On the Occurrence of Biotin in Different Fractions of Municipal Sewage

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The microbial activities which take place in sewage and wastes result in a considerable synthesis of a number of vitamins [1]. The occurrence of vitamin B_{12} and its analogues in municipal sewage sludge has been extensively studied in this laboratory [2] and by others [3,4]. It has also been found in a previous study [5,6] that certain fractions of sewage can satisfy the growth requirements of *Propionibacterium shermannii*, an organism which is known to require biotin for its growth. The occurrence of this vitamin in different fractions of municipal sewage was therefore studied. Biotin occurs in animal and plant tissues mainly in combined forms. Vigorous acid hydrolysis is necessary to release the vitamin from such forms before a microbiological assay can be made. The sewage fractions studied in this investigation were assayed for biotin activity both before and after acid hydrolysis in order to investigate the "free" forms of the vitamin which may possibly occur.

Experimental. The sewage fractions investigated were those previously studied in experiments with *Mb. omelianskii* [7] and *Propionibacterium shermannii* [5]. They are listed in Table 1. The pretreatment of the samples for the biotin determination was as follows.

Hydrolyzed samples: 50 ml sample + 10 ml 18 N sulphuric acid were autoclaved for 30 min at 121°C. 7.2 g NaOH was dissolved in each sample, 2 ml 2.5 M NaAc solution added, the pH adjusted to 4.5 and the volume to 75 ml. The samples were centrifuged for 20 min, 25 ml of the supernatant was removed, its pH adjusted to 6.8 and the resulting precipitate centrifuged off. The final supernatants were stored at −20°C until being assayed for biotin activity.

Non-hydrolyzed samples: The non-hydrolyzed samples were treated in a similar way, water being substituted for sulphuric acid and room temperature for autoclaving. All biotin values obtained were corrected for the volume changes due to pH adjustments, etc.

Biotin was determined using *Lb. arabinosus 17—5* in the turbidimetric assay method (Difco's Biotin Assay Medium). The results obtained are given in Table 1.

In Table 2, the results of experiments concerning the recovery of added biotin are recorded. The samples with added biotin were treated similarly and assayed simultaneously with the samples without added biotin in order to check the reliability of the method. In certain experiments, the samples were subjected to paper chromatography using the *sec.* butanol: water:

Table 1. Biotin content of different fractions of municipal sewage.

Sewage fraction [a]	Dry solids content [b] g/l	Biotin content μg/l [c] Non-hydrolyzed samples	Hydrolyzed samples [d]	Average biotin content μg/g dry solids
A	20	5	20	1.0
B	20	30	30	1.5
C	100	50	50	0.5
D	3.0	30	30	10.0
E	15	0	10	0.7
F	≤1	not determined	0.3	
G	≤1	» »	0.3	
H	≤1	» »	0.2	

[a]) A, raw sludge; B, sludge after one-step digestion; C, digested sludge; D, supernatant from digestion tanks; E, activated sludge; F incoming sewage; G, supernatant from sedimentation tanks; H, effluent from activated sludge tanks (= highly purified sewage).

[b]) average values of several determinations.

[c]) average values of six determinations by the turbidimetric method using *L. arabinosus* 17—5 and Difco's Biotin Assay Medium.

[d]) 3 N H_2SO_4, 30 min at 121°C.

Table 2. Typical biotin assay results using internal biotin standards.

Sewage fraction [a]	Biotin added mμg/ml	Biotin content found μg/l Non-hydrolyzed samples	Hydrolyzed samples	Recovery % Non-hydrolyzed samples	Hydrolyzed samples
C	no	47.5	49.3		
C	20	72.5	74.1	125	124
D	no	32.5	33.2		
D	20	53.0	56.2	102	111
E	no	0	9.3		
E	20	16.9	24.5	85	76

[a] *cf.* explanations to Table 1.

acetic acid system (75:25:1). The spots were located by incubating pieces of the chromatograms with the assay medium.

Results and discussion. It can be seen in Table 1 that most of the sewage fractions investigated contained considerable amounts of biotin, *viz.* 10—50 μg/l. Exceptions are of course the very dilute or purified fractions F, G, and H. In raw sewage (fraction A), about 75 % of the vitamin content occurs in a bound form. The values in Table 1 indicate that practically all of the vitamin in sewage fractions which have undergone methane fermentation occurs in a form directly available to the test organism. Further, digested sludge (sediment) contains only 0.5 μg biotin/g dry solids whereas the corresponding value for the supernatant from digestion tanks is as high as 10 μg/g. This strengthens the impression that biotin is released from the bacterial cells as the methane fermentation proceeds and the great difference between the two figures makes it hardly possible to explain this release by mere autolysis.

Activated sludge which is a product of aerobic fermentation differs markedly from the digested sludges and also to a great extent from raw sludge. All biotin in activated sludge is bound and has to be released by vigorous hydrolysis in order to become available to the test organism.

Table 2 shows recovery values for biotin added to certain sewage fractions assayed by the method used in this investigation. It can be seen in Table 2 that the recovery values varied between 76 and 125 %. However, with sewage fractions which had undergone methane fermentation, the recovery values obtained were consistently higher than 100 % whereas activated sludge always gave values lower than 90 %.

Paper chromatography of the hydrolyzed samples gave only one spot with an R_F value equal to that of crystalline biotin.

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