E where it is shown that inosine (which has previously been demonstrated to increase cellular organic phosphate(1)) sharply decreases cellular inorganic phosphate.

Summary. Movements of $P^{32}O_4$ in and out of fresh and cold-stored human erythrocytes were measured in presence and absence of inosine. I. Small amounts of hemolysis when inosine was present caused a decrease in plasma inorganic phosphate and incorporation of P^{32} into plasma organic phosphate. This formation of plasma organic phosphate decreased movement of P^{32} into the cell. II. When P^{32} was originally present in the red cell, inosine decreased the net movement from cells to plasma. We attribute this effect to utilization of inosine as substrate in the cell. III. Cells containing P^{32} during 6-day cold-storage lost 3 to 4 times as much P^{32} as cells having P^{32} for a short time. Movements from cells to plasma are described as the result of at least 2 phosphate compartments within the cell.

1. Prankerd, T. A. J., *Int. Rev. Cytol.,* 1956, v5, 279.

2. Gourley, D. R. H., *Arch. Biochem. & Biophys.,* 1952, v40, 1.

3. Kahn, J. B., Jr., *J. Pharmacol. and Exp. Therap.,* 1957, v120, 239.

4. Levi, H., *Arch. kemi, mineralogi o. geologi,* 1945, v21A, 1.

5. Mueller, C. B., Hastings, A. B., *J. Biol. Chem.,* 1951, v189, 869.

6. Tabachian, H., Altman, K. I., Young, L. E., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 712.

7. Gabrio, B. W., Hennessey, M., Thompson, J., Finch, C. A., *J. Biol. Chem.,* 1955, v215, 357.

8. Dische, Z. in *Phosphorus Metabolism,* Johns Hopkins Press, Baltimore, 1951.

9. Lionetti, F., Rees, S. B., Healy, W. A., Walker, B. S., Gibson, J. C., *J. Biol. Chem.,* 1956, v220, 467.

10. Kahn, J. B., Jr., Acheson, G. H., *J. Pharmacol. and Exp. Therap.,* 1955, v115, 305.

11. Guest, G., Siler, V., *J. Lab. Clin. Med.,* 1934, v19, 757.

12. "Student", *Biometrika,* 1908, v1, 8.

13. Martin, J. B., Doty, D. M., *Analyt. Chem.,* 1949, v21, 965.

14. Berenblum, J., Chain, E., *Biochem. J.,* 1938, v32, 295.

15. Kaplan, N. O., Greenberg, D. M., *J. Biol. Chem.,* 1944, v156, 511.

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Rate of Acetic Acid Production in the Rumen Determined by Isotope Dilution.* (25071)

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The fact that fermentation of carbohydrates in the rumen produces large quantities of short-chain fatty acids which are important sources of energy for the ruminant animal(1) has led to *in vitro*(2), isolated rumen(3), perfused rumen(4), and *in vivo*(1,5) technics of estimating the rates of production of these acids. To obtain better estimates of acetate production in the practically-fed ruminant, an isotope dilution method has been used in the work reported here.

Methods. A mature sheep fitted with a rumen cannula was maintained on an all-clover hay ration with minerals and water free-choice. *Via* a rumen cannula, 2.267 mc of sodium acetate-1-C^{14} was given 20 minutes after withdrawal of the morning hay. Samples of rumen liquor were then withdrawn through the cannula from the ventral-sac of the rumen near the ventral curvature, at approximately half-hour intervals over a 12-hour period. Immediately following withdrawal, each sample was strained through cheesecloth, the pH was lowered to approximately 1.5 with 10% H_2SO_4, and the sample was quick-frozen in dry ice and stored at 0°F until analyzed. Acetic, propionic, butyric, and the higher fatty acids were separated quantitatively by

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TABLE I. Distribution of Radioactivity/Milliliter of Rumen Liquor in Each Acid Fraction after Administration of Acetate-1-C^{14}.

Time after C^{14} acetate admin., min.	Thousands of dpm/ml of rumen liquor				
	Higher acids	Butyric acid	Propionic acid	Acetic acid	Total
15	.28	6.3	1.1	18	192
33	1.09	19.0	2.2	266	288
77	1.47	20.8	3.7	207	233
125	1.37	19.6	4.3	137	163
195	1.55	19.0	4.8	97	123
247	1.09	15.1	5.2	74	95
335	1.01	10.9	4.9	44	61
455	.65	5.9	3.3	25	35
720	.24	1.3	2.6	9	13

chromatography on Celite(6), and radioactivity of each acid fraction was determined in a liquid scintillation counter.

Results. Radioactivity was found in all 4 fatty acid fractions showing that considerable interconversion of acetic acid to butyric, propionic and higher fatty acids takes place in the rumen. Distribution of radiocarbon among the various rumen volatile fatty acids at time intervals following administration was determined and representative data are shown in Tables I and II. Total radioactivities and specific activities of the various fractions were in the order, acetic>butyric>propionic> higher acids. On the basis of the relative radioactivities of each acid when at their peaks (approximately ½ hour for acetic acid, 1 hour for butyric acid, and 3 hours for propionic acid) the percentage of acetic acid being converted to butyric would appear to be approximately 12% and to propionic acid, approximately 5%. Dilution experiments, similar to these with acetic acid, will have to be carried out with the other acids before more accurate inter-conversion data can be calculated.

TABLE II. Specific Activities (dpm/mMole) of Different Rumen Volatile Fatty Acids at Intervals following Rumen Administration of Acetate-1-C^{14}.

Time after C^{14} acetate admin., min.	Thousands of dpm/mMole			
	Higher acids	Butyric acid	Propionic acid	Acetic acid
15	95	935	57	2,390
33	369	226	108	3,369
77			260	2,633
155	480	2,204	259	2,033
221	669	1,905	280	1,314
247	500	3,246	358	1,153
335	382	1,716	265	645
455	440	1,177	266	543
720	125	335	238	206

Concentrations of the various fatty acids, expressed as millimoles of acid per liter of rumen liquor, are given in Table III. The rates of change of total radioacetic acid and of specific activity of radioacetic acid yielded straight line logarithmic functions while total acetic acid concentration yielded a straight line arithmetic function. The following regression lines were calculated for these 3 rates: (a) for disintegrations per minute of acetate per ml of rumen liquor, log y = 5.46007 – 0.002385 x; (b) for specific activity

TABLE III. mMoles/Liter of Rumen Liquor of Different Rumen Volatile Fatty Acids at Intervals after Acetate-1-C^{14} Administration.

Time after C^{14} acetate admin., min.	mMoles				
	Higher acids	Butyric acid	Propionic acid	Acetic acid	Total mMoles/l
15	2.95	6.71	19.6	77.1	106.4
33	2.64	8.41	20.7	78.9	110.6
77			14.4	78.7	
155	2.85	8.88	16.7	67.6	96.0
221	2.31	9.95	17.2	74.2	103.7
247	2.18	4.66	14.5	64.2	85.5
335	2.65	6.38	18.4	68.1	95.5
455	1.48	5.04	12.4	46.8	65.7
720	1.89	3.90	10.9	45.1	61.8

of acetate, log y = 6.57433 – 0.002123 x; (c) for total acetate concentration per ml of rumen liquor, y = 0.08152 – 0.00006292 x; where x is time in minutes following administration of radioacetate.

The rate of change of specific activity of acetate, that is, rate of dilution, was used to compute the rate of acetic acid production. The decrease in specific activity of the radioacetic acid, due to dilution by acetic acid synthesized calculated over the straight line part of the curve (33 minutes to 455 minutes), was found to be at the rate of 50% per 130 minutes. On the basis of the total acetic acid present per ml of rumen liquor at the start of this period (33 minutes after radioacetic acid administration) and this rate of dilution over the 7-hour straight line period of isotope dilution, it was calculated that 0.126 millimole of acetic acid were synthesized per ml of rumen liquor per 7 hours. This amounts to 0.00756 g or 0.0264 Kcals of acetic acid. If one assumes a rumen content of 5 liters, this then would amount to 132 Kcals, which is approximately 30% of a 140 lb. sheep's 7-hour maintenance requirement.

Summary. It has been found by the technic of isotope dilution using acetate-1-C^{14} that the normal sheep on an all-hay ration produced acetic acid in its rumen by bacterial synthesis at the rate of 0.126 millimole per ml of rumen liquor for the 7-hour period following feeding. Radioactivity was found in the other rumen volatile fatty acid (propionic, butyric and higher acids) demonstrating their formation from acetic acid in the rumen.

1. Phillipson, A. T., *J. Exp. Biol.,* 1942, v19, 186.
2. Munch-Petersen, E., *Austr. Vet. J.,* 1957, v33, 292.
3. Danielli, J. F., Hitchcock, M. W. S., Marshall, R. A., Phillipson, A. T., *J. Exp. Biol.,* 1945, v22, 75.
4. McCarthy, R. D., Holter, J. B., Shaw, J. C., Heuter, F. G., McCarthy, Jeanne, *Maryland Agr. Exp. Sta. Misc. Pub.,* 1957, v291, 33.
5. Barcroft, J., McAnally, R. A., Phillipson, A. T., *J. Exp. Biol.,* 1944, v20, 120.
6. Oppermann, R. A., Nelson, W. O., Brown, R. E., *J. Dairy Sci.,* 1957, v40, 779.

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Effect of Methylphenidate on Cardiovascular Actions of Pressor Amines. (25072)

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Fröhlich and Loewi showed that cocaine potentiated pressor responses to epinephrine (1). Later, Tainter and Chang found cocaine blocked tyramine pressor responses(2). More recently, Fleckenstein and Bass(3) and Fleckenstein and Stöckle(4) demonstrated that cocaine altered responses of cat nictitating membrane to a series of sympathomimetic amines of the phenylalkylamine type, in such a way that contractions produced by one group of amines were augmented, contractions elicited by a second group were not affected and contractions produced by a third group were decreased. Recently Maxwell, *et al.*(5) demonstrated that the central nervous system stimulant, methylphenidate, potentiates pressor actions induced by epinephrine and norepinephrine while blocking and antagonizing pressor responses to amphetamine and ephedrine, respectively. In consideration of this, and the fact that the structures and primary pharmacological characteristics of cocaine and methylphenidate are markedly different, an attempt has been made to ascertain whether the effects of methylphenidate on cardiovascular actions of various phenylalkylamines would closely parallel those of cocaine.

Materials and methods. Mongrel dogs of both sexes weighing 7 to 15 kg were used. The anesthesia was Na pentobarbital, an initial dose of 30 mg/kg intraperitoneally fol-

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