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.

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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.

cently been described.[1] Similar observations have been made on a patient sensitized to sodium sulfapyridine.[2] The specificity of the response prompted a search for the presence of antibodies specific for the sulfonamide grouping. Attempts to detect such antibodies to pure sulfonamide drug antigens have failed;[3] test antigens were therefore prepared by coupling diazotized sulfathiazole with crystalline horse serum albumin, horse serum globulin, and human serum globulin.[4] Similar azo antigens have already been employed in studies of human sulfonamide sensitivity with negative results[5, 6] although the development of specific antibodies by the rabbit immunized with sulfonamide azo proteins has been described.[3, 7]

The sulfathiazole azo proteins precipitated with some supposedly normal and several unrelated immune sera. Similar precipitation of azo proteins, in high concentration, was encountered by Landsteiner and Van der Scheer,[8] who diluted their antigens to eliminate the non-specific precipitation. Resorcinol-disazosulfathiazole (ResDST) was also synthesized[9] to determine whether exclusion of protein from the molecule would eliminate non-specific precipitation. The unrelated sera,* however, also precipitated with ResDST. The amount of precipitation depended upon the proportion in which serum and ResDST were mixed and inhibition zones were encountered with excess of antibody or antigen (Table I). With certain serum-dye ratios exposure to 37°C resulted in solution of the precipitate, the amount of precipitate in other mixtures changing in character from dispersed floccules to a cohesive disc. Precipitations of other azo proteins and horse antipneumococcal sera have already

1 Abernethy, T. J., Bukantz, S. C., and Minor, J., Presented at the Society of Clinical Investigation, Atlantic City, May 5, 1941.

2 Davidson, A. D., and Bullowa, J. G. M., *New Eng. J. Med.*, 1940, **22**, 811.

3 *Vide* 2 and Wedum, A. G., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 218, for bibliography.

4 a. Landsteiner, K., and Lampl, H., *Z. Immunitats Forsch.*, 1917, **26**, 293; b. Heidelberger, M., Kendall, F. E., and Soo Hoo, C. M., *J. Exp. Med.*, 1933, **58**, 137.

5 Schlesinger, E. R., and Mitchell, W. L., Jr., *Am. J. Dis. Child.*, 1938, **56**, 1256.

6 Wedum, A. G., *J. Bact.* (abstr.), 1941, **41**, 64.

7 Mingoia, Q., Rocha Lima, and Silva, M., *Boll. d'inst. sieroterap. milanese*, 1940, **19**, 101.

8 Landsteiner, K., and Van der Scheer, J., *J. Exp. Med.*, 1927, **45**, 1050.

9 Landsteiner, K., and Van der Scheer, J., *J. Exp. Med.*, 1932, **56**, 399.

* Rabbit anti-horse serum albumin; anti-horse serum globulin, anti-sheep cell, antipneumococcal 3 (Lederle), and anti-egg-albumin (kindly supplied by Dr. Michael Heidelberger); horse antipneumococcal 1 (Lederle) and horse anti-egg-albumin (kindly supplied by Dr. A. W. Pappenheimer, Jr.). Normal horse and rabbit sera and 2 horse antitoxins failed to precipitate with these antigens.

TABLE I.

Effect of Varying Antigen-antibody Ratios on the Amount of Precipitate Formed by Rabbit Antipneumococcal 3 Serum and Resorcinoldisazosulfathiazole.

0.2 cc of the desired serum dilution delivered, by pipet, into 0.2 cc of antigen. Readings after 1 hour at room temperature. Saline controls were negative.

Serum* dilution	Molar concentration of resorcinoldisazosulfathiazole				
	M/50	M/500	M/2,500	M/25,000	M/50,000
Undiluted	++++	+++	+±	±	±
1-5	0	+++±	++±	+	±
1-20	0	++±	++	+	±
1-80	0	+±	++	+±	±
1-320	0	±	+±	+	±
1-1280	0	0	+	+	±

*Rabbit antipneumococcal 3 serum No. 473H370h (Lederle), 9,000 units per cc (Kindly supplied by Dr. W. G. Malcolm).

been described;[10, 11, 12] rabbit antipneumococcal sera, however, failed to precipitate.[11]

It seemed of interest to determine whether the homologous pure sulfonamide drugs or their neutral azo components would inhibit the precipitation between sulfathiazole azo protein and the non-azo-specific antipneumococcal rabbit serum. M/10 solutions of the pure sulfonamide drugs can be obtained only with the soluble sodium salts which are highly alkaline in this concentration, M/10 sodium sulfapyridine having a greater titratable alkalinity than M/10 sodium sulfathiazole. The precipitation of sulfathiazole azo protein by antipneumococcal rabbit serum was completely inhibited by a saline solution equal in titratable alkalinity to M/10 sodium sulfapyridine and M/10 sodium sulfathiazole caused no greater inhibition than an equally alkaline saline solution. Simple azo components of haptens are more active inhibitors than pure haptens.[13] The inhibitory activity of a simple azo compound of sulfathiazole, ResDST, was also investigated. 0.3 cc of M/50 ResDST completely inhibited the precipitation between 0.2 cc of antipneumococcal 3 rabbit serum and 0.5 cc of 1 to 500 sulfathiazole azo crystalline horse serum albumin. This observation suggests that inhibition depends upon the chemical identity of the antigenic components of the reaction and does not necessarily indicate the presence, within the precipitating serum, of specific antibodies to the hapten.[13]

In complement-fixation studies, 2 units of complement were completely fixed by the resorcinol conjugate in the presence of anti-

10 Woolf, B., Marrack, J. R., and Downie, A. W., *J. Soc. Chem. Ind.*, 1936, **55**, 156.

11 Goebel, W. F., and Hotchkiss, R. D., *J. Exp. Med.*, 1937, **66**, 191.

12 Goebel, W. F., *J. Exp. Med.*, 1936, **64**, 29.

13 Landsteiner, K., *Bioch. Z.*, 1920, **104**, 285.

pneumococcal horse or rabbit sera. Complement fixation by atoxyl azo antigens and homologous antisera have already been described.[14]

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Excretion of Sulfathiazole in Tears.

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Seven cases of conjunctivitis occurring during the administration of sulfathiazole have been observed at the Beth Israel Hospital during the last 5 months. Quantitative determinations of the sulfathiazole concentration in the tears were made in 4 of these. Similar quantitative studies were carried out in 10 patients who were taking sulfathiazole but had no evidence of conjunctivitis (Table I). Blood sulfathiazole determinations were made in all cases at the time that the lachrymal secretion was collected.

Technic. The eyelids were first carefully cleansed with ether to rid them of sebaceous material. Tears were stimulated by the gentle application to the inner canthus of a cotton swab moistened with ether. The lachrymal secretion was then collected at the outer canthus by a fine capillary pipette. Controls had shown that this procedure did not cause any conjunctival irritation. Since sulfathiazole secretion in sweat had been demonstrated by the authors, care was exercised not to collect any tears which had overflowed on to the cheek.

The total amount of lachrymal secretion obtained from both eyes varied from 0.4 cc to 0.9 cc, and, in each case, 1.2 cc of 4 N HCl was added to the entire measured amount. This was then diluted with distilled water to a volume of 25 cc. Ten cc of this was used as a blank control. Another 10 cc was diazotized using N (1-naphthyl)

[14] Klopstock, A., and Selter, G. E., *Z. Immunitats. Forsch.*, 1928, **55**, 450; **57**, 174.