

periods indicates that even without catheterization, uniform daily samples of urine may be obtained provided the food intake is constant.

3. The H-ion concentration of the urine is approximately  $\text{PH}^+ = 8.9$  on a carrot diet, and  $\text{PH}^+ = 7.6$  on a cabbage diet. These variations correspond to the differences in the ash analyses of these two vegetables.

4. The elimination of total nitrogen on a cabbage diet is higher than on a carrot diet. The relation of the urea nitrogen to the total nitrogen excreted is constant and averages about 84% of the total nitrogen. The ammonia content of the urine is negligible.

5. The urinary excretion of phosphorus on a carrot (more alkaline) diet is greater than on a cabbage (less alkaline) diet.

6. The creatinine excretion is constant and independent of the nature or extent of the diet. The creatinine coefficient is 12 to 14.

URBANA, ILL.

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[CONTRIBUTION FROM THE LABORATORIES OF AGRICULTURAL BIOCHEMISTRY, MINNESOTA AGRICULTURAL EXPERIMENT STATION.]

## COMPARATIVE ANALYSES OF FIBRIN FROM DIFFERENT ANIMALS.<sup>1</sup>

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The ease with which fibrin can be secured and purified recommends it to the biological chemist as a valuable form of protein upon which to carry out comparative studies. However, in so far as the available literature shows, there seems to be no definite proof that the fibrins from different animals are identical in composition. Indeed certain analyses carried out by one of us indicated that perhaps there might be a difference between the fibrin procured from a European supply house and that procured from an American firm. It seemed possible that the fibrin of European origin came from horse blood,<sup>2</sup> while in all probability the fibrin prepared by American firms comes from some other source.

It seemed of sufficient worth, therefore, to prepare fibrin from some different animals and to make comparative analyses of the resulting products. The fibrins from sheep, cattle (two preparations) and swine blood were accordingly prepared and analyzed with the results shown below. Unfortunately it was not possible to prepare fibrin from horse blood at this time but one of us intends to eventually secure this material and to make the necessary analyses.

<sup>1</sup> Published with the approval of the Director as Paper No. 81, Journal Series of the Minnesota Agricultural Experiment Station.

<sup>2</sup> Samuely (Abderhalden's "Handb. Biochem. Arbeitsmethoden," II, p. 368) gives directions for the preparation of fibrin from horse blood which would indicate that this was the usual source.

### Experimental.

**The Preparation of the Fibrin.**—The fibrin was prepared by whipping freshly drawn blood<sup>1</sup> until the fibrin separated as a stringy mass. This crude fibrin was placed in water and brought at once to the laboratory, where it was thoroughly washed with distilled water to remove the blood. The washed fibrin was then passed through a food chopper which cut the material into pieces about 3 mm. long and this material was again extracted with distilled water. The fibrin was drained on cheese-cloth and the excess of moisture pressed out in a hydraulic press capable of a pressure of 400 kg. per sq. cm. The cake of fibrin was then placed in a 10% solution of sodium chloride and agitated until the particles of fibrin were entirely separated from each other. In this stage the material was allowed to stand overnight at 0° and then drained and pressed in the hydraulic press and then successively washed with water, drained and pressed until all chlorides had been removed. The pressed cake was disintegrated as completely as possible and dried in a vacuum oven at 45°. The perfectly dry product was ground to a powder in a Seck mill and this powder extracted with petroleum ether in a Soxhlet apparatus for 48 hours.<sup>2</sup>

Prepared in this manner, both preparations of fibrin from cattle blood were almost pure white in color, while those from swine and sheep blood were dark gray powders. The products contained the following percentages of nitrogen:

Cattle "A" fibrin = 15.81	Swine fibrin = 15.64
Cattle "B" fibrin = 15.82	Sheep fibrin = 15.72

Merck's "Fibrin from blood," analyses of which have already been reported,<sup>3</sup> had a nitrogen content of 15.17%.

**Method of Analysis.**—Duplicate samples of 3 g. of dry fibrin were hydrolyzed for 48 hrs. with HCl (sp. gr. 1.115) and Van Slyke's<sup>4</sup> method of protein analysis applied to the resulting hydrolysates. However, instead of recording only one fraction as "humic nitrogen," we have preferred to divide the humic nitrogen into three fractions, *i. e.*, "insoluble humic N," "soluble humic N (pptd. by Ca(OH)<sub>2</sub>)" and "humic N in phosphotungstic acid precipitate." Van Slyke recognized this latter fraction in a later paper<sup>5</sup> and unpublished work in this laboratory demonstrates the

<sup>1</sup> We wish to thank Mr. Hood, of Swift & Co., South St. Paul, Minn., for kindly furnishing us facilities for the preparation of the crude fibrin.

<sup>2</sup> Extraction with alcohol or ethyl ether was purposely avoided inasmuch as we wished to guard against any possibility of the introduction of aldehydes. It will be shown in a subsequent paper by one of us that very small quantities of aldehyde may radically alter the nitrogen distribution in a protein hydrolysate.

<sup>3</sup> Gortner, *J. Biol. Chem.*, **26**, 187 (1916).

<sup>4</sup> Van Slyke, *Ibid.*, **10**, 15-55 (1911); **12**, 275-284 (1912).

<sup>5</sup> Van Slyke, *Ibid.*, **22**, 284 (1915).

advisability of recording the "insoluble" and "soluble humin N" separately.

"The acid-insoluble humin was obtained by diluting the hydrolysate, filtering off the insoluble humin and washing it free from acid. The filtrate from the acid-insoluble humin was then concentrated as usual, to get rid of the excess of HCl, calcium hydroxide added and the ammonia distilled off. The 'acid-soluble' humin was adsorbed by or combined with the lime, and was determined in the usual manner for the humin determination."<sup>1</sup>

**The Analytical Data.**—The distribution of nitrogen in percentages of the total nitrogen is shown in Table I. These data are the average of duplicate closely agreeing determinations.

TABLE I.—NITROGEN DISTRIBUTION IN FIBRINS FROM DIFFERENT ANIMALS IN PERCENTAGES OF THE TOTAL NITROGEN.

	Cattle "A" fibrin.	Cattle "B" fibrin.	Sheep fibrin.	Swine fibrin.
Ammonia N.....	9.30	8.93	9.20	8.42
Acid-insoluble humin N.....	1.83	1.54	1.49	1.59
Acid-soluble humin N.....	1.23	1.26	1.38	1.06
Humin N in phosphotungstate ppt.	0.88	0.75	0.53	0.65
Total basic N <sup>2</sup> .....	29.76	30.02	30.01	30.31
Amino N in bases <sup>2</sup> .....	18.14	17.68	17.43	17.56
Non-amino N in bases <sup>2</sup> .....	11.14	12.35	12.58	12.77
Arginine N.....	15.12	14.99	16.12	15.19
Histidine N.....	0.11	1.10	0.74	1.36
Lysine N.....	14.23	13.50	12.85	13.59
Cystine N.....	0.30	0.43	0.30	0.17
Amino N in filt. from bases.....	56.45	56.74	55.64	56.32
Non-amino N in filt. from bases...	0.87	1.54	0.82	2.50
Total N regained.....	100.32	100.78	99.06	100.85

It appears perfectly obvious from a glance at this table that the analyses agree with each other as well as would be expected if they were all run on identical material. The only conclusion possible seems to be that these fibrins are probably identical in amino acid content. They are certainly identical in the content of the diamino acids but, of course, there is a possibility that they differ in the proportion of certain individual non-amino acids contained in the "filtrate from the bases." No method has yet been devised for quantitatively estimating the different constituents of this fraction. It appears improbable, however, that a chemical analysis will demonstrate a difference in these fibrins. In this connection it would be of interest to test the immunity relationships of the different preparations in order to determine whether or not they are actually identical biologically. It has not been possible to do this in this laboratory, but sufficient

<sup>1</sup> Gortner, *J. Biol. Chem.*, 26, 196 (1916).

<sup>2</sup> The figures reported are not included in the total N regained inasmuch as the nitrogen reported here belongs to arginine, histidine, lysine, and cystine. These data are included, however, to facilitate comparisons and calculations.

of the material is available if some one of the readers wishes to undertake the experiment.

#### Summary.

Fibrin has been prepared from the blood of cattle, sheep, and swine, and the nitrogen distribution determined by Van Slyke's method. No differences significantly greater than the expected experimental errors were found. It would thus appear that fibrin from any of these sources can be used interchangeably in experimental work without invalidating the results. Whether or not this is true for fibrins from other sources remains still an open question.

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#### NEW BOOKS.

**Some Compounds of Boron, Oxygen, and Hydrogen.** By MORRIS W. TRAVERS, D.Sc., F.R.S., N. M. GUPTA, B.Sc., AND R. C. RAY, M.Sc. London: H. K. Lewis & Co., Ltd., 1916. Pp. 48. Price, 1/-.

This little book contains an account of work done by its authors upon the products of the reaction of magnesium boride with water. The substances analyzed and otherwise investigated include  $Mg_3B_2(OH)_6$ , the main product of the reaction, two lower oxides,  $B_2O_2$  and  $B_4O_6$ , and soluble compounds like  $3H_6B_2O_2 \cdot 2(MgO \cdot H_2O)$ .

Further work remains to be done upon these compounds, and since the authors do not expect to have sufficient time at their disposal in the near future they express the hope that others will feel induced to take up the investigation.

JOEL H. HILDEBRAND.

**The Nature of Solution.** By HARRY C. JONES. D. Van Nostrand Co. 1917. Pp. xxiii + 380. Price, \$3.50.

This book, written by Professor Jones during the last summer of his life, is in essence a memorial volume, brought out under the supervision of some of his colleagues at Johns Hopkins University; there is a brief biographical sketch, and portrait, of the author, and a bibliography of his articles and books, comprising about 150 separate titles. According to the preface, "The present work is not a text-book, but a general discussion of some of the more important properties of solutions, true and colloidal. It is therefore written in a non-mathematical, indeed, largely in a semi-popular style." Its scope may perhaps best be indicated by the chapter headings: Importance of Solution (19 pp.); Earlier Views as to the Nature of Solution (31 pp.); Osmotic Pressure (23 pp.); Relation between Solutions and Gases (9 pp.); Electrolytic Dissociation (8 pp.); Diffusion (13 pp.); Depression of Vapor Tension of Solvent (19 pp.); Depression of Freezing Point (14 pp.); Electrolytes (20 pp.); Some Electrical Properties of Aqueous Solutions (46 pp.); Solution in Non-Aqueous and Mixed Sol-