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The diastatic action of Saliva

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The Diastatic Action of Saliva, as Modified by various Conditions, Studied Quantitatively.

By R. H. Chittenden and Herbert E. Smith.

The chemical changes resulting from the action of unorganized ferments are among the most interesting and important of those which occur in the animal organism. Ferment action plays such an important part in the chemical processes incident to life that definite knowledge of the conditions favorable and inimical to the action of any ferment occurring in the animal body must necessarily be of great physiological value.

Since Leuchs in 1831 discovered the diastatic action of saliva much has been learned regarding this digestive fluid, both as to its chemical action and the nature of the products formed. Still there has been lacking, until recently, definite knowledge of the conditions which influence the diastatic action of the salivary ferment, and it has been the object of the present investigation, taking advantage of previously acquired knowledge, to ascertain the exact influence of those conditions which suggest themselves as being most important in view of the destination of the ptyalin, and concerning which there has been of late a lack of agreement.

Method used in determining the rate of diastatic action.

In testing the rate of action of the salivary ferment we have in all cases employed quantitative methods, similar in their general nature to those previously used by one of us.* The amount of reducing substances formed by the amylolytic action of the ferment, which for the sake of convenience we have calculated as dextrose, admit of accurate determination by means of the improved Allihn's† method, and thus enable us to give a concise expression of the relative diastatic action, even in those cases where the differences are very slight. As recent experiments‡ have plainly indicated, the ultimate product of the dias-

* Chittenden and Griswold, Amer. Chem. Jour., iii, 305. Chittenden and Ely, ibid, iv, 107.

† Zeitschrift für analytische Chemie, 22 Jahrgang, p. 448.

‡ v. Mering and Musculus, Zeitschrift für physiologische Chemie, i, 395. O. Sullivan and E. Schultze, Berichte d. deutsch. Chem. Gesell., vii, 1047. Musculus and Gruber, Zeitschrift für physiolog. Chemie, ii, 177. v. Mering, Zeitschrift für physiolog. Chem., v, 196.

tatic action of ptyalin is dextrose; the sugar intermediate between this body and the dextrins, and which is formed in much larger quantity is maltose, with a relative reducing power of 66 as compared with dextrose, 100; while the achroodextrins and other intermediate products have very small reducing power; consequently the reducing power of a digestive mixture must necessarily express the relative diastatic action of the ferment present, since increased action means an increased formation of reducing bodies, of which the final product has the highest reducing power. In this connection it is well to remember that diastase and ptyalin both convert only a limited quantity of starch into sugar or reducing bodies,* and that no matter how great the excess of ferment or the length of time the action is continued, the percentage of starch changed into sugar does not ordinarily exceed 53 per cent.† The general method employed in our work for testing the diastatic action of saliva was as follows: the volume of the digestive mixture was in every instance 100 c.c.; the amount of starch‡ present, 1 or 2 grams, previously boiled in a definite amount of water; the temperature of digestion 38–40° C.; the length of time generally 30 minutes. When the digestion was finished, diastatic action was at once stopped by boiling the mixture; when cold, the mixture was diluted with distilled water to 200 c.c. and filtered; 25 c.c. of the filtrate or $\frac{1}{8}$th of the entire fluid was then precipitated with Fehling's solution according to Allihn's§ data and method; the reduced copper was filtered through an asbestos filter in a small weighed glass tube and ignited directly in a current of hydrogen gas and weighed as metallic copper. By means of Allihn's tables of reduction equivalents the corresponding amount of sugar, calculated as dextrose, is easily obtained, from which the percentage amount of starch converted into reducing bodies can be computed, calling dextrose $C_6H_{12}O_6$, and the starch $C_6H_{10}O_5$.‖ The following experiment illustrates the accuracy of the method and the reliance which can be placed upon it; two solutions of 100 c.c., each containing 2 grams of starch and 4 c.c. of

* Schulze and Märker, Chem. Centralbl. 1872, 823. Chittenden and Ely, Amer. Chem. Jour., iv, 120.

† Musculus and v. Mering, Zeitschrift für Physiolog. Chem., ii, p. 415.

‡ The starch was exactly neutral; made so by long and thorough washing with pure water.

§ Loc. cit.

‖ The actual amount of starch changed is, however, somewhat greater than would appear by this equation, since, as has already been mentioned, considerable of the sugar formed is maltose, which has only two-thirds the reducing power of dextrose.

filtered saliva were warmed at 40° C. for 4 hours, then examined with the following results:

	Wt. Cu in one-eighth.	Total amount of sugar.	Starch converted into sugar.
I.	0·1530 gram.	0·6248 gram.	28·13 per cent.
II.	0·1523	0·6216	27·91

Relation of dilution to diastatic action.

It is a fact well understood that the chemical action of a ferment is out of all proportion to the amount of ferment present; indeed, a given solution of a ferment can be diluted again and again without any marked difference in its chemical activity, or at least none at all proportionate to the degree of dilution. It is only when the dilution has been carried to the extreme limit that the relative power of the mixture can be taken as a measure of the amount of ferment present.

The following experiments illustrate the foregoing statement. Each digestive mixture was 100 c.c. in volume, and was warmed at 40° C. for 30 minutes. The only variations in the different mixtures consisted in the amount of saliva and starch.

SERIES I.

With 1 per cent. starch.

	Wt. Cu in one-eighth.	Total amount of sugar.	Starch converted into sugar.
20 c.c. saliva,	0·0951 gram.	0·3872 gram.	34·87 per cent.
10	0·0878	0·3584	32·26
5	0·0809	0·3296	29.67
4	0·0710	0·2904	26·14
3	0·0635	0·2608	23·48
2	0·0452	0·1880	16·92
1	0·0178	0·0792	7·23
½	0·0080	0·0408	3·66

With 2 per cent. starch.

20 c.c. saliva,	0·1784 gram.	0·7304 gram.	32.87 per cent.
10	0·1641	0·6704	30.18

SERIES II.

a. with 1 per cent. starch and 30 minutes at 40° C.

	Wt. Cu in one-eighth.	Total amount of sugar.	Starch converted into sugar.
4 c.c. saliva,	0·0721 gram.	0·2944 gram.	26·50 per cent.
2	0·0480	0·1992	17·93
1	0·0211	0·0920	8.28

b. with 2 per cent. starch and 30 minutes at 40° C.

4 c.c. saliva,	0·1006 gram.	0·4088 gram.	18·40 per cent.
2	0·0408	0·1704	7·67
1	trace		

c. with 1 per cent. starch and 10 minutes at 40° C.

	Wt. Cu in one-eighth.	Total amount of sugar.	Starch converted into sugar.
4 c.c. saliva,	0·0573 gram.	0·2352 gram.	21·15 per cent.
2	0·0213	0·0928	8·35
1	0·0091	0·0456	4·11

SERIES III.

a. with 1 per cent. starch and 30 minutes at 40° C.

	Wt. Cu in one-eighth.	Total amount of sugar.	Starch converted into sugar.
4 c c. saliva,	0·0650 gram.	0·2664 gram.	23·98 per cent.
2	0·0313	0·1336	12·01
1	0·0139	0·0644	5.79

b. with 2 per cent. starch and 30 minutes at 40° C.

4 c.c. saliva,	0·0769 gram.	0·3136 gram.	19·26 per cent.
2	0·0250	0·1080	4·86
1	0·0103	0·0504	2·27

c. with 2 per cent. starch and 4 hours at 40° C.

4 c.c. saliva,	0·1530 gram.	0·6248 gram.	28·13 per cent.
2	0·1058	0·4312	19·41
1	0·0681	0·2784	12·53

From these results it is seen that only when the dilution of normally alkaline saliva is as 1 : 50 or 100 does the diastatic action at all correspond to the amount of ferment present. The same is to be noticed in Grützner's* experiments, where the principle employed by Gruenhagen in the estimation of pepsin was used; the amount of starch dissolved by the saliva being directly proportional to the amount of ferment only when very small quantities of saliva were employed and the time limited to 10 or 15 minutes. Increasing the amount of starch beyond 1 per cent. tends to diminish somewhat the amount of sugar formed in a given time, when the dilution of the saliva is as 1 : 50 or 100, which fact agrees well with what we already know concerning the influence on ferment action of the clogging of digestive fluids in general by the products of digestion, or by the substance to be digested; series III, *a* and *b*. Increasing the length of time for the ferment to act, however, causes a corresponding increase in the amount of sugar formed, as is well seen in series III, c. It would not be at all impossible therefore by suitable dilutions to use this method as a means of determining the relative amounts of ptyalin present in different salivary or pancreatic secretions. The following results, taken from those already given, in addition to others, lends favor to this view. All the experiments were made in

* Pflüger's Archiv der Physiologie, xii, p. 294.

the usual way, and the results are expressed in percentage of starch converted into sugar.

	1	2	3
2 c.c. saliva,	12·01 per cent.	8·35 per cent.	4·73 per cent.
1	5·79	4·11	2·21
	4	5	6
1 c.c. saliva,	6·93 per cent.	26·81 per cent.*	24·00 per cent.*
½	3·56	13·72	11·34

The degree of dilution to be employed depends, of course, upon the amount of ferment present. We have usually diluted the saliva 5 or 10 times, and then added an amount of the diluted fluid corresponding to 0·5–2·0 c.c. of saliva, which in the 100 c.c. of digestive mixture makes a dilution of from 50 to 200. As we shall have occasion to state later on, neutralized saliva needs even a greater dilution. The method certainly appears as advantageous as that proposed by Dr. Roberts† a few years ago, and has the advantage of giving gravimetric results, instead of being dependent upon the disappearance of a shade of color. In using the method with different solutions it will always be found necessary to exactly neutralize the ptyalin-containing solutions, before diluting them, since variations of alkalinity, even though infinitesimal in amount, may produce discordant results. Moreover, it is better to warm the ptyalin solution with the starch for not longer than 30 minutes.

The amount of dilution which saliva will endure and still show diastatic action depends naturally upon the amount of ptyalin present in the secretion and also upon the reaction of the fluid, whether it be alkaline or neutral. The following series of experiments show the average of our results on this point.

SERIES IV.

Normally alkaline saliva, 1 per cent. starch.

	Wt. Cu in one-eighth.	Total amount of sugar.	Starch converted into sugar.
1 c.c. saliva,	0·0152 gram.	0·0704 gram.	6·33 per cent.
½	0·0057	0·0272	2·44
¼	0·0037	0·0176	1·59
$\frac{1}{10}$	trace	less than 1 per cent. of starch converted.	
$\frac{1}{20}$‡	trace		

It is thus seen that when the dilution is as 1 : 250, an appreciable

* Neutralized saliva.

† William Roberts: Jahresbericht für Theirchemie, 1881, 290.

‡ To ensure greater accuracy the saliva was diluted ten times and amounts of the diluted fluid added corresponding to the above.

amount of starch is converted into sugar in 30 minutes at 40° C. Even with a dilution of 1 : 1000 or 2000, a recognizable amount of sugar is formed under these conditions. This degree of dilution, however, cannot be considered as being the limit at which diastatic action will show itself, for with even greater dilutions, the starch is converted into soluble modifications, colored blue by iodine, without giving any recognizable amount of reducing substance; that is, in $\frac{1}{8}$th of the digestive mixture. Longer continued action at 40° C. might yield some reducing substance; it would seem, however, from our experiments, that when a certain degree of dilution is reached, the action of the small amount of ferment, in contact with the larger amount of starch (1 gram) is devoted exclusively to converting the granulose into soluble starch or other like body with non-reducing action. This agrees with the results obtained by Grützner,* who found that the nature of the products obtained by the action of ptyalin was dependent upon the intensity of the ferment action; with a small amount of ferment, erythrodextrin was the main product, while with a large amount of ferment, sugar was mainly formed. Diminishing the amount of starch in large dilutions of the saliva tends, as might be expected, to increase the amount of sugar formed.

Comparison of the diastatic action of neutralized and normally alkaline saliva.

Human mixed saliva, when freshly secreted, almost invariably possesses a distinctly alkaline reaction. Some time ago one of us published a series of experiments† on this point, in which it was shown that the average alkalinity of 51 samples of human mixed saliva, expressed as sodium carbonate, was 0·080 per cent. The extreme variations of alkalinity in the saliva from 14 individuals amounted to 0·085 per cent. calculated as sodium carbonate (0·144–0·059 per cent).

We have had occasion to make determinations of alkalinity in 15 additional samples of saliva, all collected by one person. We give the results here, as affording additional data regarding the average alkalinity of this secretion. The alkalinity is calculated, as heretofore, in the form of sodium carbonate.‡ The indicator used was delicate litmus paper.

* Pflüger's Archiv der Physiologie, xii, p. 297.

† Chittenden and Ely, Amer. Chem. Jour., iv, 329.

‡ Undoubtedly the alkaline reaction of saliva is due in part to alkaline phosphates, and probably the percentages given are only an approximation to the truth.

Filtered saliva.	0·2 per cent. HCl used in neutralizing.	Amount of alkalinity.
20 c.c.	6·25 c.c.	0·091 per cent.
40	10·70	0·078
40	12·00	0·087
25	9·10	0·116
20	6·00	0·087
20	6·25	0·091
20	6·75	0·098
20	5·30	0·077
40	12·50	0·091
20	7·00	0·102
40	12·20	0·088
20	7·80	0·113
20	6·80	0·099
20	8·30	0·120
20	7·60	0·110

Average alkalinity of the 15 samples, 0·097 per cent.

It was demonstrated some time ago by one of us* that neutralized saliva had as great a diastatic power as the unneutralized or normally alkaline. In fact, the single result which we recorded plainly indicated a greater diastatic power on the part of the neutralized saliva, since from the digestion with normally alkaline saliva, one-tenth of the mixture gave 0·0905 gram metallic copper, while the same quantity of the saliva neutralized, gave under like conditions 0·0943 gram copper; thus showing that the alkaline saliva had converted 41·58 per cent. of the starch into sugar, while the same quantity neutralized had changed 43·28 per cent. In these two experiments, however, the amount of saliva used was large, being one-fourth of the entire digestive mixture, viz., 25 c.c.

Recently Langley and Eves† have made the statement that "neutralized saliva converts starch into sugar much more actively than unneutralized saliva," without, however, giving any data. These are the only two statements recorded bearing on the relative diastatic action of the neutralized and normally alkaline secretion.

Our experiments, however, show that there is a very great difference in the action of ptyalin in neutralized and unneutralized saliva; a difference which is more manifest when the saliva is greatly diluted and seemingly out of all proportion to the amount of alkali present, in cases where the dilution is 1 : 100 or more. The following experiments show the amount of difference.

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* Chittenden and Ely, Amer. Chem. Jour., iv, 112.

† On certain conditions which influence the amylolytic action of saliva. Journal of Physiology, vol. iv, No. 1.

SERIES V.

The saliva used in this series contained 0·091 per cent. alkali, calculated as sodium carbonate:

20 c.c. of the saliva were diluted to 100 c.c. and used in *a*.

20 c.c. of the same saliva were neutralized and then diluted to 100 c.c. and used in *b*.

a. normally alkaline saliva.

	Wt. Cu in one-eighth.	Total amount of sugar.	Starch converted into sugar.
4 c.c. saliva,	0·0652 gram.	0·2672 gram.	24·05 per cent.
2	0·0282	0·1208	10·87
1	0·0094	0·0464	4.17

b. neutralized saliva.

4 c.c. saliva,	0·0867 gram.	0·3536 gram.	31·83 per cent.
2	0·0730	0·2984	26·72
1	0·0373	0·1560	14·04

The difference in diastatic action in this instance, particularly where the dilution is as 1 : 50 and 100, is very great, yet in the case of the greatest dilution of the unneutralized saliva the alkalinity of the digestive mixture is but 0·00091 per cent. calculated as alkaline carbonate. Moreover, there is a greater proportional diminution of diastatic action in this case, and also in the next greatest dilution where the amount of alkalinity is 0·00182 per cent., than in the presence of 0.00364 per cent.; a fact due either to the greater susceptibility of the ferment to alkaline carbonate in a dilute solution or else to some modifying influence of the larger amount of albuminous matter present, a point which we shall return to later.

Carrying the dilution of the saliva still further we find that the difference between the diastatic action of the neutralized and unneutralized fluid, shows itself to the limit of decisive diastatic action.

SERIES VI.

This sample of saliva contained 0·116 per cent. of alkali calculated as sodium carbonate. The percentages of starch converted into sugar during 30 minutes at 40° C. alone are given.

Amount of saliva.	Alkali in the 100 cc. of digestive mixture.	Alkaline saliva.	Neutralized saliva.
1 c.c.	0·00116 per cent.	6·33 per cent.	16·34 per cent.
$\frac{1}{2}$	0·00058	2·44	6·62
$\frac{1}{4}$	0·00029	1·54	2·07
$\frac{1}{10}$	0·00011	trace	result lost.
$\frac{1}{20}$	0·00005	trace	1.25 per cent.

Thus in a dilution of 1 : 2000 in the case of neutralized saliva, dias-

tatic action is still sufficiently pronounced to convert 1·25 per cent. starch into sugar during 30 minutes warming at 40° C.

The above results, indicative of such a marked susceptibility of the ferment in a dilute solution to the action of the alkali naturally present in saliva, suggest the possibility of there being a direct connection between the alkalinity of the natural secretion and its diastatic power. While the results already given plainly indicate that very slight changes in the alkalinity, everything else being equal, materially modify the diastatic power of the fluid; still the amount of ferment itself, as well as the amount of proteid matter, may vary in different salivas so much as to counterbalance the direct influence of changes in the alkalinity.

This, the results of our experiments seem to indicate, as we have been unable to trace out any direct connection between the natural variations of alkalinity and diastatic action.*

Influence of different percentages of sodium carbonate on the diastatic action of saliva.

In 1882, while studying the influence of peptones on the diastatic action of alkaline saliva,† data were then obtained showing a constant diminution of diastatic action in the presence of the alkaline carbonate: the conversion of starch into sugar being diminished in proportion as the percentage of alkali was increased. The digestions at 40° C. were then continued for 45 minutes and the ptyalin was present in large amount, 25 of the 100 c.c. of digestive mixture being undiluted, unneutralized saliva, thus making a very powerful diastatic fluid. We give the data then obtained in the percentage of starch or glycogen converted into sugar.

a. Influence of 0·05 per cent. sodium carbonate.

	Saliva alone.	Saliva + Na_2Co_3 = 0·05%.	Difference.
Glycogen,	28·68 per cent.	20·20 per cent.	8·48 per cent.

b. Influence of 0·15 per cent. sodium carbonate.‡

	Saliva alone.	Saliva + Na_2Co_3 = 0·15%.	Difference.
Starch,	40·23 per cent.	17·48 per cent.	22·75 per cent.
"	37·15	14·72	22·43
"	37·55	15·48	22·07
"	38·36	13·57	24·79
Glycogen,	28·68	9·40	

* Compare Chittenden and Ely, Amer. Chem. Jour., iv, 329.

† Chittenden and Ely, Amer. Chem. Jour., iv, 121.

‡ The alkalinity is somewhat greater, owing to the unneutralized alkali of the saliva.

c. Influence of 0·30 per cent. sodium carbonate.

	Saliva alone.	Saliva + Na_2Co_3 = 0·30%.	Difference.
Starch,	40·27 per cent.	10·83 per cent.	29·44 per cent.
"	40·23	9·87	30·36
"	37·15	9·52	27·63
"	38·80	9·79	29·01
"	37·55	10·01	27·54
"	38·36	9·60	28·76
Glycogen,	29·11	6·93	

The action of the sodium carbonate is here very marked and very constant.

We have repeated this series of experiments in part, varying the conditions only by using neutralized saliva, so that the percentages of alkali present might be exact.*

SERIES VIII.

Per cent. Na_2Co_3.	Starch converted.	Difference.
0	41·16 per cent.	
0·005	39·47	1·69 per cent.
0·025	34·84	6·32
0·050	29·81	11·35
0·150	17·88	23·28
0·300	10·88	30·28

It is evident from these results that the presence of a definite percentage of sodium carbonate will produce approximately a constant diminution in the diastatic action of the saliva. This result, however, is constant only when the saliva acts in the above dilution. Diminish the amount of ferment—or rather dilute the saliva—and then the above percentages of alkali produce quite a different result. The above results were obtained where the dilution of the saliva was as 1 : 4. Adding now neutralized saliva to the alkaline mixtures of starch and water in such proportion that 10 c.c. of the original saliva are present in 100 c.c. of digestive mixture; i. e., a dilution of 1 : 10, the results are different.

The following figures were obtained with the above dilution, the mixtures being warmed at 40° C. for 30 minutes.

SERIES IX.

Per cent. Na_2Co_3.	Wt. Cu in one-eighth.	Total amt. sugar formed.
0	0·0998 gram.	0·4064 gram.
0·005	0·0898	0·3664
0·025	0·0437	0·1816
0·050	0·0277	0·1184
0·100	0·0182	0·0808
0·300	0·0105	0·0504
0·500	0·0091	0·0448

* The standard solutions of sodium carbonate were made from the chemically pure, anhydrous salt.

These figures lead to the following percentages of starch converted into sugar under the different degrees of alkalinity.

Per cent. Na_2Co_3.	Starch converted.	Difference.
0	36·57 per cent.	
0·005	32·98	3·59 per cent.
0·025	16·35	20·22
0·050	10·66	25·91
0·100	7·27	29·30
0·300	4·53	32·04
0·500	4·03	32·54

By comparing the two preceding columns of differences it is very manifest that the alkaline carbonate has a much greater retarding action on the more dilute saliva than on the stronger solution; very noticeably so in the mixtures containing 0·025 and 0·050 per cent. of the alkaline salt.

By diluting neutralized saliva still more, and then using quantities of the fluid equal to 2 c.c. of the original saliva, making in the 100 c.c. of digestive mixture a dilution of 1 : 50, even 0·005 per cent. of sodium carbonate is sufficient to retard the diastatic action of the ferment almost completely; thus, in one experiment with the above amount of saliva in the presence of 0·005 per cent. sodium carbonate but 4·03 per cent. of the starch was converted into sugar in 30 minutes at 40° C., while the same amount of saliva alone converted 27·08 per cent. of the starch into sugar. By increasing the percentage of alkaline carbonate the diastatic action was stopped completely.

It is thus evident that the percentage of alkaline carbonate which absolutely or to a certain extent hinders the diastatic action of saliva can be designated only for a definite mixture, and not in a general sense. Langley and Eves* state that sodium carbonate of 0·0015 per cent. causes a retardation in the action of ptyalin; our experiments with unneutralized saliva diluted, plainly show that even much smaller percentages of alkalinity may decidedly retard the action of the ferment, while in similarly diluted saliva 0·005 per cent. of sodium carbonate may prevent diastatic action almost entirely.

Again Langley and Eves† state that the "amylolytic action of saliva becomes less the more alkaline salt there is in the solution, the rate of decrease is, however, slow compared with that which occurs when hydrochloric acid is added in similarly increasing quantities." The rate of decrease, however, as our experiments plainly show, is dependent greatly upon the amount of dilution.

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* Journal of Physiology, vol. iv, No. 1. † Ibid.

Destruction of salivary ptyalin by sodium carbonate.

To how great an extent is the retarding influence of sodium carbonate due to destruction of the ferment? Langley and Eves* state that "sodium carbonate has a very slight destructive action on ptyalin, its retarding power is out of all proportion to its power of destruction."

The following experiments demonstrate the exact action of the sodium carbonate.

SERIES X.

70 c.c. of filtered saliva (the same saliva as used in Series IX), were exactly neutralized with 0·2 per cent. HCl and diluted to 140 c.c.

The following mixtures were then prepared:

	1	2	3	4	5
Diluted saliva,	20 c.c.	20 c.c.	20 c.c.	20 c.c.	20 c.c.
Na_2CO_3 sol.,	0	20 " 0·1%	10 " 0·6%	20 " 0·6%	20 " 1·%
H_2O,	20 "	0	10 "	0	0
Per cent. Na_2Co_3,	0	0·05	0·15	0·30	0·50

These were warmed at 40° C. for 30 minutes, then neutralized with the amounts of dilute acid given below, water and starch added, and the mixtures again warmed at 40° C. for 30 minutes.

	1	2	3	4	5
HCl 0·2 per cent.,	0	6·88 c.c.	20·6 c.c.	41·3 c.c.	68·8 c.c.
Starch + H_2O,	60 c.c.	53·20	39·4	18·7	20·0
	100 c.c.	100 c.c.	100 c.c.	100 c.c.	128·8 c.c.

	Wt. Cu in one-eighth.	Total amount sugar.	Starch converted.
1	0·0998 gram.	0·4064 gram.	36·59 per cent.
2	0·0991	0·4032	36·30
3	0·0992	0·4040	36·40
4	0·0474	0·1968	17·71
5	0·0278	0·1192	10·73

In the above digestive mixtures the ultimate dilution of the saliva is the same as in series IX, 1 : 10, and being the same saliva, the above results are directly comparable with those of series IX. Warming saliva of the above strength with 0·05 and 0·15 per cent. sodium carbonate for 30 minutes causes no destruction of the ptyalin whatever, as the results of experiments 2 and 3 indicate, consequently any diminished diastatic action in the presence of the above percentages of alkaline carbonate must be due to a simple retardation of

* Journal of Physiology, vol. iv, No. 1.

the action of the ferment and not to its destruction. On the other hand, 0·3 and 0·5 per cent. sodium carbonate under like conditions and with the same strength of saliva cause a marked destruction of the ferment, as the results of experiments 4 and 5 plainly show.

We have repeated the above series of experiments with a saliva, neutralized and diluted 5 times, using in each experiment 10 c.c. of the diluted fluid, equal to 2 c.c. of the original saliva. The only other deviation from the conditions already given consisted in warming the saliva with the alkaline carbonate for 1 hour instead of 30 minutes. We will not give the details of the experiment, as the results were mostly negative. With this amount of saliva, 0·15 per cent. sodium carbonate almost completely destroyed the ferment in 1 hour's warming at 40° C., and even 0·05 per cent. of the alkaline carbonate showed under these conditions a very great destructive action; thus, after heating the diluted saliva with 0·05 per cent. sodium carbonate for 1 hour at 40° C., and then neutralizing the mixture it was able in 30 minutes to convert but 5·69 per cent. of starch into sugar, while the same quantity of saliva simply warmed with water, converted under like conditions 27·08 per cent. of starch into sugar.* Under these circumstances, then, the destructive action of dilute sodium carbonate is very great. To what is due this great difference in the action of sodium carbonate of the same strength? Probably to the presence of the larger amount of albuminous matter which in the less diluted saliva possibly combines with the alkaline carbonate. It would follow, moreover, from our results, that any proteid compound formed, has in itself no destructive action on the ferment, even to a slight extent. 0·005 per cent. sodium carbonate causes no destruction of the ferment in 1 hour's warming at 40° C.; that is, in saliva of this dilution.

Influence of proteid matter on the diastatic action of saliva in neutral solutions.

It was formerly demonstrated by one of us† that the presence of 1 per cent. peptone tended to increase the diastatic action of saliva in a neutral solution to such an extent that on an average about 4 per

* The amount of destruction produced in saliva of this solution by the above percentage of sodium carbonate does not appear to be constant, since we have found in several cases a much greater diastatic action after an hour's warming at 40° C. than in the above instance, due probably to the larger amount of ptyalin or proteid matter present.

† Chittenden and Ely, Amer. Chem. Jour., vol. iv, 107.

cent. more starch was converted into sugar during 45 minutes at 40° C.; this with 25 c.c. of saliva in 100 c.c. of the digestive mixture. This effect we attributed to a direct stimulating action on the part of the proteid matter. Langley and Eves,* however, object to this conclusion, although they bring forward no facts to prove the contrary. Considering that litmus will not detect less than 0·001 per cent. acid or alkali they state that there may be in the neutralized fluid an excess of acid or alkali to this extent, and if, as may well be the case, ptyalin acts best in a neutral solution, the effect of the peptone might be due to its putting *hors de combat* the slight excess of acid or alkali which remains on apparent neutralization. But as Langley himself has shown, the proteid matter naturally present in 25 c.c. saliva, or even much less, is far more than sufficient to combine with and render inert any such amount of free acid or alkali. We see no other possible explanation of the action of peptones on the diastatic action of saliva in a neutral solution than a direct stimulation of the ferment. Moreover, Langley and Eves have found that when neutralized saliva is diluted a hundred times, peptone is still able to increase the rate at which it converts starch into sugar, from which they are forced to conclude that the small amount of acid or alkali which may be present, cannot exert, in such a dilution, any retarding influence. We present the following additional results confirmatory of our previous experiments.

In our present experiments we have, however, used much less saliva, and also smaller percentages of peptone.

SERIES XI.

20 c.c. of filtered saliva were neutralized and then diluted to 100 c.c.

0·8 gram of pure albumin-peptone was dissolved in water, made exactly neutral with Na_2Co_3 and the solution diluted to 100 c.c.

10 c.c. of the diluted saliva were employed in each digestion, and of the peptone solution quantities equivalent to 0·05, 0·1, and 0·2 gram of peptone. Length of digestion, 30 minutes.

Per cent. peptone.	Wt. Cu in one-eighth.	Total amt. sugar.	Starch converted.
0	0·0834 gram.	0·3400 gram.	30·61 per cent.
0·05	0·0875	0·3568	32·11
0·10	0·0868	0·3544	32·01
0·20	0·0873	0·3560	32·04

Here, with the smaller amount of ferment, the increase is not so great as with the larger quantity of saliva and with the longer

‡ Journal of Physiology, vol. iv. No. 1.

period of digestion; still, the amount of starch converted is increased on an average about 1·50 per cent. It is interesting to note that under these conditions the full effect of the proteid matter is produced by even 0·05 per cent. Langley and Eves found the maximum effect with saliva ten times diluted to be produced by about 0·1 per cent. peptone. In our experiment, however, the dilution of the saliva in the digestive mixture is 1 : 50.

Influence of proteid matter on the diastatic action of saliva in alkaline solutions.

It was previously demonstrated by one of us that the presence of 1 per cent. peptone in a digestive mixture containing 25 per cent. saliva and 0·3 and 0·15 per cent. sodium carbonate respectively, tended to nearly double the diastatic action, bringing it up almost to the action of saliva unmixed with alkaline carbonate.

We give here a few additional experiments bearing on this point.

The very noticeable difference in the action of small percentages of sodium carbonate on the diastatic activity of moderately dilute and very dilute saliva at once suggests the possibility of some connection between the dilution and the reduced percentage of proteid matter. What, now, is the influence of small amounts of peptone on very weak alkaline solutions of saliva? We will give the results of one series of experiments in answer to this question.

Series XII.

20 c.c. of saliva with an alkalinity equal to 0·110 per cent. sodium carbonate were diluted to 100 c.c., 10 c.c. of the diluted saliva were used in each digestion of 100 c.c.; consequently the alkalinity of the digestive mixture was equal to 0·0022 per cent. sodium carbonate. Neutral peptone was added in varying quantities. The mixtures were warmed at 40° C. for 30 minutes.

Per cent. peptone.	Wt. Cu in one-eighth.	Total amt. sugar.	Starch converted.
0	0·0761 gram.	0·3104 gram.	27·94 per cent.
0·05	0·0823	0·3352	30·18
0·10	0·0841	0·3424	30·82
0·20	0·0853	0·3480	31·33

The same saliva neutralized converted 30·61 per cent. of the starch into sugar; consequently the neutral peptone (0·2 per cent.) caused the alkaline saliva to show a diastatic action considerably greater than the neutral saliva, but not equal in this case to the action of the same percentage of peptone on the neutralized saliva. Compare series xi, made with the same saliva.

Still other experiments of the same nature have shown like results, and even more marked. Thus, while neutral saliva without peptone converted in one instance 18·16 per cent. starch into sugar, a like quantity of the normally alkaline saliva (=0·002 per cent. Na_2Co_3 in the digestive mixture) with 0·1 per cent. peptone converted 31·90 per cent. starch into sugar.

Increasing the percentage of carbonate to a point where previous experiment had shown almost complete stopping of the action of the ferment, it was found that 0·1 per cent. of neutral peptone would, in the above dilution, bring the diastatic action up, almost to that of the neutral saliva.

SERIES XIII.

Thus, 20 c.c. of saliva were neutralized and diluted to 100 c.c., 10 c.c. used in each digestion.

	0 Na_2CO_3 0 Peptone.	0·005% Na_2CO_3 0 Peptone.	0·005% Na_2CO_3 0·10% Peptone.
Wt. Cu in one-eighth,	0·0803 gram.	0·0181 gram.	0·0708 gram.
Total amt. sugar,	0·3272	0·0800	0·2896
Starch converted,	29·45 per cent.	7·20 per cent.	26·07 per cent.

With 0·025 and 0·050 per cent. sodium carbonate, 0·1 per cent. peptone availed but little: there was slight diastatic action, but not enough sugar formed to make the determination of it of any value. These results would seem to indicate that one action of the peptone in an alkaline solution is to combine with the alkaline carbonate and form a compound of quite different power: thus, with 0·050 per cent. sodium carbonate a corresponding larger percentage of peptone is required to increase the diastatic power. In addition to this action, however, there is still manifest the direct stimulating action of the proteid matter on the ferment; seen in one case in the increased percentage of sugar formed in the alkaline solution over the amount formed in neutral solution by the same saliva under like conditions.

As to the union of peptone and the alkaline carbonate we have a strong indication of a combination in that the presence of peptone tends to diminish somewhat the destructive action of small percentages of sodium carbonate in diluted saliva.

Thus, while 10 c.c. of neutralized, dilute saliva (1 : 5) warmed for 1 hour with 0·05 per cent. sodium carbonate converted after neutralization 25·05 per cent. starch into sugar, the same amount of saliva warmed for the same length of time with the same percentage of sodium carbonate, plus 0·4 per cent. peptone converted after neutralization 32·68 per cent. of the starch.

The peptone present had evidently in some way prevented the destructive action of the alkaline carbonate, and the most plausible explanation seems to be the probable formation of an alkaline-proteid body.

Influence of free acid and of acid-proteid matter on the diastatic action of saliva.

The influence of dilute acid solutions on the diastatic action of saliva is naturally a point of considerable physiological importance. In view of the rapid passage of the salivary secretions into the stomach, we need to have accurate knowledge of the exact influence of free acid and acid-reacting fluids on the ferment and its diastatic activity.

In considering this question we do not need now to take into account the older observations of Jacubowitsch, Lehmann, Schiff, Watson, Brücke, Hammarsten and others, since these led to no agreement of opinion and more recently acquired knowledge has rendered necessary different methods of procedure.

In 1881 it was announced by one of us* that the ferment of saliva was destroyed on being warmed for two hours with gastric juice containing 0·2 per cent. hydrochloric acid; also that the same treatment with 0·2 per cent. hydrochloric acid alone caused great destruction of the ferment, so that on neutralization diastatic action was greatly diminished. At the same time it was pointed out that much smaller percentages of acid, even 0·025 per cent.,† diminished the diastatic action of the ferment very materially. Shortly after this, similar results were obtained independently by Langley,‡ who in an interesting paper on the destruction of ferments in the alimentary canal, pointed out that ptyalin from the parotids of a rabbit was destroyed by digestion with a small amount of gastric juice, and also that weak solutions of the ferment were more or less destroyed by heating at 40° C. with 0·014 per cent. hydrochloric acid. In comparing these latter experiments with the preceding it is to be remembered that the former were made with 25 c.c. of filtered human saliva, a much stronger solution doubtless, both as regards the ferment and the albuminous matter present.

Later it was pointed out by one of us,§ that peptones have a very

* Chittenden and Griswold, Amer. Chem. Jour., vol. iii, 305.

† Irrespective of the proteid matter.

‡ Journal of Physiology, vol. iii, No. 3.

§ Chittenden and Ely, Amer. Chem. Jour., vol. iv, 114.

decided influence on the diastatic action of saliva in acid solutions; that while the presence of 0·025 per cent. hydrochloric acid prevented the conversion of but 3·50 per cent. of the starch into sugar, the presence of 1 per cent. peptone allowed the conversion of 48·85 per cent. of the starch, 7 per cent. more than the saliva alone would convert under like conditions; a fact which would indicate something more on the part of the proteid matter, than a mere union of the peptone and acid. Undoubtedly there was a combination of the peptone and acid, but in addition there was manifested the direct stimulating action of the proteid matter. At the time these experiments were made, however, we were unaware of Danilewsky's* method of testing for free acid with tropæolin 00, by which he proved the union of acids with various forms of proteid matter; compounds acid to test papers, but not containing free acid. Falk† likewise noticed the influence of peptones on diastatic action, in an acid solution of malt infusion; thus by adding a small amount of 0·0135 per cent. hydrochloric acid to an infusion of malt and this to some starch paste, no reaction for sugar could be obtained, but by adding the same proportion of acid and some peptone, then the sugar reaction soon appeared. This fact Falk considered as evidence of the union of the acid and peptone.

In view of these results we have repeated some of our previous work, under different conditions, trying many additional experiments, especially as in a recent paper on the amylolytic action of saliva, Langley and Eves‡ have arrived at some conclusions not in accord with our results.

a. Influence of acid-proteid matter.

We have used the tropæolin test for the detection of free acid, whenever it has been necessary in our work, employing the method as recommended by Danilewsky. The tropæolin 00 was dissolved in methyl alcohol (saturated solution) and when a test for free acid was to be made, drops of the alcoholic solution were allowed to evaporate on a porcelain plate at 40° C., and then while still at 40° C., a drop of the fluid to be tested was added and allowed to dry. Free hydrochloric acid causes the dry residue to take on a violet color. We have made a number of trials to ascertain how small a percentage of free hydrochloric acid can be detected by this test. Using a

* Centralbl. Med. Wiss., 1880.

† Virchow's Archivs, lxxxiv, 1881, p. 130. ‡ Loc. cit.

standard solution of hydrochloric acid of known strength,* we have found that 0·003 per cent. of this acid can be detected with certainty, a drop of such a mixture giving a distinctly recognizable violet color. A smaller percentage cannot be recognized and we have therefore invariably deducted the above amount in our various tests for free acid.

The amount of proteid matter naturally present in saliva and which is capable of combining with acids, is apparently quite constant. Langley and Eves found as a mean of several observations that 5 c.c. of filtered, neutralized saliva contained proteids capable of combining with 2 c.c. of 0·1 per cent. hydrochloric acid. We have found as a mean of eight determinations that 20 c.c. of filtered, neutralized saliva contained proteids capable of combining with 7·74 c.c. 0·1 per cent. hydrochloric acid. In an attempt to ascertain approximately how much proteid matter this amount of acid signified, we took the results of our experiments with peptones, in which we found that 1 gram of pure neutral peptone required 48·0 c.c. 0·1 per cent. hydrochloric acid to saturate it. Consequently 1 c.c. of 0·1 per cent. acid would combine with 0·0208 gram peptone, and assuming that the combining power of the proteids present in saliva is the same as that of peptones, the 20 c.c. of saliva would contain 0·16099 gram proteid matter, equal to 0·804 per cent.; a result which at once shows that the combining power of the proteids of saliva and peptone must be quite different, or as is more probable, that considerable of the acid added, is used up in reacting with the phosphates of the alkalies present in the saliva.

Saliva, as a rule, does not contain much more than 0·5 per cent. solid matter, and Hammerbacher has found in human mixed saliva 0·139 per cent. albumin and ptyalin.†

A comparison of the diastatic action of neutral saliva considerably diluted, and similarly diluted saliva in which the proteids present have been saturated with acid, shows at once that acid-proteid matter, even though present in but small quantity, has a distinctly stimulating action on the salivary ferment.

The following experiments will illustrate this point and also show the extent of the stimulation.

SERIES XIV.

A. 40 c.c. filtered saliva were neutralized and then diluted to 200 c.c.

* All of our standard acid solutions were of exactly the strength specified, as was proved by titration with standard solution of silver nitrate.

† Jahresbericht für Thiercheime, 1881, 269.

B. 40 c.c. of the above diluted saliva required 6·8 c.c. 0·05 per cent. HCl to saturate the proteids present = 0·0074 per cent. combined HCl.

Two digestions each were made with *A* and *B*, using quantities of the above salivas equivalent to 4 and 2 c.c. of the original saliva.

	Wt. Cu in one-eighth.	Total amt. sugar formed.
20 c.c. *A*,	0·0913 gram.	0·3720 gram.
23·4 *B*,	0·0987	0·4016
10 c.c. *A*,	0·0850 gram.	0·3472 gram.
11·7 *B*,	0·0940	0·3832

	Starch converted.		Starch converted.
20 c.c. *A*,	33·49 per cent.	10 c.c. *A*,	31·26 per cent.
23·4 *B*,	36·15	11·7 *B*,	34·49
Increase,	2·66 per cent.	Increase,	3·23 per cent.

It is seen that the addition of the acid in this instance causes a very decided increase in the diastatic activity of the saliva. The amount of combined acid present in the 100 c.c. of digestive mixture in the two cases was 0·0017 and 0·0008 per cent. respectively, yet the presence of this small amount of combined acid manifestly acts as a stimulant to the diastatic ferment.* Even still smaller percentages of acid-proteid matter have an equally decided action on the salivary ptyalin. The following series of experiments illustrate this point and at the same time are confirmatory of the preceding one.

SERIES XV.

A. 40 c.c. filtered saliva were neutralized and diluted to 200 c.c.

B. 50 c.c. of the above diluted saliva required 4·75 c.c. 0·05 per cent. HCl to saturate the proteids. The solution was distinctly acid to litmus paper and contained 0·0043 per cent. combined HCl.

Four digestions were made with both *A* and *B*, using quantities of saliva in each case equivalent to 4, 2, 1 and 0·5 c.c. of the original saliva.

	Wt. Cu in one-eighth.	Total amt. sugar formed.
20 c.c. *A*,	0·0925 gram.	0·3768 gram.
21·9 *B*,	0·0959	0·3912
10 c.c. *A*,	0·0827 gram.	0·3368 gram.
10·95 *B*,	0·0876	0·3576
5 c.c. *A*,	0·0671 gram.	0·2744 gram.
5·5 *B*,	0·0751	0·3064
2·5 c.c. *A*,	0·0305 gram.	0·1296 gram.
2·75 *B*,	0·0375	0·1568

* Doubtless these percentages of combined acid are too high, since as before mentioned some of the acid added probably reacts with the phosphates naturally present in the saliva.

	Starch converted.		Starch converted.
20 c.c. *A*,	33.85 per cent.	10 c.c. *A*,	30·32 per cent.
21·9 *B*,	35·22	10·95 *B*,	32·19
Increase,	1·37 per cent.	Increase,	1·87 per cent.
5 c.c. *A*,	24·69 per cent.	2·5 c.c. *A*,	11·68 per cent.
5·5 *B*,	27·58	2·75 *B*,	14·10
Increase,	2·89 per cent.	Increase,	2·42 per cent.

Here the same results are to be seen as in the preceding experiment, although the amount of proteid matter is much less. In both series of experiments it is to be noticed that as the percentage of combined acid is diminished the difference between the diastatic activity of the neutral solution and the corresponding acid solution is increased, at the same time it is to be seen that in the first series of experiments where the percentage of proteid matter is larger there is a greater increase in the conversion of starch with the 23·4 c.c. of acid-reacting saliva than with the 21·9 c.c. of the acid-reacting fluid of the second series of experiments with its smaller percentage of proteid matter.

In the last series of experiments where 21·9 c.c. of *B* are used the amount of combined acid in the digestive mixture is but 0·00094 per cent. HCl, so that where the smaller amounts of acid-reacting saliva are used the percentage amount of combined acid is very small indeed.

Increasing the amount of saliva used and thereby the percentage of acid-proteid matter brought us finally to a point where the acid-proteid matter failed to stimulate the diastatic action of the ferment and even began to show a tendency to retard its action. The following series of experiments, using saliva wholly undiluted, illustrates this point.

SERIES XVI.

100 c.c. of filtered saliva were neutralized requiring 32 c.c. 0·2 per cent. HCl=*A*.

52·8 c.c. *A*=40 c.c. of the original saliva required 12·15 c.c. 0·1 per cent. HCl to combine with the proteids, making saliva *B*; the fluid was distinctly acid to litmus and contained 0·0187 per cent. combined acid. Three digestions were made with both *A* and *B*, using quantities of the fluids equal to 20, 10 and 5 c.c. respectively of the original saliva.

	Wt. Cu in one-eighth.	Total amt. sugar.
26·4 c.c. *A*,	0·1083 gram.	0·4408 gram.
32·48 *B*,	0·1065	0·4336
13·2 c.c. *A*,	0·1024 gram.	0·4168 gram.
16·24 *B*,	0·1087	0·4424
6·6 c.c. *A*,	0·0948 gram.	0·3864 gram.
8·12 *B*,	0·1031	0·4192

	Starch converted.	Combined HCl in the 100 c.c. digestive mixture.
26·4 c.c. *A*,	39·68 per cent.	0
32·48 *B*,	38·96	0·00608 per cent.
Decrease,	0·72 per cent.	
13·2 c.c. *A*,	37·52 per cent.	0
16·24 *B*,	39·73	0·00304 per cent.
Increase,	2·21 per cent.	
6·6 c.c. *A*,	34·79 per cent.	0
8·12 *B*,	37·74	0·00152 per cent.
Increase,	2·95 per cent.	

In this series of experiments where the percentage of combined acid in the digestive mixture is much greater than before, the same increase in diastatic action is noticed. With the largest quantity of saliva however where the amount of combined acid is 0·006 per cent. we seem to have reached a point where the acid-proteid matter ceases to stimulate and begins to retard the action of the ferment. That this is actually the case we have proved by another experiment confirmatory of the preceding one, using in the digestion however two grams of starch instead of one.

Thus while an amount of neutral saliva, equal to 20 c.c. of the original secretion converted 39·08 per cent. starch into sugar, the same amount of saliva having all of its proteid matter combined with acid converted under the same conditions 38·21 per cent. of the starch, a decrease of 0·87 per cent; in this case however the amount of combined acid present in the 100 c.c. of digestive mixture was 0·008 per cent.

It thus seems plainly proven that up to a certain percentage the presence of acid-proteid matter in the saliva tends to decidedly stimulate its diastatic action. We cannot therefore agree with Langley and Eves that ptyalin acts best in every instance in a neutral solution, for our results certainly show an increased action of the ferment in the presence of the acid-proteids, except where the latter are present in comparatively large amount.

The only possible fallacies which suggest themselves here are traces of undetectable alkali in the starch and the presence of phosphates of calcium or magnesium. This result moreover makes clear many statements previously recorded which would otherwise be difficult of explanation. Thus it has been recorded by Astaschewsky,* that the saliva of the parotid gland possesses a very faint acid reac-

* Centralbl. med. Wiss., 1875, 15.

tion and that the maximum of the diastatic action of parotid saliva corresponds with the strongest acid reaction; but in these observations doubtless the acid reaction was in every case due to acid-proteids and not to free acid. Again it was found by one of us* that the presence of 0·005 per cent. HCl decidedly increased the diastatic action of saliva, but while the observation was correct the result was wrongfully attributed to 0·005 per cent. free acid when it should have been attributed to the same percentage of combined acid, where doubtless the proteid matter was not wholly saturated. Likewise Watson's† oft-quoted result, where the addition of a drop of strong acid to saliva gave him an increased diastatic action, was doubtless due to the acid-proteid matter formed and not to free acid, though it may have been due to partial or complete neutralization.

We endeavored to ascertain whether the acid-proteid matter formed by the addition of acid to undiluted saliva would have any destructive action on the diastatic ferment when warmed at 40° C. Of course only a slight action, if any could be expected, still it seemed of sufficient importance to warrant the experiment. Accordingly two mixtures were prepared as follows:

	A.	*B.*
Saliva,	20 c.c.	20 c.c.
HCl 0·2% to neutralize,	6·8	6·8
" " combine with proteids,		3·2
H_2O,	13·2	10
	40·0 c.c.	40·0 c.c.
	Neutral.	0·016% HCl combined

These two solutions were warmed at 40° C. for 1 hour, then neutralizing and equalizing‡ mixtures were added, after which starch and water to 100 c.c. The results were in *A* a conversion of 38·68 per cent. of the starch into sugar, and in *B* a conversion of 38·26 per cent., so that while there may have been some little destruction of the ferment, it is plain that the diminished action noticed in the two preceding cases in the presence of the larger percentages of acid-proteid matter was probably due to simple retardation, since the percentage of combined acid was not more than half that in the above experiment.

We have studied the influence of acid-proteid matter on salivary

* Chittenden and Griswold, Amer. Chem. Jour., vol. iii, 312.

† Jour. Chem. Soc., 1879, 543.

‡ Equivalent amounts of standard acid and sodium carbonate solutions, so that *A* for example might contain the same amount of sodium chloride as *B*.

digestion still further by experimenting likewise with peptones, and in studying the influence of acid-peptones on the action of the ferment we have been impressed with the striking effect of very minute quantities of acid on the ordinary action of peptones, increasing it very decidedly. It has already been shown that the presence of 0·05, 0·1 and 0·2 per cent. of neutral peptone produces, in neutral solutions, a like increased diastatic action; with 0·5 per cent. peptone the increase is as much as with 0·2 per cent.; that is, in the case of saliva considerably diluted. With acid-peptones, however, the effect produced is quite different, and the amount of combined acid necessary to produce this different effect is quite small.

Peptones as usually prepared contain a small amount of combined acid. The sample we used required per gram 0·014 gram Na_2Co_3 to make it neutral; this would be equivalent to 0·00964 gram HCl. Consequently the percentage of combined acid in the peptone, assuming it to be hydrochloric acid, would be 0·964 per cent. With such an acid-peptone the following experiments were tried.

SERIES XVII.

20 c.c. saliva were neutralized and diluted to 100 c.c.; of this solution 10 c.c., equal to 2 c.c. of original saliva were used in each digestion. Four experiments were tried, in three of which 0·050 gram, 0·100 gram and 0·200 gram of the above acid-peptone were added. Following are the results, after warming the mixtures at 40° C. for 30 minutes.

Per cent. peptone.	Wt. Cu. in one-eighth.	Total amt. sugar formed.	Starch converted.
0	0·0766 gram.	0·3128 gram.	28·16 per cent.
0·05	0·0873	0·3560	32·05
0·10	0·0897	0·3656	32·91
0·20	0·0929	0·3784	34·21

Comparing these results with those obtained by similar percentages of neutral peptone the difference is sufficiently striking, and yet the percentage of combined acid in the digestive mixture, where there is present 0·20 gram of acid-peptone, is but 0·0019 per cent. calculated as HCl.

Experimenting with peptones completely saturated with acid, and in this case with what was known to be hydrochloric acid, results similar to the above were obtained, with, however, several suggestive deviations. The following series of experiments will serve to illustrate the main points of interest.

SERIES XVIII.

40 c.c. filtered saliva were neutralized and diluted to 200 c.c.; 10 c.c. of this diluted fluid were used in each experiment.

A standard solution of peptone saturated with hydrochloric acid was also prepared.

The following percentages of peptone and combined acid were contained in the different digestive mixtures of 100 c.c.

	1	2	3	4	5	6	7
Peptone,	0	0	0·01%	0·020%	0·040%	0·060%	0·080%
Combined HCl,	0	0·0006%*	0·00057%	0·00115%	0·0023%	0·00345%	0·0046%

Following are the results of the digestions:

	Wt. Cu in one-eighth.	Total amt. sugar.	Starch converted.
No. 1	0·0872 gram.	0·3560 gram.	31·85 per cent.
2	0·0896	0·3656	32.91
3	0·0901	0·3672	33·06
4	0·0935	0·3808	34·28
5	0·0892	0·3640	32·77
6	0·0775	0·3160	28·45
7	0·0495	0·2048	18·43

It is to be noticed, first, that in this series of experiments the peptones, being completely saturated with acid, are present in much smaller percentages proportionally than the combined acid is, and the effect produced is a diminished diastatic action in the case of Nos. 6 and 7, in the presence of an amount of combined acid which, in the case of the proteids naturally present in saliva, has no retarding action whatever, but on the contrary a stimulating action. The addition of a larger amonnt of peptone to Nos. 5, 6 and 7, for example, the percentage of acid remaining the same, tends to bring up the diastatic action very decidedly.

It would appear from these results, moreover, assuming that the combining power of peptone is the same as the proteids present in saliva, that the presence of say 0·003 per cent. combined HCl in the form of saturated acid-peptone has a retarding action, while the same percentage of combined HCl in the form of saturated salivary proteids, has, in the case of saliva of the same dilution, a decided stimulating action. At the same time it is to be remembered that when acid is added to saliva some considerable acid may be used by the inorganic salts with formation of acid phosphates, for example. These results, moreover, indicate that such is doubtless the case. Increasing the percentage of peptone to say 1 per cent. admits of the addition of larger amounts of hydrochloric acid, without partic-

* To saturate the proteids naturally present in the saliva.

ularly retarding the action of the ferment; thus, as Langley and Eves state, "0·0075 per cent. hydrochloric acid may be added to saliva diluted 10 or 20 times, provided 1 per cent. peptone be present, and yet its action on starch be about equal to that of the saliva without peptone or acid."

Again it would appear that small percentages of acid-proteid matter, either peptones or the albuminous bodies present in saliva, tend to increase the diastatic action not only beyond the natural action of the saliva, but also somewhat beyond the action of the saliva plus the same percentage of neutral peptone. Thus, while the presence of a few hundredths of 1 per cent. of neutral peptone in saliva diluted 1 : 50 caused about 1·5 per cent. increased conversion of starch, acid-peptone caused in 30 minutes 2·17 per cent. increased conversion. Again, as has been seen, the acid-proteids of saliva cause a like increase. Large percentages of acid-proteids, however, in which the albuminous matter is completely saturated, distinctly retard the diastatic action.

These results harmonize in a general way with the previous results obtained by one of us,* in which it was found that the presence of 1 per cent. peptone in an acid-reacting fluid, which by itself almost completely stopped the diastatic action of the saliva, increased the diastatic action of the ferment above the action of the neutral saliva and also above the action of the neutral saliva plus the 1 per cent. of neutral peptone.

We next endeavored to ascertain how much of the retarding action of the acid-peptone is due to destruction of the ferment. Without giving details we have found that with saliva ten times diluted there is a noticeable destruction of the ferment in the presence of 0·028 per cent. of combined acid, although it is not great. In this case it is to be understood that the amount of peptone present is only such as would furnish this percentage of combined acid. The following percentages of starch converted (after neutralization and equalization) show the amount of destruction under the different conditions.

SERIES XIX.

Length of time at 40° C.	Per cent. combined HCl.	Per cent. peptone.	Starch converted.
	0	0	31·65 per cent.
30 min.	0·014	0·25	31·18
30	0·028	0·50	30·82
60	0·028	0·50	29·74
30	0·057	1·00	27·73

* Chittenden and Ely, loc. cit.

It is thus manifest from our results that the retarding influence of the larger percentages of acid-peptones is out of all proportion to their power of destruction. Still larger percentages of acid-saturated peptones produce a much greater destruction. Thus, by warming 10 c.c. of a neutral dilute saliva (1 : 5) with a solution of peptone saturated with acid, in such proportion that the mixture contained 0·430 per cent. combined HCl, there was in 30 minutes an almost complete destruction of the ferment.

b. Influence of free acid.

In view of the fact that some time ago one of us* was of the opinion that small percentages of acid† tended to increase the diastatic action of saliva, it was of especial interest now to ascertain definitely whether free acid when present in small percentages does invariably retard diastatic action. Langley and Eves state that "although saliva neutralized to litmus sometimes shows an increase of action on the addition of 0·0005 to 0·001 per cent. HCl; yet if the proteids of the saliva be saturated with acid, there is a diminution of its amylolytic action, although no free acid is present in the saliva." This we cannot regard as correct without qualification, since our experiments appear to show that saliva with its proteid matter saturated with acid has a greater diastatic action in a given time than saliva simply neutralized, provided the percentage of acid-saturated proteids is not too large. The same investigators further state "that 0·0015 per cent. HCl distinctly diminishes the amylolytic action of pytalin," and "since 0·0015 per cent. HCl increases amylolytic action it seems very unlikely that 0·005 per cent. should increase it;" but as Langley and Eves, in studying the influence of free acid, apparently used diluted, neutralized saliva, in which the proteids present were not combined with acid, depending simply upon dilution to avoid the influence of these bodies, it seems to us a little uncertain whether their results are strictly accurate on this point, since saliva even very much diluted does contain some proteid matter. They, however, state in this connection that "we have often found that solutions which we have thought carefully neutralized have been increased in action by the presence of still smaller percentages of acid, viz: 0·0005 to 0·0010 per cent." Here, however, so far as their results show, the observed increase of activity may have been due

* Chittenden and Griswold, loc. cit.

† Considered as 0·005 per cent., although we now know the above figure could not represent free acid, owing to the proteid matter of the saliva.

to the small amount of acid-proteid matter present, certainly could not have been due wholly to free acid.

We have tried a large number of experiments on this point in a variety of ways, all of which tend to show that a very small trace of free acid, when the amount of acid-proteids is not large, does, seemingly, slightly increase the diastatic action of the ferment. It is, perhaps, questionable, however, whether in the use of such small percentages of acid, the results are to be strictly depended upon. The presence of a small amount of phosphate in the starch or a trace of alkali, not to be detected by litmus, might easily neutralize the small amount of acid added. Again, non-saturation of the proteids to only a very slight extent might effect the result. We subjoin two or three of our experiments.

SERIES XX.

20 c.c. filtered saliva were neutralized and then sufficient acid added to combine with the proteids present; the mixture then diluted to 100 c.c. The solution contained 0·0114 gram combined HCl, but no free acid. *A*.

20 c.c. of the same filtered saliva neutralized, and the proteids just saturated with acid. 3·1 c.c. 0·1 per cent. HCl were then added and the mixture diluted to 100 c.c. The solution contained 0·0114 gram combined HCl, and in addition 0·0031 gram free HCl. The solution gave a distinct violet with tropaeolin 00. *B*.

Digestions were made, using 1 gram starch in a volume of 100 c.c. Time, 30 minutes. Following are the results.

Amount diluted saliva.	*A*. Wt. Cu in one-eighth.	*A*. Total amt. sugar.	*B*. Wt. Cu in one-eighth.	*B*. Total amt. sugar.
20 c.c.	0·0988 gram.	0·4024 gram.	0·0972 gram.	0·3960 gram.
10	0·0917	0·3736	0·0921	0·3752
5	0·0826	0·3368	0·0861	0·3512

Amount diluted saliva.	Starch converted. *A*.	Starch converted. *B*.	Free HCl in *B*.
20 c.c.	36·23 per cent.	35·65 per cent.	0·00062 per cent.
10	33·53	33·78	0·00031
5	30·32	31·62	0·00015

Here there can be no question but that there was free acid in *B*. The saliva gave a distinct reacion with tropaeolin 00 and the starch used was apparently neutral. In this instance 0·0006 per cent. free acid slightly diminished the diastatic action, while 0·0003 per cent. slightly increased it.

A second experiment of like nature gave the following results:

SERIES XXI.

20 c.c. filtered saliva were neutralized and the proteids exactly saturated with HCl, then diluted to 100 c.c. The solution contained 0·0073 per cent. combined HCl, but no free acid. *A.*

20 c.c. of the same saliva neutralized and the proteids saturated by the addition of the same amount of acid as in *A;* 1·2 c.c. 0·1 per cent. HCl were then added, so that a distinct tropaeolin reaction could be obtained in the 41 c.c. of fluid. The fluid was diluted to 100 c.c. and then contained 0·0012 per cent. free HCl. *B.*

20 c.c. of the same saliva, neutralized and the proteids exactly saturated with acid; then enough more acid added to give a distinct tropaeolin reaction, after which the solution was diluted to 100 c.c. The 100 c.c. of fluid contained exactly 0·003 gram HCl. *C.*

A drop of the latter fluid on being tested gave a distinct violet with tropaeolin 00.

Following are the results of digestions made with the foregoing solutions of saliva.

Amount of diluted saliva.	Starch converted.		
	A.	*B.*	*C.*
20 c.c.	35·65 per cent.	35·58 per cent.	35.36 per cent.
10	33·71	33·27	34·14
5	28·81	29·53	30·32

Here it is seen, as before, that the smaller percentages of free acid arising from the use of 5 and 10 c.c. of saliva, show a distinctly increased diastatic activity, while with 20 c.c. the results are very nearly identical; too large an amount of free acid to increase the action and yet not enough to materially diminish it.

We next tried the influence of *increased* percentages of free acid on the action of ptyalin.

SERIES XXII.

30 c.c. filtered saliva were neutralized and the proteids just saturated with acid, then diluted to 150 c.c.; 10 c.c. of this diluted saliva equal to 2 c.c. of the original saliva were used in each digestion. Following are the results, after warming with starch at 40° C. for 30 minutes in the presence of the percentages of free acid specified. The acid solutions were mixed with the starch previous to the addition of the saliva.

Per cent. free acid.	Wt. Cu in one-eighth.	Total amt. sugar.	Starch converted.
0	0·0919 gram.	0·3744 gram.	33·71 per cent.
0·0006	0·0924	0·3768	33·92
0·0010	0·0773	0·3152	28·37
0·0020	0·0166	0·0744	6·69
0·0030	trace		

Here a slight increase is noticed with 0·0006 per cent. followed at 0·002 per cent. by a rapid fall in diastatic action.

With stronger solutions of ptyalin, like results were obtained as follows:

SERIES XXIII.

Filtered saliva was neutralized and the proteids just saturated with HCl. An amount of this fluid equivalent to 5 c.c. of the original saliva was used in each digestion. In this amount there was present 0·00266 gram combined HCl, but no free acid whatever.

Following are the results of digestions with this saliva in the presence of the percentages of free acid specified.

Per cent. free acid.	Wt. Cu in one-eighth.	Total amt. sugar.	Starch converted.
0	0·0956 gram.	0·3896 gram.	35·07 per cent.
0·0005	0·0966	0·3936	35·43
0·0010	0·0867	0·3536	31·80
0·0020	0·0162	0·0728	6·55
0·0030	trace		

Increasing now the amount of saliva still further, so that the percentage of combined acid reaches a point where its retarding influence begins to be felt, the presence of the smallest amount of free acid then causes at once a decided decrease in diastatic action. Thus, using the same saliva as was employed in the preceding series, only in such quantity that 20 c.c. of original saliva were present in each digestive mixture, it was found that the free acid produced a much greater retarding effect than before. The percentage of combined hydrochloric acid, in the form of acid-proteids, contained in each digestive mixture was 0·01064 per cent. Following are the results of the diastatic action.

Per cent. free acid.	Wt. Cu in one-eighth.	Total amt. sugar.	Starch converted.
0	0·0972 gram.	0·3960 gram.	35·65 per cent.
0·0005	0·0830	0·3384	30·46
0·0010	0·0410	0·1712	15·41
0·0020	0·0061	0·0328	2·95
0·0030	trace		

This result accords with the statement made by Langley and Eves, "that if the proteids of saliva be saturated with acid there is a diminution of its amylolytic action, although no free acid is present in the saliva. This diminution is made more marked by the addition of the smallest quantity of hydrochloric acid." The above quantitative results plainly testify to the accuracy of the latter part of their statement. As to the action of the acid-saturated proteids that is wholly dependent upon the percentage present.

c. Destructive action of free acid.

It has been clearly shown* that acid approximating to the strength of the acid of the gastric juice has a destructive action on the salivary ferment. Smaller percentages of acid have a like destructive action. It has at the same time been shown that the presence of very much smaller percentages of free acid stops the amylolytic action of the ferment. Is this stopping of the amylolytic action in every case due to destruction of the ferment, or simply to the retarding action of its presence? Langley, by using an aqueous extract of the parotid of rabbits, with but little proteid matter, concluded that the presence of 0·014 per cent. hydrochloric acid is sufficient to destroy all but the merest trace of ferment in five minutes at 39° C. This before the action of acid-proteids was known. Chittenden and Ely by experimenting with human saliva came to the conclusion "that there may be in the presence of a *very dilute* acid, a simple stopping of the diastatic action, without destruction of the ferment;" in other words, the retarding influence of very small percentages of free acid is not necessarily due to destruction of the ferment. Langley and Eves criticising this conclusion state "that since Chittenden and Ely apparently used unneutralized saliva and took no account of the proteids present, it seems to us probable that not only was there no free hydrochloric acid in their experiments, but that even the proteids were not saturated with acid." In the article to which they refer it is, however, explicitly stated in a foot note† that the saliva was neutralized and then an amount of acid added to equal 0·025 per cent. Unfortunately, we did not then know of the action of acid on the proteids of the saliva; consequently, the above percentage must have been mainly in the form of combined acid. Still, the smaller percentages of free acid do not show great destructive action; their power of retarding the action of the ferment is out of all proportion to their power of destruction. Amylolytic action is almost entirely stopped by the presence of 0·002 per cent. free hydrochloric acid, but warming saliva at 40° C. with 0·002 or even 0·005 per cent. hydrochloric acid for 30 minutes causes little if any destruction of the ferment. On neutralization, diastatic action goes on as vigorously as ever.

This is well illustrated by the following experiments:

* Chittenden and Griswold, loc. cit.; Chittenden and Ely, loc. cit.: Langley, loc. cit.

† Amer. Chem. Jour., vol. iv, p. 119.

SERIES XXIV.

20 c.c. of filtered saliva were neutralized, the proteids just saturated with acid and the mixture diluted to 100 c.c. The solution contained 0·007 per cent. combined HCl.

10 c.c. of this diluted saliva were warmed with the specified percentages of acid for a definite time, then neutralizing and equalizing mixtures were added and the diastatic action determined.

Following are the results.

Length of time at 40° C.	Per cent. of free HCl.	Starch converted.
30 minutes,	0	32·63 per cent.
30	0·001	34·08
30	0·002	31·38
60	0·002	32·48
30	0·005	31·27
30	0·010	4·60
30	0·030	Complete destruction.

Although the results are for some reason a little irregular it is very evident that up to 0·005 per cent. of free acid there is, under these conditions, no particular destruction of the ferment. With 0·010 per cent. on the other hand the destruction is very great.

As to the bearing which these results have on the possible amylolytic action of saliva in the stomach, it is plain that when the fluids of the stomach acquire an acid reaction due to the presence of free hydrochloric acid ptyalin will soon be destroyed. In the first stage of digestion, however, when there is no free acid, the conversion of starch into sugar can undoubtedly go on, and at this stage of the process the proteid matter present may act as a shield to protect the ptyalin and at the same time to stimulate it in its action, but as the acid-proteids increase in amount and come nearer and nearer to their saturation point it is possible that diastatic action may entirely stop even before free acid makes its appearance. Certainly all salivary ptyalin must ultimately be destroyed in the stomach.

General conclusions.

1. The diastatic action of saliva can be taken as a definite measure of the amount of ferment present only when the dilution of the saliva in the digestive mixture is as 1 : 50 or 100. The limit of dilution at which decisive diastatic action will manifest itself with formation of reducing bodies is 1 : 2000–3000, under the conditions previously given.

2. The diastatic action of neutralized saliva is greater than that of normally alkaline saliva. The difference is particularly noticeable

where the dilution is as 1 : 50 or 100, and is apparently out of all proportion to the amount of alkalinity.

3. Sodium carbonate retards the diastatic action of ptyalin in proportion to the amount of alkaline carbonate present. The percentage of alkaline carbonate, however, which hinders diastatic action can be designated only for definite mixtures and not in a general sense, being dependent upon the dilution of the saliva and the consequent change in percentage of proteid matter.

4. The destructive action of sodium carbonate is modified materially by the dilution of the saliva; becoming greater the more the fluid is diluted. This result is due not to simple dilution but doubtless to the diminished amount of proteids.

5. Neutral peptone has a direct stimulating effect on the diastatic action of neutral saliva.

6. The presence of small percentages of neutral peptone tends to raise the diastatic action of normally alkaline saliva, to a point even beyond the action of the neutralized fluid; due in part doubtless to a loose combination of the alkali with the proteid matter, and also to a direct stimulation of the ferment. Likewise peptone tends to diminish in a similar manner the retarding action of the various percentages of sodium carbonate. To accomplish this, however, the amount of peptone must be proportionate to the percentage of alkaline carbonate.

7. Peptone tends to prevent the destructive action of dilute sodium carbonate on salivary ptyalin, thus giving proof of the probable formation of an alkaline-proteid body.

8. Saliva with its proteid matter saturated with acid appears to have a greater diastatic action than when simply neutralized; except when the acid-proteids thus formed are above a certain percentage. Small percentages of peptone saturated with acid, similarly increase the diastatic action of neutralized saliva up to a certain point. Increasing the percentage of acid-proteids finally causes a diminution of diastatic activity.

9. The retarding influence of acid-proteids is out of all proportion to their power of destruction. Large percentages however of acid-proteids may cause almost complete destruction of the ferment.

10. The most favorable condition for the diastatic action of ptyalin, under most circumstances, appears to be a neutral condition of the fluid together with the presence of more or less proteid matter. The addition of very small amounts of hydrochloric acid, however, to *dilute* solutions of saliva, giving thereby a *small percentage* of acid-

proteids, appears to still further increase diastatic action. Under *such conditions* a minute trace of free acid appears to still further increase the action.

11. 0·003 per cent. free hydrochloric acid almost completely stops the amylolytic action of ptyalin. The larger the amount of saturated proteids the more pronounced becomes the retarding action of free acids.

12. The retarding effects of the smaller percentages of free acid are not due wholly to destruction of the ferment. Pronounced destruction takes place with 0·005–0·010 per cent. free hydrochloric acid.

13. Proteid matter, in influencing the diastatic activity of salivary ptyalin, acts not only by combining with acids and alkalies, but apparently also by direct stimulation of the ferment.

Sheffield Laboratory of Yale College, Jan., 1885.