There are, then, at least 2 factors required for the growth of *T. geleii* in addition to thiamin and possibly riboflavin or other known vitamins. Further attempts at purification of these factors are in progress. Meanwhile, the protein-free hay fractions are being used to determine the amino-acid requirements of the organism.

Gelatin (vitamin-free Harris) was made up in 1% solution containing the 2 fractions from hay plus thiamin and pyridoxin. While growth was obtained in the sixth transplant in this medium, it is far from equal to that in crude casein. No growth at all occurred in second transplant in a solution containing the ten essential amino acids (for the rat) in the proportions found in 1% casein. Both of the hay fractions, thiamin and pyridoxin were also present. There are 5 possible reasons for the failure of the organism to grow in the amino acid solution: 1. Polypeptides are required; 2. some supplement is lacking; 3. an essential amino acid is lacking; 4. the medium contains a toxic substance; 5. the osmotic pressure of the medium is too high. This last possibility seems the most likely, since toxicity is eliminated by the fact that the addition of 1 ml of the amino acid solution to 5 ml of 1% gelatin plus supplements makes possible excellent growth.

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Concentration of Free Sulfanilamide, Sulfapyridine and Sulfathiazol in Material Drained from Human Biliary Tract.

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Two grams of sulfanilamide, sulfapyridine and sulfathiazol were ingested in successive experiments by 4 patients from whom bile was draining through tubes inserted in the bile duct after operations upon the biliary tract. Intervals of 3 or 4 days separated the experiments upon each subject. A satisfactory test with sulfapyridine was not obtained in one instance. Specimens of bile were collected for 4 successive 4-hour periods in each experiment. The first period preceded, and the others followed the ingestion of the drug. Samples of urine were collected simultaneously with the bile specimens after the drug had been given, and a sample of blood was drawn at the midpoint of each of these 3 4-hour periods. The free compounds were determined in the specimens of bile by the diazo technic of Brat-

ton and Marshall[1] in the presence of acetone. Since the usual methods for clarifying solutions, including the one used upon bile by Hubbard and Anderson[2] precipitated a rather high proportion of added sulfathiazol, the bile was clarified by the use of 2 successive precipitations with barium—first in alkaline solution as the phosphotungstate and then in acid solution as the sulfate. The sulfonamide drugs could be determined with an accuracy of about 5%, or, if present in very low concentrations, to the nearest 0.1 mg per 100 cc by this procedure. Control specimens, analyzed as a part of each experiment, gave no red color by the technic. The concentrations of the drugs in the urine were determined after dilution, one part to 100, by the same diazo technic. Blood analyses were carried out as described by Bratton and Marshall.[1]

The results upon the blood and bile of different subjects were qualitatively similar, except for such variations in actual blood concentrations as would be expected from the varying ease of absorption of the different compounds studied.[3] The actual relationships between the concentrations in blood and bile were sufficiently alike in the studies upon the different patients to justify presenting an average of the figures. Although the excretion in the different experiments varied rather markedly, corresponding, at least in some instances, with the excretion of total fluid,[4] the results have also been com-

TABLE I.
Excretion of Free Sulfonamide Compounds.
Average for 3 patients.

Compound	Period, 4 hr	Blood, mg/100 cc	Bile, mg/100 cc	Urine	
				Vol., cc	Drug, mg
Sulfanilamide	1st	5.5	3.6	359	102
	2d	3.9	2.6	181	100
	3d	2.8	2.3	250	111
Sulfathiazol	1st	2.9	0.1	115	102
	2d	3.1	0.3	197	131
	3d	2.1	0.4	417	217
Sulfapyridine	1st	1.4	0.7	387	26
	2d	1.4	1.3	212	55
	3d	1.2	1.1	230	44

1 Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.

2 Hubbard, R. S., and Anderson, R. K., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 487.

3 Goodman, L., and Gilman, A., *The Pharmacological Basis of Therapeutics*, New York, 1941, pp. 1014, 1068, 1087.

4 Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, W. C., *J. Pharm. Exp. Therap.*, 1937, **61**, 191.

bined. In Table I are given the average values for the 3 patients who received all 3 of the compounds studied.

The table shows the lack of a quantitative relationship between the amounts of the drugs present in the blood and urine which has repeatedly been demonstrated, and suggests, particularly in the experiments upon sulfanilamide, that the rate of excretion of these substances is relatively constant when the urine volume is constant.[4] The excretion of the drugs into the bile shows marked qualitative differences, which, as already stated, was present in each of the individual experiments. Considerable amounts of sulfanilamide were present in the bile. These paralleled approximately the concentrations in the blood, but the values of the ratio $\frac{\text{Free sulfanilamide in bile}}{\text{Free sulfanilamide in blood}}$ increased during the later parts of the experiments as previously reported.[2] The concentration of sulfathiazol, in the bile, on the other hand, was very low throughout the periods of study, and increased rather than diminished in spite of the drop in the concentration in the blood. This increase in concentration, although slight, was much beyond the limit of accuracy of the methods, and was shown in all the experiments carried out. The results with sulfapyridine were more irregular than were those obtained with the other two drugs, and perhaps are associated with the effect of the known irregularities in the absorption of this compound[5, 6] upon the blood concentrations. The figures obtained in these studies resemble, the authors believe, those obtained with sulfanilamide rather than the results with sulfathiazol.

The results with sulfathiazol are difficult to interpret. It has been reported that this drug enters the spinal fluid in relatively low concentration but diffuses fairly readily into other body fluids.[7] It is possible that this relative failure of passage into the liver is an expression of a general relatively low diffusibility of this compound. Against such an assumption are the figures upon the urinary excretion of this substance shown in the table and included in the literature.[8] It is possible that there is a precipitation or alteration of the compound involved in the loss, but if this is so the loss must be during the formation of the bile, for no significant change in the

5 Long, P. H., and Feinstone, W. J., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 486.

6 Brown, W. H., Thornton, W. B., and Wilson, J. S., *J. Clin. Invest.*, 1939, **18**, 803.

7 Sadusk, J. F., Jr., and Hershfeld, J. W., quoted by Goodman, L., and Gilman, A., *The Pharmacological Basis of Therapeutics*, New York, 1941, p. 1088.

8 Sadusk, J. F., Jr., Blake, F. G., and Seymour, A., *Yale J. Biol. Med.*, 1940, **12**, 681.

sulfathiazol content occurred when bile was stored for several days. It was not possible to test the material for the presence of acetylated sulfathiazol by the methods available. It seems improbable although not absolutely impossible, that acetylsulfathiazol might have been present in bile in spite of the practically complete absence of acetylsulfanilamide in such material.[2] The authors are not able to give a satisfactory interpretation of this finding, but they do wish to point out one result of the difference in concentration of sulfanilamide and sulfathiazol in the bile. When relatively large amounts of a readily absorbed substance enter the bile and are returned to the intestine the time during which the compound remains in the body is prolonged. Excretion by the liver, therefore, will tend, at least slightly, to prolong the period during which sulfanilamide remains in the body as compared with the time sulfathiazol remains in the body.

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Ineffectiveness of Adrenal Cortical Hormone on Serum Complement of the Guinea Pig.*

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Partial adrenalectomy increases the susceptibility of guinea pigs and rats to anaphylactic shock.[1, 2, 3] The lethal dose of antigen has been found to be inversely proportional to the degree of adrenal insufficiency. Wolfram and Zwemer[4] report that "cortin" protects normal sensitized guinea pigs against fatal anaphylaxis. In the dog the severity of anaphylactic shock is said to be lessened by the previous injection of cortical hormone.[5] Perla and his coworkers[6]

* The expenses of this investigation were provided by the Proctor Fund, Harvard University.

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2 Flashman, D. H., *J. Inf. Dis.*, 1926, **38**, 461.

3 Wyman, L. C., *Am. J. Phys.*, 1929, **89**, 356.

4 Wolfram, J., and Zwemer, R. L., *J. Exp. Med.*, 1935, **61**, 9.

5 Dragstedt, C. A., Mills, M. A., and Mead, F. B., *J. Pharm. and Exp. Ther.*, 1937, **59**, 359.

6 Perla, D., Freiman, D. G., Sandberg, M., and Greenberg, S. S., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 397.