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Changes in Fatty Acid and Cholesterol Synthesis in Rat Liver Slices due to Aromatic Amines formed During the Degradation of Some Herbicides

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Herbicides of the phenylurea, acylanilide and phenylcarbamate type are widely used in the cultivation of vegetables, fruits and grain. These compounds are relatively non-persistent (KAUFMAN and PLIMMER 1972) because each contains a -CO-NH-bond that can be hydrolyzed. Although certain soil bacteria can degrade some of the substituted anilines formed by hydrolysis (KEARNEY and KAUFMAN 1972), others are tightly bound by soil components and are rather persistent. In the case of 3,4-dichloraniline, which is formed from the herbicides propanil (BARTHA and PRAMER 1967) and diuron it could be demonstrated (BARHTA et al. 1968), that in the soil further reactions can occur to give azo compounds having structures related to known carcinogens.

Aniline and substituted anilines are rather lipophilic compounds and after entering a higher organism they may affect the metabolism and/or the composition of membranes. We have investigated the effect of some substituted anilines on the biosynthesis of fatty acids and of cholesterol in rat liver tissue, the main organ for the synthesis of these lipids.

MATERIALS AND METHODS

Aniline, 4-chloraniline, 3,4-dichloraniline and 2-aminoaniline were from Merck Darmstadt, 3-chloraniline, 4-chlor-3-nitroaniline and 3-amino-1,2,4-triazole from Fluka AG, Neu-Ulm. 1-^{14}C-acetic acid (sodium salt, 61 mCi/mM) was from Amersham-Buchler, Braunschweig; all biochemicals were purchased from Boehringer, Mannheim.
Female rats (Sprague Dawley), weighing 150-200 g, were from Gassner, Sulzfeld and were fed on a standard diet (Eggersmann, Rinteln). Eight rats were kept together in a cage for one week prior to use.
The liver was removed under ether anaesthesia, the organ was rinsed in an icecold NaCl solution (0,9%). Slices (about 0.5 mm thick) were cut with a razor blade, 510-550 mg liver slices from the same liver were used each for the experiment and for the control. The interval between cutting the slices and starting the incubation was always 10 min.

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The slices were incubated (according to NUMA et al.1961) in glass-stoppered tubes (35 mL), in a solution of 4.0 mL of Krebs Henseleit buffer, pH 7.2, and 10 µmoles of sodium acetate containing 8.5 x 10^5 cpm of 1 ^{14}C sodium acetate. The addition of cofactors such as NADP, Glucose-6-phosphate or ATP did not increase the synthesis of fatty acids or cholesterol. The amine (in concentrations up to 1.0 mg/mL incubation medium) was added where indicated. Test and control experiments were each performed in quadruplicate with rats from the same cage. Incubation was started by the addition of liver slices and was continued for 60 min at 37°C with vigorous shaking. The incubation was stopped by the addition of 1 mL of 10 M KOH, 1 mg of cholesterol (2 mg/mL ethanol) was added and the solution was saponified in a boiling water bath for 60 min.

To extract cholesterol (according to ENTENMAN 1957) after cooling 5 mL of water and 10 mL of ethanol were added and the solution was extracted three times with petrol ether (30 - 50°C). The combined petrol ether extracts were filtered and dried with anhydrous sodium sulfate. The petrol ether phase was evaporated in vacuo and the residue was dissolved in 2 mL of toluene scintillation liquid.

After extraction of cholesterol the aqueous phase was diluted with 70 mL of water, acidified with sulfuric acid and fatty acids were extracted with petrol ether as described for the extraction of cholesterol.

RESULTS AND DISCUSSION

Since after the in vivo administration of the amines (in the drinking water, in the food or after injection) the food intake of the animals was reduced indicating toxic effects of these compounds under in vivo conditions, the experiments were carried out with liver slices. Fatty acid synthesis as well as cholesterol synthesis are sensitive to the nutritional state of the animals and are reduced by fasting (NUMA et al.1961, ROMSOS and LEVEILLE 1957, HAMPRECHT 1969). Using liver slices, no such indirect effects can occur. As was shown previously (c. f. NUMA et al.1961) liver slices are well suited to investigate lipid biosynthesis from labeled acetate.

When rat liver slices were incubated with acetate as described in the Materials and Methods section, synthetic rates of 974±41 and 93±4 nmoles/g wet wt./h/37°C were obtained for fatty acids and cholesterol, respectively. As can be seen from Figure 1 and Table 1, the addition of aniline or monosubstituted anilines to the incubation medium stimulated fatty acid synthesis from acetate up to fourfold, monochloranilines were most effective. For comparison, 3-amino-1,2,4-triazole (herbicide) or tween 40 (detergent) were used, these compounds did not significantly alter the synthesis of fatty acids from acetate (Table 1). This indicates that the stimulatory effect appears to be specific for these monosubstituted anili-

nes. Disubstituted anilines had only a small stimulatory effect with low concentrations or were inhibitory (Fig.2).

TABLE 1

Synthesis of fatty acids (fa) and of cholesterol (chol) from ^{14}C-acetate in rat liver slices. For further experimental details see Materials and Methods.

Compound (0.5 mg/mL incub. med.)	ac → fa (% of control)	ac → chol
none (control)	100*	100**
3-methyl-aniline (from methiuron)	282	139
3-(trifluormethyl)-aniline (from Fluometuron)	288	96
2-amino-aniline (from Topsin, fungicide)	132	106
3-amino-1,2,4-triazole (amitrol, herbicide)	118	124
Tween 40	129	105

* 974±41 nmoles/g wet wt./h/37°C

** 93±4 nmoles/g wet wt./h/37°C

With the monosubstituted anilines cholesterol synthesis from acetate remained almost unchanged (Fig. 1, Table 1), while disubstituted anilines were inhibitory (Fig. 2). When ^{14}C-mevalonic acid instead of acetate was used as the precursor for cholesterol synthesis, the pattern of the curves (not shown) almost paralleled those of cholesterol synthesis from acetate.

These experiments clearly demonstrate that some anilines in rat liver slices stimulate the incorporation of labeled acetate into fatty acids. The concentration of the chloranilines in the incubation mixture giving the highest stimulatory effect (Fig. 1) was about 1-2 mmolar (0.5-1 mg/mL), but their concentration inside the cell as well as their mode of action is not known. The stimulatory effect of these compounds was obtained only when liver slices were used. Fatty acid synthesis in the cytoplasmatic fraction alone (NUMA et al. 1961), however, remained almost unchanged after the addition of the amines (MESSNER, unpublished results). This indicates that the amines may

affect the transport (permeability) of acetate into the liver cell leading to increased synthetic rates. Further, these compounds may directly inhibit the carnitine transport system involved in transferring the fatty acids from the cytoplasm into the mitochondria. This, in turn, would lead to decreased degradation rates and hence to increased radioactivity in the fatty acid fraction.

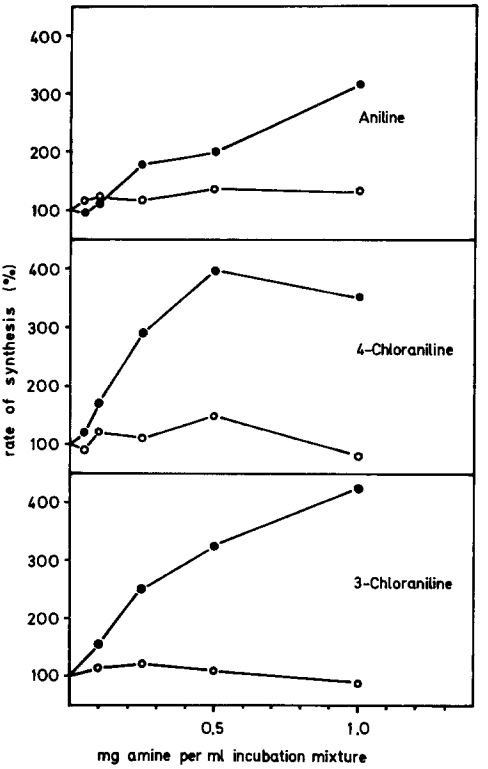

Fig. 1. Effect of aniline and chloraniline on the biosynthesis of fatty acids and cholesterol in rat liver slices. Rat liver slices were incubated with ^{14}C-acetate and the amine as indicated and the synthesis of fatty acids (●——●) and of cholesterol (o——o) was measured as described in Materials and Methods. Control experiments were without the amine and were set 100 percent (values see text). Each point is the average of four individual experiments.

On the other hand, cholesterol synthesis and most of the enzymes being involved in this process are bound to the endoplasmatic reticulum (DUGAN and PORTER 1976). Since the formation of cholesterol was not markedly affected

as compared to that of fatty acids (Table 1, Fig. 1), these compounds may be less effective on some intracellular particles and/or membranes. The mode of action of disubstituted amines differs from that of monosubstituted anilines, they were especially inhibitory on cholesterol synthesis (Fig. 2).

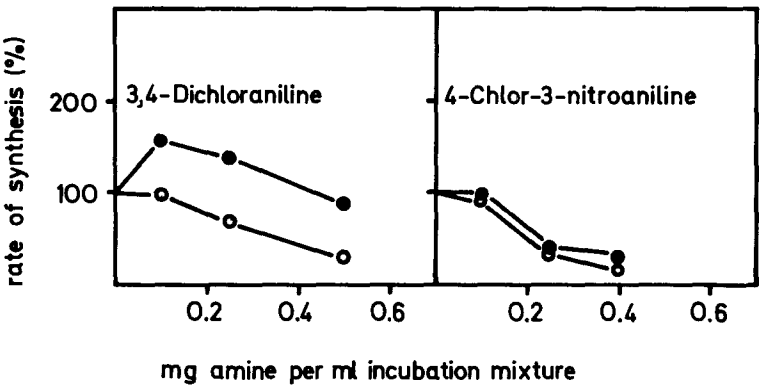

Fig. 2. Effect of disubstituted anilines on the biosynthesis of fatty acids and cholesterol in rat liver slices. Further details see legend to Figure 1.

While caution must always be applied in drawing conclusions about the in vivo situation from in vitro results, our results indicate that aromatic amines formed from some herbicides may severely alter the hepatic lipid metabolism.

REFERENCES

BARTHA, R., A. B. LINKE and D. PRAMER: Science 161, 582 (1968).

BARTHA, R. and D. PRAMER: Science 156, 1617 (1967).

DUGAN, E. R. and J. W. PORTER in: The Enzymes of Biological Membranes (A. MARTONOSI, ed.) Vol. 2, 161, J. Wiley and Sons, London (1976).

ENTENMAN, D.: Methods in Enzymol. 3, 299 (1957).

HAMPRECHT, B.: Naturwissenschaften 56, 398 (1969).

KAUFMAN, D. D. and J. R. PLIMMER in: Water Pollution Microbiology (R. MITCHELL, ed.) p. 173, John Wiley Inc., New York (1972).

KEARNEY, P. C. and D.D. KAUFMAN in: Degradation of Synthetic Organic Molecules in the Biosphere, p. 166, Printing and Publishing Office, National Academy of Sciences, Washington, D. C. (1972).

NUMA, S., M. MATSUHASHI and F. LYNEN: Biochem. Z. 334, 203 (1961).

ROMSOS, D. R. and G. A. LEVEILLE in: Modification of Lipid Metabolism (E. G. PERKINS and L. A. HING, eds.) p. 127, Academic Press, New York (1975).