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Free Volatile Acidity of Blood and Tissues Following Ingestion of Ethyl Alcohol.*

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Is the acidosis of alcoholic intoxication due to the accumulation of acetic acid as well as the production of abnormal quantities of lactic acid?[1] The presence of acetic has never been demonstrated. Analyses of tissues by means of the writer's recently published procedure for the determination of minute quantities of volatile acids[2] appeared to answer the question affirmatively. However, a subsequent study of the analytical method, as applied to tissues, has shown that a substantial part of the acid so determined is derived by hydrolysis of the "bound" volatile acids by the reagents during the steam distillation. By carrying out the steam distillation with a minimum of phosphoric acid and tungstate, it was found that normal tissues contain only traces of *free* volatile acid and that the latter are not apparently increased after ingestion of ethyl alcohol.

Analytical Procedure. The sample† is frozen and crushed by the method of Graeser, Ginsberg and Friedemann.[3] It is immediately swept into a small aluminum scoop; it is rapidly weighed. It is then transferred to the steam distillation flask, after which the scoop is again weighed. Analysis of 10 to 15 g samples is recommended. Fifteen cc of phosphoric acid solution,‡ 15 cc of 10% Na_2WO_4 and 10 cc of distilled water are then added. The flask is closed by means of a soft rubber stopper and the contents are mixed by violent shaking. The steam distillation and redistillation from acid-$MgSO_4$-HgO are carried out as already described. The dis-

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1 Himwich, H. E., Nahum, L. H., Rakieten, N., Fazikas, J. F., DuBois, D., and Gildea, E. F., *J. Am. Med. Assn.*, 1933, **100**, 651.

2 Friedemann, T. E., *J. Biol. Chem.*, 1938, **123**, 161.

† In this laboratory all animals are anesthetized by means of pentobarbital sodium (Nembutal). Tissues are removed as soon as possible after the full effect of the anesthetic is noted. The anesthetic necessary for this work was donated by the Abbott Laboratories, North Chicago, Illinois.

3 Graeser, J. B., Ginsberg, J. E., and Friedemann, T. E., *J. Biol. Chem.*, 1934, **104**, 149.

‡ 55 cc of syrupy, 85% phosphoric acid are diluted to a volume of 1,000 cc.

tillate is aerated *15 minutes* by means of a rapid stream of CO_2-free air. Three drops of a 1% alcoholic solution of phenolphthalein are added. The acid is titrated by means of 0.01 *N* NaOH.

Free volatile acids of normal tissues. Representative data from 2 normal dogs are shown in Table I. The blanks in Expt. 1 required 0.57, 0.52, 0.58, 0.50 cc 0.01 *N* NaOH. The volatile acids from 10 to 15 g of tissue, with the exception of brain, required only a few drops more than the blank. The following are representative titrations of duplicate samples: 10 cc of blood required 0.60 and 0.58 cc of 0.01 *N* NaOH; 17.0 and 15.5 g of skeletal muscle, 0.59, 0.62; 16.5 and 15.5 g of liver, 0.70, 0.66; 17.0 and 15.5 g of kidney, 0.74, 0.90; 12.9 and 13.9 g of brain, 2.90, 2.90. Note that the variations in the titration of duplicates are within the errors of titration of the blanks. It should be noted further that these low results were obtained by the direct distillation of the tissue with steam. Very slightly lower results are obtained when protein-free filtrates are distilled.

Similar small yields of volatile acids have been obtained from tissues of other animals. The results vary slightly in each animal, but they agree, within the limit of error of the method.

In a few experiments, tissues were kept 30 minutes at room temperature before freezing in liquid nitrogen. An increase of volatile acidity was noted in most instances. In Exp. 3, for example, the volatile acidities of the immediately frozen samples and of the samples which were frozen after 30 minutes of incubation at room temperature, were as follows: skeletal muscle, 0.04, 0.07; heart muscle, 0.05, 0.24; liver, 0.08, 0.25; kidney, 0.24, 0.31; brain, 1.65, 2.01.

TABLE I.

Free Volatile Acids of Tissues.

Cc *N* or mM of free volatile acid per kg of tissue. The results also represent the cc of 0.01 *N* NaOH (minus the blank) required to titrate the volatile acids from 10 g of tissue.

	Normal animal		Normal animal after ethyl alcohol ingestion
	Exp. 1	Exp. 2	Exp. 3
Blood	0.05	0.12	0.05
Muscle, skeletal	.04	.04	.03
" heart	.11	.05	0
" stomach	.15	.11	.16
" diaphragm	.10		
Spleen	.03		
Liver	.09	.08	.15
Kidney	.17	.24	.19
Testicle	.08		
Brain	1.77	1.65	1.58

Free volatile acids following the ingestion of alcohol. 4.0 g of ethyl alcohol per kilo of body weight were given by stomach tube to a fasting normal male dog which weighed 29 kg; 150 cc of 96% "grain" alcohol were dissolved in 400 cc of water. One-half, or 200 cc of solution, was given at 8:45 A.M.; the other half was given at 9:20 A.M. Although the animal was greatly under the influence of the alcohol, it was conscious up to 10 A.M., at which time the anesthetic was given. The venous blood at this time contained 560 mg of alcohol per 100 cc. The results are shown in the last column of the table. The volatile acidity of all tissues, within the limit of experimental error, was the same as in the normal animals.

The writer's (unpublished) study of the volatile acids of tissues has shown that large quantities of these acids can be obtained by hydrolizing tissues with a 2 *N* H_2SO_4 for a period of about 6 hours. Acetic acid constitutes at least 95% of the volatile acids so obtained. It is not apparently obtained from the fat. The rate of liberation of this "bound" volatile acid parallels the rate of hydrolysis of the polysaccharide (total hydrolizable reducing substances-free sugar—glycogen). It is, therefore, most likely present in tissue as an acetyl radical attached to the polysaccharide; the polysaccharide, in turn, is combined with protein. The total volatile (acetic) acid content of tissues expressed as mM or cc *N* per kilo is approximately as follows: blood, 4 to 5; skeletal muscle, 4 to 5; liver, 6 to 8; kidney, 8 to 10; brain, 18 to 20. By comparing these data with those shown in the table, it is evident that the free acids constitute only about 1 to 3% of the total volatile acids of the tissue. Brain tissue is an exception; it apparently contains considerable quantities of free volatile acids and large quantities of easily hydrolyzed esters. Its content of total volatile acids also is unusually large.

The intermediary production of acetic acid and other volatile acids from the aerobic metabolism of carbohydrate and sugar metabolites, fats, and amino-acids by tissue slices in the Warburg apparatus has been abundantly demonstrated by the work of many investigators. Tissues, especially liver tissue, contain alcoholases.[4] The oxidation of alcohol by tissue slices, especially of liver, readily yields acetic acid.[5] Since these acids are formed in considerable quantity in the intermediary metabolism, why are they found free in such small concentration in tissue? The answer is that acetic acid is burned with great rapidity (author's unpublished data); it is removed as rapidly as it is formed in the intact normal animal. The maximum rate of

[4] Reichel, L., and Köhle, H., *Z. physiol. Chem.*, 1935, **236**, 158.

[5] Leloir, L. F., and Muñoz, J. M., *Biochem. J.*, 1938, **32**, 299.

oxidation of ethyl alcohol (about 4.5 mM per kilo per hour) is less than one-half that of the maximum rate of oxidation of sugar in the normal animal. The rate of acid production, assuming that all of the alcohol (4.5 mM) is converted into acetic acid, is relatively small and is well within the capacity of the animal to remove all of the acid by oxidation.

Summary. 1. A procedure is described for the determination of the free volatile acids of tissues. 2. Normal tissues of the dog, with the exception of brain, contain only traces, from 0 to 0.25 mM or cc *N*, of free volatile acidity per kg. This constitutes from 1 to 3% of the total volatile acids which can be obtained by hydrolysis with 2 *N* H_2SO_4. About 10%, from 1.5 to 2.0 mM, of all of the volatile acids of the brain are present either as free acid or in some form which is readily hydrolized. 3. The metabolism of ethyl alcohol in the dog does not result in an increase of the free volatile acids of the blood and tissues.

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Evidence of Local Protection Against Infection with Type I Pneumococcus.*

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The production of local protection or "local immunity" to microorganismal infection was first fully described by Wassermann and Citron[1] and interpreted by them as an *"Umstimmung"* or "retuning" of the local cells. This phenomenon has subsequently been observed but differently explained, especially by Besredka,[2] Gay,[3] and Opie.[4]

Bull and McKee[5] and recently Walsh and Cannon[6] demonstrated in rabbits a definite specific resistance of the upper respiratory tract to pneumococcal infection subsequent to specific local intranasal instilla-

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1 Wassermann, A., and Citron, J., *Z. f. Hyg. u. Infectionskr.*, 1905, **50**, 331.

2 Besredka, A., *Compt. Rend. Soc. de Biol.*, 1923, **88**, 1273.

3 Gay, F. P., *The Newer Knowledge of Bacteriology and Immunology*, Jordan, E. O., and Falk, J. S., University of Chicago Press, 1928, 881.

4 Opie, E. L., *J. Immunol.*, 1929, **17**, 329.

5 Bull, Carroll G., and McKee, C. M., *Am. J. Hyg.*, 1929, **9**, 490.

6 Walsh, T. E., and Cannon, P. R., *J. Immunol.*, 1936, **31**, 331.