

sent the officers of the association in working out with the Ecological Society of America a symposium for the Ottawa meeting. Other suggestions for cooperating with Canadian scientists for the purpose of developing a strong program were approved.

*Richmond Meeting:* Dr. McKinley presented the plans developed by Dr. Walter L. Treadway, Assistant Surgeon General of the U. S. Public Health Service, for a symposium on mental health. The executive committee approved the plans and invited Dr. Treadway to organize and take charge of the symposium, with the assistance in administrative details of the secretary of the Section on Medical Sciences.

*Milwaukee Meeting:* The dates set for the Milwaukee meeting were June 19 to 24, 1939.

#### MISCELLANEOUS REPORTS

Dr. Harvey Fletcher, who was the representative of the association at the Nottingham meeting of the British Association for the Advancement of Science, sent in an interesting report on the methods of our English cousins of conducting general scientific meetings. He commented particularly and approvingly of the interesting general addresses and the enjoyable social functions. Dr. Caldwell presented a report by Dr. Harry A. Carpenter, who represented the association at the Tokio, Japan, Conference of the Science Section of the World Education Federation. Dr. Caldwell was directed to express to Dr. Carpenter the appreciation of the executive committee for representing the association at the Tokio meeting. Dr. Caldwell made a report on plans for weekly broadcasts on science under the auspices of the association, and the permanent secretary was authorized to attempt to put the plans into effect. Dr. Caldwell reported on the Indianapolis program of the American Science Teachers Association and also on the formation of branches of the association. A report from Dr. Gregory D. Walcott, chairman of the Committee on Source Books, was presented; the permanent secretary was directed to express to the committee the association's appreciation of the splendid progress made.

#### APPOINTMENTS OF REPRESENTATIVES

Dr. Ward was appointed the representative of the association in any group discussions pertaining to the Rocky Mountain Tunnel Project. Dr. Malcolm H. Soule was appointed the representative of the association at the Third International Congress for Microbiology, to be held in New York City in the summer of 1939. Dr. Franz Boas was appointed the representative of the association at the International Congress of Anthropology and Ethnology, to be held in Copenhagen, Denmark, from August 1 to 6, 1938. Dr. Selskar M. Gunn and Dr. Reginald R. Gates, of the University of London, King's College, were appointed representatives of the association at the twenty-fifth session of the Indian Science Congress, to be held in Calcutta, India, from January 3 to 8, 1938.

#### MISCELLANEOUS ACTIONS

The executive committee voted that the inscription on the medal accompanying the Theobald Smith Award, the first of which is to be presented to Dr. Robley D. Evans at Indianapolis, shall be: "Awarded in (year) to" on the first line, with the name of the recipient immediately below. Following the report that the bibliography on "Conserving Our Natural Resources" is now ready for free distribution, the executive committee passed a vote of thanks and appreciation to Dr. Joseph Wheeler, Chairman of the Committee on Popular Science Reading Lists. Three fellows were elected upon recommendations of the Sections on Physics and Medical Sciences. The Association for Symbolic Logic, having 196 members, of whom 48 are members of the association and 32 are fellows, was admitted to the relationship of an affiliated society. The executive committee voted its appreciation to Dr. W. W. Campbell for his letter on general operations of the association, in which he expressed the opinion that two meetings per year are inadvisable and that the programs should have more general papers and fewer that are narrowly technical.

F. R. MOULTON,  
*Permanent Secretary*

## SPECIAL ARTICLES

### THE NATURE OF PAPAIN ACTIVATION

AMONG the enzymes that digest high molecular proteins and therefore are designated proteinases, the papainases distinguish themselves by the property of being activated in the presence of SH compounds or of HCN. This activation was considered for some time to be due to a reduction process.<sup>1</sup> Doubts regarding this interpretation arose when it was discovered that activated papain is capable of digesting simple

substances such as hippurylamide<sup>2</sup> and that this digestion can be activated and inhibited by processes which can scarcely be regarded as reductions and oxidations. Thus the hydrolysis of hippurylamide or of carbo-benzoylisoglutamine was inhibited by phenylhydrazine, while the digestion of albumin peptone was activated by the same reagent.<sup>3</sup> This contrasting

<sup>2</sup> M. Bergmann, L. Zervas and J. S. Fruton, *Jour. Biol. Chem.*, 111: 225, 1935.

<sup>3</sup> M. Bergmann and W. F. Ross, *Jour. Biol. Chem.*, 114: 717, 1936.

<sup>1</sup> T. Bersin, *Ergebnisse der Enzymforschung*, 4: 68, 1935.

behavior toward two substrates led to the hypothesis that papain and other papainases are constituted of two partial enzymes which are inactive when associated with each other, but which dissociate in the presence of activators and thus become active.<sup>4</sup>

The experimental basis of this hypothesis seemed unsatisfactory because of the heterogeneous nature of albumin peptone which was employed as one of the substrates. This peptone is a mixture of various protein split-products and frequently contains substances which may influence the activity of the enzyme. We have now found that data similar to those obtained with albumin peptone can be gotten with a well-crystallized synthetic substance, benzoyl arginine amide. The hydrolysis of this synthetic compound is activated by the addition of phenylhydrazine. However, this activation is observed only if the papain preparation is a natural, unpurified extract which contains SH compounds as natural activators. Addition of phenylhydrazine to purified papain, in which these accompanying activators are absent, produces no activation towards either albumin peptone or benzoyl arginine amide. Addition of HCN or a similar activator to the purified papain is necessary to restore the activation by phenylhydrazine (Table I). A similar effect was

Synthetic substrates were also used to study the effect of several purification procedures on the activity of papain. It was observed that essentially all the proteolytic activity toward both gelatin and carbobenzoxyisoglutamine is precipitated between 0.5 and 0.7 saturation with respect to ammonium sulfate.<sup>6</sup>

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# OSMOTIC EFFECTS OF DEUTERIUM OXIDE (HEAVY WATER) ON LIVING CELLS

In considering the permeability of erythrocytes to D<sub>2</sub>O<sup>1</sup> it occurred to me that D<sub>2</sub>O, because its vapor pressure was less than that of H<sub>2</sub>O, would cause osmosis in the same way that concentrated solutions would. The vapor pressures<sup>2</sup> of D<sub>2</sub>O and H<sub>2</sub>O were 15.06 and 17.36 mm respectively at 20°, and H<sub>2</sub>O in a 7.43 M ideal solution would have a vapor pressure equal to that in pure D<sub>2</sub>O, i.e., 15.06 mm. In this case we might suppose that the rate of osmosis is roughly parallel to the difference of equilibrium vapor pressures, i.e., fugacities of the two types of water. I have found that plasmolysis of plant cells and hemolysis of erythrocytes occurs, although osmosis is so transient that in my experiments hemolysis is incomplete, and plasmolysis is expressed only by shrinkage of cell and cell wall together.

I have studied the shrinkage preceding plasmolysis in *Nitella clavata* A. Br., using a method used by Collander and Bärlund<sup>3</sup> for penetration of alcohol into similar material, *Chara*. We have isolated terminal "leaves" 5–12 mm long, attached to a node, and left them in double distilled water for about a day to recover. A leaf was fixed by dropping wax (3 parts cacao, 1 beeswax) on the basal end, meantime supporting the free end with wet filter paper, in a glass cell 20 × 6 mm, and 4 mm deep. About 0.4 ml of water was put in, and the filter paper removed. The cell was put on a mechanical stage under a 16 mm objective (Bausch and Lomb) and No. 8 periplan (Leitz) oculars containing an ocular micrometer. One of the divisions of the scale of this micrometer measured 7.2 μ in the focal plane. Using 1 ml pipettes water was removed and replaced several times to make sure of the regular behavior of the leaf. When the leaf

TABLE I  
ACTIVATION OF PAPAIN BY VARIOUS REAGENTS

Enzyme preparation	Substrate		
	Albumin peptone	Benzoyl arginine amide	Carbo-benzoxyisoglutamine
Natural papain .....	.04	.01	.09
Natural papain + phenylhydrazine .....	.26	.33	.05
Purified papain .....	.00	.02	.03
Purified papain + phenylhydrazine .....	-.02	-.03	.03
Purified papain + phenylhydrazine + HCN .....	.65	.16	.01
Purified papain + phenylhydrazine + cysteine .....		.60	.48

The splitting is expressed as the increase in cc of 0.01 N KOH per 0.2 cc of the reaction mixture. An increase of 1 cc for the synthetic compounds corresponds to 100 per cent. splitting of one peptide linkage. Time interval, 24 hours. Temperature, 40° C. pH, 5.0.

reported<sup>5</sup> for the splitting of carbobenzoxyisoglutamine where the presence of a large concentration of cysteine effected an activation of purified papain + phenylhydrazine. These experiments tend to show that in the behavior of papain toward various substrates there is no absolute differentiation but rather a relative one which depends upon the nature of the substrate. In view of this finding, the hypothesis of a two-enzyme system in papain becomes at present superfluous.

<sup>4</sup> M. Bergmann and J. S. Fruton, *SCIENCE*, 84: 2169, 1936.

<sup>5</sup> M. Bergmann, J. S. Fruton and H. Fraenkel-Conrat, *Jour. Biol. Chem.*, 119: 35, 1937.

<sup>6</sup> A. K. Balls, H. Lineweaver and R. R. Thompson (*SCIENCE*, 86: 379, 1937), used this property for the isolation of papain crystals.

<sup>1</sup> S. C. Brooks, *Jour. Cell. Comp. Physiol.*, 7: 163–171, 1935.

<sup>2</sup> G. N. Lewis and R. T. MacDonald, *Jour. Am. Chem. Soc.*, 55: 3057–59, 1933.

<sup>3</sup> R. Collander and H. Bärlund, *Acta bot. Fenn.*, No. 11, 1–114, 1933.