plasmosis from the acute lesions to final disseminated calcification, this furnishes strong supporting evidence of the etiological relationship.

*Summary.* We report the first direct isolation of *H. capsulatum* from natural sources; *i.e.*, soil from a storm cellar in which severe pneumonitis was contracted by soldiers in 1944. This organism was also isolated from soil from the storm cellar following animal inoculation on three occasions. This evidence and that obtained by studies of the men involved in the pneumonitis epidemic indicates that the epidemic was caused by H. *capsulatum.*

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1. Emmons, C. W., *Pub. Health Rep.,* 1949, v64, 892.

2. Ajello, L., Zeidberg, L. D., *Science,* 1951, v113, 662.

3. Furcolow, M. L., Larsh, H. W., *et al.,* unreported studies.

4. Stewart, R. A., Meyer, K. F., PROC. SOC. EXP. BIOL. AND MED., 1931-32, v29, 937.

5. Grayston, J. T., Loosli, C. G., Alexander, E. R., *Science,* 1951, v114, 323.

6. Cain, J. C., Devins, E. J., Downing, J. E., *Arch. Int. Med.,* 1947, v79, 626.

7. Commission on Acute Respiratory Diseases in collaboration with Mickle, W. A., Jr., *Arch. Int. Med.,* 1947, v80, 203.

8. White, F. C., Hill, H. E., *Am. Rev. Tuberc.,* 1950, v62, 1.

9. Furcolow, M. L., *Postgraduate Med.,* 1950, v8, 15.

10. ———, *Pub. Health Rep.,* 1950, v65, 965.

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## Effect of Deficiencies of Sodium and Potassium on Motility of the Intestine of the Rat.* (19585)

H. S. PERDUE AND P. H. PHILLIPS.

*From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.*

Workers in this laboratory frequently have observed that animals receiving potassium deficient diets had distention of the stomach and of the small intestine. Also, many rats which were fed a potassium deficient diet containing 19% cellulose died of intestinal obstruction. These are evidences of decreased intestinal tone and motility.

Gazes *et al.*(1) reported that isotonic potassium chloride administered intravenously stimulated intestinal motility in human beings and dogs with adynamic ileus. Webster *et al.* (2) observed that rats fed a diet containing 0.01% potassium exhibited decreased intestinal tone and finally died of a condition resembling paralytic ileus. Since the observations of these workers were essentially qualitative, quantitative observations of the effect of dietary potassium deficiency on intestinal motility are of interest. The possible interrelationship of sodium with potassium in this regard also was investigated since increased levels of sodium have been shown to increase the mortality and the severity of the heart lesions of potassium deficient rats(3).

*Experimental procedure.* Groups of 5 adult female albino rats weighing from 180 to 220 g each were placed on each of the 4 experimental diets employed. The diets were essentially the same as the low sodium and potassium diets of Grunert and Phillips(4). The deficient diets contained 0.005% sodium when sodium was deficient and 0.005% potassium when potassium was deficient. The control diet contained 0.25% sodium and 0.5% potassium. After 6 weeks on the experimental

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TABLE I. Summary of Results.

| Na and K content of diet | | Distance bolus was propelled in 10 min | | % of length of small intestine bolus propelled in 10 min | |
|---|---|---|---|---|---|
| % Na | % K | Avg, cm | Range | Avg | Range |
| .005 | .005 | 49 | 45.7– 50.8 | 45.2 | 41.8– 48.7 |
| .25 | .005 | 49 | 43.3– 53.3 | 50.9 | 46 – 58.8 |
| .005 | .5 | 101 | 97.7–104 | 97 | 95 – 98 |
| .25 | .5 | 104 | 93 –105 | 98 | 97.5–100 |

diets the propulsive motility of the intestine of each of the rats was determined by means of a modification of the technic of Macht(5). The procedure used was as follows: The rats were fasted for 24 hours and then each rat was given 2 ml of a 10% suspension of charcoal in a 10% aqueous solution of gum acacia by stomach tube. No anesthesia was used at any point in the experiment. Exactly 10 minutes after the administration of the charcoal and gum acacia each of the rats was killed by a blow on the head followed by exsanguination. The small intestine was then dissected out from the pyloric sphincter to the ileo-cecal junction, laid out in a straight line, and its length and the maximum progress of the charcoal from the pyloric sphincter measured. The exsanguination and dissection took about 30 seconds.

*Results.* Potassium deficiency or a combined deficiency of sodium and potassium lowered the propulsive motility of the small intestine of the rats by about 50% under the conditions of this experiment (Table I). Sodium deficiency alone was without effect on intestinal motility as determined by this technic.

*Discussion.* Fenn(6) states that although potassium is not regarded as a humoral agent for neuromuscular transmission, it plays an important role at the neuromuscular junction. Since the peristaltic contractions of the small intestine are dependent upon the intrinsic nerve plexuses of Auerbach and Meissner(7), a decrease in the potassium content of the serum or of the muscle cells may cause a decrease in peristaltic activity by affecting the normal function of the neuromuscular junction. Meyer *et al.*(3) have reported a decrease in serum potassium in rats which received a diet containing 0.005% potassium and 0.25% sodium, but rats receiving a diet containing 0.005% potassium and 0.005% sodium did not exhibit hypopotassemia. In view of the decrease in propulsive motility of the intestine of the rats which received the diet containing 0.005% potassium and 0.005% sodium, serum potassium concentration does not appear to play a direct role in this phenomenon. The potassium content of visceral muscle may affect the neuromuscular junction or it may affect the ability of the muscle cells to respond to stimulation by affecting the enzyme systems involved in muscle contraction. Thus, potassium is known to be necessary for the phosphorylation of creatine in connection with pyruvate oxidation *in vitro*(8) and potassium deficiency may affect the functioning of this enzyme system *in vivo*.

*Summary.* Maintenance of adult female albino rats on a diet containing 0.005% potassium for 6 weeks resulted in a 50% decrease in intestinal propulsive motility. Reduction of the sodium content of this diet from 0.25% to 0.005% did not result in an increase in intestinal motility. Feeding a diet containing 0.005% sodium and 0.5% potassium did not alter the intestinal propulsive motility of the albino rats when compared with observations of control animals on a diet containing 0.5% potassium and 0.25% sodium.

1. Gazes, P. C., Richardson, J. A., and Cotton, M. deV., *J. Lab. and Clin. Med.*, 1951, v37, 902.

2. Webster, D. R., Henrikson, H. W., and Currie, D. J., *Ann. Surg.*, 1950, v132, 779

3. Meyer, J. H., Grunert, R. R., Zepplin, T. M., Grummer, R. H., Bohstedt, G., and Phillips, P. H., *Am. J. Physiol.*, 1950, v152, 182.

4. Grunert, R. R., and Phillips, P. H., *J. Biol. Chem.*, 1949, v181, 821.

5. Macht, D. I., PROC. SOC. EXP. BIOL. AND MED.,

1930, v30, 1272.
6. Fenn, W. O., *Physiol. Rev.*, 1940, v20, 377.
7. Best, C. H., and Taylor, N. B., 1945, *The Physiological Basis of Medical Practice*, The Williams and Wilkins Co., Baltimore, page 501.
8. Boyer, P. D., Lardy, H. A., and Phillips, P. H., *J. Biol. Chem.*, 1943, v149, 529.

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## Vitamin $B_{12}$ Biosynthesis after Oral and Intravenous Administration of Inorganic $Co^{60}$ to Sheep.* (19586)

R. A. MONROE, H. E. SAUBERLICH,† C. L. COMAR, AND S. L. HOOD.

*From the University of Tennessee-Atomic Energy Commission Agricultural Research Program, Oak Ridge*

The literature through 1950 concerning the chemistry, physiological role, and clinical use of vit. $B_{12}$ has been reviewed comprehensively by Smith(1), Ungley(2), and Marston(3). The essentiality of cobalt in ruminant nutrition has been recognized for many years(4,5) and a number of distribution studies using inorganic radioactive cobalt have been reported(6-12). Also, some information is available regarding the distribution of vit. $B_{12}$ labeled with radioactive cobalt(12-14). Nevertheless, the relationship between the dietary need of inorganic cobalt and the physiological role of vit. $B_{12}$, especially concerning the factors involved in $B_{12}$ biosynthesis, has remained little understood.

The present work extends the information obtained in earlier radiocobalt studies by partition of the labeled cobalt into 3 distinct fractions of the excretions and tissues—namely, vit. $B_{12}$-like, inorganic, and bound. These data have yielded evidence concerning the degree and possible sites of vit. $B_{12}$ biosynthesis in sheep.

*Methods. Treatment of animals.* Four normal wether lambs weighing about 80 lb each were placed in metabolism stalls. Each of the animals received 2 mc of $Co^{60}$ chloride solution containing about 1.6 mg of cobalt. Two were dosed orally by gelatin capsule and 2 received an intravenous (jugular) injection. The metabolism stalls and the technics for handling the animals, administering the activity, and collecting samples have been described by Hansard, *et al.*(15). *Collection of samples.* Feces and urine were collected continuously and samples were taken daily over a period of a week. The urine was collected under toluene and both feces and urine samples were refrigerated with toluene added. At the end of 7 days the animals were sacrificed and various tissues were sampled for analysis. Aliquots of each sample were counted for total radioactivity. In addition, butanol-soluble and water-soluble phases of similar aliquots were separated and counted as described in detail below. In all samples except urine some residual activity was also obtained in the insoluble fraction.

*Counting technics.* Several counting technics were employed in the work, depending on the sample size and the amount of radioactivity present in the sample. With large amounts of activity, accurate counts were obtained by measuring a few grams of fresh sample using an unshielded Geiger-Müller tube with an 89.5 mg/cm$^2$ aluminum absorber, thus recording only gamma activity. With somewhat less activity, it was necessary to count without an absorber. In this case, a wet ash digest was prepared, to assure reproducible geometry, and measured with the unshielded thin-window tube. Still smaller amounts of activity were counted by using dry ashed samples and a shielded thin-window G-M tube.

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† Oak Ridge Institute of Nuclear Studies Fellow on leave from Alabama Polytechnic Institute.