factor. In view of the fact that a thromboplastin with high and constant activity can readily be prepared, there is no longer any valid justification for attempting quantitative studies of prothrombin with preparations of variable and uncertain potency.

Summary. The intramuscular injection of sodium citrate in dogs and rabbits does not change the prothrombin level of the blood.

13018

Determination of Pantothenic Acid in Normal Blood and Urine by Microbiological Technic.

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In an earlier report from this laboratory[1] a technic was described for the assay of pantothenic acid based upon the growth response of the bacterium *Proteus morganii* to the vitamin. The application of this technic to the assay of pantothenic acid in natural substances, particularly blood and urine, is described in this communication.

Methods. The detailed procedure for the microbiological assay method employed has been given elsewhere.[2] Briefly, it consists of employing a pantothenate-free assay medium in which *Proteus morganii* will not grow. Addition of calcium pantothenate (Merck) in concentrations above 0.0001 μg per ml of medium permits growth of the test organism. Within limits the amount of growth is proportional to the pantothenate concentration. The increase in growth can be easily measured by employing a turbidimetric method. By plotting the turbidity values obtained against the concentration of pantothenate used, a "standard curve" is obtained.

To determine the pantothenic acid content of a specimen, an aqueous extract is prepared and autoclaved, and then a known quantity of this is incorporated in the assay medium. After inoculation with the test organism, the medium is incubated for 24 hours at 37°C and the turbidity is measured. Reference is then made to

* We wish to thank Merck & Company, Inc., for generously supplying the synthetic calcium pantothenate employed in this study.

1 Pelczar, M. J., Jr., and Porter, J. R., *J. Bact.*, 1941, **41**, 23.

2 Pelczar, M. J., Jr., and Porter, J. R., *J. Biol. Chem.*, 1941, **139**, 111.

the "standard curve," and the pantothenate content of the extract is calculated.

Urine specimens were collected over a 24-hour period from 9 apparently normal people. These samples were collected at 4 different times during the experiment and precautions were taken to avoid bacterial contamination. To determine the pantothenate content of urine, a 1:100 dilution was made, and quantities of this dilution ranging between 0.1 ml-0.3 ml were incorporated in the assay medium.

Blood was obtained from normal individuals by pricking the finger tip and exactly 0.5 ml was added to 4.5 ml of distilled water. It was necessary to clarify this material prior to making additions to the medium. This was done by first heating the samples to boiling, then adding approximately 0.5 g of kielselguhr. After a few minutes the kieselguhr and adsorbed substances were removed by centrifugation. Following centrifugation the clear supernatant was removed, sterilized by autoclaving, and 0.4 ml added to the assay medium.

Results. Values obtained for the pantothenate content of normal blood and urine are presented in Tables I and II.

The results given in Table I for the pantothenate concentration of normal blood as determined by this investigation are not in agreement with the values obtained by Stanbery, Snell and Spies[3] who em-

TABLE I.
Pantothenic Acid Content of Normal Blood.

Source of specimen	Pantothenic acid concentration in blood (Specimens obtained on different days)	
	I	II
	μg/ml	μg/ml
1	.054	.062
2	.070	.068
3	.070	.052
4	.047	.040
5	.099	.097
6	.081	.046
7	.032	.033
8	—	.030
9	.036	.054
10	.071	.058
11	.082	.080
12	.057	.060
13	.068	.084
14	.047	—
15	.031	.064
16	.048	.080
17	.049	—

[3] Stanbery, S. R., Snell, E. E., and Spies, T. D., *J. Biol. Chem.*, 1940, **135**, 353.

TABLE II.
Pantothenic Acid Content of Normal Urine.

Source of Specimen	Pantothenic acid content of 24-hr specimens of urine collected on different days							
	I		II		III		IV	
	Vol.	Mg Pantothenic Acid	Vol.	Mg Pantothenic Acid	Vol.	Mg Pantothenic Acid	Vol.	Mg Pantothenic Acid
A	1950	4.39*	2050	4.27	1850	4.31	2250	3.26
B	1450	3.70	1425	3.98	—	—	1400	4.69
C	1900	5.05	—	—	—	—	—	—
D	2000	3.97	1900	2.60	1900	1.46	2650	3.71
E	1400	3.22	1150	2.76	1400	3.20	1400	3.36
F	1050	5.08	1200	4.86	1000	4.55	1250	4.44
G	1150	5.98	1350	4.59	1900	6.79	1450	4.13
H	800	2.74	1250	1.74	1000	2.55	1350	2.35
I	—	—	1000	4.30	700	2.31	1050	3.99

*Average results of repeated determinations.

ployed a different microbiological assay technic. They found that the concentration of this substance in normal whole blood ranged from 0.19-0.32 μg per ml and the average value obtained was 0.225 μg per ml. Our experiments indicate that a much lower level of this vitamin is present in normal blood. We have found that the content per ml of blood varies between 0.030-0.099 μg for the group of individuals studied and the average value, calculated from the results in Table I, is 0.059 μg per ml. We have also observed considerably more variation in specimens from the same individual, taken at different times, than was found by Stanbery, Snell and Spies.[3] The discrepancy between the results obtained in this investigation and those reported by Stanbery, Snell and Spies may be due to determination of the free pantothenic acid in the one instance and to the total pantothenate (free and combined) in the other.

The amount of pantothenic acid present in 24-hour specimens of urine obtained at 4 different times from 9 persons, varied from 1.46 mg to 6.79 mg. The calculated average was 3.81 mg. At present we are unaware of other similar data for comparison. Spies, Stanbery, Williams, Jukes and Babcock,[4] in an investigation on the relationship of pantothenic acid to nutrition in the human, measured the normal level of the vitamin in blood and urine as well as conditions influencing deviations from the normal. However, actual values for the individual determinations were not reported.

Evidence for the specificity of this assay procedure was obtained

[4] Spies, T. D., Stanbery, S. R., Williams, R. J., Jukes, T. H., and Babcock, S. H., *J. A. M. A.*, 1940, **115**, 523.

by recovery experiments. Known amounts of synthetic Ca-pantothenate were added to alkali-treated (pantothenate-free) urine and to other urine specimens whose pantothenic acid content had been previously determined. These materials were then assayed and the resulting values compared to the calculated amount of the vitamin which should be present. It will be seen that the recovery values presented in Tables III and IV are in very close agreement with the

TABLE III.
Quantitative Recovery of Pantothenic Acid from Alkali Treated Urine.

Alkali treated urine specimens added to 10 ml of assay medium	μg pantothenic acid added to 10 ml of assay medium	Nephelometer reading	Pantothenic acid equivalent	% recovery
ml				
	.0	0	.0	—
A—.05	.002	23	.0019	95
	.004	42	.004	100
	.0	0	.0	—
B—.05	.002	22	.0018	90
	.004	42	.004	100
	.0	0	.0	—
C—.05	.002	24	.002	100
	.004	42	.004	100

TABLE IV.
Quantitative Recovery of Pantothenic Acid from Untreated Urine.

Additions made to 10 ml of assay medium		Total pantothenic acid content in assay medium		
Urine specimen (pantothenic acid content previously assayed) μg	Synthetic pantothenic acid, μg	Calculated pantothenic acid content, μg	Amt pantothenic acid as determined by assay, μg	% recovery
.0045	.001	.0055	.0051	92
	.002	.0065	.0061	94
.0033	.001	.0043	.0044	102
	.002	.0053	.0057	107
.0022	.001	.0032	.0030	94
	.002	.0042	.0043	102

actual amounts calculated to be present. Similar experiments with blood showed that the adsorption procedure using kieselguhr did not lower the pantothenic acid content of the blood; the recovery of added Ca-pantothenate was quantitative. Additional evidence for the specificity of this technic is presented elsewhere.[2]

Summary. A microbiological technic, employing *Proteus morganii*

as the test organism for the assay of pantothenic acid, has been applied to evaluating the level of this vitamin in blood and urine.

It was found that the pantothenate content of blood from 17 normal persons ranged between 0.030 and 0.099 μg per ml of blood and averaged 0.059 μg per ml. The pantothenate content of 24-hour urine specimens from 9 persons ranged from 1.46 mg to 6.79 mg and averaged 3.81 mg.

13019 P

Experimental Alimentary Azotemia: Comparative Effects of Red Cells and Plasma.

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In a previous paper,[1] the authors showed in experimental animals that the ingestion of whole blood produces a marked rise in the urea nitrogen content of the recipient's blood. This observation agreed with that of Schiff, *et al.*,[2] Kaump and Parsons,[3] and Yuile and Hawkins[4] and indicated that the azotemia occurring in clinical cases of bleeding peptic ulcer might also be caused by blood absorption.

In an attempt to find what element in the blood caused the rise, 5 dogs were given beef plasma by stomach tube in the first series of experiments and in the second series of experiments 5 dogs were given beef cells in the same manner. In all 10 experiments blood samples were taken every day at 2 a.m., 8 a.m., 12 noon, 4 p.m., and 8 p.m., one day before giving the substance to be tested and for at least 2 days thereafter. In all instances the dogs given cells showed a much more marked elevation in blood urea nitrogen than did the plasma fed dogs. A typical curve is shown in Chart 1.

Effect of hemoglobin. Administration of a pure hemoglobin solution to 2 dogs produced a rise in blood urea nitrogen comparable to that produced by cells alone.

Effect of iron. To rule out the action of the iron in the hemo-

1 Chunn, C. F., and Harkins, H. N., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 569.

2 Schiff, L., Stevens, R. J., Goodman, S., Garber, E., and Lublin, A., *Am. J. Dig. Dis.*, 1939, **6**, 597.

3 Kaump, D. H., and Parsons, J. C., *Am. J. Dig. Dis.*, 1940, **7**, 191.

4 Yuile, C. L., and Hawkins, W. B., *Am. J. Med. Sci.*, 1941, **201**, 162.