrogens (Lacassagne) |
|-----------------------|--------------------------|-----------------|
| Sex and strain        | Incidence of mammary cancer in strain | Substance injected | Site and character of sarcoma |
| Male R3               | High                      | Equilenin benzoate | Right axilla: polymorphous, largely spindle-cell |
| Female 30             | Low                       | Oestrone, equilin and equilenin | Right axilla, polymorphous-cell |
| Male 17nc             | Low                       | Equilin           | Right axilla, spindle-cell |
| Female 17 nc          | Low                       | Equilin           | Right axilla, spindle-cell |

at a distance, in the wall of the bladder. No such tumours have been found in mice of these strains injected with other hormones such as testosterone, or with the solvent used (olive oil + 10 per cent ethylene glycol). Lacassagne has described also (284; cf. 161) 13 tumours, apparently lymphosarcomas, occurring in mice (9 males and 5 females) of both high-cancer and low-cancer strains, which had been injected with oestrone together with one or more of the following: anterior pituitary extract, equilin, equilenin, progesterone. Eleven of these tumours were in the position of the thymus, and 3 (the total thus appears to be 14) seemed to arise in lymph nodes. In the mice bearing these latter tumours the thymus was atrophied. Tumours of this type are the most frequent, after those of the mamma, to arise in mice, but no sarcoma, lymphoid or spindle-celled, has occurred spontaneously in the lines in question during the last five years. Hence the tumours described appear to be due to the substances injected.

Loeb, Burns, Suntzef and Moskop (298) state that “in vagina and cervix there occurs a rhythmic development of squamous epithelium and keratin at the time of proestrus; but there takes place in addition an irregular down-growth of the epithelium into the underlying connective tissue which is greater in the sexually mature than in the immature mouse, and which reaches a maximum in old age. In all strains of mice long continued injection of large amounts of estrin increases the length and the frequency of these processes, and we may therefore assume that ovarian hormones are concerned also in their normal development. Now there occurred in one animal spontaneously, and in a considerable number of animals under the influence of long continued injection of large doses of estrin, a further localized down-growth of epithelium into the connective tissue, which assumed microscopically either the character of precancerous proliferation or of an early stage of cancer. In quite a good many animals these changes were multiple; they were found in the vagina, the cervix, and in the beginning of the uterus... There can be little doubt that
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 reversible changes of this kind do, in the end, become non-reversible if the stimulation is continued still longer." Of one such case these authors say (297): "If such a condition had been observed in a human being it would have been called malignant."

The same authors record that "sarcomas were observed by us to develop in six among the large number of injected mice which we studied. . . . In five of our cases the sarcoma developed at or near the site of the injections. . . . In three cases, sarcoma formation followed long continued injections of estrin; in three other cases it followed injections of other substances. In a recent instance it was observed by Burns and Suntzeff in a mouse injected, likewise over a long period of time, with a liver extract prepared for the treatment of pernicious anemia." (Cf. Schabad: Compt. rend. Soc. d. biol. 126: 1180, 1937.) These sarcomas occurred in low-tumour as well as in high-tumour strains. The authors conclude that "these tumours developed in response to non-specific irritations."

Heiman (272), in describing a case of spontaneous mammary carcinoma in a rabbit, refers to conditions (simple hyperplasia, chronic cystic mastitis, papillary cystadenoma) induced in the mammary glands of 7 old female rabbits receiving injections of antuitrin S and theelin (a proprietary preparation of oestrone) for from two months to two years, identical with non-malignant areas in the spontaneous case. "It has, however, been impossible to produce malignant changes in the induced growth in spite of prolonged injections."

Lacassagne and Nyka (287) compared the action of oestrone or oestradiol upon the pituitary in four lines of mice in which the incidence of mammary cancer is known. In one line (17nc), in which mammary cancer does not now occur either spontaneously or as a result of treatment with oestrogens, the pituitary showed adenoma-like nodules in 6 mice, and in a seventh a tumour of malignant appearance. No such neoplastic growth was found in three other lines (one with a high, two with no incidence of mammary cancer).

Araki (quoted by Kinost, 280) found adenomatous enlargement of the islets of Langerhans, of the adrenal cortex, and of the chromophobe tissue of the pituitary, in mice receiving injections of follicular hormone.

Zondek (372) grafted a sarcoma produced originally by 3:4-benzpyrene, which had undergone several passages in rats, into rats of either sex dwarfed (body weight 90 to 100 gm. as against 170 gm. for controls) by injections of dimenformon (oestrone) and found the growth of the tumour to be as rapid in the dwarfs as in the controls. "At five weeks the tumours were enormous compared with the size of the rat. . . . The injections of dimenformon were continued after the inoculation." The author concludes that "the growth hormone of the anterior pituitary has no important effect upon the growth of malignant tumours."

Mohs (304) found that spontaneous grafted adenofibromas of the mammary glands of rats did not contain demonstrable amounts of oestrogen, while very large doses of oestrone were necessary to cause recognisable amounts to appear in such tumours, which even then did not show more than did the muscle of the same rat. "Hence, adenofibroma does not owe its growth power to an ability to concentrate estrin within itself."
Gilmour (263) applied oestrone (0.01 per cent in chloroform) to the interscapular region of mice twice weekly, and after 2 to 25 such applications inoculated tumours (mammary carcinoma 63 and 113, Crocker sarcoma, skin carcinoma 2146). From fourteen to seventeen days later the average weight of the tumours was 0.67 gm. in the treated mice and 0.9 gm. in the controls, a significant difference, which was as distinct in animals receiving the fewest applications as in those receiving the most, and was similar in the two sexes. “A consideration of the body weights of the animals shows that the oestrone group suffered a general retardation in bodily growth, which was confirmed at autopsy, when the thin, badly nourished condition of the oestrone-painted mice was in striking contrast to the fat, well nourished bodies of the controls.” Hence, although oestrone can induce normal female mammae, and mammary cancer in males, the mammary cells when they have become malignant do not respond to the stimulating action of oestrone. These results confirm those of Bischoff and Maxwell (Am. J. Cancer 27: 87, 1936).

Cramer and Horning (236) have described a golden brown material apparently consisting of necrotic tissue impregnated with lipoid which appears in the adrenal medulla, at first as separate lobules, then as a continuous band between the medulla and cortex and extending later into the medulla and to a less extent into the cortex. This process was observed (a) in male and female mice of mixed strains after prolonged application of oestrogenic hormones to the skin; (b) spontaneously, in mice of both sexes of the R III strain of Dobrovolskaïa-Zavadskai'a (“The brown degeneration is always fully developed in the mice of the D.Z. strain before mammary cancer appears, that is to say in the females developing mammary cancer spontaneously and in the males developing mammary cancer after oestrin.”), and (c) in 4 of 7 mice with spontaneous mammary tumours belonging to mixed strains of low cancer incidence. The relation of this change to the development of mammary cancer is uncertain. “Further observations on the adrenals of other inbred strains with a high and a low incidence of mammary cancer should settle the question decisively, and one of the objects of this communication is to draw the attention of other observers working with different inbred strains to this problem. . . . From a study of the endocrine organs in a number of mice which had been painted with benzpyrene, dibenzanthracene or tar, and which had developed cutaneous cancer, we can affirm that these substances do not produce the profound changes in the endocrine apparatus corresponding to those found after treatment with oestrin.” (Cf. Dobrovolskai'a-Zavadskai'a: Compt. rend. Soc. de biol. 125: 877, 1937; Lacassagne and Raynaud, ibid. 124: 1183 and 1186; Burrows: J. Path. & Bact. 43: 121, 1936.) An interesting discussion of the relation of oestrogens to carcinoma of the mamma has been published by Cramer (235).

VII. The Combined Administration of Oestrone and Carcinogenic Compounds

Gilmour (263) applied 3:4-benzpyrene (0.3 per cent in benzene) twenty times to the interscapular skin of mice of mixed strain (Series A and B); in one-half of these (Series A) oestrone (0.01 per cent) also was applied to the
TABLE XI: Tumour Incidence in Mice Painted with Benzpyrene and Oestrone (A) and Benzpyrene Alone (B) (Gilmour)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Negative</th>
<th>Papillomata</th>
<th>Carcinomata</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>23</td>
<td>77</td>
</tr>
<tr>
<td>B</td>
<td>30</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>A</td>
<td>18.5</td>
<td>28.5</td>
<td>53</td>
</tr>
<tr>
<td>B</td>
<td>47</td>
<td>33</td>
<td>20</td>
</tr>
</tbody>
</table>

rump, both paintings taking place twice weekly until the end of the experiment. Some of the results, which show that oestrone enhanced the carcinogenic action of the hydrocarbon both as to time of induction and number of tumours, are given in Table XI. In Experiment I the first papilloma appeared in the oestrone-treated animals (series A) four weeks earlier than in those painted by benzpyrene alone (series B), and 14 mice of series A bore papillomas when the first ones appeared in series B; the first malignant tumour appeared in Series A after eight weeks (this appears to be the shortest recorded time in which an epithelioma has been produced by a hydrocarbon) and in series B after fourteen weeks, when there were already 7 malignant tumours in series A. The retardation of growth in body weight in series A is shown in a graph.

Two A mice, a male and a female, developed mammary adenocarcinoma after eight and a half months painting with oestrone, and these two produced no skin cancers (cf. Cramer: J. Path. & Bact. 43: 77, 1936). No mammary tumours appeared in these mice, which were of mixed strain, after application of 3:4-benzpyrene only. Cf. Maisin and Coolen (171), who observed mammary tumours in about 20 per cent of mice painted with methylcholanthrene (15 in 67 mice) or with 3:4-benzpyrene (23 in 85 mice). “Ce pourcentage est fortement supérieur à celui que l'on observe chez les souris de cet âge dans notre élevage. Au moment de l'apparition du cancer, des souris sont à peine âgées d'un an.” It would be of great interest to know whether such tumours have been obtained, and in what strains, by other workers. See also Schabad (337), p. 70, above.

[J. A. Schockaert (Bruxelles-méd. 15: 1010, 1935) found that oestradiol (20 international units of the benzoate in oil subcutaneously, weekly), caused tar cancer to develop more rapidly in 150 mice than in 150 controls receiving oil only, 25 per cent of cancers as against 4 per cent after four months, and 65 per cent as against 27 per cent after five and a half months. See also Schockaert and van Damme: Second International Cancer Congress, 1936, vol. II, p. 56. Cailliau and Juster (Bull. de l'Assoc. franç. pour l'étude du cancer 23: 128, 1934), speaking of the effects of injection of oestrin in rabbits during painting with tar, say: "en six semaines, des nodules épithéliomateux, contrôlés par la biopsie, du volume d'un pois ou d'une fève, apparaissent, le lapin témoin restant au stade de la peau rugueuse et alopecique."

Perry and Ginzton (323) applied 1:2:5:6-dibenzanthracene (0.3 per cent
in benzene) and six weeks later oestrone (0.1 per cent "pure crystalline theelin" in benzene) twice weekly to the nape of female mice (unpedigreed albino) as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal mice: 1 : 2 : 5 : 6-dibenzanthracene</td>
</tr>
<tr>
<td>II</td>
<td>Normal mice: 1 : 2 : 5 : 6-dibenzanthracene + theelin (about 125 rat units per dose)</td>
</tr>
<tr>
<td>III</td>
<td>Castrated mice: 1 : 2 : 5 : 6-dibenzanthracene</td>
</tr>
<tr>
<td>IV</td>
<td>Castrated mice: 1 : 2 : 5 : 6-dibenzanthracene + theelin (about 125 rat units per dose)</td>
</tr>
</tbody>
</table>

The numbers in each group are not stated, but 150 mice in all were used. "To reduce the hazard of benzene poisoning, the 1:2:5:6-dibenzanthracene for Groups II and IV was subsequently put into solution with the theelin. After the development of pyometra, the interval between applications of theelin was extended to a week. Treatment was continued throughout the life of the animal." Some of the mice "were known to be of a non-tumor bearing strain, and it is not likely that the others were of tumor-bearing strains. That no carcinomas of the breast developed in Group I would indicate that those occurring in the other groups were not spontaneous. . . . Papillomas of the skin, uterus, vagina, lung, kidney, stomach, colon, and bladder occurred, as well as cutaneous horns, sebaceous cysts, adenomas of the lung and maxillary sinus, cystadenomas of the meibomian gland and of the breast, cysts of the ovary and para-urethral glands, carcinomas of skin, breasts, ureters, lung, stomach, and colon, myofibroma, hemangioma, lymphoblastoma and thymoma.

"Skin papillomas were practically universal, as is common when the skin is painted with a carcinogenic substance. . . . The occurrence of papillomas in the viscera seems reasonably explained as a systemic effect of the carcinogenic substances. There were many sebaceous cysts; and many of the skin carcinomas arose in such cysts. . . . Many secondary papillomas developed about the face and a few on other parts of the body. Those of the face were noticeable for the distribution, which was the same as that observed in human basal-cell carcinomas, i.e., about the eyes, ears and nose. Perhaps because the face was so readily scratched, these lesions were almost universally subject to suppuration." The epitheliomas were of basal-cell, squamous, and mixed types. The incidence of carcinomas of the skin is summarised in Table XII.

"The actual numbers of skin carcinomas in Groups II and IV were small because of the mortality from benzene poisoning, pyometra, and carcinoma of the breast before time for the development of carcinoma of the skin."

Carcinoma of the mamma occurred in 17 mice as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Mice</th>
<th>Carcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (25)</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>II (14)</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>III (37)</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>IV (15)</td>
<td></td>
<td>7</td>
</tr>
</tbody>
</table>

Ten of the tumours appeared before the sixth month. "Thirteen of the tumors were of the hemorrhagic type and were readily identified. . . . Eleven of the carcinomas of the breast were on the shoulder; the others in the inguinal region. This would indicate that the areas adjacent to and draining the

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6 The authors do not state the stage of the experiment to which these figures refer.
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lymphatics from the painted area received proportionally larger doses than were received systemically, and that the biological response was somewhat proportional to the dosage."

Seven breast tumors metastasized: 5 to the lung, 1 to the axillary lymph nodes, and 1 to both lungs and lymph nodes. In 4 instances the mammary carcinomas were multiple."

The authors record other conditions of the breast (alveolar hyperplasia, adenoma, intraductal papilloma and cystadenoma) of some of which photomicrographs are given, but do not state their distribution in the four groups.

<table>
<thead>
<tr>
<th>Number of mice when first carcinoma of the skin was recorded</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of carcinomas of the skin</td>
<td>18</td>
<td>3</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>Number of carcinomas of the skin</td>
<td>9</td>
<td>2</td>
<td>14</td>
<td>1</td>
</tr>
</tbody>
</table>

Epidermoid carcinoma of the cervix occurred in 3 mice: 1 in Group II, which lived ten weeks longer than the others in the group, and 2 in Group IV, of which 1 lived two weeks longer than the others. It is probable, therefore, that more of these tumours would be obtained if early death from pyometra could be avoided. In the case of one of these tumours, which had caused considerable destruction of tissue, the question of the origin from the cervix or from the vagina could not be decided. Photomicrographs of these tumours are given, but these are not correlated with the data for individual tumours in the text. Of the 3 mice with uterine tumours, 2 showed mammary cancer also ("in the mouse with advanced carcinoma of the uterus the breast carcinoma was large") and the third cystic hyperplasia of the breast.

"Hyperplasia of the uterus and vagina was universal in our groups of mice receiving theelin. Cystic hyperplasia and papillomas occurred frequently" (Cf. Lacassagne (160) and McEuen (170) and results quoted in the section "Oestrogens in relation to Tumours" above).

"Animals treated with only 1:2:5:6-dibenzanthracene developed carcinomas of the skin, lung, breast, stomach, and colon. These are the first carcinomas of the breast and alimentary tract to be produced with 1:2:5:6-dibenzanthracene. Hyperplasias and papillomas occurred in the viscera. Myofibromas, lymphoblastoma, and thymoma were also produced. . . . As we were not able to treat groups of mice with theelin alone, we cannot evaluate the proportionate action of the two chemicals. More tumours, and more varieties of tumour developed, and developed earlier, in the groups receiving both substances." (Cf. Gilmour, 263.)

Kinosita (280) states that the introduction of "butter-yellow" (see Table I), in cholesterol or olive oil, into the uterus of the mouse intensifies the epithelial metaplasia produced by oestrone.

7 With regard to this question of lymph drainage cf. Polettini (325). In his mice, painting on the sacrum was followed by the development of 4 mammary cancers, all of which were in the left inguinal region. On the occurrence of mammary tumours after applications to the skin cf. Maisin and Coolen (171).
VIII. Tumours of the Lung

Andervont (198) injected 1:2:5:6-dibenzanthracene in lard subcutaneously in strain A mice which give induced lung tumours more readily than do many other strains, and have a high incidence of spontaneous lung tumours, and in strain M mice which are not known to have a high incidence of such tumours. In a comparative experiment upon two strains the following results were obtained (Table XIII).

<table>
<thead>
<tr>
<th>Table XIII: Response of Strain A and Strain M Mice to Subcutaneous Injection of 1:2:5:6-Dibenzanthracene in Lard (Andervont)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain A</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Number of mice injected</td>
</tr>
<tr>
<td>No tumours</td>
</tr>
<tr>
<td>Lung tumours only</td>
</tr>
<tr>
<td>Subcutaneous tumours only</td>
</tr>
<tr>
<td>Tumours in subcutaneous tissue and lungs</td>
</tr>
</tbody>
</table>

* The figures bracketed refer to surviving mice killed after 4 months to terminate the experiment.

The lungs of strain A mice appear to be a more delicate test-object than the subcutaneous tissues for the carcinogenic action of 1:2:5:6-dibenzanthracene, for the lung tumours appear earlier than the subcutaneous tumours. Thus in one experiment 28 female A mice received 0.8 mg. of the hydrocarbon subcutaneously; three months later 26 were alive and of these all but one showed lung tumours, while none had a subcutaneous tumour. The normal incidence of lung tumours is shown in Table XIV.

<table>
<thead>
<tr>
<th>Table XIV: Incidence of Lung Tumors in Mice Receiving 1.6 mg. 1:2:5:6-Dibenzanthracene Subcutaneously and in Normal Controls (Andervont)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of mice</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Injected mice</td>
</tr>
<tr>
<td>Controls</td>
</tr>
</tbody>
</table>

This paper contains a valuable summary of the literature relating to induced lung tumours in mice.

Andervont (199) bred hybrids between two strains of mice—A, which has a high incidence, and C57 black, which has a low, if any, incidence of spontaneous lung tumours—and tested some of these hybrids by subcutaneous injections of 1:2:5:6-dibenzanthracene in lard, while others served as controls. At that time no lung tumours had been induced by such injections in C57 blacks. The results are shown in Table XV.

The high proportion of induced lung tumours in both hybrid generations shows that this susceptibility is inherited in a dominant manner. The lung tumours in a high proportion of the injected mice were multiple, while the
controls showed two single tumours only. (On the subject of single and multiple lung tumours see Lettinga, p. 91, below.)

In further experiments Andervont (200) transplanted to the subcutaneous tissues of normal mice of strain A, or of a strain derived partly from A, pulmonary tumours from seven strain A mice which had received subcutaneous or intraperitoneal injections of 1:2:5:6-dibenzanthracene in lard, in cholesterol, or as choleic acid. Grafts into other strains were unsuccessful. The tumours have undergone from 3 to 23 passages in the subcutaneous tissue; in several tumours the rate of growth increased in later passages. Three of the tumours consisted mostly of carcinoma in the primary state and in the earlier passages, and all three changed into sarcoma in subsequent passages. None of the grafted tumours shows any tendency to metastasise to the lungs. It is not, of course, possible to define exactly the part played by the hydrocarbon in the origin of the primary tumours, but four of these "arose in strain A mice which were less than nine months old and were, in all probability, induced by injections of the carcinogenic agent. . . . Transplantations of spontaneous pulmonary tumors are in progress. . . ."

Andervont and Lorenz (202) injected into mice of several pure strains 1:2:5:6-dibenzanthracene either (a) in dog serum, or in horse serum saturated with cholesterol, intravenously and subcutaneously, or (b) in lard, subcutaneously. Lung tumours (adenocarcinomas) appeared both in the A strain, which produces many spontaneous lung tumours, and in the C₃H strain which produces few if any.

The distribution of tumours in C₃H mice is shown in Table XVI. Evidently the lard solution tended to produce local tumours; and the dog-serum dispersion to produce tumours at a distance.

The injection of either the serum dispersions or the lard solution was followed in some mice, including those of the C₃H series, by the development of single and multiple tumours of the liver, in one case 22 × 15 × 15 mm. in size, described as hepatomas.

Andervont (201) followed the suggestions given by the experiments described in the preceding paragraphs by injecting subcutaneously into strain A mice 1:2:5:6-dibenzanthracene in five different media (see Table XVII). The horse-serum dispersion was the most effectual in producing lung tumours.
and in this case the nodules were so numerous that the number 30+ is given as an approximation.

Silk threads impregnated with about 1 mg. of 1:2:5:6-dibenzanthracene were drawn through and left in the lungs of 35 mice (strains A, C,H, C57). Tumours (adenoma, adenocarcinoma, squamous-cell carcinoma; generally two or all three of these in one section) were obtained in the lungs of mice of all three strains within three months, though C57 mice are very resistant to the induction of lung tumours by the subcutaneous injection of lard-dibenzanthracene. Portions of some of the tumours appeared to be sarcomatous. A tumour (adenoma and squamous-cell) which was transplanted had become wholly sarcomatous by the sixth passage.

Andervont and Lorenz (203) injected mice subcutaneously with (a) a horse-serum dispersion of 1:2:5:6-dibenzanthracene or (b) the same with addition of charcoal. In C,H mice (b) produced tumours at the site of injection more rapidly than (a), while (a) induced lung tumours and (b) did not. "These results suggest that charcoal held the carcinogenic compound at the site of injection."

In strain A mice, which are especially susceptible to the induction of lung tumours by subcutaneous injection of dibenzanthracene in lard or in glycerin, the results showed that "of the 10 dibenzanthracene charcoal animals, 5 were found to be free from lung tumours and 5 had but a single lung tumour, while of the 8 crystal-injected mice, all had large multiple lung tumours."

When injected intravenously, in A mice, both (a) and (b) induced lung tumours, the former more rapidly, while charcoal alone had little, if any, such action. No mention is made of the appearance of tumours in any other organ as a result of these injections into the blood stream.

A very important study of the production of lung tumours in mice receiving
graded amounts of $1:2:5:6$-dibenzanthracene subcutaneously has been carried out by Lettinga (292). For quantitative comparison he classified mice bearing primary lung tumours into those showing many tumours (a figure of the lungs of one such mouse shows over 40 tumours), two tumours, or one tumour. The results (Table XVIII) show that nearly all mice receiving $5 \times 0.5$ mg. of the hydrocarbon, or more, produced many lung tumours (Series A and B), while no mouse receiving less than this amount (Series C to W) produced more than two tumours. Further, many of the series receiving the smaller amounts did not show distinctly more lung tumours than did the control mice, of which 4 out of 49, or 8 per cent, bore a single tumour. This abrupt transition from a small to a large number of lung tumours suggests very strongly an overflow of carcinogenic material either from the actual site of injection or from some organ (e.g. the liver; cf. Peacock, 180) where these compounds undergo chemical change.

<table>
<thead>
<tr>
<th>Series</th>
<th>Number of mice injected</th>
<th>Number of mice showing lung tumours</th>
<th>Amount of $1:2:5:6$-dibenzanthracene injected (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Many tumours</td>
<td>Two tumours</td>
</tr>
<tr>
<td>A</td>
<td>9 ♀</td>
<td>9</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td>8 ♀</td>
<td>7</td>
<td>—</td>
</tr>
<tr>
<td>C</td>
<td>10 ♀</td>
<td>—</td>
<td>2</td>
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On the subject of this section, see also Schabad (337) under "Action of Carcinogenic Compounds in Different Species and Tissues," p. 70, above.

**IX. Tissue Culture and Cytology**

Doljanski and Halberstaedter (243) have published a well illustrated account of a pure strain of malignant mesenchymal cells propagated in tissue culture from the fourth grafted generation of a sarcoma of the rat produced by $3:4$-benzpyrene. The cells retain their malignancy after prolonged cultivation *in vitro*; a single culture, on reinoculation into a rat, forms a tumour identical in microscopic structure with the original tumour, and such tumours may be again transmitted by grafting from rat to rat.
Ruffilli and Napoleone (336) give a fully illustrated account of the growth in tissue culture of a mesodermal tumour produced in a fowl by injection of 3:4-benzpyrene in lard. The cultures resembled those stimulated to growth by embryonal extract, and the curves of growth showed no sign of the S-shape assumed by normal cultures, which suggests an inhibiting factor.

Warren H. Lewis (296) observed pinocytosis (enclosure of fluid by pseudopodia) in cells in tissue cultures from malignant tumours of the rat and mouse. "In a recent study of 160 dibenzanthracene mouse tumors pinocytosis was seen in the malignant cells in cultures from 90 of the tumors and here again it would probably have been seen in many others had more series of cultures been made and examined especially for this purpose." The fluid enters the cells as globules which move centrally. Many sarcoma cells show at times considerable intracellular digestion which is similar to that seen in normal macrophages.

Belkin and Shear (213) have studied the staining with neutral red of fresh preparations of cells from sarcomas of the mouse, including those produced by 1:2:5:6-dibenzanthracene.

X. Carcinogenic Compounds and Viruses

Andrewes, Ahlström, Foulds and Gye (204) found that in rabbits injected intramuscularly with tar, and then intravenously with the Shope virus of infectious fibroma, there was a generalised fibromatosis which was not found in rabbits not treated with tar. Virus localised at the site of the tar produced in one experiment a transplantable fibroma and in another a polymorphous-cell sarcoma. 3:4-Benzpyrene was found to affect the response of rabbits to intravenous or intradermal inoculations of the virus much as tar does, and further experiments should show whether this effect is a specific one for carcinogenic compounds. These experiments are an extension of those of Rous and Kidd (Science: 83: 468, 1936).

Lacassagne and Nyka (286) give further details (see 75) of the production of tumours by 3:4-benzpyrene upon the pinna of rabbits with and without destruction of the hypophysis. Intravenous injection of an infective extract of Shope papilloma greatly intensified the growth of the tumours in the normal rabbits, but had little or no effect in the absence of the hypophysis.

Foulds (258) found that non-infective extracts of a tumour induced in a fowl by 1:2:5:6-dibenzanthracene when injected into a rabbit caused the production of a serum which neutralised filtrates of Rous sarcoma I, and he gives reasons for deducing the presence of a virus in the non-filterable tumour. "It is concluded that in virus tumours an intracellular virus-protoplasm complex is formed, and filterability depends on the dissociation of the complex in a particular way."

J. W. Beard and R. W. G. Wyckoff (212) ground with sand 5–10 gm. of glycerolated infective wart tissue of the Shope papilloma from cotton-tail rabbits and subjected a saline extract to ultra-centrifugation (two hours, maximum gravity \( \times 60,000 \)) after clearance at low speed; the spinning was repeated three or four times until all light weight impurities and fine colloidal matter had been eliminated. Sixty grams of wart (from 3 natural tumours.
and 2 induced by inoculation) was used. "The heavy protein from each sample sedimented with the sharp boundary that characterizes a single molecular species." In every instance the sedimentation constant was the same. If this protein has about the same shape in solution as that of the tobacco mosaic virus (Wyckoff, Biscoe and Stanley: J. Biol. Chem. 117: 57, 1937) the molecular weight would be rather more than 20 million and the particle about 40 millimicrons in diameter. The substance contained about 15 per cent nitrogen, gave positive reactions with biuret, Millon, and xanthoproteic tests, and coagulated at 66–67° (papilloma extracts lose activity between 67° and 70° Shope). The minimum of purified protein needed to produce warts in seventeen days was 10⁻² to 10⁻⁸ gm., whereas 10⁻⁵ to 10⁻⁸ gm. of total protein from the saline extracts was required for comparable infection. "The heavy protein was several thousand times as infectious as the wart tissue from which it was derived."

Active extracts of cotton-tail rabbit papilloma produce in domestic rabbits exuberant growths which usually yield no active virus (Shope); 10 grams of such tissue, found in repeated tests by Shope to be non-infectious, yielded no heavy protein on ultra-centrifugation.

Later in the year (368) Wyckoff reported that over 100 mg. of this protein had been prepared and many of its biological, chemical and physical properties determined; the same paper contains a review of the subject of ultra-centrifugal purification of proteins. The papilloma protein is found in a concentration of about 0.05 per cent in the infected tissue (Wyckoff, 367).

In a later paper (369) Wyckoff and Beard compare the influence of pH on (a) the infectiousness of the virus and (b) the stability of the protein molecule. On the acid side of pH 7 the virus activity remains high until it is lost suddenly between 2.9 and 3.3, and exactly at this point the protein molecule splits. At pH 1.85 the inactive protein sediments sharply as a single molecular species. Between 3.3 and 7, on both sides of the isoelectric point, the protein has a principal component with a faint secondary boundary, and virus activity persists, but at pH above 7 activity is gradually lost, while above 10.1 the virus solutions become inactive immediately, and the molecule fragments at the same pH.

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