On the Probable Existence of Emulsin in Yeast.

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The observations of Treub,* Greshoff,† Jouck,‡ and other investigators have established the fact that hydrocyanic acid is furnished by a comparatively large number of plants belonging to a wide range of natural orders. Dunstan and Henry have applied to this process the name "cyanogenesis," and have shown that in many plants the production of hydrocyanic acid is due to the interaction of a cyanogenetic glucoside with a specific enzyme. Thus in *Lotus arabicus*, the hydrocyanic acid is produced by the decomposition of the glucoside lotusin by the enzyme lotase,§ in *Sorghum vulgare* by the action of emulsin on the glucoside dhurrin,‖ and in the seeds of *Phaseolus lunatus* as the result of the decomposition of phaseolunatin by the enzyme emulsin.¶ The same authors have indicated that similar actions probably take place in cassava (*Manihot utilissima*), *Lotus australis* and *Chailletia cymosa*, all of which have been found to yield hydrocyanic acid when crushed in presence of water.**

The isolation of these cyanogenetic glucosides is often a matter of considerable difficulty, because, as a rule, they are only soluble in water and aqueous alcohol, and it is therefore a troublesome operation to separate them from the associated sugar (usually dextrose) and pectous matter which are also, in general, only soluble in the same solvents. In a few cases it has been found possible to remove dextrose from such mixtures by the action of phenylhydrazine, but this process usually leads to the loss of a portion of the glucoside, owing to partial condensation with the reagent.†† Some cyanogenetic glucosides are also slightly soluble in ethyl acetate, and this solvent

* 'Annales du Jardin Botanique de Buitenzorg,' vol. 9, p. 259.
† 'Berichte,' 1890, vol. 23, p. 3548.
‡ 'Inaug. Dissertat.,' Strassburg, 1902.
§ 'Phil. Trans.,' B, 1901, vol. 194, p. 518.
‖ *Ibid.*, A, 1902, p. 399.
¶ 'Roy. Soc. Proc.,' 1903, vol. 72, p. 285.
** 'Phil. Trans.,' A, 1902, vol. 199, p. 399, and 'Bulletin of the Imperial Institute,' 1903, vol. 1, pp. 12 and 112.
†† Dunstan and Henry, 'Phil. Trans.,' A, 1902, vol. 199, p. 402.

has been employed for the isolation of mandelonitrile glucoside,* and of dhurrin and phaseolunatin.†

With a view to devising a general process for the isolation of cyanogenetic glucosides we have, at Professor Dunstan's suggestion, investigated more thoroughly the properties of some of the known glucosides of this type, and the present paper contains an account of a number of results obtained in attempting to remove dextrose from mixtures of this sugar with cyanogenetic glucosides, by fermentation with yeast.

Action of Yeast on Amygdalin.

As a preliminary experiment, a solution of a mixture of equal quantities of amygdalin and dextrose in water was mixed with a small quantity of ordinary pressed yeast and allowed to stand in a warm place. Fermentation took place, carbon dioxide was evolved, and after several days a distinct odour of oil of bitter almonds was observed. This unexpected decomposition of the glucoside rendered necessary confirmatory experiments with amygdalin alone.

Amygdalin (2 grammes) was dissolved in 100 c.c. of water and about 6 grammes of ordinary pressed yeast added, together with a few drops of toluene to render the mixture antiseptic. The experimental flask was plugged with cotton wool and kept, together with a control flask containing a solution of amygdalin in water without yeast, at 40°. In the flask to which yeast had been added, the odour of benzaldehyde was observed after three days. This rapidly increased in intensity. After standing for two days more the mixture was distilled until free from hydrocyanic acid and the distillate, previously rendered slightly alkaline with potash, was extracted with ether. The oily residue left on distilling off the solvent had a strong odour of benzaldehyde, and the presence of this substance was proved by its conversion into dibenzylideneacetone (melting point, 112°), by condensation with acetone in presence of potash, and into benzaldehyde phenylhydrazone by the action of phenylhydrazine.

The aqueous solution left after extraction with ether was diluted to a known volume with water, and a portion examined for prussic acid with positive results. An aliquot part of the whole was then titrated with standard silver nitrate solution by Liebig's method, and by this means it was found that 33 per cent. of the amygdalin originally present in the solution had been decomposed by the yeast. The control flask was similarly examined, and it was found that in it no decomposition had occurred. A second experi-

* Fischer, 'Berichte,' 1895, vol. 28, p. 1509.

† Dunstan and Henry, *loc. cit.*

ment carried out on similar lines with another portion of the same specimen of yeast showed that after 11 days 67 per cent. of the amygdalin had been decomposed, after which the action appeared to cease. The residue left in the flask after distilling off the benzaldehyde and hydrocyanic acid was evaporated to dryness on the water-bath and extracted with alcohol. This solution was decolorised with animal charcoal and the solvent distilled off, leaving a quantity of unchanged amygdalin. This was extracted with ether. On evaporation of the solvent a minute quantity of a crystalline substance which was not amygdalin was obtained. The quantity of this material isolated was so small that it was impossible to further examine it, and its formation was not observed in any further experiment. It is possible that this substance was benzoic acid, since Herzog has shown* that salicyl alcohol is oxidised to salicylic acid by yeast, and it is conceivable that some of the benzaldehyde formed in this case may have similarly been oxidised to benzoic acid. No benzoic acid was obtained, however, by the action of yeast on small quantities of benzaldehyde suspended in water.

The residue contained no dextrose, this having been changed by the zymase of the yeast forming alcohol and carbon dioxide.

This decomposition of amygdalin by yeast does not seem to have been observed previously, though Bourquelot has ascribed such an action to *Aspergillus niger*† and Gerard to *Penicillium glaucum*.‡

These experiments having established the fact that the hydrolysis of the amygdalin was brought about by the yeast, it was next sought to determine to which constituent of the yeast this hydrolytic action was due.

Fischer§ has shown that a preparation of invertase obtained by washing yeast with water has the power of partially hydrolysing amygdalin, forming one molecule of dextrose and one molecule of mandelonitrile glucoside according to the equation

$$\underset{\text{Amygdalin.}}{C_{20}H_{27}O_{11}N} + H_2O = \underset{\substack{\text{Mandelonitrile}\\ \text{glucoside.}}}{C_{14}H_{17}O_6N} + \underset{\text{Dextrose.}}{C_6H_{12}O_6}.$$

This experiment was repeated and no difficulty was experienced in obtaining mandelonitrile glucoside by this means, and under no circumstances was any formation of hydrocyanic acid or benzaldehyde observed when such yeast washings were added to a solution of amygdalin. The complete hydrolysis of this glucoside by yeast cannot therefore be due to the action of invertase.

* 'Zeit. Physiol. Chem.,' 1903, vol. 37, p. 396.

† 'Compt. Rend. Soc. Biol.,' 1893, pp. 653 and 804.

‡ *Ibid.*, 1893, p. 651.

§ 'Berichte,' 1894, vol. 27, p. 2989; 1895, vol. 28, p. 1809.

The washed yeast so prepared had, moreover, lost none of its activity, and the following experiments, showing the rate at which *washed* yeast decomposes amygdalin, may be quoted as illustrative of this. For each experiment 2 grammes of amygdalin were dissolved in 50 c.c. of water contained in a small flask, and about 5 grammes of washed yeast were added to the solution. A number of flasks were prepared and kept at 40° in a water bath. At intervals a flask was taken out and the hydrocyanic acid formed estimated. The odour of benzaldehyde was first observed after the flasks had been kept at 40° for 74 hours.

Time after commencement of action.	Amount of hydrocyanic acid formed.	Amygdalin decomposed.
hours.	gramme.	Per cent.
2	0·006	5·5
24	0·012	10·5
48	0·016	14·0
72	0·019	16·0
120	0·038	31·8
168	0·052	42·0
240	0·085	70·8

The last observation probably represents the limit of decomposition, since another flask examined 48 hours later gave practically the same result. It will be observed that this limit is a little higher than that (67·8 per cent.) obtained with the unwashed yeast. A number of similar experiments were also made with the "Zymin" or "Acetondauerhefe," described by Albert, Buchner, and Rapp,* which is prepared by digesting brewers' yeast with acetone until the cell walls are ruptured and the contents egested. This material was also found to decompose amygdalin, though much less rapidly than pressed yeast. Thus, in one experiment 2 grammes of "Zymin" were added to an aqueous solution of 1 gramme of amygdalin and the mixture kept at about 40°; the odour of benzaldehyde was first observed after 74 hours, and at the end of 90 hours the hydrocyanic acid formed was estimated. This amounted to 0·0057 gramme, corresponding to 8·7 per cent. of the glucoside. A second experiment gave 0·0059 gramme of acid, equivalent to 9·8 per cent. of amygdalin, after 114 hours.

Experiments with Yeast Juice.

The action of yeast juice (Buchner's zymase)† on amygdalin was also investigated. For a liberal supply of this material prepared from fresh

* 'Berichte,' 1902, vol. 35. p. 2376.

† *Ibid.*, 1897, vol. 30, p. 117.

brewers' yeast by a slight modification of Buchner's process we are indebted to Dr. A. Harden of the Lister Institute of Preventive Medicine, to whom we take this opportunity of expressing our thanks.

In the experiments with yeast juice on amygdalin, the flasks were plugged with cotton wool, their contents having been previously rendered antiseptic by the addition of a few drops of toluene. The temperature used was that found experimentally to be the best, viz., 40°.

Three different preparations of yeast juice were employed, and these, as the following table shows, varied considerably in activity.

Yeast juice.	Weight of amygdalin used in 20 c.c. of water.	Amount of yeast juice added.	Time required for commencement of decomposition.
	gramme.	c.c.	hours.
Specimen No. 1.....	1	5	23
„ No. 2.....	1	5	36
„ No. 3.....	1	5	120

Dilution of the juice had no very striking effect on the rate of decomposition, but the addition of a little water seemed to slightly accelerate the activity, and further addition of water to slightly diminish it, as the following table shows.

Weight of amygdalin used.	Volume of yeast juice.	Volume of water used.	Time required for commencement of decomposition.
gramme.	c.c.	c.c.	hours.
1	5	5	22·5
1	5	10	21
1	5	15	21·5
1	5	20	23
1	5	25	24

The products of the action of yeast juice on amygdalin are the same as those of yeast itself, viz., benzaldehyde, hydrocyanic acid, alcohol and carbon dioxide.

Influence of Hydrocyanic Acid on the Glucosidolytic Action of Yeast Juice.

That hydrocyanic acid of moderate strength has comparatively little disturbing action on the glucosidolytic activity of yeast is obvious from the fact that the decomposition of amygdalin can proceed to the extent of 70 per cent. of the amount of glucoside used. It will be seen from the following

table that hydrocyanic acid exerts very little, if any, inhibiting action on the glucosidolytic activity of yeast juice, even in comparatively strong solutions. On the contrary Buchner has shown* that this acid lessens the activity of the zymase in yeast juice.

Weight of amygdalin used.	Volume of yeast juice added.	Volume of 1 per cent. solution of hydrocyanic acid added.	Length of time during which action proceeded.	Amygdalin decomposed.
gramme.	c.c.	c.c.	hours.	Per cent.
1	5	—	36	12
1	5	5	36	11·5
1	5	10	36	12
1	5	20	36	11

It is well known that the activity of the zymase of yeast juice decreases when the juice is kept,† and this is generally ascribed to the action of the proteolytic enzyme (endotryptase) first detected in yeast by Hahn and Geret.‡ It was consequently considered advisable to ascertain whether the glucosidolytic action of yeast juice similarly diminishes on keeping. The juice was kept at the atmospheric temperature and its activity towards amygdalin tried at intervals.

Juice.	Time of standing.	Time required to initiate the decomposition of amygdalin.
	hours.	hours.
Specimen No. 1	24	22
„ No. 1	47	23
„ No. 1	120	26
„ No. 2	24	40
„ No. 2	120	56

It is evident from these results that the glucosidolytic activity undergoes slight diminution when the yeast juice is kept, but this diminution is in no way comparable with that which the activity of the zymase undergoes under the same conditions. For purposes of comparison, some experiments were made to ascertain whether the commoner proteolytic enzymes interfered to any extent with the action of the emulsin of almonds. For this purpose 1 gramme of Merck's emulsin was thoroughly mixed with 100 c.c. of water,

* 'Berichte,' 1898, vol. 31, p. 2672.

† Albert and Buchner, 'Berichte,' 1900, vol. 33, p. 971.

‡ 'Berichte,' 1898, vol. 31, p. 202.

and to measured portions of the mixture a weighed quantity of commercial preparations of pepsin, trypsin, and papain were added. After a certain period had elapsed, portions of these mixtures were allowed to act on solutions of amygdalin, and the amounts of hydrocyanic acid formed after definite periods were determined.

As a control experiment, 20 c.c. of the emulsin mixture were allowed to stand, and at intervals 2 c.c. were added to 1 gramme of amygdalin dissolved in 20 c.c. of water, and the amount of the glucoside decomposed estimated after a certain lapse of time.

Control Experiments with Emulsin.

Time allowed to stand.	Time of action on amygdalin.	Amygdalin decomposed.
hours.	hours.	Per cent.
—	18	65·7
25	18	63·2
50	18	56·8

Experiments with Emulsin and Proteolytic Enzymes.

Proteolytic enzyme.	Weight of proteolytic enzyme.	Volume of emulsin solution.	Time of proteolytic action.	Time of action of emulsin on amygdalin.	Amygdalin decomposed.
	gramme.	c.c.	hours.	hours.	Per cent.
Pepsin	0·2	3	26	24	None
		3	50	[2—3 days]	,,
	0·2	5	25	24	2·0
		5	50	48	None
	0·2	20	24	18	32·0
		20	50	24	8·7
		20	106	24	None
Trypsin	0·2	3	26	24	47·3
		3	50	24	33·0
	0·2	5	25	24	51·3
		5	50	24	38·0
Papain	0·2	5	24	18	60·7
		5	50	24	58·3

These results show that, of the three proteolytic ferments used, pepsin exerts a powerful destructive action on the activity of the emulsin of almonds, trypsin a well-marked action, whilst papain affects it slightly. It will be observed that the slight action exerted by trypsin on emulsin is similar to that of the proteolytic enzyme of yeast on the glucosidolytic constituent of yeast, and this is in conformity with Kutscher's observation* that the proteo-

* 'Zeit. Physiol. Chem.,' 1901, vol. 32, p. 59.

lytic enzyme of yeast resembles trypsin in its action. (*Compare also Hahn and Geret.**)

Effect of Antiseptics on the Glucosidolytic Action of Yeast.

The activity of the glucosidolytic constituent of the yeast is not affected by antiseptics, and in this respect, as also in its behaviour towards dilute mineral acids and alkalis, by which its action is totally inhibited, it resembles the emulsin of almonds.

The experiments recorded in the following table were carried out under comparable conditions at 40°. In each case 1 gramme of amygdalin was used, and the hydrocyanic acid formed was estimated by Liebig's method.

Substance.	Amount of antiseptic.	Weight of yeast used.	Time.	Hydrocyanic acid formed.	Amygdalin decomposed.
		grammes.	hours.	gramme.	Per cent.
(Control)	—	3	90	0·0063	10·5
Chloroform	1 c.c.	3	90	0·0060	10·0
Toluene.....	1 ,,	3	90	0·0061	9·8
Phenol	0·02 gramme	3	90	0·0060	10·0

Action of Yeast on Glucosides.

The results of the experiments already described indicate that the action of yeast on amygdalin is probably due to the presence in the yeast-cells of an enzyme of the type of emulsin, and it was considered desirable to ascertain whether or not the glucosidolytic constituent could be definitely identified with this enzyme.

The identification of an enzyme is at present a somewhat difficult problem, since it is as yet impossible to isolate bodies of this type in a pure state. Recourse must therefore be had to the investigation of the specific action of the ferment, and especially to the range of temperature over which it is active, the nature of the substances it decomposes, and the characters of the decomposition products. For this purpose the action of yeast on a number of glucosides other than amygdalin was examined. The principal results obtained were as follows:—

Salicin.—Yeast decomposes salicin in precisely the same manner as emulsin, forming saligenin. The latter was isolated, and identified by means of its melting point (82°), and its characteristic colour reaction with ferric chloride. It might have been expected that some salicylic acid would have been formed, due to the further action of the yeast on the saligenin,† but no

* 'Zeit. Biol.,' 1900, vol. 40, p. 117.

† Compare Herzog, *loc. cit.*

trace of this acid could be detected. The dextrose first formed in this action is changed by the zymase of the yeast forming alcohol and carbon dioxide.

Mandelonitrile Glucoside.—Fischer has shown that this glucoside is readily hydrolysed by emulsin. It is also hydrolysed by yeast; thus, when yeast was added to a solution of 0·5 gramme of the glucoside dissolved in 10 c.c. of water and the mixture kept at 40°, the odour of benzaldehyde was observed after 36 hours, and after 72 hours 40 per cent. of the glucoside present had been decomposed. The dextrose first formed was decomposed by the zymase of the yeast.

Other Glucosides.—In the same way it was found that arbutin and phaseolunatin were decomposed by yeast, whilst quercitrin, digitalin and sinalbin were unattacked by it.

It seemed possible that this action of yeast on glucosides might be due to the direct fermentation of the sugar residues present in these substances by the zymase contained in the yeast or its preparations, with the result that the molecule underwent total disruption, the products other than sugars being non-fermentable and, therefore, remaining intact. This explanation of the action is, however, not permissible in view of the fact that the action of yeast is restricted to certain types of glucosides, and that it does not decompose digitalin or quercitrin, although these contain residues of the fermentable sugars, digitalose and rhamnose respectively. Moreover, Fischer has asserted that disaccharides are never fermented directly by yeast, and that the latter only attacks hexoses produced by the preliminary decomposition of disaccharides by hydrolytic enzymes such as invertase, maltase and lactase, and bearing in mind the analogy in constitution between glucosides and the disaccharides established by Fischer, it is probable that the same rule holds good with regard to glucosides.

Fractionation of Yeast Juice by Heat Coagulation.

Wroblewski* has shown that when yeast juice is heated, coagulations of proteid matters occur at certain definite temperatures, and that in particular the filtrate from the coagulate produced at 41° is practically free from zymase.† Wroblewski's experiments were repeated, small tubes containing the yeast juice being heated gradually to various temperatures between 40° and 70°. The coagulates obtained at these various temperatures were filtered off and the activity of the filtrate in each case towards amygdalin and dextrose was determined. The principal coagulates were found to be produced at 48°, 55°,

* 'Berichte,' 1898, vol. 31, p. 3218, and 'Journ. prakt. Chem.,' 1901, vol. 64, p. 1.

† Compare Buchner, 'Berichte,' 1899, vol. 32, p. 2086.

58°, and 65°. These temperatures differ somewhat from those given by Wroblewski, being on the whole a few degrees higher, but agreement in this respect is scarcely to be expected in experiments of this kind, since the formation of the various precipitates is probably due to the coagulation in turn of different proteid matters, including the enzymes, and the temperatures at which the coagulates are formed probably depends to some extent on the concentration of the various proteids in the yeast juice, and these, in turn, will vary with the previous history of the yeast from which the juice is prepared. The results obtained in one set of these experiments are shown in the following table:—

Temperature to which juice was heated.	Time of heating.	Change observed.	Activity of filtrate towards—	
			Glucose.	Amygdalin.
degrees.	minutes.			
41	90	None	Active	Active
42	20	None		
46	18	Turbidity		
48	25	Voluminous precipitate...	Slight	Benzaldehyde formed after 47 hours
50	20	None	None	Active
53	40	Slight flocculence		
55	30	Voluminous precipitate...	...	Benzaldehyde formed after 45 hours
56	20	None		
58	30	Voluminous precipitate...	...	Benzaldehyde formed after 34 hours
63	15	Turbidity		
66	25	Voluminous precipitate...	...	Benzaldehyde formed after 58 hours
70	20	None	...	Benzaldehyde formed after several days
70—72	20	None	...	No action

It was observed in all the sets of heat coagulation experiments that the activity of the yeast juice towards amygdalin increased as each of the first few coagulates was removed. This increase in activity is no doubt due in part to the gradual concentration of the glucosidolytic enzyme in the liquid as the result of continued heating, but it may also be due in part to the removal of other enzymes, especially the endotryptase which, as has been shown, appears to gradually destroy the glucosidolytic enzyme. The maximum activity of the juice towards amygdalin is reached when it has been heated at 58° (according to Hahn and Geret* the endotryptase is destroyed when the yeast juice is heated at 60°), further heating diminishes the activity, which finally disappears at about 70°. Yeast juice which has been heated to 70° still hydrolyses sucrose and must, therefore, contain invertase.

* *Loc. cit.*

Fractional Precipitation of Yeast Juice with Alcohol.

Attempts were made to isolate a definitely active preparation of the enzyme by treating yeast juice with successive small quantities of alcohol and collecting the several precipitates so produced. It was found, however, that these precipitates exhibited, in a less degree, all the activities characteristic of yeast juice itself. Recourse was, therefore, had to the alcoholic precipitation of yeast juice which had been heated previously to 58° and subsequently filtered. The precipitate so obtained was washed with dilute alcohol, spread on glass, and dried by exposure over desiccating agents under reduced pressure. This preparation contained the glucosidolytic enzyme and invertase, but was free from endotryptase and zymase.

As in all the previous experiments in which the action of yeast or yeast preparations on amygdalin was investigated, no dextrose could be found among the hydrolytic products, this having been decomposed by the zymase, it was thought worth while to investigate more fully the action of this new preparation on amygdalin. About 0·1 gramme was added to 20 c.c. of a 2-per-cent. solution of amygdalin in water and the mixture maintained at 40°. The odour of benzaldehyde became noticeable after 90 hours. The action was allowed to proceed for some time and then the benzaldehyde was extracted with ether and identified by conversion into dibenzylideneacetone. The presence of hydrocyanic acid among the hydrolytic products was proved by the application of the usual tests. To the liquid, left after removal of benzaldehyde, phenylhydrazine acetate was added and the mixture warmed at 100°. After about 20 minutes the phenylosazone which had separated was collected, washed, and recrystallised from alcohol. It melted at 205°. A specimen of phenylglucosazone prepared at the same time melted at 205°.

Identification of the Glucosidolytic Enzyme of Yeast.

The data afforded by the results of the experiments already described are that the glucosidolytic enzyme of yeast hydrolyses amygdalin, salicin, arbutin, phaseolunatin, and mandelonitrile glucoside, but does not attack sinalbin, digitalin, or quercitrin. The temperature at which its activity is destroyed is about 70°, and it is most active at 40°. Its activity is inhibited by the presence of small quantities of alkalis or acids, but not by antiseptic agents.

A comparatively large number of glucosidolytic enzymes have been described, but of those which hydrolyse glucosides containing the —CN group or —CNS group only the following are known:—

Emulsin, which decomposes amygdalin, dhurrin, phaseolunatin and gynocardin.

Lotase, which hydrolyses lotusin and appears to be otherwise inactive.

Gynocardase, which hydrolyses gynocardin.

Myrosin, which hydrolyses sinigrin (potassium myronate) and sinalbin and appears to be otherwise inactive.

Of these four enzymes it is possible that gynocardase may prove to be identical with emulsin,* and assuming this, it is evident that the activity of each of the three remaining enzymes is associated with a particular type of glucoside; thus lotase reacts with lotusin, which differs from all the known cyanogenetic glucosides in having the —CN group attached to the sugar residue; myrosin reacts only with glucosides having the —CNS group attached to the non-sugar portion of the molecule, and emulsin decomposes glucosides having the —CN group associated with the non-sugar portion of the molecule. Power and Gornall have, however, obtained from the seeds of *Taraktogenos Kurzii,* a preparation which is stated to decompose both potassium myronate and amygdalin.† This may be due to the simultaneous presence in this preparation of two ferments, one of the emulsin and the other of the myrosin type.

It will be seen that the range of activity of the glucosidolytic enzyme of yeast coincides with that of emulsin in so far as the nature of the glucosides decomposed is concerned. As regards the influence of change of temperature on the activity of emulsin scarcely any observations are on record, and it was considered worth while to ascertain whether, like the yeast enzyme, emulsin ceases to be active at about 70°. It is difficult to secure strictly comparable conditions for such experiments, since there is no known means whereby equivalent solutions of the two materials can be procured; thus, in some comparative experiments, it was found that 1 c.c. of a liquid obtained by shaking up 1 gramme of Merck's emulsin with 100 c.c. of water when added to 10 c.c. of a 10-per-cent. solution of amygdalin in water decomposed 85 per cent. of the glucoside in 20 hours, whilst 5 grammes of yeast, under the same conditions, decomposed only 6·5 per cent. of the amygdalin in the same time, whence it would appear that if the glucosidolytic activity of yeast is due to the presence of emulsin, the specimen of yeast used in this instance could have contained only 0·0001 of that contained in the specimen of commercial emulsin used.

It was, however, satisfactorily established that a remarkable diminution in the activity of emulsin can be brought about by heating, and that total disappearance of activity takes place at about the same temperature as that observed in the case of yeast. The results recorded in the following table

* Power and Lees, 'Journ. Chem. Soc.,' 1905, vol. 87, pp. 354 and 357.

† 'Journ. Chem. Soc.,' 1904, vol. 83, p. 841.

were obtained with a liquid containing 1 gramme of Merck's emulsin thoroughly mixed with 100 c.c. of water:—

Temperature to which liquid was heated.	Time of heating.	Time required for decomposition of amygdalin by 1 c.c. of the liquid after heating.
degrees.	hours.	minutes.
64	2	30
65	1	30
66	1	40
68	1	40
69	1	90
70	75 minutes	150
71	30 „	30 hours
71	45 „	no action

These results show that although the temperature at which emulsin becomes inactive is practically identical with that at which the activity of the glucosidolytic enzyme of yeast ceases, emulsin may be exposed to the same temperatures as yeast juice for a much longer time before its glucosidolytic activity is destroyed. This is no doubt due to the fact that the concentration of the emulsin in the preparation used in this set of experiments was much greater than in the yeast juice used in the experiments described on p. 577.

It has been shown, therefore, that the glucosides which are decomposed by yeast are those which are attacked by emulsin, and further that the conditions under which these decompositions are effected by yeast, especially as regards temperature, are those which are operative in the case of emulsin. Taking all these facts into consideration there seems to be little room for doubt that the glucosidolytic activity of yeast is due to the secretion of emulsin in the cells of the plant.