A Note on the Distribution of β-Alanine

JAY R. SCHENCK.* (Introduced by V. du Vigneaud.)

From the Department of Biochemistry, Cornell University Medical College, New York City.

Previous investigations of the distribution of β-alanine in various natural sources have been limited by the lack of sensitivity of the methods of analysis used. With the development of the diphtheria bacilli growth assay of β-alanine,[1] a method which is sufficiently sensitive to detect 5 γ of the compound, it became possible to reinvestigate the problem more satisfactorily.

Kaplansky[2] used the method of Abderhalden and Fodor[3] for the detection of β-alanine to show that β-alanine was not present in muscle proteins. The method consisted of esterification of the protein hydrolysate and subsequent distillation of the esters. The characteristic odor of the acrylic ester formed by decomposition of the β-alanine ester was used for detection of β-alanine. By this method Kaplansky was able to find no β-alanine in extracted muscle, although some β-alanine not detected by this method was undoubtedly present in the hydrolysate since we have found that three extractions are not sufficient to remove all the β-alanine from muscle.

In the present study, a number of pure proteins, namely, zein, alpha wheat globulin, gliadin, canteloupe seed globulin, Bence-Jones protein, edestin (hemp seed), silk fibroin, egg albumin, and insulin were assayed and found to contain no β-alanine. Recently Pollack[4] has found by assaying with a yeast-growth method that silk fibroin, horse hemoglobin, egg albumin, gelatin, casein, and lactoglobulin were substantially free from β-alanine. It may be concluded that β-alanine is not a general constituent of proteins.†

Many attempts to isolate and estimate carnosine from various tissues may be found in the literature. Fewer attempts have been made to isolate anserine. By separating the tissues and assaying them individually for β-alanine, it would be possible to discover any considerable store of anserine or other β-alanine-containing compound. Table I shows the results of β-alanine assays of tissues obtained from a rat. Both the concentration and total amount of β-alanine are included. The liver and muscle tissues account for 90% of the total β-alanine and it is apparent that no large store of β-alanine compound exists in other tissues.

TABLE I.
Distribution of β-Alanine in the Rat.
(70 g female.)

	Mg per 100 g moist tissue	Mg in entire organ
Skeletal Muscle	25	4.1
Liver	26	0.9
Bones	0.8	0.04
Brain and Spinal Cord	2	0.03
Skin, Hair and Fat	0.6	0.08
Heart	<7	<0.02
Viscera (except liver)	2	0.27

Hydrolysis increases the activity of muscle extracts due to the liberation of free β-alanine which is more active than carnosine by the growth test. However, no such increase is observed upon hydrolysis of liver

* Abbott Laboratories Research Fellow.

1 Schenck, J. R., *J. Biol. Chem.*, in press.

2 Kaplansky, S., *Z. physiol. Chem.*, 1926, **158**, 19.

3 Abderhalden, E., and Fodor, A., *Z. physiol. Chem.*, 1913, **85**, 112.

4 Pollack, M. A., *J. Am. Chem. Soc.*, 1943, **65**, 484.

† A preparation of phaseolin contained 0.07% β-alanine, but after some purification, the assay indicated only 0.02%; this amount was not further reduced by dialysis. The molecular weight of phaseolin is not known, but a molecular weight of 500,000 would be required by this low β-alanine content. It is more reasonable to consider the β-alanine as an impurity.

extracts. Smorodinzew[5] was unable to isolate carnosine from liver extracts. Furthermore, liver extracts were found to be more resistant to nitrous acid than solutions of β-alanine, carnosine, or muscle extracts, indicating that the amino group of β-alanine of the liver is protected. In view of these observations, the presence of much carnosine or anserine in liver is highly unlikely and the entire β-alanine content is probably in the form of pantothenic acid. The average values reported here are higher than the pantothenic acid assays reported by Strong, Feeney, and Earle,[6] and by Rohrman, Burget, and Williams,[7] but comparable to those of Williams and coworkers.[8,9]

Assays for β-alanine of tissues from the rat and the cat are presented in Table II. From this table it is apparent that there is considerable difference in the β-alanine content of different tissues, but that a specific tissue of one species has a fairly uniform β-alanine content in a number of individual animals. For example, in the rat, the gastrocnemius muscle contains about 100 mg % β-alanine, whereas the pectoralis minor contains about 5 mg %. The gastrocnemius muscle of the cat is about 4 times as rich in β-alanine as the corresponding tissue of the rat. Liver and kidney have a much lower β-alanine content than most muscle tissue.

TABLE II.
β-Alanine Content of Hydrolyzed Tissue.
(Mg per 100 g of moist tissue.)

Rat No.	Muscle: Gastrocnemius	Muscle: Pectoralis Minor	Liver	Kidney
105	—	—	28	5
118	—	—	26	—
130	—	—	19	5
162	100	6	< 2	—
196	105	—	15	<2
228	85	4	23	5
230	60	—	21	6
231	90	6	9	1
232	75	8	29	—
233	108	—	—	<1
235	—	—	—	5
239	95	4	—	<1
240	90	—	< 4	—
241	115	—	6	—
242	127	—	5	—

Cat No.	Gastrocnemius Muscle	Liver
III 41	—	25
III 50	650	—
IV 74	400	—
IV 95	350	—
IV 103	310*	—
V 52	—	9
V 75	500	6

* Autolyzed before hydrolysis.

Summary. The diphtheria bacilli growth test for β-alanine has been applied to a number of proteins and animal tissues. Skeletal muscle and liver were shown to be the richest animal sources of β-alanine, and β-alanine was shown to be absent from a number of purified proteins.

5 Smorodinzew, J., *Z. physiol. Chem.*, 1914, **92**, 214.

6 Strong, F. M., Feeney, R. E., and Earle, A., *Ind. Eng. Chem.*, Anal. Ed., 1941, **13**, 566.

7 Rohrman, E., Burget, G. E., and Williams, R. J., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 473.

8 Wright, L. D., McMahan, J. R., Cheldelin, V. H., Taylor, A., Snell, E. E., and Williams, R. J., *The University of Texas Publication*, No. 4137, 1941, 38.

9 Mitchell, H. K., and Isbell, E. R., *The University of Texas Publication*, No. 4237, 1942, 37.