THE DETERMINATION OF TURNOVER RATE AND POOL SIZE OF ACETATE IN THE GOAT RUMEN BY THE ISOTOPE DILUTION METHOD

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同位元素稀釋法에 依한 염소胃의 Acetate 含量 및 吸收率의 測定

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(Received June 26, 1960)

摘 要

本實驗의 目的은 炭酸基에 放射性同位元素 C^{14}를 標識한 sodium acetate ($CH_3C^{14}OONa$)를 使用하여 염소의 胃內에 存在하는 acetate의 胃壁으로 부터의 吸收率과 胃의 acetate의 平均含量을 測定하는데 있다. C^{14}로 標識된 sodium acetate(specific activity 1.35×10^8 cpm./g.)를 給飼 3時間後의 염소의 胃內에 注入하고 注入 2分後부터 約 2分間隙으로 胃 內容物을 抽出하여 column chromatography를 利用하여 acetate를 分離定量한 後 그의 specific activity를 測定하였다. 注入後 3分頃까지는 胃內에 存在하는 acetate에 依한 標識 acetate의 稀釋으로 말미암아 specific activity는 急激히 減少되어 갔고 3分後 부터는 減少度가 比較的 緩慢하였으나 亦是 계속적으로 減少되어 갔다. 稀釋完了後의 이 specific activity 減少는 胃壁을 通한 acetate의 吸收와 胃 內容物로부터의 acetate 生成으로 因한 것으로서, 이 減少率로 부터 acetate의 胃壁吸收速度를 推定할 수 있다.

上記 specific activity의 減少 graph로 부터 推定된 胃內 acetate의 量은 本實驗의 諸條件下에서는 約 30g이었으며 胃內 acetate의 specific activity가 1/2로 減少되는데 要하는 平均時間은 約 4分이었다. 이는 胃內에 存在하는 acetate量의 約 切半은 4分동안에 胃壁을 通過함을 意味한다.

Introduction

It is well known that volatile fatty acids (VFA) play an important role in the nutrition and metabolism of ruminant. These VFA are produced in large quantities within the rumen by microbial action on cellulose, starch, and protein, and reach the liver directly by the portal vein, according to McCarthy,et al (1958), and the subject has been widely reviewed by Edwards (1955), Owens(1954), and Phillipson and Cuthberston(1956). McCarthy, Shaw, McCarthy, and Lakshmanan(1955) reported that in the goat rumen relative rates of production of the VFA are acetate> propionate> butyrate> valerate. Holter, Lakshmanan, and Shaw (1959) reported that acetate accounts for over 90% of the concentration in the rumen. Thus acetate has an important role in the metabolism of carbohydrates and in the formation of ketone bodies in ruminant. Moreover, the production of acetate in the stomach is much greater in the ruminant than in other animals. Therefore, one of the first requirements for an understanding of the biochemistry of ruminant metabolism is a knowledge of quantities and proportions of the VFA produced and absorbed and the form in which these rumen substances enter the blood of the host animal. For these measurements, various methods were employed and the level of these VFA reported by different workers

varies considerably, perhaps as a result of the use of different extraction techniques, as criticized by Phillipson(1958). Furthermore, these results were no more than the measurement of the VFA level in the blood. Duclaux(1874), Friedeman(1938), McClendon(1944), and McAnally(1944) reported distillation procedures for the removal of the VFA from blood. However, seperation of individual acids was not put on a practical basis until the introduction of partition chromatography by Martin and Synge(1941). Subsequently, reliable column partition chromatographic techniques have been reported by Elsden(1946), Moyle, Baldwin, and Scarisbrick(1948), Keeney(1955) and Wiseman and Irvin(1957) and have been used for the resolution of blood acids, but quantitative recovery of the VFA from blood is still dependent upon the efficiency of extraction.

Annison(1954) found 3.3-7.9mg./100ml. (mg.%) total VFA calculated as acetate in the venous blood of the cow 2-4 hrs. post feeding. Similar results have been found with the venous blood of the goat by Annison(1954) and by Phillipson(1958). McClymont(1949) reported 8-12mg. % of acetate in the arterial blood of cows 2-4 hrs. after feeding. The concentration of the VFA (calculated as acetate) in the portal blood of sheep lies in the range of 5-22mg. %, according to Craine (1952) and others(Ref., 19-25). Contrast to these data for the VFA level in the blood, there is little information as to the production and absorption of VFA in the rumen. It is, therefore, strongly desirable to obtain a general value of VFA in the rumen in view of the dynamic nature of this system where production and absorption occur continuously and simultaneously. This study was undertaken to establish the turnover rate and the pool size of acetate in the goat rumen by isotope dilution method.

Experimental Materials and Methods

1 g. of labeled sodium acetate($CH_3C^{14}OONa$) was diluted with distilled water to 10cc, of which 9cc were put directly into an unanesthetized male goat rumen weighing approximately 70 lbs., 3 hours postfeeding. The specific activity(S.A.) of the acetate was 200 microcuries/g. which gave, on a Geiger counter, 1.5×10^8 cpm. Therefore, the amount of radioactivity introduced into the goat rumen was 1.35×10^8 cpm.

Beginning 3 minutes after the introduction of the labeled sodium acetate, a number of samples of rumen fluids were taken out at intervals of approximately 2 minutes. The samples thus taken out were filtered and the filtrates were centrifuged for an hour (5,000 rpm), the supernatant of which was taken for the sample of partition column chromatography to seperate the VFA in it. Johns-Mansville CELITE analytical filter aid (diatomaceous silica earth) was used for the absorbent with alphamine red as internal indicator. A mixture of redistilled acetone and n-hexane was used as the solvent. For the development of butyric and higher acids the ratio of acetone and hexane was 5 : 95; for propionic, 10 : 90; for acetic, 25 : 75. Higher acetone concentration means faster band movement and less spreading of bands.

The amount of acetic acids in the solution seperated, by the chromatographic method was determined by titrating with KOH solution. The solution of acetic acid, then, were put on the hotplate at low temperature to dryness. An aliquot amount of distilled water was added to the dried matter and known amounts of the solution were put in stainless steel planchets for the measure-

ment of the radioactivity. The measurement of the specific activity of the samples was done by a Geiger-counter of windowless gas-flow type at Geiger-region(1,250 V.). An automatic sample changer was used. The counting rates were constantly regulated to give approximately 4,000 cpm.

Results

The changes in S. A. of the samples taken out from the goat rumen at various time intervals are given in Table I. It is shown, from the table, that the change in S.A. during the first 3 minutes after first sampling is much greater than afterward; the S.A. is reduced to about 1/10 in a minute during the first 3 minutes while only 1/3 to 1/2 is reduced in a minute after that period. This is undoubtedly because of the mixing of the radioactive acetate introduced and non-radioactive acetate already present in the rumen. Therefore, the degree of dilution of the radioactivity determines the size of the effective acetate pool of the goat rumen. It should be, however, borne in mind that the change in S.A. in the first three minutes is not entirely due to the uniform mixing between the introduced acetate and the effective acetate, because absorption of the acid by

Table 1. Changes in Specific Activity with Time.

Time after Injection	Time Interval	Specific Activity		Mean	Standard Deviation
2min.	2min.	A_1	122,576 cpm		
		A_2	99,380		
		A_3	102,522		
		A_4	102,466	106,736±4,616.5	9,333
3	1	B_1	13,820		
		B_2	14,691		
		B_3	13,104		
		B_4	13,661	13,819±284.5	569
6	3	C_1	5,469		
		C_2	4,934		
		C_3	4,562		
		C_4	4,585	4,888±183.0	366
9	3	D_1	2,481		
		D_2	2,594		
		D_3	2,658		
		D_4	2,623	2,589±33.0	66
12	3	E_1	2,807		
		E_2	2,912		
		E_3	2,730	2,816±43.3	75
14	2	F_1	2,371		
		F_2	2,286		
		F_3	2,357	2,338±21.4	37
16	2	G_1	1,000		
		G_2	1,003		
		G_3	931	978±19.1	33

the rumen during the mixing should also be taken into consideration. Therefore, the change in S.A. in the first few minutes after the introduction of the radioactive acetate is the results of both dilution by mixing and absorption. It is obvious, however, that the change in S.A. by the absorption from the rumen is very small compared to that by mixing, because the area in which the radioactive acetate is contact with the wall of the forestomach of the host animal before the uniform mixing is completed is not so wide as it is after the completion of the mixing. The author considered the change in S.A. in this period as a full resultant of the dilution by mixing, taking the effects of absorption negligible, with the hope that further studies may clarify this point.

The decline in S.A. after the completion of the mixing is due to the absorption from the wall of the rumen and to dilution of remaining acetate by newly produced acetate. In the table 1 the rate of change in S.A. may be distinctly divided into two. The great difference between these two rates makes it possible to assume that the uniform mixing has satisfactorily completed in the first 6 minutes after the introduction of the labeled acetate, and the author assumed that after this period the S.A. decreased entirely because of the absorption of rumen acetate. Under this assumption, fig. 1 was drawn which shows the changes in specific activity by mixing (B), and by absorption and production (A).

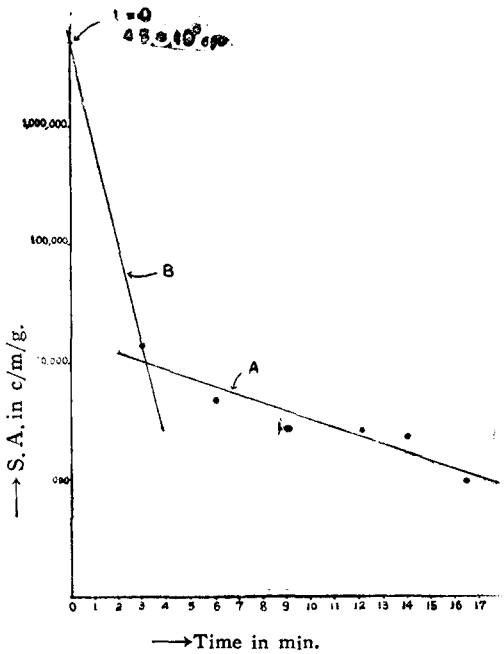

Fig. 1 The change in specific activity with time. A denotes the turnover rate and B, the degree of dilution.

From fig. 1 the time in which the S.A. at a given time is reduced to 1/2 its initial value was determined and is shown in table 2. It is seen that the half-life of the rumen acetate is roughly 4 minutes; half the amount of the rumen acetate at a given time will have been absorbed in 4 minutes.

Table 2. The Turnover Rate Determined from the Decay Curve

Specific Activity	Time(min. & sec.)	Time Interval (min. & sec.)
10,000	3:12	4:12
5,000	7:24	
8,000	4:35	4:00
4,000	8:35	
6,000	6:12	4:12
3,000	10:24	
5,000	7:24	4:06
2,500	11:30	
4,000	8:36	4:14
2,000	12:50	
2,000	12:50	4:10
1,000	17:00	

When the decay curve for the specific activity of acetate is extrapolated to the ordinate, the value at t=0 (t=time) will be an approximation of the degree of dilution of radioactive acetate by that of the effective acetate pool. Thus the dose in total counts per gram of acetate at zero time will approximate the size of the acetate pool in gram. The S.A. at zero time as determined from the extrapolated value, in fig. 1, is found to be about 4.5×10^6 cpm/g., which corresponds to 1/30 the introduced dose, meaning that a thirty-time dilution of acetate has occured. Since the amount of acetate initially introduced into the rumen was 0.9 g.,the acetate pool size of the goat may be said to reach 27g.

Discussion

That large quantities of organic acids are produced within the rumen by microbial action is well established fact. The measurement of these values has, however, been not satisfactory until many kinds of radioactive isotopes of biological importance were commercially available. The measurement by isotopic methods can measure only a rough general value which is the results of the equilibrium between absorption and production of the acetate within the rumen, because there is a dynamic situation in the rumen in which acetate produced is promptly absorbed by the hepatic blood and new acetate is constantly produced; there is continuous turnover of acetate in the rumen. It is this general value that concerns us. The dose of acetate obtained in the present study is no more than the value only obtainable at those conditions as the present experiment was carried out and with different conditions the value undoubtedly will vary, if not significantly. Assuming that this value may represent the general dose of acetate and furthermore that an average adult goat contains about 2*l*. of rumen fluid in it, one may conclude that the acetate pool size of the goat lies close to 1.5g. % or to a concentration of about 0.2 mol. Formerly it was thought that no real absorption of substances was taken place in the rumen; however, it is proved by recent studies that some organic acids such as VFA move directly from the rumen to the liver by way of the portal vein. The auther, however, has not yet been informed as to its moving rate, i.e., absorption rate. According to the results of the present experiment, the half-life of acetate is shown to be about 4 minutes, but it is clear that this value varies more or less in accordance with time and conditions. One may, however, conclude from these results that the turnover rate of acetate is very high and that this high rate means the constant movement of acetate, and presumably other organic acids including VFA as well, from the stomach to the liver. According to McCarthy, Shaw, and Lakshmanan(1958), the acetate formed at the rumen reaches extrahepatic tissues through liver as such with no transformation on the way or in the liver. Since the fact means that the acetate formed at the rumen is concerned directly with the respiratory metabolism in the tissues, the quantity of the acetate formed in the rumen and its quantity of absorption influence immediately the tissue metabolism. The auther assumed the peroid of the first 3 minutes in fig. 1 as a necessary duration for uniform mixing. Though it is just an assumption, there will be no remarkable difference in actual value even if some deviation in time around the 3 minutes is accepted. Therefore the decline of S.A. from 6 minutes after the introduction of the acetate was considered to be entirely due to the absorption of the acetate by the rumen wall.

A more detailed analysis of the decay curves shown in fig. 1 of the mixing and absorption of

labeled acetate should be undertaken in further studies, thereby the factors of declining S.A. may be clarified.

Summary

The object of the present experiment was to determine the turnover rate and the pool size of acetate in the goat rumen by the method of isotope dilution.

180 microcuries of labeled acetate ($CH_3C^{14}OONa$) were put directly into an unanesthetized goat rumen. Beginning 2 minutes after the introduction of the labeled sodium acetate, samples of rumen fluid were taken out at intervals of approximately 2 minutes. The specific activity contained in the acetate of the fluid was counted by a Geiger counter. The degree of dilution was found to be about 30; it was, therefore, estimated that the amount of acetate present in the goat rumen is about 27g. The time in which the specific activity of the acetate in the goat rumen is reduced to half its initial value was approximately 4 minutes.

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