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Toxicity of Acetates for Rats.

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In investigations of the intermediary metabolism of acetate using the heavy carbon isotope as a tracer element it became necessary to know what acetate doses could be tolerated by rats for a two-week period. While a number of investigators, including Deuel and Milhorat,[1] Taistra,[2] Daken,[3] Lusk,[4] McKay, Barnes, Carne and Wick,[5] Lamb and Evvard,[6] have given what may be considered massive doses of acetates without lethal effects, there are few reports of lethal effects produced by acetates. Recently Woodward, Lange, Nelson and Calvery[7] determined the acute toxicity of sodium acetate for rats and found a minimum lethal dose of 55 millimols per kg. Sollman[8] added acetic acid to the drinking water of rats and found that with an intake of less than 3.5 millimols per kg per day from drinking water of 0.25% acetic acid there were no significant disturbances of weight or appetite. Higher dosages reduced both. There is no information in the literature on toxic doses administered over time periods of 7 to 14 days. Nor is there information on relative toxicities of acid, sodium and ammonium salts. For metabolic investigation this information is necessary.

Experimental. Female rats usually in the weight range of 170-200 g were used. They were raised on a stock diet of casein 15, whole milk powder 10, NaCl 0.8, $CaCO_3$ 1.5, butter 5.2, whole ground wheat 67.5, cod liver oil 1.0. The rats were allowed free access to this diet and water throughout the experiments. During the experimental period they were given by stomach tube a 1.0 N solution of the acetates twice a day with doses 8 hr apart. Daily weighings were made and the physical condition of the animals noted.

Results. The summarized results are given in Table I. Data for each rat are given in column 2 where the daily dosage in millimols per kg is given in parentheses followed by the number of days survival for each rat. In the right hand column of the table there is calculated the percentage of the total metabolic rate contributed by acetate combustion assuming a normal metabolic rate of 30 calories per day per 200 g rat.

The results of the table indicate that a dosage of 30 millimols per kg of acetic acid can be tolerated for a 2-week period while 40 millimols per kg day will cause death in 3 to 5 days usually. Sodium acetate is considerably less toxic, the maximum tolerable dose being 70 to 80 millimols per kg per day. Ammonium acetate of 60 millimols per kg day is the maximum dosage which can be tolerated by rats for a 2-week period.

Discussion. It is to be noted that when high doses were used the mortality was high on the second, third or fourth day. If the animal could survive the period there was a good possibility of surviving the 14-day period. For investigations on metabolism of acetates using heavy carbon the procedure is recommended of starting with a high dosage of ordinary acetate for 7 days before using the rare material on the survivors.

Deuel and Milhorat[1] and MacKay,

1 Deuel, H. J., and Milhorat, A. T., *J. Biol. Chem.*, 1928, **78**, 299.

2 Taistra, Sophie A., *J. Biol. Chem.*, 1921, **49**, 479.

3 Dakin, H. D., *J. Biol. Chem.*, 1907, **3**, 57.

4 Lusk, Graham, *J. Biol. Chem.*, 1921, **49**, 453.

5 MacKay, E. M., Barnes, R. H., Carne, H. O., and Wick, A. N., *J. Biol. Chem.*, 1940, **135**, 157.

6 Lamb, A. R., and Evvard, J. M., *J. Biol. Chem.*, 1919, **37**, 329.

7 Woodward, G., Lange, S. W., Nelson, K. W., and Calvery, H. O., *J. Ind. Hyg. Tox.*, 1941, **23**, 78.

8 Sollman, Torald, *J. Pharm. Exp. Therap.*, 1920-21, **16**, 463.

TABLE I.
Survival Times and Weight Changes of Rats Receiving High Dosages of Acetate.

Substance fed	(Daily dosage millimols per kg), No. of days survival	% metabolic rate due to acetate combustion
Acetic Acid	(30) 14, 14, 14; (40) 2, 2, 3, 3, 3, 3, 4, 4, 5, 5, 14, 14; (50) 2, 2, 3, 3, 3, 3, 4, 5, 5, 5, 6, 6; (60) 2, 2, 2, 2	4.2 to 8.4
Sodium Acetate	(30) 4, 14, 14; (40) 8, 13, 14; (50) 14, 14, 14; (60) 14, 14, 14; (70) 14, 14, 14; (80) 2, 14, 14; (90) 1, 3, 7, 14, 14; (100) 1, 2, 2, 14; (110) 1	4.2 to 15.4
Ammonium Acetate	(30) 14, 14, 14; (40) 8, 14, 14; (50) 4, 14, 14; (60) 1, 14, 14; (70) 1, 1, 4	4.2 to 9.7
$NaHCO_3$	(100) 14, 14, 14; (150) 1, 1, 1	
Water	14 (volume equal to largest dosage)	

Barnes, Carne and Wick[5] have reported gastrointestinal irritation with acetic acid and have used preferably sodium acetate. A difficulty in the use of the sodium salt is that excess sodium remains after combustion of the acetate radical and alkalosis develops. Alkalosis, however, is not the toxic factor since a dosage of 100 millimols per kg-day of NaOAc produced death in 1 to 2 days of 3 of a group of 4 rats while 100 millimols per kg-day of $NaHCO_3$ produced no fatalities in a group of 3 rats in 14 days. Alkalosis due to the sodium salt can be prevented by using the free acid or the ammonium salt. These two acetates while not producing alkalosis are both irritant to the alimentary tract and probably produce their toxic symptoms by injury to this organ.

When sodium acetate is given in 2 daily doses 70-80 millimols per day can be tolerated for 14 days. The total daily tolerable dose exceeds the single toxic dose of 55 millimols as found by Woodward, Lange, Nelson and Calvery.[7]

The main toxic symptoms observed just before death were loss of weight, blistering of the paws, and reddish appearance of nose and whiskers.

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Isolation of *Staphylococcus albus* from Hemolymph of the Roach, *Blatta orientalis.*

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Previous examinations of hemolymph from the oriental roach, *Blatta orientalis,* have demonstrated that bacterial infections of the body fluid are associated with high total hemocyte counts[1] and high percentages of mitotically dividing hemolymph cells.[2] Since it is known that some insect leucocytes are phagocytic, the above responses may be interpreted as a mechanism whereby the numbers of hemocytes are increased for defense purposes. Thus a larger number of ingesting phagocytes would be available for combatting the invading organism. In addition, other symptoms indicated that presence of

1 Tauber, Oscar E., and Yeager, J. F., *Ann. Ent. Soc. Am.,* 1935, **28**, 229.

2 Tauber, Oscar E., *Ann. Ent. Soc. Am.,* 1940, **33**, 113.