SECTIONS

TOXICITY AND ANTIPYRETIC PROPERTIES OF SOME HALOGENATED ACETANILIDS.*

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Investigations of the past few years have begun to direct attention to further study of compounds of fluorine both chemically and physiologically. Inorganic fluorides both soluble and insoluble have, in spite of their high toxicity, been also investigated physiologically; thus, a 1 per cent solution of sodium fluoride has been tried in certain parathyroid conditions (1) and intravenous injections of calcium fluoride have been tried for decreasing the phosphorous in the urine (2).

G. Litzka (3) has studied the toxicity and antithyroideal activity of 3-fluorotyrosine in a series of papers. Henne (4), working with fluorinated hydrocarbons to be used as refrigerants, has shown that as the hydrogens of methane are successively replaced by fluorine beyond the introduction of the first fluorine atom, the stability of the compound increases and the toxicity decreases. Animals were able to exist in an artificial atmosphere of 80 per cent fluoroform and 20 per cent oxygen.

Vliet and Volweiler (5) have prepared some mercurated benzotrifluorides which are relatively non-toxic and are claimed to have antiseptic activity.

Lehmann (6) studied the effect of the introduction of fluorine into the side chain of five aromatic compounds and compared their activities with those of the parent hydrocarbons and with the analogous chloro-compounds. He found that the introduction of fluorine and chlorine in the side chain of tolulene and *m*-toluidine increased the toxicity of the compound over that of toluene, the fluorine more so than the chlorine. *m*-Trifluorotoluidine could be used as a narcotic for frogs.

The action of fluorobenzene, *p*-fluorotoluene and *p*-fluoroacetanilid was studied by K. Lang (7). He found that the fluorine was apparently not stored in the tissues of the body with the possible exception of some accumulation in the heart. The pharmacological action of the substances gave no evidence of a specific effect of fluorine, the compounds resembling in activity the corresponding non-halogenated compounds. The fate of fluorobenzene and *p*-fluoroacetanilid in the body could not be determined with certainty, the fluorine appearing in unidentified organic compounds, but *p*-fluorotoluene was found in the urine as *p*-fluorobenzoic acid.

Recently there has appeared an investigation of some pressor compounds containing fluorine. H. L. Hansen (8) reported the preparation of several chloro- and fluoro-adrenalones and found that, while both types of halogenated derivatives were far inferior to the parent substance, the fluoro-derivatives were less active than the corresponding chloro-derivatives. He made no statement of the relative toxicities of the compounds.

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In the present investigation, the antipyretic properties of *m*- and *p*-fluoroacetanilid and *p*-chloroacetanilid and the relative toxicities of acetanilid and *p*-fluoroacetanilid were studied. A review of the literature showed that the laboratory tests on the toxicity of acetanilid have been carried out using most of the common laboratory animals and the tests of antipyretic activity have been carried out on rabbits and white rats. The present writers have used cats ranging from 2.0 to 2.5 Kg. for the toxicity studies and from 1.5 to 3.0 Kg. for the tests of antipyretic activity.

The *m*- and *p*-fluoroacetanilids were prepared by the method of Schiemann and Pillarsky (9) and the *p*-chloroacetanilid from *p*-chloroaniline and acetic anhydride. All of the compounds were recrystallized and melted at the temperatures reported in the literature.

It was thought desirable to note whether or not there would be any effect on circulation and respiration when the fluoroacetanilid was given intravenously. For this purpose, the carotid blood pressure and respiration (by tracheal canula) were simultaneously recorded in the usual manner during the perfusion of solutions of either acetanilid or *p*-fluoroacetanilid (0.5 Gm. per 100 cc.) in 10 per cent alcohol into the femoral vein. It was necessary to use 10 per cent alcohol because of the low solubility of *p*-fluoroacetanilid in water.

It was found that after some solution had been run in, it was no longer necessary to administer ether. This was, presumably, the effect of the alcohol being given since the same was true for the control with 10 per cent alcohol alone. No very significant differences between the alcohol

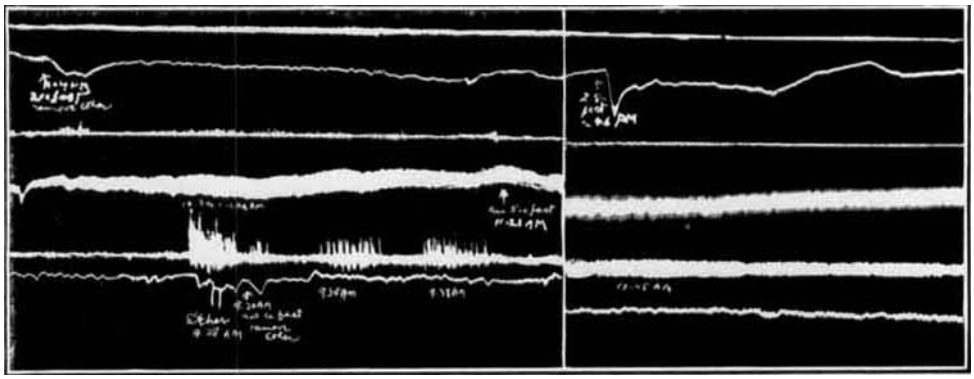

Fig. 1.—The upper tracing of each pair shows respiration, the lower shows carotid blood pressure (mercury manometer). The pairs reading from top to bottom are for *p*-fluoroacetanilid for 10 per cent alcohol alone and for acetanilid. Ether anesthesia. The first section was taken, 10 minutes after the beginning of the experiment and the second 65 minutes later.

and the alcoholic solutions of the acetanilids can be shown. However, after about 10 cc. of the 10 per cent alcohol had been run in, the pulse pressure of the cat had doubled and, after about 40 cc., had tripled the original value, while when either the solution of acetanilid or *p*-fluoroacetanilid was injected the pulse pressure remained unchanged or increased by about one-half its original value until close to death. The rapid injection of 5 cc. of 10 per cent alcohol caused only a slight fall in blood pressure whereas 3 cc. of the solutions of acetanilid or *p*-fluoroacetanilid produced sharp transient falls in blood pressure. These facts are offered for comparison with the report of L. F. Herz (10) who stated that acetanilid when injected intravenously in 35 per cent alcohol produced a tracing identical with that given by 35 per cent alcohol alone. Obviously the effect of the 35 per cent alcohol was sufficient to obscure any effect the acetanilid may have had. The tracings would seem to indicate that both acetanilid and *p*-fluoroacetanilid partly prevented the increase in pulse pressure normally caused by the alcohol.

In the experiments on acute toxicity, cats of both sexes in the weight range 2.0 to 2.5 Kg. were used. The calculated dose was given by capsule and the time recorded. Based on a number of observations, *p*-fluoroacetanilid appeared to show toxic symptoms (stiffening of the hind legs, loss of sense of balance) more rapidly than did acetanilid. With both compounds the animals died of respiratory failure. The dose affected liver function, the liver showing a peculiar mottling. In the advanced stages there was relaxation of the abdominal muscles and the animals lay

down breathing heavily. Near death, convulsions were sometimes observed. *p*-Fluoroacetanilid differentiated itself from acetanilid in that there was little or no dilatation of the pupil and little salivation. Both compounds produced cyanosis.

From the standpoint of the oral dose of acetanilid or *p*-fluoroacetanilid necessary to produce death, there is little from which to choose. Acetanilid regularly produced death (5 out of 5 cats) when given orally in capsules containing a dose of 0.25 Gm. per Kg. cat. At this same dose level, *p*-fluoroacetanilid caused death in 3 out of 6 cats while doses of 0.275 Gm. per Kg. were fatal in all cases. S. T. Helms (11) stated that he used all the usual laboratory animals and that doses of 1000 mg. acetanilid failed to produce death in any case.

To determine whether there was any difference in chronic toxicity, some cats were given daily doses of acetanilid and *p*-fluoroacetanilid by capsule. The daily administration of 0.03 Gm. per Kg. cat (one-eighth the dose of acetanilid found to be fatal) of acetanilid or *p*-fluoroacetanilid for 3 weeks produced very little noticeable effect. There was practically no loss in weight by either of the cats. The dose was doubled (one-fourth the fatal dose of acetanilid). After 5 more weeks neither cat showed very much change, a small weight loss having occurred, which, in the case of the cat receiving acetanilid, was slightly greater. The daily dose was raised to one-half

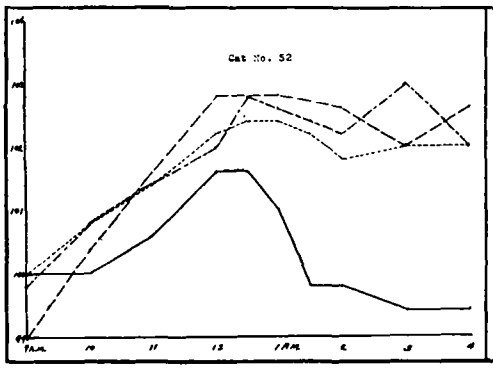

Fig. 2.

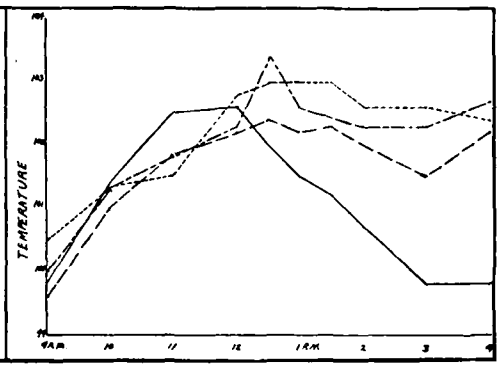

Fig. 3.

Fig. 2.—The course of the fever is shown by — — —, the effect of the dose of acetanilid by ———, the effect of the same dose of *p*-fluoroacetanilid . . . . . , and of double this dose by — - — - —.

Fig. 3.—The effect of the dose of acetanilid is shown by ———, the effect of the same dose of *p*-fluoroacetanilid by . . . . . , the effect of the same dose of *m*-fluoroacetanilid by — - — - —, and of *p*-chloroacetanilid by — — —.

the fatal dose of acetanilid when the decline in weight became more evident and the cats were definitely depressed at all times. Five days later the cat receiving the *p*-fluoroacetanilid died. The kidneys showed extensive hemorrhage, the liver the characteristic mottled appearance. The teeth showed no mottling. The cat receiving acetanilid lived for an additional 34 days. Its liver and kidneys showed similar effects. A pair of cats was started with a daily dose of either compound of one-half the fatal dose. The cat receiving *p*-fluoroacetanilid died after the second dose, the cat receiving acetanilid after the fifth dose. Another cat given the same daily dose of *p*-fluoroacetanilid died after the third dose.

The antipyretic activities of acetanilid and *p*-fluoroacetanilid were compared in the following manner. The cats were given an intraperitoneal injection of 10 cc. of a 5 per cent solution of dried egg albumin in normal saline solution at 9 A.M. The rectal temperature was taken before the injection and every hour until noon. At 12 noon a capsule containing the desired dose of acetanilid or *p*-fluoroacetanilid (0.025 Gm. per Kg. cat) was given orally. The rectal temperature was taken every half hour for 2 hours and then every hour for 2 hours. In all cases, the fall in temperature produced by acetanilid was quite marked while that by *p*-fluoroacetanilid was insignificant. To rule out any resistance of the cats to one or the other of the compounds, the doses of the drugs were reversed after a suitable rest period. Since the doses of *p*-fluoroacetanilid quite uniformly produced little or no fall in temperature, the dose in a series of trials was raised to 0.05 Gm. per

Kg. cat. This dose of *p*-fluoroacetanilid elicited light toxic symptoms in some of the cats but produced no greater fall in temperature than the original dose. Figure 2 shows the characteristic results obtained throughout.

In this connection it should be noted that it was often possible to use the same cat on successive days or sometimes on the second day after a previous use. However, if a week or more intervened between the trials on the cat, the results could not be relied upon. A reaction, apparently to the protein, made itself evident. To obviate this difficulty when the above procedure became impractical, the following modification was adopted. Five cats were injected with the 5 per cent solution of albumin and the temperatures recorded as before. At noon, capsules of *m*-fluoroacetanilid (0.025 Gm. per Kg.) were given to all cats. The average temperatures for the five cats were then plotted. The same procedure was also followed with *p*-chloroacetanilid. Figure 3 indicates the results obtained. For the purpose of comparison, the data obtained for acetanilid and *p*-fluoroacetanilid have been recalculated on the above basis and shown also.

DISCUSSION.

The slight difference in the acute toxicity of *p*-fluoroacetanilid as compared to acetanilid may be dependent on the lower degree of solubility of the former. The resulting slower absorption may also account for the more rapid death of cats receiving repeated large doses of *p*-fluoroacetanilid, the accumulation being greater. Apparently a certain degree of tolerance is established after repeated dosage as is evident from the fact that cats having received small doses of both compounds for a period withstood the dosage of 0.125 Gm. per Kg. for a longer time. Substitution of chlorine or fluorine in the nucleus very markedly reduces the antipyretic activity of acetanilid and, in the case of *p*-fluoroacetanilid, reduces the toxicity but little.

SUMMARY.

1. *p*-Fluoroacetanilid shows no greater acute toxicity for cats than does acetanilid.

2. *p*-or *m*-Fluoroacetanilid or *p*-chloroacetanilid show very little or no antipyretic activity.

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