maceutical dealers, because these preparations are made by very different processes of extraction, concentration or activation, which leave, probably, mixtures of ferments in widely different proportions in the finished products, and unknown amounts of inorganic salts.

(5) There is evidence to suggest that the products sold as trypsins or pancreatins contain at least two different enzymes reacting in different ways with proteins. The effects observed in any case are mixed effects depending on the proportions in which the enzymes are present. These enzymes possess different degrees of thermostability.

(6) The desirability of a more rational definition of trypsin is pointed out. The definition should include a statement of the essential points of manufacture and should be authorized by some responsible body such as a pharmacopoeial revision committee. Since what is called trypsin is prepared for the use of medical men, these users are entitled to the fullest knowledge concerning the composition and properties of the product. There is no excuse for secrecy here and products should be made to conform to interchangeable standards.

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STUDIES ON ENZYME ACTION. XI. SOME EXPERIMENTS WITH CASTOR BEAN UREASE.

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Takeuchi,[1] in 1909, found in soy beans an enzyme, urease, capable of hydrolyzing urea into ammonia and carbon dioxide. Keisel[2] and Zemplen[3] then showed that ureases are present in a number of plants. The action of the soy bean urease under various conditions was studied by Takeuchi, Armstrong and Horton,[4] Armstrong, Benjamin and Horton,[5] and more recently by Van Slyke, Zacharias, and Cullen.[6] The application of the soy bean urease to the quantitative determination of urea was first proposed by E. K. Marshall, Jr.[7]

In view of the interest which has been developed in connection with the soy bean urease and its application to analytical work, some experiments

[1] *J. Coll. Agric., Tokyo*, **1**, 1 (1909).

[2] *Z. physiol. Chem.*, **75**, 169 (1911).

[3] *Ibid.*, **79**, 229 (1912).

[4] *Proc. Roy. Soc. Lond.*, (*B*) **85**, 109 (1912).

[5] *Ibid.*, **86**, 328 (1913).

[6] *Proc. Soc. Exp. Biol. Med.*, **11**, 155 (1914).

[7] *J. Biol. Chem.*, **14**, 283 (1913); **15**, 487, 495 (1913); **17**, 351 (1914); **18**, 53 (1914) (with D. M. Davis). For the quantitative estimation of urea by urease, cf. also Plimmer and Skelton, *Biochem. J.*, **8**, 70 (1914).

with the urease which was shown to exist in castor beans[1] were carried out and will be described in this paper.

Two castor bean preparations were used: Preparation A, consisting of ground, cold pressed, castor beans[2] containing probably about 5% of oil; and Preparation B, consisting of husk- and oil-free castor beans, ground to pass through a 40-mesh sifter. In the experiments, the castor bean preparation was mixed with the urea solution and water or salt solution and after about 1 cc. toluene had been added, allowed to remain in the incubator at 38–40° for the length of time indicated. The ammonia formed was removed by aeration[3] for two or three hours after enough 2 *N* NaOH solution had been added to make the mixture about 1 *N* (with respect to the NaOH). The ammonia was absorbed in the usual way in a definite volume of standard sulfuric acid which was afterwards titrated with standard NaOH solution, with alizarin sulfonic acid as indicator. Preliminary experiments showed that neither the urease preparations nor the urea solutions evolved ammonia in the aeration with normal NaOH solution as described. The urea used contained nitrogen (Kjeldahl determinations) corresponding to 93.8% pure urea, which was therefore used as the basis for calculating the contents of the solutions. The detailed results of the experiments will not be given here but only the percentage amounts of urea hydrolyzed under the conditions of the experiments. The castor bean preparations show very much less activity than do soy bean preparations, so that the reactions were allowed to run considerably longer times than is customary with the latter. For instance, 2 cc. of a soy bean extract prepared according to Marshall's method, 100 cc. water, 28.1 mg. urea, after 4 hours gave 13% hydrolysis, while 10 cc. of the filtrate from 10 g. of Preparation A and 100 cc. water, with 28.1 mg. urea and 5 cc. water, after 18 hours showed 10% hydrolysis. Also, 0.1 g. husk- and oil-free, ground, soy beans hydrolyzed 47 mg. urea in 50 cc. of water completely in 4.5 hours, while 0.2 g. Preparation B hydrolyzed only 3.4% of the urea under similar conditions in 25 hours.

In the results which follow, the description of the experiments will be given and then the percentage hydrolyses of the urea.

Preparation A, 0.5 g.; 25 cc. solution; 28.1 mg. urea; 22 hours.

Water, 4.5%; 0.002 *N* NaOH solution, 3.5%; 0.002 *N* HCl solution, 1.3%; 0.004 *N* solution, 2.5%; 0.004 *N* HCl solution, 1.0%.

Dilute HCl and NaOH solutions retard the action, the former to a greater extent than the latter. The retarding actions of strong acids and alkalies, in more than very small quantities, on the action of the soy bean urease, were studied by Takeuchi, Armstrong, Marshall, and Van Slyke.

[1] IV paper of this series, THIS JOURNAL, **35**, 292 (1913).

[2] Supplied by the Baker Castor Oil Company, New York.

[3] Kober, THIS JOURNAL, **30**, 1131 (1908); **32**, 689 (1910); Folin and Farmer, *J. Biol. Chem.*, **12**, 499 (1912).

Preparation A, 1 g.; 20 cc. solution; 28.1 mg. urea; 22 hours.

Water, 12%; 0.004 *M* $MnSO_4$ solution, 10%; 0.075 *M* Na_2HPO_4 solution, 14%; 0.04 *M* $MgSO_4$ solution, 12%; 0.075 *M* KH_2PO_4 solution, 3%; 0.075 *M* NaF solution, 2%.

The disodium phosphate exerted a small accelerating action, while the magnesium and manganous sulfates caused very little change. This latter is of interest in showing that the hydrolytic action of the urease of castor beans is different from the hydrolytic actions of the lipases of castor beans, which are very much accelerated by manganous and magnesium sulfates.[1] On the other hand, the retarding influence of sodium fluoride is shown both with the urease and the lipase.[1] Takeuchi found a similar action with the soy bean urease.

Preparation B, 0.5 g.; 15 cc. solution; 28.1 mg. urea; 19 hours.

Water	21%	0.0007 *N* NaOH sol.	16%	0.007 *N* Na_2CO_3 sol.	21%
0.0007 *N* HCl sol.	19%	0.007 *N* NaOH sol.	15%	0.07 *N* Na_2CO_3 sol.	20%
0.007 *N* HCl sol.	10%	0.07 *N* NaOH sol.	9%	Water + 1 g. $CuSO_4.5H_2O$	1%
0.07 *N* HCl sol.	6%	0.0007 *N* Na_2CO_3 sol.	18%	Water + 1 g. $Pb(NO_3)_2$	3%

In the most dilute (0.0007 *N*) solutions, the HCl exerted a smaller retarding effect than the NaOH; in the more concentrated, the reverse was the case, the actions then being similar to those observed with Preparation A. Sodium carbonate appeared to exert very little influence on the reaction, due, perhaps, to compensating actions in which accelerating effects of carbon dioxide, similar to those observed by Armstrong with the soy bean urease, may play a part. The inhibiting actions of copper sulfate and lead nitrate are very marked and similar to the results obtained by Takeuchi with copper sulfate and soy bean urease.

Preparation B, 0.5 g.; 25 cc. solution; 28.1 mg. urea; 18 hours.

0.1 *M* Na_2HPO_4 solution	23%	0.002 *M* $MnSO_4$ solution	15%
0.1 *M* KH_2PO_4 solution	6	0.05 *M* $MgSO_4$ solution	18
0.1 *M* NaF solution	5		

The results are similar to those obtained with Preparation A. No experiments with water alone were carried out with this series, but to judge from a number of other experiments, 20% of the urea would have been decomposed with no salt present. The slightly alkaline disodium phosphate accelerated the reaction, the acid monopotassium phosphate retarded it almost as much as did the sodium fluoride, while the manganous and magnesium sulfates showed small retarding actions. That manganous sulfate in more concentrated solution exerted considerable retardation is shown by the following results:

Preparation B, 0.5 g.; 20 cc. water (with added salt); 28.1 mg. urea; 23 hours.

No $MnSO_4.4H_2O$ added	19%	0.05 g. $MnSO_4.4H_2O$ added	10%
0.005 g. $MnSO_4.4H_2O$ added	15	0.04 g. $MnSO_4.4H_2O$ added	6

[1] Cf. V paper of this series, THIS JOURNAL, **35**, 601 (1913) and the references given there.

The following tables show the results obtained when the quantity of urea, of water, and of the duration of the reaction were varied.

Preparation B, 0.5 mg.; 100 cc. water; duration of reaction, 24 and 48 hours; amounts of urea, as stated.

	470	375	282	188	94	47 mg. urea
24 hours..	0.8	1.2	1.6	2.5	5.2	11.2% urea hydrolyzed
48 hours..	1.3	1.9	2.6	4.3	8.2	14.8% urea hydrolyzed

For the 24 hours reactions, the absolute quantities of urea decomposed increased with increasing dilution of the solution or decreasing amounts of urea present. For the 48 hours actions, the absolute quantities of urea which were decomposed increased as the dilution increased, down to the concentration of 188 mg., and then decreased on further dilution. The percentage decomposed, however, increased throughout with decreasing concentration. With the soy bean urease, Armstrong and Horton found that increasing the concentration of the urea retarded the reaction, and Marshall, that the velocity of hydrolysis increased with dilution to a maximum and, on further dilution, decreased.

Preparation B, 0.5 g.; 56.3 mg. urea; duration of reaction and amount of water, as stated.

	100 cc.	80 cc.	60 cc.	40 cc.	20 cc.	10 cc. water
21 hours..	4	6	6	7	9	9% urea hydrolyzed
45 hours..	8	11	12	14	14	15% urea hydrolyzed
69 hours..	13	17	23	25	26	27% urea hydrolyzed

The amount of urea hydrolyzed is very nearly proportional to the time of reaction in most of these experiments. Practically no difference is observable between the results for the most concentrated solutions. The experimental error is comparatively large here, so that the calculation of reaction velocity constants would furnish conclusions of questionable value. The relation between any two of these results does not come out clearly, but the trend of the changes when all of the experiments are considered is quite clear.

Preparation B, 0.5 g.; 15 cc. water; duration of reaction and amount of urea, as stated.

	20	43	68	91	115	139	163	187 hours
14.1 mg. urea.....	30	70	88	95	95	89	..	..% urea hydrolyzed
28.1 mg. urea.....	16	39	..	54	62	65	74	..% urea hydrolyzed
56.3 mg. urea.....	10	22	29	34	37	43	40	44% urea hydrolyzed

With the smallest quantity of urea, the reaction proceeded so far that in most of these results comparison with the rest is not feasible. The products of the reaction, as well as the deterioration of the enzyme due to long contact with water, probably affect these results. These secondary influences show themselves in the third series, where the reaction apparently comes to a stop. All that can be said with regard to these results is that for the first two time periods, the amounts of urea decomposed are nearly proportional to the durations of action, but that variations

occur for greater durations, due to complicating or secondary reactions.

A series of experiments was carried out in which 10 g. of Preparation A were extracted with 100 cc. of solution, filtered through paper, and 10 cc. of the filtrates tested, each with 28.1 mg. urea, 5 cc. of added water, for 18 hours.

Extracting sol.: Water, 1 *M* NaCl, 0.05 *M* $MgSO_4$, 0.1 *M* Na_2HPO_4, 0.1 *M* KH_2PO_4.

% Urea hydrolyzed:	10	6	8	7	1

Water extracted more urease apparently than the disodium hydrogen phosphate solution, although the solid preparation tested directly with the solutions showed greater activity with the latter than with the former. From the previous results it was to be expected that the monopotassium phosphate extract would show little activity, and that the other salt extracts less activity than the aqueous extract.

Conclusions.

Castor bean preparations hydrolyzed much less urea under comparable conditions than did similar soy bean preparations. This indicates that the urease of castor beans is less active than that of soy beans, or that less urease is present in castor beans than in soy beans.

The action of acids, bases, and salts on the hydrolysis of urea by castor bean urease was studied. Relations, similar to those observed by others with soy bean urease, were found.

[CONTRIBUTION FROM THE NEVADA AGRICULTURAL EXPERIMENT STATION.]

ENZYMES PRESENT IN ALFALFA. ALFALFA INVESTIGATION, V.

BY C. A. JACOBSON AND AUGUST HOLMES.

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The present work is a continuation of that begun by one of us in Prof. Hedin's laboratory in Uppsala, on the enzymes present in alfalfa seeds.[1]

The same general methods of work there used have been followed in the present investigation, which embraces the enzymes of the green as well as of the dried stems and leaves and of the fresh roots. In addition to the enzymes tested for and determined in the seeds, we have included three carbohydrases not infrequently encountered in juices and extracts of plants, namely, maltase, lactase, and pectinase. All standard solutions employed in the investigation were derived titrimetrically from normal hydrochloric acid, which had been standardized, gravimetrically, against silver.

All experiments were run in duplicate, of which only the mean will be recorded.

[1] THIS JOURNAL, **34**, 1730 (1912).