shown that sera of rabbits immunized against unheated aqueous extracts of Brown-Pearce carcinoma give cross reactions with pus of rabbits, though not with aqueous extracts of normal organs.

However, if future experiments should conclusively demonstrate the presence of specific tumor antibodies, it would still be necessary to exclude the obvious likelihood (Dmochowski[3]) that the antigenic substance is part of the cellular elements of the tumor, before the virus analogy could logically be pressed. The specificity of tumors such as the one under consideration is comparable to the specificity of transplants in which genetic factors strongly influence the course of growth. These genetic differences must be reflected in chemical differences. Thus it would seem possible that genetic differences between the Brown-Pearce tumor and its host could exist and result in the formation of antibodies. Due to more rapid growth, such antibodies might be more readily formed by a tumor than a transplant.

13048

Rumen Synthesis of the Vitamin B Complex on Natural Rations.*†

M. I. WEGNER, A. N. BOOTH, C. A. ELVEHJEM AND E. B. HART.

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

During the past several years considerable work demonstrating the synthesis of the vitamin B complex in the rumen of polygastric animals has been reported.[1-4] The above mentioned work showed conclusively the synthesis of the vitamin B complex in the rumen when the animal was fed a ration devoid or nearly so of the B complex. What the picture would be if the animal was fed a natural ration containing the members of the B complex has as yet received

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1 Bechdel, S. I., *et al.*, *J. Biol. Chem.*, 1928, **80**, 231.

2 McElroy, L. W., and Goss, H., *Proc. Am. Soc. Biol. Chem.*, 1940, **34**, lxv; *J. Biol. Chem.*, 1939, **130**, 437.

3 Wegner, M. I., *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 769.

4 Johnson, P., *et al.*, *J. Dairy Sci.*, 1941, **24**, 57.

TABLE I.
Composition of the Grain Mixtures.

Trial No.	Basal grain mix,* lb	Linseed oil meal, lb	Urea, lb	% protein (N x 6.25)
1	80	—	—	11.3
2	78	—	2.0	17.1
3	64.5	15.5	0.6	17.1
4	50	29	1.2	23.3
5	37	41	2.0	31.1
6	74.5	—	5.5	31.2

*Consists of a 50-50 mixture of ground corn and oats to which 1% salt and 1% bone meal were added.

little attention.[5] The opportunity to investigate this question presented itself during a study of the problem of inorganic nitrogen utilization by ruminants. We had available for this study a heifer with a rumen fistula from which samples of the rumen ingesta could be removed at will. The object of the work reported in this paper was to determine whether adding urea, linseed oil meal or both to the grain mixture of a basal ration in varying proportions had any influence on the synthesis of the B complex in the rumen.

The Holstein heifer used weighed approximately 1000 pounds and had a rumen fistula equipped with a rubber plug which insured almost anaerobic conditions in the rumen and prevented leakage or loss of rumen contents. The basal ration fed consisted of 15 pounds of corn silage, 4 pounds of timothy hay, and 4 pounds of grain mixture (½ ground corn, ½ ground oats). The amount of corn silage and timothy hay fed remained constant throughout the duration of the experiment. The only variable was in the grain mixture to which urea or oil meal, or both were added as listed in Table I.

The animal was fed daily at 8 a.m. and 8 p.m. Samples were taken only after an equilibrium period on each individual grain ration of a week or more. All samples were obtained 6 hours after feeding (2 p.m.). The animal always consumed all of the ration within one hour after feeding. The sampling was done by hand in such a manner as to obtain a representative aliquot of the rumen material. Four to 5 kilos of the wet material were obtained at each sampling. The samples were taken on alternate days until about 20 kilos of the wet material (13 to 15% dry matter) had been obtained. The ingesta were stored in a dark-cold room at 5-10°C in crocks for 4 to 8 days, having first been treated with 95% ethanol so that the final concentration of alcohol was approximately 50%. Then the wet material was dried in a dark drying room (temperature 40-50°C) in shallow enamel lined pans for 24-40 hours. The dry ma-

[5] Hunt, C. H., *et al.*, *J. Nutr.*, 1941, **21**, 85.

terial was next finely ground in a Wiley mill and stored in glass jars in the cold room until needed for assay. The assays were run on a composite of all samples taken for a particular trial. All of the above mentioned steps were taken in order to standardize the conditions with each ration that was tried.

The methods of assay used were as follows: Thiamin, chick;[7] riboflavin, microbiological;[8] nicotinic acid, chemical[9] and microbiological;[10] pyridoxine, rat;[11] pantothenic acid, microbiological;[12] biotin, microbiological.[13]

Examination of Table II shows only a slight variation in the B complex content with the various grain mixtures fed. The greatest variation was in the protein or nitrogen content of the rations.

Table III lists the 6 components of the vitamin B complex assayed for in the rumen ingesta. As can be observed, nearly all the factors are higher in the ingesta than in the feed. Data are also

TABLE II.
B Complex Content of Rations and Ration Constituents Fed.
(Gamma per gram dry matter.)

Material assayed	Thiamin	Ribo-flavin	Pyrido-xine HCl	Ca Panto-thenate	Nicotinic acid		Biotin
					Chemical	Bacterial	
Basal grain mix	7-8.0	2.28	2-3	16.0	177	20.0	.091
Timothy Hay	2.0	4.86	3-4	10.0	190	23.0	.032
Corn Silage	<0.5	9.92	1-2	4.7	303	15.0	.080
Linseed oil meal	5-6.0	<0.3	5-6	23.0	156	43.0	.166
Trial 1 (calc. ration content)	3.3	5.68	2.5	10.2	225	19.0	.068
Trial 2 (calc. ration content)	3.3	5.68	2.5	10.2	225	19.0	.068
Trial 3 (calc. ration content)	3.2	5.40	3.0	11.6	220	20.5	.073
Trial 4 (calc. ration content)	3.1	5.23	3.5	13.0	215	22.0	.078
Trial 5 (calc. ration content)	3.0	5.10	3.8	14.0	211	23.1	.081
Trial 6 (calc. ration content)	3.25	5.62	2.5	10.2	224	19.0	.068
Synthetic Calf Ration	0	<0.4	<1.0	<3.4	60	<1.0	<.018

[7] Arnold, A., and Elvehjem, C. A., *J. Nutr.*, 1938, **15**, 403.

[8] Snell, E. E., and Strong, F. M., *J. Ind. and Eng. Chem., Anal. Ed.*, 1939, **11**, 346.

[9] Melnick, D., and Field, H., Jr., *J. Biol. Chem.*, 1940, **134**, 1.

[10] Snell, E. E., and Wright, L. D., Reported to Federation of Am. Soc. for Exp. Biol., April 15-19, 1941 (Chicago).

[11] Conger, T. W., and Elvehjem, C. A., in press.

[12] Strong, F. M., *et al.*, Reported to Am. Chem. Soc. Div. Biol. Chem., 1940 (Detroit).

[13] Lampen, J. O., *et al.*, Reported to Div. of Biol. Chem. of Am. Chem. Soc., Detroit, Mich., Sept. 9-13, 1940, p. 21.

TABLE III.
B Complex Content of Rumen Contents.
(Gamma per gram dry matter.)

Material assay	Thiamin	Ribo-flavin	Pyrido-xine HCl	Ca Panto-thenate	Nicotinic acid		Biotin
					Chemical	Bacterial	
Heifer—Trial 1	4-5	24.6	4-5	26.6	530	50	.080
" " 2	2-3	26.7	8-9	31.0	568	56	.125
" " 3	4-5	22.7	7-8	29.2	466	63	—
" " 4	3.5-4.5	16.3	10.3	27.6	444	58	—
" " 5	4-5	11.6	8.5	31.2	438	68	—
" " 6	—	13.6	—	24.4	—	66	—
" " 6 (dried at pH 4.7)	—	12.9	—	8.0	—	—	—
Calf—Trial 1	4-5	29.0	3-4	33.0	520	54	.091
Calf—On synthetic ration	10-12	18.6	7.0	55.5	220	73	.087
Calf—On synthetic ration (plus 200 mg B_1 per day)	>20	26.5	11-12	82.5	187	100	.250

given in Table III on the effect of an acid pH during drying on the amounts of riboflavin and pantothenic acid in the ingesta. In addition, the question of variation in the vitamin content of the rumen material of 2 different animals receiving the same ration was investigated, and the results included in Table III.

From the data it can be seen that as the nitrogen level of the rations being fed was increased there was little change in the vitamin levels with the exception of the riboflavin values which decreased. No explanation for the decrease is readily apparent. The increase in the components of the B complex found in the ingesta compared to the rations fed are not absolute values. That is, a number of variables will affect the values obtained. Since the factors assayed for are water soluble, the concentration might be greater, the higher the moisture content of the samples taken. This is due to the fact that analyses are based on dry matter. All our samples were very uniform in moisture content from trial to trial. Another factor that would cause variations is the time of sampling after feeding since bacterial activity and leaching do influence the vitamin content.[5]

We feel that the increase in B vitamins in the ingesta as contrasted with the ration fed is due to a synthesis and not to a concentration effect. The first reason for this conclusion is that the vitamin increases in the ingesta over the ration are not all of the same magnitude. Further evidence is the decrease in flavin with an increase of protein in the ration while the other factors show little change. Lastly, since the B vitamins are water soluble a decrease due to leaching rather than an increase would be expected in the ingesta if no synthesis took place.

The values obtained from the rumen content when the basal ration alone was fed, as compared to the other rations used, gives a clearer picture of any changes produced since the variable of rumen digestion and absorption is the same in each case and is therefore eliminated.

The duplication of these results with another animal was tried. Fortunately, another Holstein calf (400 pounds) with a rumen fistula was available. Trial 1 was repeated using this animal and the results obtained show that the figures are of the same magnitude as those found in the heifer.

Since our last report[5] a new method for the determination of nicotinic acid became available and we are therefore including two sets of figures for this substance. We feel that the bacterial method gives a more accurate estimation of the biological activity of the rumen ingesta for this factor.

For comparison, values previously reported[3] by feeding a "synthetic" ration to a calf are also included.

Treating the sample of rumen ingesta at a pH 4.5-5.0 did not enhance the flavin value as was reported by McElroy and Goss.[6] In fact, the acid had a destructive action on the pantothenic acid. However, the possibility exists that the differences are due to the types of rations used.

Summary. Six members of the vitamin B complex in the rumen ingesta of a heifer fed a ration composed of natural feeds were determined. In most cases higher values were found in the rumen ingesta than in the ration fed. With the exception of flavin, variation of the amount of urea or protein in the grain mixture of the ration had little if any effect on the vitamin content of the ingesta.

13049 P

Non-Specific Precipitation of Sulfathiazole Azo-Conjugates by Immune Sera; Inhibition and Complement-Fixation.

SAMUEL C. BUKANTZ AND THEODORE J. ABERNETHY. (Introduced by Michael Heidelberger.)

From the Parmelee Pneumonia Research Laboratory, Emergency Hospital, Washington, D.C.

A case of acquired sulfathiazole sensitivity exhibiting a specific response to administration of single doses of sulfathiazole has re-

[6] McElroy, L. W., and Goss, H., *J. Nutr.*, 1940, **20**, 541.