IV. DEVELOPMENT OF LIVER TUMORS IN PURE STRAIN MICE FOLLOWING THE INJECTION OF 2-AMINO-5-AZOTOLUENE

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The preceding studies on carcinogenesis (Shear, 1936, a, b) dealt with the production of tumors by hydrocarbons related to 1:2-benzanthracene. The finding of Kennaway¹ and his colleagues that hydrocarbons are carcinogenic was the culmination of a search for the active agent in coal tar which began when Yamagiwa and Ichikawa (1915) announced the experimental production of skin tumors with coal tar.

The success of these Japanese investigators was, in turn, the outgrowth of numerous attempts on the part of many previous investigators to induce malignancy in experimental animals by the administration of a wide variety of substances. Among the substances used by Yamagiwa and Ichikawa (1915, 1918) in attempting to produce malignant growths was scarlet red; this was used alone and in combination with tar. Hyperplasia was produced by scarlet red but, for various reasons, its use was discontinued and coal tar was used alone. Scarlet red was also injected into the wall of hens’ oviducts by Yamagiwa and Ohno (1918) in an attempt to produce glandular cancer; carcinoma was obtained in 3 of the 41 hens, and hypertrophy in many others. One tumor contained scarlet red in the center of the neoplasm.

This use of scarlet red originated with B. Fischer (1906). After testing many substances in earlier attempts to produce malignant growths, he injected a solution of scarlet red into the ears of rabbits. The atypical epithelial proliferation which resulted was difficult to distinguish, histologically, from cancer, but the growths always receded. Fischer stated that this was the first instance in which tumor-like proliferation had been produced by a chemical compound, and suggested that this finding threw a new light on the genesis of malignant growths, especially on the genesis of paraffin cancer, chimney sweeps’ cancer, and similar occupational cancers.

The publication of this paper by Fischer thirty years ago created a sensation. A large number of workers immediately repeated Fischer’s work, many of them (Stahr, 1907; Hayward, 1909; Stoebber, 1909, 1910; Katz, 1910; Benthin, 1911) referring to it as a sensational finding. That the atypical epithelial proliferation produced by scarlet red bore a strong resemblance, histologically, to malignant tissue was amply confirmed by the above mentioned authors and by others (Helmholtz, 1907; McConnell, 1907; Werner, 1908; and Haga, 1913, who reviewed in detail the already extensive literature).

¹ This work has recently been reviewed by Cook (1936).
Since the proliferation produced by scarlet red was not observed to develop to the stage of malignancy, Fischer suggested that this compound might possibly be useful in stimulating healing of epithelium. This suggestion was adopted by Schmieden (1908), who employed a salve containing scarlet red to accelerate wound healing in patients. This use of scarlet red rapidly found wide acceptance in clinical practice (Hayward; Davis, 1910, 1911; Stoeber; Grossmann, 1910; Katz; and Schmieden and Hayward, 1911, who reviewed the work since the appearance of Fischer's paper, giving a bibliography of 94 references).

Although it was generally agreed that scarlet red had a stimulating effect on the healing of wounds, it was considered somewhat objectionable because it stained the clothes and bedding. Hayward tested a variety of compounds related to scarlet red and found that amidoazotoluol \(^2\) was the active part of the scarlet red molecule. He employed a salve containing amidoazotoluol and reported that it was as efficacious as scarlet red.

That o-amidoazotoluol had an effect on epithelial tissue similar to that of scarlet red was also found by Stoeber, Davis, Katz, Michaelis (1911), Benthin, Schmieden and Hayward, and Haga. Epithelial proliferation was obtained, in experimental animals and in patients, at least as rapidly as with scarlet red. Davis stated: “The consensus of opinion is that there is no danger of producing malignant growths by the clinical use of these substances. My own experience has convinced me of this, and although occasionally there is an overgrowth of epithelium, this soon assumes the level and the appearance of the normal skin.” Schmieden and Hayward also discussed the possibility of inducing malignant growths by the clinical use of scarlet red and of amidoazotoluol but stated that, in spite of the similarity of the microscopic picture to carcinoma, true malignant growths had not as yet been reported either clinically or in experimental animals following the use of these compounds.

Haga, however, found that when the proliferation produced by these substances is pronounced, it becomes atypical. In the skin and tongue he obtained a picture resembling squamous-cell epithelioma and, in the stomach, one like adenocarcinoma. Haga produced proliferation in the mucous membranes, in the mammary glands, in the endothelium of the lymphatics, and in the epithelium of the bile ducts.

A great deal of work was done with scarlet red, o-amidoazotoluene, and similar substances, between 1906, when Fischer published his paper, and 1915. When, in the latter year, Yamagiwa and Ichikawa reported that they had succeeded in producing true malignant growths in rabbits by application of

\(^2\) This compound, ortho-amidoazotoluol, is preferably designated as 2-amino-5-azotoluene.
coal tar, the attention of those interested in carcinogenesis appears to have shifted from the azo compounds to coal tar.

Some years later, Schmidt (1924), who was employing sudan dyes to stain in vivo fats fed to mice, noted the deepest coloration in the fat of the bile ducts. In addition, he observed that feeding of scarlet red produced epithelial proliferation in the liver. Large liver masses were noted which, upon histological examination, were seen to be due to extensive proliferation of liver cells. Some of the liver tissue was considered to be in part adenomatous and in part sarcomatous. Schmidt concluded that these masses were liver adenomas and that their formation was related to the excretion of the dye into the bile ducts. In one case, an infiltrating sarcoma in the neck was noted; Schmidt could not decide whether it was a spontaneous tumor or whether it was produced by the treatment. The production of liver adenomas in mice by feeding scarlet red has been confirmed by Korteweg (1932).

The effect of o-amidoazotolouol, the active group of the scarlet red molecule,

\[
\text{amidoazobenzol} \quad \text{amidoazotolouol} \\
\text{monoacetyl-amidoazotolouol} \quad \text{diacetyl-amidoazotolouol} \\
\text{Biebrich’s Scarlet R} \quad \text{(medicinal)}
\]

on internal organs was studied by Yoshida (1931, 1932a). A solution of this compound in oil was injected subcutaneously into guinea-pigs; the injections were repeated at intervals for five months. The thyroids showed degenerative atrophy followed by atypical epithelial regeneration in squamous-cell form which later showed cornification.

After finding that o-amidoazotolouol produces metaplastic changes in thyroid epithelium, Yoshida (1932, b) studied the comparative effects of related compounds. Solutions of the following substances were injected subcutaneously into guinea-pigs (the nomenclature is the one employed by Yoshida).

In four to five weeks amidoazobenzol produced the same kind of pronounced degeneration in the thyroid that amidoazotolouol produced in the same time; but whereas the amidoazotolouol produced epithelial regeneration in addition to epithelial damage, the amidoazobenzol produced only epithelial damage and no regeneration.

\[^3\text{Since much of the work with this compound has been published in journals that are not readily available in this country, these papers are cited here in some detail.}\]
The diacetyl compound, during the six weeks the experiment lasted, produced changes similar to that produced by the amidoazotoluol; it acted more slowly than the latter, however, and larger doses were required. The monoacetyl compound and the scarlet red were without effect in the short period that the test lasted.

The solutions of amidoazobenzol and of amidoazotoluol were injected subcutaneously into mice, also. Here, too, there was a pronounced difference in their effects. The latter compound produced a remarkable proliferation of liver tissue in about two weeks. The proliferation was so pronounced after four weeks that white irregular patches could be seen macroscopically. The histological picture resembled that of adenoma. At the same time there was an accompanying increase in connective tissue. Amidoazobenzol was more toxic than the corresponding toluol compound; the animals died for the most part in two or three weeks showing no liver tissue proliferation, whereas the azotoluol produced proliferation even in those cases in which large doses caused death in two to three weeks.

Yoshida (1932, c) next reported that, upon repetition of the experiment in which the olive oil solution of amidoazotoluol was injected into guinea-pigs, the same changes in the thyroid were obtained. Furthermore, in one animal which had received repeated injections over an entire year, the thyroid contained foci of cells which, histologically, strongly resembled squamous-cell epithelioma. Yoshida then administered the compound by mouth to other guinea-pigs and to rats. Oral administration also produced these pathological changes in the thyroid. A remarkable effect, moreover, was produced in the livers of the rats, especially in the interstitium; there was bile-duct and connective-tissue proliferation. The picture was said to resemble that of the early stages of annular liver cirrhosis.

Yoshida (1933) later stated that these changes in the interstitium were obtained only irregularly, and were apparently due to unknown factors. The regular noteworthy changes occurred almost always in the liver parenchyma of the rats and mice. In this paper Yoshida reported that typical liver-cell carcinoma was obtained in 4 rats of 26 that had been fed o-amidoazotoluol for more than nine months.

In the feeding experiment a 5 per cent solution of this compound in olive oil was incorporated in the rice diet so that each gram of food contained 1 mg. of the azotoluol. After about two months an active hyperplastic proliferation of the liver cells was noted at the periphery of the acini. The liver-cell proliferation paralleled the duration of the feeding, assuming an increasingly atypical character. In about five to eight months adenomatous changes were noted. Finally, the 4 rats that lived more than nine months all developed typical hepatomas. The histological picture resembled that of human hepatoma. In no case was the development of hepatoma preceded by liver cirrhosis.

Yoshida also administered this compound subcutaneously for long periods. After 448 days he obtained one hepatoma, but in this case he found a worm in the tumor. This was never noted in the hepatomas obtained in the feeding experiments.

Having produced liver tumors in 4 rats with o-amidoazotoluol, Yoshida
next fed this compound to a large number of rats. The compound, dissolved in olive oil, was mixed with the diet and fed to 360 white rats, divided into four groups. The dosage was varied, as well as the duration of the administration of the compound. The first tumor was obtained in 200 days. After 250 days of continuous feeding, all of the rats had liver carcinoma. Some of the rats had, in addition, bile-duct carcinoma as well. Metastases to the omentum and to the periportal lymph nodes were noted. In 2 instances metastasis to the lungs occurred.

The hyperplasia of the liver cells began after fifteen days in the periphery of the acini; by the thirtieth day there were definite hyperplastic foci. This hyperplasia was stated to be neither regenerative nor reparative in the usual pathological sense, inasmuch as no serious damage had preceded it; furthermore it was not accompanied by inflammation. No increase in connective tissue was noted either. These foci developed during the next few months into parenchymatous liver adenomas, Yoshida stated. After several months more of proliferation, these adenomas became carcinomatous. A total of 83 liver carcinomas was obtained; in 26 cases bile-duct carcinoma was also present.

In one of the groups, the feeding of the compound was interrupted after stated intervals. Administration for four and a half months was found to be sufficient for the production of tumors, for withdrawal of the o-amidoazotoluol from the diet after 135 days did not interfere with the subsequent development of liver carcinoma.

Comparative tests were also made with 2 related compounds,

\[ \text{p-amidoazotoluol} \]

\[ \text{p-amidoazobenzol} \]

These compounds were fed to rats for about fifteen months. Neither carcinoma nor hyperplasia resulted.\(^4\)

Yoshida also noted, in a few of the animals fed with o-amidoazotoluol, papilloma formation in the forestomach and in the urinary bladder, the latter occasionally presenting the picture of early squamous-cell epithelioma. However, most of the animals did not show these changes.

**Experimental**\(^5\)

When Yoshida (1934, a) reported that liver-cell carcinomas were regularly produced in rats that had been fed o-amidoazotoluol, experiments were undertaken in this laboratory with this compound. The specimen employed (2-amino-5-azotoluene) was obtained from the Eastman Kodak Company; it

\(^4\) The statement in the second paragraph of section VI on page 529 (Yoshida, 1934, a), that these two substances produce cancer, is apparently a clerical error. In the next paragraph Yoshida stated that cancer was not produced. Miura (1935), in a summary of the work of Yoshida, also stated that these two compounds failed to produce cancer.

\(^5\) These findings were reported (Shear, 1936, c) at the Washington meeting of the Federation of American Societies for Experimental Biology, March 26-28, 1936.
had a m.p. of 99–101° and was used without further purification. The solid compound, moistened with glycerol as a lubricant, was introduced subcutaneously in the axillary region of 23 pure strain mice. Two varieties of mice were used: 4 Strain M ("Leaden") females and 19 Strain A mice of both sexes.

The experiment was started in December 1934. During the course of a year the injection was repeated 6 times, at intervals of about two months; approximately 10 mg. was given at each injection. The compound was introduced in the solid state so as to have a large reserve subcutaneously from which the dye would slowly but continuously enter the circulation. The injected solid was palpable for many weeks after each injection. That it did enter the circulation was evident from the fact that the urine was often seen to be colored by dye.

The mice tolerated the compound well. During the first nine months 7 mice died without showing any macroscopic changes in the liver. In the tenth month, ulceration of the skin over the site of injection occurred in 2 mice; these lesions subsequently healed.

During the eleventh month 2 mice died. At autopsy one was macroscopically negative; the other had a single large tumor in the liver. During the next month 3 more mice died: 2 of the animals were decomposed; the liver of the third mouse was studded with multiple tumors.

Of the 11 surviving animals, 4 had palpable masses in the liver at the end.
of a year. Autopsy revealed, in all 4 cases, markedly enlarged livers containing numerous tumors of various sizes. The remaining 7 mice were killed during the 14th month; all showed multiple tumors in the liver.

Figure 1 is a photograph of one of these mice taken at autopsy. It shows the greatly enlarged liver characteristic of the mice that had been treated
FIG. 3A. PRIMARY LIVER-CELL TUMOR, LOW POWER (AUTHOR'S PHOTO, PREPARED WITH ADVICE AND AID OF PROF. S. B. WOLBACH)

FIG. 3B. SAME TUMOR AS FIG. 3A. × 300 (CROCKER LAB. PHOTO NO. 6074)
FIG. 4A. ADENOCARCINOMA OF THE LARGE BOWEL. × 250 (CROCKER LAB. PHOTO NO. 6068)

FIG. 4B. ADENOCARCINOMA OF THE LARGE BOWEL. × 100 (CROCKER LAB. PHOTO NO. 6069)
with the dye for long periods, and innumerable tumors of various sizes. Figs. 2 and 3 (A and B) are photomicrographs of sections from these tumors. According to Prof. S. B. Wolbach, of the Department of Pathology of the Harvard Medical School, the tumors produced in these mice are liver-cell carcinomas.

In one mouse bearing multiple liver-cell carcinomas a large tumor was found in the intestines. This was diagnosed by Professor Wolbach as an adenocarcinoma of the large bowel (Figs. 4 A and B).

*Transplants*: Transplantation was carried out with 5 tumors from 4 of these mice. Two of the mice were Strain M females, one was a Strain A male, and the fourth was a Strain A female. One tumor was taken for transplant from each of the first 3 mice; 2 tumors were taken from the Strain A female. Fragments of each tumor were implanted subcutaneously in the groin of several mice of the same strain in which the tumor had arisen. Negative results were obtained with the transplants of the 3 liver tumors from the Strain A mice.

Explants of the tumors from the 2 Strain M mice, however, took successfully. In the case of one of these tumors, the implants grew well in all of the 3 implanted mice; at the end of six weeks the transplanted tumors had an average diameter of about 10 mm. This tumor has been repeatedly transplanted subcutaneously and is now in its 10th generation. After several transfers, the tumors grew faster. Histologically, the transplants all retained

6 The "average diameter" (Shear, 1936, d) is defined as the arithmetical mean of the 3 diameters of the tumor.
their characteristic liver-cell nature as can be seen on comparing the original tumor (Fig. 5) with the 4th generation transplant (Fig. 6). The figures show histologically what was noted macroscopically, i.e., that following repeated transplantation the tumors grew more rapidly.

Explants of the tumor from the other Strain M mouse gave rise to slowly growing tumors in 2 of 3 mice. This tumor was successfully carried for 2 generations and was then discontinued. Here, too, the transplanted tumors retained their characteristic liver-cell nature.

As a further check on the malignancy of these tumors, normal livers from Strain M mice were also transplanted in the same way into other Strain M mice. 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 tumors. However, no growths were observed in four months. Analogous transplants of tissue from livers of Strain A mice also gave negative results.

**DISCUSSION**

Yoshida produced liver-cell carcinomas in rats fed over a long period with a diet which contained 2-amino-5-azotoluene. The carcinogenic action of this compound has been confirmed in this laboratory by the production of liver-cell carcinomas in 13 pure strain mice in which the compound had been introduced subcutaneously, as a solid. Two of these tumors were successfully transplanted for several generations.
It is of interest that no tumors occurred 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. Some of the dye was obviously absorbed into the general circulation, for it produced tumors in the liver; furthermore, the urine was dyed yellow. Apart from one adenocarcinoma of the large bowel, there was no evidence of tumor in other organs at autopsy. The results of histological examination of other tissues will be reported at a later date.

While this experiment was in progress, a number of other reports on the action of this compound were published in Japan. Yoshida (1934, b) injected an oil solution of the dye subcutaneously into 30 white rats; the injections were repeated at intervals of two to four weeks. Only 6 of the rats lived more than 200 days. Of these survivors, only one developed a hepatoma; in addition it developed an adenocarcinoma of the skin. A second mouse had a typical skin cancrum.

Yoshida (1935, a) next fed the dye to 23 rabbits for almost two years without obtaining typical hepatomas; 7 of the long-lived rabbits developed annular liver cirrhosis, but Sugiura (1936) pointed out, in an abstract of Yoshida's report, that liver cirrhosis is not uncommon as a spontaneous disease in old rabbits.

In a histological study of the urinary bladders of the rats that had been fed with this compound and had developed hepatomas, Yoshida (1935, b) obtained microscopic and macroscopic evidence of epithelial proliferation. In one case a papilloma was transformed into an infiltrating squamous-cell epithelioma. The changes in the bladder were much less regularly obtained than those in the liver. In the controls no epithelial proliferation was observed in the bladder. Parasites were never seen in or near the growths.

The administration of glucose, Yoshida (1935, c) found, did not affect the production of liver-cell tumors. In this experiment, a 30 per cent glucose solution was injected subcutaneously twice weekly to rats fed with the dye. In one rat liver cirrhosis was noted after thirty-four days. Of the many hundreds of rat livers studied, this was the only instance in which liver cirrhosis was noted, Yoshida stated.

In the same laboratory, Nishiyama (1935, a) fed this azo dye to 22 rats and concurrently injected daily a 25 per cent glucose solution subcutaneously. Of the 7 rats that lived more than 200 days, 5 developed invasive sarcomas at the site of injection. Nishiyama (1935, b) also fed the azo dye to 40 mice. Only 7 mice survived eleven months on this diet. In 6 of these 7 mice multiple tumor nodules were found in the liver which, on comparison histologically with those obtained in the rat experiments, were considered to be hepatomas.

Iikubo (1935, 1936) attempted to transplant liver tumors produced in rats by 2-amino-5-azotoluene. The original rats and the new hosts were of unspecified lineage. The transplants were introduced subcutaneously, intraperitoneally and intrahepatically. Most of the transplants failed to establish themselves. In some cases, however, the transplants grew; one tumor has been carried for 15 generations.

In another interesting paper from this same laboratory, Otsuka (1935) reported that mice fed with diazo-aminobenzol developed papillomatous
growths in the fundus of the stomach. Of the 30 mice in this experiment, 11 lived more than ten months; all 11 showed distinct epithelial proliferation. Sometimes numerous mitoses were obtained but, in general, carcinomatous degeneration was not observed. In no case were parasites found in or near the papillomatous growths.

The fate of 2-amino-5-azotoluene in the body has been studied by Hashimoto (1935), who also worked in this same laboratory. He found that this dye, when fed to rabbits, is first reduced and then excreted in the urine as the acetylated compound.

The picture presented at autopsy by the pure strain mice bearing the multiple liver tumors is similar to that seen in patients with multiple liver tumors, according to Professor Wolbach. Whether the findings obtained with this dye have any connection with clinical hepatomas or other clinical tumors it is not yet possible to say. It may be mentioned, however, that 2-amino-5-azotoluene has been used commercially to dye leather which, on coming in contact with human skin, has produced dermatitis; Schwartz (1936) has identified this dye as the substance responsible for the dermatitis. Derivatives of this substance are also being used in a number of salves to promote the healing of wounds.  

In recent years data have been accumulating which raise the question as to whether pigments play a significant rôle in the genesis of cancer. For example, Sequeira and Vint (1934), who examined 482 malignant tumors occurring in East African negroes, found 184 cancers of the skin and adjacent mucous membranes; of these, 52 (or 11 per cent of all the tumors) were melanomas. They stated that there seems to be no doubt that malignant melanoma is more common in Kenya and Tanganyika than in Great Britain. Horgan (1935) reported that, in spite of previous statements to the contrary, melanomas are not uncommon in the colored races. He examined 438 malignant tumors occurring in Sudanese and found that 31 (or 7 per cent) were melanomas. He cited other data, from Nigeria, in which 10 of 90 malignant tumors were melanomas. He concluded that a tumor which constitutes from 7 to 12 per cent of all malignant tumors should be regarded as comparatively common. Smith and Elmes (1934) analyzed 500 tumors in natives of Nigeria and found 40 melanotic sarcomas, i.e., 8 per cent of all tumors; 94 of the 500 tumors were in the skin (i.e., 19 per cent of all tumors). Vint (1935) stated that malignant growths of the skin are of the utmost importance in the East African native, constituting 36 per cent of 277 carcinomas. Under skin growths, he included all tumors of "the covering membranes"; among them were 59 melanomas.

Of more direct interest in connection with the experimental production of liver carcinoma by a dye are the recent reports that there is a strikingly high incidence of primary liver carcinoma among pigmented peoples. Cazanove (1931) found a high incidence of cancer of the liver among the natives of

\footnote{Personal communication from Dr. L. Schwartz. See also U. S. Dispensatory, 21st Edition (1926).}
the French colonies in West Africa and Indo-China. Smith and Elmes found 55 tumors of the liver in 500 tumors (i.e., 11 per cent) occurring in East African natives. The primary liver tumors were found in children as young as three and four years of age. Of the 55 liver tumors, 32 were undoubtedly of primary hepatic origin. Smith and Elmes stated: "This number, which is 6.4 per cent. of the present series, though a conservative estimate, seems to be exceptionally high in view of the statistics quoted by Ewing (1928) where 1.3 per cent. is regarded as being excessive."

Vint listed 38 liver carcinomas, of which 31 cases were liver-cell growths and 7 were of bile-duct origin; these constituted 14 per cent of all carcinoma cases in his series (East African natives). Berman (1935) has reported that among South African natives there is a high incidence of primary carcinoma of the liver, especially in the young male adult. In 116 Bantu males and 99 Bantu females in a Johannesburg hospital, the incidence of liver carcinoma was 32 per cent and 5 per cent respectively. Of 253 male Bantu mine laborers that had carcinoma, 229 or more than 90 per cent had liver carcinoma. Furthermore, the tribes from Portuguese East Africa, constituting only 35 per cent of the miners, contributed 76 per cent of the carcinoma cases. According to Dr. Joseph Gilman, although the high incidence of liver tumors among these negroes has not yet been satisfactorily accounted for, it appears possible that it may be correlated with the depth of pigmentation of the skin.

Similar data have recently been published regarding Asians. Snijders (1924) stated that, as regards tumors in the Javanese and Chinese of Sumatra, "The most striking feature is the high frequency of primary cancer of the liver." Strong and Pitts (1930) found eight times as many liver carcinomas among Chinese as among whites, in performing autopsies in western Canada (1 liver carcinoma among 909 white autopsies, as compared with 8 among 115 Chinese autopsies). The relatively small numbers of Chinese admitted to their hospital, they stated, "only further emphasize the strikingly high incidence of primary carcinoma of the liver among these people." While these authors were inclined to associate the frequency of liver carcinoma in the Chinese with parasitic infection, especially with liver flukes, they made no mention of finding such infestation in the livers of any of their patients.

In an extensive series of cases in Singapore studied clinically and post mortem, Tull (1932) recorded 134 cases of primary liver carcinoma. Most of the liver tumors occurred in Chinese (126 cases of the 134). Tull considered the possible rôle of liver flukes in the genesis of these tumors, because of the frequency of liver infestation with flukes in the natives of South China. He found, however, that a considerable proportion of the Chinese patients had been resident for many years in Singapore, where these flukes are not endemic; several of the Chinese had been in Singapore all their lives. He found that in his cases flukes were not commonly present.

As had been found among the African natives, Tull found that among the Chinese there were many cases at comparatively early ages. He concluded that: "Primary carcinoma of the liver is comparatively frequent among natives of certain provinces in Southern China."

* Personal communication.
Whether skin pigments are involved in the genesis of skin or liver tumors clinically remains for further work to determine. As regards certain types of synthetic benzene derivatives, however, the situation is clearer. The compound 2-amino-5-azotoluene, which is used commercially under several names, is capable of producing liver tumors. The compound styryl 430, which was used to treat trypanosome infection, was found by Browning, Gulbransen and Niven (1936) to produce sarcomas at the site of injection. The substances responsible for producing so-called "aniline cancer" of the bladder in workers in chemical plants have not yet been identified.

The question arises as to whether other synthetic benzene derivatives are carcinogenic. A wide variety of such compounds are used commercially in the preparation of materials that come into intimate contact with the body. Twenty years ago Salant and Bengis (1916) investigated the fate of some azo-benzene derivatives in the body. They studied Sudan III, benzene-azo-β-naphthylamine, benzene-azo-β-naphthol, benzene-azo-dimethylaniline, and aminoazobenzene. They found that a number of these dyes were excreted in the urine, in the form of glucuronates.

These authors stated: "Although synthetic dyes have played an important part in the study of biology and medicine, the behavior of many of these compounds in the body is still imperfectly understood and the action of some of them entirely unknown. Owing to their extensive employment in numerous industries, and especially in the preparation of foods, this lack of satisfactory information frequently proved to be a matter of serious import as questions regarding their effect on health were often raised, but no definite answer could be given in the present state of our knowledge." As regards the effect of such compounds on tumor genesis, the statement of Salant and Bengis is applicable today.

**Summary**

1. Subcutaneous administration of 2-amino-5-azotoluene ("o-amidoazo-toluol") in pure strain mice gave rise to multiple primary liver-cell carcinoma in all animals that survived for one year.
2. Several of the liver tumors were successfully transplanted subcutaneously and retained their liver-cell character after repeated transplantation.
3. Similar transplantation of normal liver tissue gave negative results.

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