The Carcinogenic Activity of 2-Acetaminofluorene

III. Manner of Administration, Age of Animals, and Type of Diet

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Wilson, DeEds, and Cox (4) have shown that 2-acetaminofluorene (AAF), when incorporated into the diet and fed to rats for some time, produced hyperplastic and carcinomatous lesions in the animals. At the time that report was written, certain animals that had received the compound by various other routes of administration were still alive and well. The authors drew the tentative conclusion that “the failure to produce tumors in the parenterally treated rats [suggests] that continued administration by way of the gastrointestinal tract is necessary for carcinogenic activity.” The preliminary work on this phase of the subject was continued and expanded, and the results form the subject matter for this paper.

EXPERIMENTAL

Single oral dose of AAF.---A single, large dose of AAF by mouth does not lead to tumor formation. This experiment has been reported in some detail previously (5) and it need not be enlarged upon here. The important point is that AAF is carcinogenic when given by mouth only when the administration is continued for a period approaching 25 days. Longer administration increases the incidence of tumors and decreases the time required for initiation of the lesions.

Subcutaneous implantation of crystalline AAF.---A previous report (4) told of the implantation of approximately 0.5 gm. of crystalline AAF in the subcutaneous tissue of the groin of 5 female rats. (By mistake they were described as males in the earlier paper). After 14 months there were no gross signs of tumor. The animals finally were autopsied 504 to 728 days after the date of implantation. Two of them developed subcutaneous tumors in the mammary region, one had several growths in the uterus, and all 5 had a few small cystic areas in the liver suggesting mild chronic changes due to AAF. Microscopical examination of the tissues revealed that all showed changes like those seen when the compound was administered by mouth. The extent of the changes was about that of the group which received 0.125 per cent in the diet for 25 days (5). Four of the livers showed small cysts. Two of 3 ovaries examined showed proliferation of abnormal stromal cells, and one contained a cyst lined by a single layer of partly ciliated cuboidal epithelial cells. One animal had a carcinoma of the lung composed of rather small epithelial cells in a dense fibrous stroma. Some of the cells were in small clusters but no gland formation was seen. The same animal had a well differentiated adenoma of the uterus. The other animal from which the uterus was examined showed marked nodular proliferation of small glands beneath the endometrium. One animal had 5 subcutaneous nodules, one at the site of implantation. As the section of the latter was not specifically indicated, it is not certain which of the 5 nodules in the sections represented this lesion. However, 3 were adenomas, probably of mammary origin, one was a hyperplastic mammary gland; the fifth, a fibroma, may have arisen from the subcutaneous tissue, although it could also have been a mammary tumor. No other subcutaneous tumors were seen in any of these animals. The 4 bladders which were examined showed no lesions.

Subcutaneous injection of AAF solution.---It was previously mentioned (4) that 6 C57 mice, injected subcutaneously with AAF dissolved in sesame oil on 2 occasions, 42 days apart, appeared normal up to the time of death 10 months later. The seventh animal was autopsied 23 months after the first injection. There were no signs of tumors.

Five male rats were used for injections of AAF dissolved in propylene glycol. Injections of 20 mgm. of AAF in 0.4 ml. were made four times in a 6 month period. One animal which died after 238 days had myocarditis and renal lesions suggesting...
chronic glomerulonephritis. There were no tumors. The rest lived for periods up to 614 days. One rat had leukemia. Another showed an infiltrating tumor of the lung composed of somewhat irregular spindle cells in a collagenous stroma. This has been classified as fibrosarcoma. It may have been the primary tumor, but there was a smaller subcutaneous nodule composed of similar tumor tissue. Another animal showed no lesions, and the fifth, from which only a section of bladder was studied microscopically had a small myoma of the bladder. Three bladders and four livers from these animals showed none of the changes usually seen after administration of effective doses of AAF so it is concluded that the compound given subcutaneously in propylene glycol in this quantity had little or no carcinogenic effect. The presence of leukemia in one animal which received propylene glycol alone and of a lung carcinoma and an adrenal adenoma in another animal similarly treated provides further doubt that the AAF administered in propylene glycol produced any lesions.

Installation of powdered AAF into the auditory canal.—We (4) have previously described subcutaneous epidermoid carcinomas of the side of the face and suggested that they may have arisen from the auditory canal or accessory structures such as sebaceous ducts. Bielschowsky (1) has definitely called the lesion a carcinoma arising from the ductus acousticus externus. While it was our belief that AAF had to be taken by mouth in order to be effective, it was possible that the animals got some of the compound into their ears while eating and that this might have caused the tumor in the head. To test this possibility, 6 male rats were used. Powdered AAF was blown into the ears weekly for 13 months. One animal died 21 months from the start and the others were killed and autopsied after 24 months. There was no gross suggestion of tumors in any tissue of any of the rats. Tissues from 5 of them were studied microscopically. They showed no liver or bladder lesions, or any of the other lesions common in AAF-treated animals. Two, however, had carcinoma of the lung. It seems likely that these were not specific results of the treatment, although some of the powder was visible in the air and a possible local effect from inhalation of the powder cannot be disregarded.

Effect of enriching the diet.—At about the time these experiments were in progress, it was being shown that the adequacy of the diet was of great importance in determining the carcinogenic effectiveness of \( p \)-dimethylaminobenzene. In particular, the addition of yeast to the diet decreased the incidence of hepatomas (see reference 2, p. 197). Since the liver tumors of the AAF animals frequently resembled certain of those produced by \( p \)-dimethylaminobenzene, it was decided to supplement an AAF diet with foods of a high vitamin content. To 79 parts of the regular diet were added 1 part of cod liver oil, 5 parts of dried brewers' yeast, and 15 parts of wheat germ. Sugiura and Rhoads (3) showed later that for a maximum effect, 15 per cent of yeast had to be incorporated into the rice diet containing the azo compound. However, since the regular diet used in the AAF studies seemed satisfactory from all viewpoints, as contrasted with the poor quality of the rice diet, it is probable that 5 per cent of yeast was an adequate addition.

Eleven rats, 6 females and 5 males, were placed on this enriched diet when they were about 45 days old. The concentration of AAF in the diet was 0.125 per cent for 41 days; it was then reduced to 0.031 per cent until the 143rd day, and afterwards omitted entirely. The vitamin supplements were continued after 215 days. By the 174th day of the experiment, 4 of the rats had developed external tumors and postmortem examination revealed possible thickening in the bladders and a somewhat nodular and cystic appearance of the livers. A fifth tumor was evident before the experiment was accidentally terminated. There was no indication that the dietary supplements had modified the types of lesions or changed the time at which they made their appearance. However, another study still in progress suggests that the diet may be of great importance in regard to the carcinogenicity of AAF.

Effect of the age of the rats.—Most of the animals used in these investigations were young, and were started on the experiment shortly after weaning. The relation of the age of the experimental animals to the carcinogenic effect of AAF was studied using 8 male and 6 female rats, aged 440 to 618 days. They were fed AAF in a concentration of 0.031 per cent in the diet for 274 days. In this length of time, 10 of the 14 animals had developed tumors and 3 of the others had organs, which although not normal, could not from gross appearance be considered tumorous. No histological examination was made. AAF was as effective in these older animals as in younger rats, but probably no more so. Because of the small number of animals, no conclusion is justified, but it is interesting to note that only 1 head tumor and 1 tumor of the mammary type were found in this group of 14 rats.

DISCUSSION

The earlier suggestion that AAF must be ingested for a considerable period is substantiated.
Installation of the powder into the ear was without effect, and although this was done but once a week, some of the compound presumably dissolved in the ear wax with resulting continuous exposure. The rats given AAF in propylene glycol subcutaneously developed a few tumors that were not typical of the lesions produced by AAF. The interpretation of these findings was complicated by the exhibition of several neoplasms in rats receiving only propylene glycol. Those rats in which subcutaneous implantations of AAF crystals were made developed lesions similar to those produced by oral administration for a barely effective period of time. The lesions were minimal, and a long time was required before they made an appearance.

It was suggested in the first paper (4) that the effective carcinogenic agent probably was not 2-acetaminofluorene, but 2-aminofluorene, deacetylation having taken place in the gastrointestinal tract. It is possible that the crystalline AAF was gradually hydrolyzed within the body producing the minimal lesions; or the continued presence of AAF in the body might have caused these changes. How long the AAF remained in the body is not known. The lumps at the site of implantation disappeared within a few weeks. Since AAF is soluble in fats and fat solvents, this disappearance was in all probability due to solution. Whether there was hydrolysis, or excretion or other metabolic change, it is not known. In any case, the development of tumors was not typical in that the incidence was low and the time required for development was long.

Apparently AAF must be taken by mouth for a period of time (at least 25 days) in order to produce cancer effectively. Introduction of AAF parenterally may lead to minimal changes after a prolonged time. It is possible that the effective carcinogen, even in this case, is aminofluorene, the acetyl group having been hydrolyzed off in the tissues instead of in the alimentary tract.

The carcinogenic action of AAF appeared at first to be much less subject to the nutritional status of the animal than is that of p-dimethylaminoazobenzene since the latter compound is most effective on a nutritionally unsatisfactory rice diet, and slight modifications of the diet greatly decrease the number of tumors developing. Addition of vitamin-rich foods to the already satisfactory diet did not change the incidence or time of development of AAF neoplasms. However, there is some recent evidence, still incomplete, that diet may be of considerable importance.

Spontaneous benign mammary tumors develop in about 3 per cent of the breeding female rats of the colony, especially in the older animals. This being the case, a more rapid or more severe development of cancer might be expected were older animals to be given AAF. However the effect on the older animals seemed comparable to that on young rats. The one possible difference, the apparent decrease in number of tumors of head and breast, is interesting, particularly in regard to the latter since these rats, some of which had been breeders, were at an age when spontaneous benign tumors might be expected to appear.

SUMMARY

1. A single, large dose of 2-acetaminofluorene (AAF) by mouth did not lead to the production of tumors. No carcinomas of the acoustic canal, and no lesions in other organs clearly attributable to AAF developed when powdered AAF was introduced into the external ear.

2. Subcutaneous injection of AAF dissolved in propylene glycol led to a few changes, some of them tumorous, which were not clearly the result of AAF. Implantation of crystalline AAF subcutaneously caused minimal changes after a prolonged time.

3. The most effective way found so far to produce cancerous lesions in rats from AAF is to administer the compound by mouth for a considerable time.

4. Enrichment of the diet by the incorporation of cod liver oil, yeast and wheat germ, did not change the incidence or time of development of AAF lesions. However, recent evidence indicates that the character of the diet is important.

5. Age of the experimental animal did not influence the time of development of AAF tumors.

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REFERENCES


3. SUGIURA, K., and RHOADS, C. P. Experimental Liver Cancer in Rats and its Inhibition by Rice-Bran Extract, Yeast, and Yeast Extract. Cancer Research, 1:3-16, 1941.


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