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Deacylation of N^4-n-Acylsulfanilamides and N^4-n-Acylsulfanilylhydroxamides *in vitro*.*

MATHIAS F. F. KOHL AND LAURA M. FLYNN.
(Introduced by P. A. Shaffer.)

From the Departments of Biochemistry and Pharmacology, School of Medicine, Washington University, St. Louis, Mo.

The acyl derivatives of sulfanilamide are of interest because sulfanilamide is in part acetylated in the animal body before excretion,[1] the acetylated form having only slight therapeutic activity. Higher acyl derivatives have nevertheless been shown to possess therapeutic activity comparable to sulfanilamide. Miller, Rock and Moore[2] synthesized a series of N^4-n-acylsulfanilamides, the therapeutic activity of which appears to increase with the length of the acyl group, up to 6 carbons, beyond which it falls off rapidly. N^4-n-acylsulfanilylhydroxamides are found to possess therapeutic activity[3] and high bacteriostatic value *in vitro*.[4] The water solubility of these compounds decreases with lengthening of the carbon chain.

Aberhalden[5, 6] and Bergman[7-9] described a group of enzymes known as acylases which split acylated amino acids. More recently Michel, Bernheim and Bernheim[10] have described an acylase which splits acetanilid. This enzyme, which they believe identical with the earlier described acylase, is found in high concentrations in liver and kidney of dog, cat, rabbit, ox and mouse.

We have studied the deacylation *in vitro* by rat liver of N^4-n-acylsulfanilamides and the analogous N^4-n-acylsulfanilylhydroxamides in which the NH_2-group of the sulfonamide is replaced by an

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1 Harris, J. S., and Klein, J. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 78.

2 Miller, E., Rock, H. J., and Moore, M. L., *J. Am. Chem. Soc.*, 1939, **61**, 1198.

3 Cooper, F. B., Gross, P., and Lewis, M., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 491.

4 Main, E. R., Shinn, L. E., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 593.

5 Aberhalden, E., and Ehrenwall, E., *Fermentforsch.*, 1931, **12**, 223, 376.

6 Aberhalden, E., and Heumann, J., *Fermentforsch.*, 1931, **12**, 572.

7 Bergman, M., Zervas, L., and Fruton, J. S., *J. Biol. Chem.*, 1935, **111**, 225.

8 Bergman, M., Zerbas, L., and Ross, W. F., *J. Biol. Chem.*, 1935, **111**, 245.

9 Bergman, M., and Ross, W. F., *J. Biol. Chem.*, 1935, **111**, 659.

10 Michel, H. O., Bernheim, F., and Bernheim, M. L. C., *J. Pharmacol. Exp. Therap.*, 1937, **61**, 321.

–NHOH group. On the hypothesis that a free N^4-amino group is essential for activity it seemed important to learn the extent to which these therapeutically active acyl compounds are deacylated in the animal body. Since the analytical method available (determination of free and total sulfanilamide after hydrolysis) does not distinguish other acyl compounds from the acetyl compound appearing in urine, it seemed preferable to study the hydrolysis by tissue brei with which acetylation does not occur to confuse the results.

Methods. Extracts of liver tissue made by grinding liver with sand and an equal volume of water were strained through cheese cloth. Weights of the acyl compounds equivalent to 1 mg of unacylated compound were added to 20 cc of liver brei representing 5 g of liver in M/20 PO_4 buffer (pH 7.5). Two drops of toluene were added as a preservative. The mixtures were shaken at 37.5°C. Aliquot samples were removed at intervals for analysis. Proteins were removed with 10% trichloracetic acid (or by alcohol) and filtrates analyzed for free and total sulfanilamide by Marshall's method. Colorimetric determinations were made with an electrophotometer. Percentage of hydrolysis was calculated from the free amine found.

Comparative Hydrolysis of Sulfanilamide Derivatives. We find that the ease and rate of hydrolysis of these acyl sulfanilamide derivatives by liver brei vary with the length of the C-chain in the fatty acid. Acetyl sulfanilamide is decomposed only slowly while long acyl groups are broken off more easily. The ease of hydrolysis parallels the therapeutic activity of these compounds (reported from other laboratories[2, 3]) which permits the view that the acyl derivatives become active after hydrolysis.

Similar experiments were performed using as substrates acetanilid and 4:4′-acetylamino diphenyl sulfone. Using the same samples of liver brei in tests with the three substrates, acetanilid consistently showed a percentage of hydrolysis higher than that of 4:4′-acetylamino diphenyl sulfone and acetylsulfanilamide.

Tables I, II and III represent the results of typical experiments with N^4-acyl sulfanilamides, N^4-n-acylsulfanilylhydroxamides, acetanilid and 4:4′-acetylamino diphenyl sulfone as substrates.

In vivo deacylation of acylated sulfanilamide derivatives has been shown by others from blood and urine analyses. Nitti, Bovet and Hamon[11] found that the formyl, acetyl, propionyl and butyryl derivatives of 4:4′-diamino diphenyl sulfone were rapidly hydrolyzed in the body to 4:4′-diamino diphenyl sulfone. Cooper, Gross and

[11] Nitti, F., Bovet, D., and Hamon, Y., *Compt. rend. soc. biol.*, 1938, **128**, 26.

TABLE I.
Deacylation of Acylsulfanilamides† by Liver Suspension.

Substrate	% hydrolysis 2 hr	5 hr	8 hr
Acetylsulfanilamide	9.1	9.1	8.1
Butyrylsulfanilamide	14.1	27.8	30.4
Valerylsulfanilamide	21.7	24.0	25.0
Caproylsulfanilamide	38.3	41.0	41.9
Heptanoylsulfanilamide	63.9	80.1	89.6

TABLE II.
Deacylation of Acylsulfanilylhydroxamides† by Liver Suspension.

Substrate	% hydrolysis 2 hr	4 hr	6.5 hr
Acetylsulfanilylhydroxamide	6.6	8.1	9.6
Valerylsulfanilylhydroxamide	11.5	15.9	22.7
Caproylsulfanilylhydroxamide	21.8	28.8	41.3
Heptanoylsulfaniiylhydroxamide	43.1	62.9	74.5

TABLE III.
Deacylation of Other Compounds by Liver Suspension.

Substrate	% hydrolysis 4 hr	8 hr
Acetylsulfanilamide‡	5.4	9.4
4:4′-Acetylaminodiphenylsulfone‡	10.6	15.0
Acetanilid	58.0	72.0

† These acyl compounds were synthesized and presented to us through the courtesy of Sharp and Dohme, Technical Division, Glenolden, Penn.

‡ These compounds were synthesized and presented to us through the courtesy of Monsanto Chemical Company, St. Louis, Mo.

Lewis[8] in their recent study of N^4-n-acylsulfanilylhydroxamides found that mice given 50 mg oral doses of the valeryl, caproyl and heptanoyl compounds showed approximately 10 mg % of diazotizable material (calculated as sulfanilamide) in the blood 2 hours later.

Conclusions. The ease of deacylation of N^4-n-acylsulfanilamides and N^4-n-acylsulfanilylhydroxamides *in vitro* by liver brei is found to increase with the length of the acyl group.