6. Salk, J. E., Youngner, J. S., and Ward, E. N., *Am. J. Hyg.*, 1954, v60, 214.
7. Melnick, J. L., and Opton, M. E., *Bull. World Health Org.*, 1956, v14, 129.
8. Salk, J. E., Lewis, L. J., Youngner, J. S., and Bennett, B. L., *Am. J. Hyg.*, 1951, v54, 157.
9. Fulton, F., and Dumbell, K. R., *J. Gen. Microbiol.*, 1949, v3, 97.
10. Morgan, P., Carter, J. S., and Wenner, H. A., *J. Lab. and Clin. Med.*, 1953, v42, 665.
11. Svedmyr, A., Enders, J. F., and Holloway, A., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 296.
12. Chin, T. D. Y., Beran, G. W., and Wenner, H. A., *Am. J. Hyg.*

Received April 2, 1957. P.S.E.B.M., 1957, v95.

Enzymatic Release of Pantetheine from Coenzyme A in Animal Tissues And Its Microbiological Determination. (23190)

R. L. WOLFF, S. DUBOST AND J. J. BRIGNON (Introduced by Raymond Cahen)
Laboratoire de Chimie Biologique, Faculté de Médecine, Nancy, France.

Microbiological determination of pantothenic acid(1) or of pantetheine(2) released by enzymatic hydrolysis, has been recommended as a routine method for determination of coenzyme A in animal tissues. The first of these two methods requires the use of 2 enzymes, liver enzyme and intestinal phosphatase. With one enzyme, phosphatase, CoA is split into pantetheine. As the activity coefficient of pantetheine for growth of *Lactobacillus arabinosus* amounts to 20-40% (on a molecular basis) of that of pantothenic acid, microbiological determination of pantetheine, in the presence of preformed free pantothenic acid, becomes very difficult with *L. arabinosus*. With *Lactobacillus helveticus*, another difficulty arises owing to stimulation of the activity of pantetheine by small amounts of pantothenic acid present in the mixture.

Experiments are here reported pointing to a modification of the basal medium for *Lactobacillus arabinosus* 17-5 whereby pantothenic acid and pantetheine, in equimolecular amount, display an identical activity as growth factors. Each of the two factors is devoid of any stimulatory activity towards the other. This method has been checked with the double enzyme method of Novelli and Lipmann.

Method. The basal medium, for *L. arabinosus* 17-5, has been described by Skeggs and Wright(3). It was modified by addition of 50 ml of a 20 mg/ml solution of cysteine hydrochloride to 250 ml of the original medium and was adjusted to pH 6.6-6.8. The tubes were incubated in glass jars at 37°C, for 24 hours in an atmosphere of pure CO_2. *Substrates.* The Ca-pantothenate was of commercial origin. The pantetheine was used as its S-benzoyl derivative,* the identity of the activity for the 2 compounds having been demonstrated. It had been prepared according to Schwyzer(4). *Intestinal phosphatase* was prepared from calf intestinal mucosa according to the first steps of the method described by Schmidt and Thannhauser(5). The trypsin digested mucosa, after filtration, was dialyzed against tap-water for 72 hours and was kept in the freezer at –20°C. *Phosphatase activity* of the solution corresponded to 45 Thannhauser units per ml. Two ml of aqueous tissue extracts, prepared according to the procedure of Kaplan and Lipmann(6), were incubated for 3 hours at 37° with 0.4 ml phosphatase, 0.1 ml 0.12 M $NaHCO_3$ and 47.5 ml H_2O before the assay.

Results. The action of cysteine and CO_2 on growth of *L. arabinosus* in the presence of varying amounts of S-benzoylpantetheine or pantothenic acid is shown in Fig. 1 (I-IV). Cysteine, in the amount of 10 mg per tube of 5 ml, considerably increases activity of pantetheine but not that of pantothenic acid. In an atmosphere saturated with CO_2, activity of either growth factor is slightly increased but that of pantothenic acid to a lesser degree

* Obtained through the courtesy of Dr. Schwyzer, Ciba Inc., Basel, Switzerland.

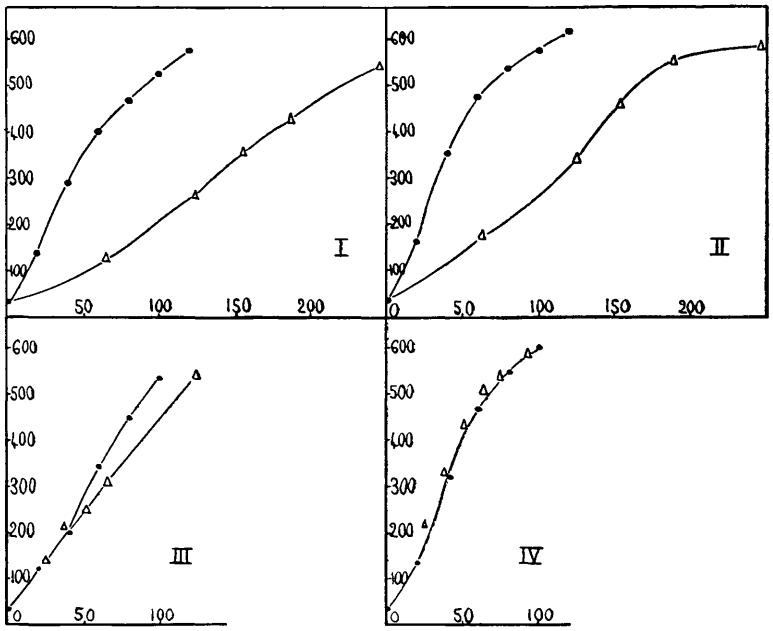

FIG. 1. Growth curves of *Lactobacillus arabinosus* in presence of S-benzoylpantetheine (△) or pantothenic acid (●). Influence of cysteine (10 mg/tube of 5 ml) and CO_2. Abscissa: Ca-pantothenate or S-benzoylpantetheine (on an equimolecular basis). Ordinate: Units (Meunier). I = Atmosphere: air; II = Atmosphere: CO_2 100%; III = Atmosphere: air + 10 mg cysteine per tube; IV = Atmosphere: CO_2 100% + 10 mg cysteine per tube.

than that of pantetheine. Under the combined influence of cysteine and CO_2, growth stimulation of *L. arabinosus* by pantetheine or pantothenic acid becomes identical. In contrast to *L. helveticus*, no potentiation occurs in the presence of the 2 growth factors, the total activity being (on a molecular basis) the sum of each individual activity.

Table I shows the results of microbiological determination of CoA in several tissues of rats, rabbits and guinea pigs. The two methods give identical results.

TABLE I. Determination of CoA Bound Pantetheine Released after Incubation of Tissue Extract with Intestinal Phosphatase and Calculated as Ca-pantothenate. (The number in the table corresponds to the sum: performed pantothenic acid + liberated pantetheine.)

Tissue	Liver		Heart		Kidney		Spleen	
Method	a	b	a	b	a	b	a	b
No. of rats	9	9	6	6	7	7	5	5
Mean	68.5	65.5	32.8	33.4	34.9	32.5	9.1	8.5
S.E. of mean	4.37	3.76	4.88	4.58	2.82	3.71	1.36	1.02

Tissue	Muscle		Brain		Testicles		Adrenals	
Method	a	b	a	b	a	b	a	b
No. of rats	2	2	3	3	2	2	1	1
Mean	9.8	11.5	8.5	8.6	13.1	13.1	26	30

Animal	Rabbit		Guinea pig	
Method	a	b	a	b
Liver	42.7	42.7	52.1	53
Kidney	41.8	40.8	29.1	27.8
Heart	21.5	19.4	10	9.4
Brain	10.7	11.1		
Adrenals			10.6	8.8

a = present method; b = method of Novelli and Lipmann.

According to Marnay(7), Cheldelin *et al.* (8) and Atkin *et al.*(9), heart and muscle contain considerable amounts of "conjugated pantothenic acid" of a different kind of CoA bound pantothenic acid. The conjugate is split by autolysis, by takadiastase, mylase P, etc. The amount of this "conjugated pantothenic acid" exceeds by far the amounts of CoA bound pantothenic acid as measured by the double enzyme method. It is interesting to note that the non-purified phosphatase used in these experiments is devoid of any "pantothenic conjugates" splitting activity other than CoA.

Summary. (1) A method has been described whereby pantetheine and free pantothenic acid may be determined microbiologically in a medium in which the 2 factors exert (on a molecular basis) the same effect on *Lactobacillus arabinosus*. The action of the 2 growth factors is additive. (2) The CoA bound pantothenic acid is liberated as pantetheine by incubation of an aqueous tissue extract with intestinal phosphatase and can be determined in the presence of preformed free pantothenate. (3) The results of this method, applied to several tissue extracts, agree with those of Novelli and Lipmann.

1. Novelli, G. D., Kaplan, N. O., and Lipmann, F., *J. Biol. Chem.*, 1949, v177, 97.

2. Dubost, S., Brignon, J. J., and Wolff, R. L., *C. R. Soc. Biol.*, 1955, v149, 1008.

3. Skeggs, H. R., and Wright, L. D., *J. Biol. Chem.*, 1944, v156, 21.

4. Schwyzer, R., *Helv. Chim. Acta*, 1952, v35, 1903.

5. Schmidt, G., and Thannhauser, S. J., *J. Biol. Chem.*, 1943, v149, 369.

6. Kaplan, N. O., and Lipmann, F., *ibid.*, 1948, v174, 37.

7. Marnay, C., *Bull. Soc. Chim. Biol.*, 1953, v35, 220.

8. Cheldelin, V. H., Eppright, M. A., Snell, E. E., and Guirand, B. M., Univ. Tex. Publ., 1942.

9. Atkin, L., Williams, W. L., Schultz, A. S., and Frey, C. N., *Ind. Eng. Chem. Anal. Edit.*, 1944, v16, 67.

Received April 3, 1957. P.S.E.B.M., 1957, v95.

Action of Protamine on Renal Potassium Excretion in the Rabbit. (23191)

ERNEST C. FOULKES,* IRWIN B. HANENSON,† AND BENJAMIN F. MILLER

May Institute for Medical Research, Jewish Hospital Assn., and Departments of Physiology and Medicine, University of Cincinnati College of Medicine.

Recent evidence has shown that protamine is an inhibitor of active potassium transport in kidney cortex slices of the rabbit(1). Sodium transport in that system is not affected. In this report, we describe some effects of protamine on renal electrolyte excretion in the rabbit. It was the purpose of these experiments to determine whether the effects of protamine in the whole animal parallel those observed in kidney slices from the same species.

Methods. Male albino rabbits weighing 7 to 8 lb were placed on a standard solid rabbit ration.‡ Prior to the experiment water or 0.45% saline was administered by stomach tube. Intravenous injections and infusions were given through a marginal ear vein. Urine was collected from an indwelling catheter by gentle suction with a syringe. The bladders of several animals sacrificed at the end of the experiment were examined for residual urine but were invariably found to be empty. Animals were restrained on their backs and blood collected from the heart at approximately the midpoint of each clearance period. The filtration rate was measured with creatinine administered by constant intravenous infusion or by subcutaneous injection one hour before the beginning of the experiment. Electrolyte determinations were carried out on a Baird flame photometer on suitable dilutions of serum and urine with lithium as internal standard. Creatinine was estimated as total chro-

* Established Investigator, Amer. Heart Assn.

† Eda K. Loeb Memorial Scholar, May Institute.

‡ Obtained from Rockland Farms, New City, N.Y.