arising when drugs or additives are adsorbed at a solid membrane interface, when impurities are solubilized from a solid membrane, or when pore size of a membrane influences diffusion through it.

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Distribution of Tritiated Derivatives of Fluorene in the Rat

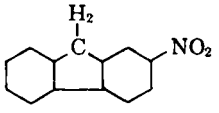
By F. E. RAY and O. O. WEJEBE

By the study of tritiated derivatives we have found that the liver carcinogen, 2-acetylaminofluorene, is concentrated to a greater extent in the liver of the rat than 2-acetylaminofluorenone, which produces only an occasional liver tumor. 2-Nitrofluorene, which also produces few, if any, liver tumors, gives a concentration that is intermediate. 2-Nitrofluorenone, which has not been tested for carcinogenicity, gives a concentration in the liver similar to 2-nitrofluorene. The highest concentrations in the liver result when the compounds are given by intraperitoneal injection.

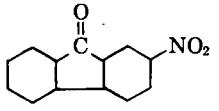
IN 1940, THE U. S. Department of Agriculture proposed the use of 2-acetylaminofluorene (AAF) as an insecticide; but before releasing it for field tests, it was sent to the Regional Laboratory in California for the determination of toxicity. After an extensive series of tests, Wilson, De Eds, and Cox (1) reported it to be carcinogenic to rats and mice. While this ended its career as an insecticide, it became of fundamental importance in the study of the etiology of cancer.

Whereas the carcinogenic hydrocarbons produce tumors almost exclusively at the site of application, acetylaminofluorene produces a wide variety of tumors distant from the site of application. The principal organ of attack, however, is the liver. It has been shown to be carcinogenic to mice, rats, rabbits, hamsters, dogs, and fowl. It is indeed fortunate that this compound was thoroughly tested *before* being released for general use.

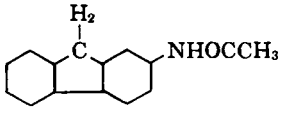
It seems reasonable to assume that a compound that has a specific effect on an organ must have an affinity for that tissue. In previous work we found this postulate to be true: a derivative causing gastric cancer localized in stomach tissue to a greater extent than a closely related nongastric carcinogen (2). Because 2-acetylaminofluorene (III) causes many liver tumors, but 2-acetylaminofluorenone (IV) does not, but is carcinogenic to other tissue (3, 5), one might expect a greater concentration of the former in the liver. To determine if this thesis is correct, radioactive derivatives of these compounds were administered and their distribution studied.



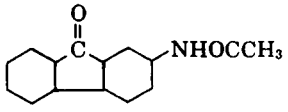
2-Nitrofluorene
(2-NF)
I



2-Nitrofluorenone
(2-NFone)
II



2-Acetylaminofluorene
(AAF)
III



2-Acetylaminofluorenone
(AAFone)
IV

Recent work by Ray, Cromer, Aycock, and Pitzer (2) has shown that it is practical to pre-

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pare radioactive 2-nitrofluorene and 2,7-dinitrofluorene by exposing the compounds to the action of tritium(H_2^3) for a number of days, and that isotopically pure amines are conveniently prepared from the corresponding nitro compounds. The reduction, catalytically with hydrogen and platinum, acetylation, and subsequent purification readily eliminate radioactive impurities produced during the irradiation with tritium. This simplifies, to a considerable extent, radiotracer studies with these compounds and their derivatives. It seemed worthwhile, therefore, to use the tritiation technique to compare 2-nitrofluorene (2-NF) (I) and 2-nitrofluorenone (2-NFone) (II). This was followed by a comparison of 2-acetylaminofluorene (AAF) (III) and 2-acetylaminofluorenone (AAFone) (IV).

EXPERIMENTAL

The nitro compounds, I and II, were tritiated by exposing 1 Gm. of each to 15 curies of tritium for 2 weeks at 27° and 666 mm. in 45 ml. of methyl chloride and 15 ml. of methanol. In two separate experiments the specific activity of 2-nitrofluorene after recrystallization from glacial acetic acid to constant radio-solubility was 173 μc./mg. and 194 μc./mg., respectively: 2-nitrofluorenone gave 74 μc./mg. and 84 μc./mg. It will be noted (formulas I and II) that the fluorenone has seven-ninths (78%) as many hydrogens to be replaced by tritium; however, the two aliphatic hydrogens replaced by oxygen are considerably more active than the remaining seven aromatic hydrogens and, therefore, more easily tritiated, giving a ratio of about one to four. The nitro compounds were administered as a basis of comparison with the amines.

Emulsions were made that contained equimolar amounts of the substances—0.24 mc. per cubic centimeter for 2-nitrofluorene (m.p. 156°) and 0.09 mc. per cubic centimeter for 2-nitrofluorenone (m.p. 218°). One milliliter dose was administered by stomach tube or by intraperitoneal injection. On reduction with platinum catalyst and hydrogen, followed by acetylation and purification to constant radio-solubility, yielded 2-acetylaminofluorene-H^3 (m.p. 192°) of specific activity, 26.2 μc./mg. Similarly, 2-acetylaminofluorenone-H^3 (m.p. 225°) gave 2.69 μc./mg., a ratio of about one to ten. The lower activity resulted because base acetylaminofluorenone was added in the purification process.

TABLE I.—RADIOACTIVITY PRESENT AT THE END OF 6 HOURS[a] (2-NITROFLUORENONE AND 2-NITROFLUORENE)

Tissue	2-Nitrofluorenone, %	2-Nitrofluorene, %
Liver	1.14	1.19
Kidney	0.21	0.18
Urine	4.98	2.09
Blood	0.61	1.05
Stomach	2.76	2.29
Stom. cont.	16.36	17.98
Spleen	0.03	0.04

[a] Oral administration.

Procedure.—Young adult Wistar rats weighing 170 Gm. each were used. Equimolar amounts of the two compounds (0.045 *M*) were heated with 1 ml. of 95% ethyl alcohol and made up to 10 ml. and homogenized with 1% aqueous methylcellulose solution. One milliliter of each was then fed *via* stomach tube. Another milliliter of each was diluted to 500 ml. with 95% ethyl alcohol to be used as a standard. The animals were housed in metabolism cages permitting the separate collection of urine and feces. At the end of the designated time, they were killed with ether and their organs removed. The tissues were treated with 10x portions of 1% sodium hydroxide, allowed to stand overnight in the cold room, homogenized, and made up to volume. Aliquots were then counted three or more times to 100,000 or 30 minutes in a dioxane-naphthalene-DPO-POPOP scintillator. Samples of the standard were included in every count. Corrections were made for background and quenching, and the results expressed as per cent of administered dose (68×10^6 to 155×10^6 counts/minute). The efficiency of the Tri-Carb scintillation counter used was 33.21 to 34% (737,000–755,000 counts/minute/microcurie).

RESULTS AND DISCUSSION

A comparison of the amount of radioactive material found at 6 hours after oral administration in the liver shows no substantial difference between the nitrofluorene and nitrofluorenone compounds (Table I, 1.14 and 1.19%). Little difference is found in the spleen, kidneys, and in the stomach and contents. The percentage of fluorenone derivative excreted in the urine is over twice that of the fluorene, indicating more rapid elimination of the former. This is in harmony with the lesser amount of fluorenone in the blood.

Table II gives the results of the 24-hour experiment in which the same compounds were administered intraperitoneally. Here again there is no real difference in the localization of the two compounds in the liver (7.04 and 6.72). Again, somewhat more of the fluorenone derivative seems to be excreted in the urine (13.82 to 9.80); this may thus indicate a real difference. Now the small difference in the percentages in stomach tissue has become substantial in favor of 2-nitrofluorene. While the amount of the two compounds in the blood is almost equal, a real difference seems to exist in favor of the fluorene derivative in the spleen. The somewhat slower elimination of 2-nitrofluorene and consequent greater concentration in blood, spleen, and stomach tissue may indicate that some hydrogen

TABLE II.—RADIOACTIVITY PRESENT AT THE END OF 24 HOURS[a] (2-NITROFLUORENONE AND 2-NITROFLUORENE)

Tissue	2-NFone, %	2-NF, %
Liver	7.04	6.72
Urine	13.82	9.80
Stomach	0.32	1.17
Stom. cont.	1.60	0.60
Intestine	16.47	18.07
Kidney	0.68	1.00
Spleen	0.50	1.70
Blood	1.36	1.53

[a] Intraperitoneal injection.

TABLE III.—2-ACETYLAMINOFLUORENE AND 2-ACETYLAMINOFLUORENONE[a]

	Oral 12 Hr.		Oral 24 Hr.		i.p. 24 Hr.	
	AAFone	AAF	AAFone	AAF	AAFone	AAF
Liver	1.63	1.80	0.68	1.16	3.75	13.46
Urine	16.61	13.06	15.33	19.63	22.43	24.91
Stomach	0.31	1.31	1.66	0.09	0.15	0.45
Stom. cont.	16.03	18.74	19.18	0.05	5.86	3.97
Intestine	36.86	48.64	16.41	57.67	20.81	25.85
Kidney	0.24	0.22	0.15	0.10	0.91	1.72
Spleen	0.03	0.02	0.03	0.001	0.08	0.43
Blood	0.39	0.59	0.57	1.27	0.46	0.51
Body fluids	0.88	0.76	0.32	0.15	4.30	1.83
Carcass	7.98	8.94	5.29	6.62	15.16	15.71

[a] Values are per cent of administered dose.

binding of fluorene occurs at the 9-position (=CH_2 group), although the effect may be purely a negative electrostatic influence of the C:O group. Later in this paper we will attempt to decide which effect is dominant.

The data for AAF and AAFone are given in Table III. At 12 hours following oral administration there seems to be only a slight difference in the percentages in the liver. Twenty-four hours after oral administration, however, there is almost twice as much AAF as AAFone in the liver (1.16/0.68). This is more dramatically shown in the 24-hour experiment after intraperitoneal injection (6) of the two compounds. Here the ratio in the liver is 13.46/3.75.

We find that AAF is eliminated to a somewhat greater extent in the urine than is AAFone, but this difference is not great enough to be significant. There are, however, much less of the nitro compounds eliminated in the 24-hour i.p. group, which is directly comparable to the 24-hour i.p.-AAF-AAFone group. It has been shown by Morris, Dubnik, and Johnson (4) and by Miller, *et al.* (5), that 2-nitrofluorene is carcinogenic but much less so than AAF, both in the number of tumors produced and the per cent of rats developing tumors. 2-Nitrofluorene, like 2-aminofluorenone, produces few, if any, liver tumors. It seems, therefore, that liver tumorigenesis is favored by the simultaneous presence of the amino and methylene groups. On this basis we predict that 2-nitrofluorenone would be still less carcinogenic than 2-nitrofluorene.

A comparison of the amounts of 2-nitrofluorene and 2-acetylaminofluorene excreted in the urine at the end of 24 hours after i.p. injection (Tables II and III) shows that 25% of the AAF is eliminated *versus* 8% of NF. Using N^{15}-labeled 2-NF (7) and the oral route of administration, Dyer (8) compared 2-NF with AAF and found that a total of 68% of NF material was excreted in urine *versus* 81% of AAF. We thus find the same order of elimination. This indicates that the amine is more readily metabolized than the nitro compound and suggests that reduction of the nitro group is a prerequisite to carcinogenesis (9).

Although a minimum concentration in a tissue such as liver seems necessary for activity, this is not, of course, the sole requisite. This is illustrated by the fact that the 24-hour i.p. animals given the nitro compounds showed a considerable concentration in the liver. This was in excess of the 24-hour i.p. animals with AAFone, but only about half that of AAF itself (Table III). Whether this indicates that the nitro compounds *would* produce liver tumors if administered *via* the intraperitoneal route remains to be demonstrated. That the i.p. route is effective in carcinogenesis of related compounds has been demonstrated by Morris, Wagner, Ray, Stewart, and Snell (6).

CONCLUSION

These experiments offer evidence in favor of our thesis that a carcinogen that attacks a particular organ has a certain affinity for that organ compared to a carcinogen that seldom if ever produces tumors in that organ. We find that 2-acetylaminofluorene has a greater affinity for the liver than 2-acetylaminofluorenone or 2-nitrofluorene, neither of which produces more than an occasional tumor in the liver.

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