ACTION OF FORMALDEHYDE ON ENZYMES AND ON CERTAIN PROTEIDS.

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It is a well known fact that formaldehyde, even in very small amount, will exert a peculiar action on proteid material, hardening it or otherwise altering its chemical and physical properties. Fibrin, when allowed to stand in a solution of formaldehyde even as dilute as 1:500, soon becomes hardened and resists, as will be presently shown, the action of proteolytic enzymes, and although it may finally be digested the process is nevertheless greatly retarded. Again, egg albumen or blood serum to which a small amount of formaldehyde has been added will not coagulate when heated; and numerous other instances might be cited showing the changes that take place when formaldehyde is present.

Formaldehyde also has powerful germicidal properties, and even in small amount will inhibit the growth of micro-organisms. In stronger concentration it will promptly destroy bacteria.

The exact chemical nature of the soluble ferments, or enzymes, is far from being understood. Some authorities claim they are proteid-like substances. When obtained in the purest condition possible they contain nitrogen, and many give some of the proteid reactions. Certain of the purified ferments, however, fail to give the common proteid reactions. When such reactions are given by a ferment presumably pure, it is still impossible to determine whether these are due to contamination with proteid material, or whether they are due to the ferment itself.

The experiments to be described were carried on with a view of determining whether formaldehyde exerts any action on some of the more common soluble ferments, especially those concerned in the processes of digestion. Accepting the common view that enzymes are

proteid compounds, it might be expected that they would undergo alteration in the same way as ordinary proteid substances. Such change in the composition of the enzyme would be shown by a decreased or wholly suppressed ferment action. It should be borne in mind, however, that a decrease in ferment action, or its entire absence, may be due to the action of the formaldehyde on the substance to be digested. Thus, fibrin which has been kept in a solution of formaldehyde 1:100 for a day is scarcely affected by a solution of pepsin-hydrochloric acid, even after being kept at a temperature of about 38° for several hours. On the other hand, formaldehyde may be added to the digestive fluid in the proportion of 1:100, and at the end of several weeks this solution will be found to be still active; in fact, it will dissolve fresh fibrin nearly if not quite as rapidly as a fresh solution of pepsin-hydrochloric acid.

The literature contains but very little concerning the effect of formaldehyde on digestive ferments. According to Loew,* pepsin and disastase lose their activity when left in contact with formaldehyde for one day; the amounts of substances used were 1 gramme of ferment, 10 grammes of water, and 5 cc. of 15 per cent formaldehyde solution (=5 per cent formaldehyde in the mixture). Other ferments, emulsin, papaïn, tryspin, used in the form of crude products, soon give, like many other proteid substances, precipitates with formaldehyde which are very difficultly soluble in acids and in alkalis. Simons † found that formaldehyde has no apparent effect on peptic digestion, but has a very depressing action on the pancreatic ferment (trypsin), even one part in 2000 parts of the solution being sufficient to distinctly retard digestion. We have been able to confirm this statement. Maybery and Goldsmith ‡ tried the action of several antiseptics on peptic digestion. Their results show that the greater the amount of formaldehyde used the greater will be the percentage of fibrin undigested in a given time. In Wurtz's Dictionnaire de Chimie, Deuxième Supplément, vol. iii, p. 313, the action of formaldehyde on proteid material and on ferments is considered very briefly.

* *Journ. f. prakt. Chem.*, 1888, xxxvii, 101.
† *J. Am. Chem. Soc.*, 1897, xix, 744.
‡ *J. Am. Chem. Soc.*, 1897, xix, 889.

The statement is made that the soluble ferments (diastase, pepsin, pancreatin, etc.) are modified completely by formaldehyde, when this is used in solution of sufficient concentration. This statement, as will be presently shown, does not hold true for pepsin, malt diastase and rennin.

In view of the rather incomplete knowledge in regard to the action of formaldehyde on digestive ferments, it was deemed advisable to make a systematic study of the subject. The ferments pepsin, rennin, pancreatin and papaïn were used; these were ordinary commercial preparations, and were found by control experiments to be more or less active. The pancreatin, however, possessed very little diastatic power, and consequently an aqueous extract of fresh pancreatic gland was used for the purpose of observing the effects on the diastatic as well as on the tryptic ferments. The commercial preparations of ptyalin and malt diastase were found to possess but very little activity, and hence saliva and malt were employed instead. Inasmuch as the preparations referred to are far from being pure ferments and contain more or less foreign material, it is obvious that the ratio between the amounts of formaldehyde and ferment cannot be expressed. All that can be given are the amounts of materials used, the dilution, the amount of formaldehyde, the conditions, and the results obtained. In all the tests the conditions most suitable to the normal action of the ferments, *i. e.* temperature, reaction, etc., were adhered to as closely as possible. When ferments having similar properties were compared, the tests were made under conditions as nearly alike as possible. Several series of tests were made with each ferment; the conditions were varied somewhat with each series, as will be seen from the detailed experiments. When doubtful results were obtained, the tests were repeated. In general, the work was carried on in the following manner: A certain amount of the commercial ferment, or of the solution of the ferment, was dissolved in distilled water, and this solution was divided into portions; to each a definite amount of "formalin" was added, so that the liquid contained known amounts of formaldehyde, varying usually from 1:100 to 1:000. These mixtures of formaldehyde and ferment were allowed to stand in corked

flasks at ordinary room temperature. From time to time portions were tested in order to determine whether the activity had been affected, and if so to what extent. The formalin used was approximately a 40 per cent solution, as determined by analysis; it contained only a trace of free acid, too small to be estimated. Fresh, well washed fibrin from the blood of steers was used in testing the proteolytic ferments, and freshly prepared one or two per cent starch paste, made of potato starch, for the diastatic experiments. The experiments were carried on at a temperature of 37°-40°, except in the case of malt diastase, where the temperature of 57°-60° was employed.

ACTION OF FORMALDEHYDE ON FIBRIN.

Benedicenti * gives a review of the work that has been done on the action of formaldehyde on various proteid substances, and also the results of several series of experiments carried on by himself. He confirms the statements that had been made, that formalhehyde hardens proteids and renders them incapable of swelling in dilute hydrochloric acid, and of being digested by pepsin-hydrochloric acid or by pancreatin juice.

Several series of tests were made in order to determine how much formaldehyde must be used and for how long a time it must be allowed to act in order that fibrin would become so altered that it would be incapable of being digested.

Experiment I.—A few small shreds of fibrin were added to portions of 15 cc. each of an active solution of pepsin. Formaldehyde was then added in the proportions of 1:100, 1:250, 1:1000, and 1:2500. The tubes were corked and set aside for 24 hours at ordinary temperature. An equal volume of 0.5 per cent hydrochloric acid was then added to each tube and the tubes were placed in a water-bath maintained at a temperature of 38°-40°, and examined at intervals during the next 24 hours. Table I shows the changes that took place. Tube C served as a control.

In this experiment the fibrin and pepsin were both subjected to the action of the formaldehyde. The fibrin alone was affected.

Experiment II.—Fibrin was allowed to stand in solutions of formaldehyde 1:500 and 1:1000 at ordinary temperature. At the end of three

* *Archiv f. Anat. u. Physiol.* (Physiol. Abtheilung), 1897, p. 219.

days small pieces were removed and squeezed thoroughly, then subjected to the action of a stronger solution of pepsin-hydrochloric acid than that used in the preceding experiment. At the end of three-quarters of an hour the fibrin that had been exposed to the weaker solution of formaldehyde was dissolved almost completely, and disappeared entirely a few minutes later. That which had been exposed to the stronger solution of formaldehyde dissolved more slowly. At the end of an hour it was swollen, and the liquid was slightly cloudy; after another hour it had entirely dissolved.

TABLE I.

MIXTURES OF PEPSIN, FIBRIN AND FORMALDEHYDE—24 HOURS AT ORDINARY TEMPERATURE, THEN DIGESTED AT 38°–40°.

Tube No.	Form-aldehyde.	Examined at the end of			
		1 hour.	2½ hours.	4 hours.	24 hours.
1.	1:100	No change.	No change.	Fibrin slightly swollen; liquid clear.	Fibrin nearly dissolved.
2.	1:250	Fibrin slightly swollen; liquid clear.	Fibrin dissolving slowly; liquid cloudy.	Fibrin dissolving slowly.	Fibrin nearly dissolved.
3.	1:1000	Fibrin swollen; liquid cloudy.	Fibrin nearly dissolved.	Fibrin dissolved.	
4.	1:2500	Fibrin dissolving; liquid cloudy.	Fibrin almost dissolved.	Fibrin dissolved.	
C.	.....	Fibrin dissolved.			

Similar results were obtained with an aqueous extract of pancreatic gland. The solution used was the one described in Experiment XVIII. Fibrin, which had been exposed to the action of formaldehyde 1:1000 for 24 hours at room temperature, was digested nearly as rapidly as fibrin which had not been exposed to formaldehyde; that which had stood in formaldehyde 1:500 for the same length of time was digested more slowly, though it did not dissolve completely.

Fibrin, which had stood in formaldehyde 1:1000 for 24 hours at ordinary temperature, or for only a few hours at 40°, was digested completely by the commercial pancreatin, though much more slowly than fresh fibrin. When, however, the action of formaldehyde had been continued for a few hours longer at 40°, the pancreatin had no effect.

The results with papaïn will be described later. It might be said here, however, that a strong solution of the ferment had no effect on fibrin that had been exposed to formaldehyde 1:1000 for a few hours at room temperature, or even for half an hour at 40°.

These results show that formaldehyde, even in very small amount, will alter fibrin in the course of a few hours so that it will offer considerable resistance to the action of proteolytic ferments. The action of the formaldehyde is more marked at a temperature of 40° than at ordinary room temperature. Although the fibrin may be digested the process is retarded. Peptic digestion was affected the least.

ACTION OF FORMALDEHYDE ON MILK.

Pottevin* observed that formaldehyde retards the coagulation of milk by rennet, and that rennet and sucrase on contact with strong solutions of formaldehyde become inactive. He also found that inversion of sugar by sucrase or even by acids was retarded or suppressed by formaldehyde. Weigle and Merkel † found that the precipitate of casein in milk containing formaldehyde is coarsely flocculent and voluminous, and that the digestion of milk and egg albumen is prevented by formaldehyde. The action of formaldehyde on the proteids of milk, as well as on other proteid substances, has been studied by numerous other investigators.

Milk is not coagulated by formaldehyde. If formaldehyde is added to milk in the proportion of 1:500, the latter will be altered within a few hours so that it will not be coagulated by an active solution of rennin. If less formaldehyde is added coagulation will take place, but slowly.

The rennet used was Liquid Rennet, made by John Wyeth and Bro. When diluted with four parts of water (1-4), 1 cc. of this dilute rennet added to 10 cc. of fresh milk and kept at 40° would give a solid coagulum in about three-quarters of an hour to an hour. When diluted with an equal volume of water (1-1), 1 cc. would coagulate 10 cc. of milk in about ten minutes, or if the milk were previously warmed to 40°, in two or three minutes.

Experiment III.—Formalin was added to portions of 240 cc. of fresh milk in flasks in the following proportions:

Flask 1	received	24	cc. formalin (40 per cent) =	formaldehyde	.....	1:25
" 2	"	6	"	"	.....	1:100
" 3	"	1.2	"	"	.....	1:500
" 4	"	.6	"	"	.....	1:1000
" C	"	no formalin and served as a control. Fresh milk was always used in the control tests.				

* *Ann. Inst. Pasteur*, 1894, viii, 796.

† Forschungsberichte über Lebensmittel. München, 1895, pp. 91-94.

The flasks containing the milk and formaldehyde were corked and set aside in a cool place. At stated intervals portions of 10 cc. were taken out of each flask, 1 cc. of the dilute rennet (1-4) added, and the tubes then placed in the water-bath and kept at 38°-40°. If no coagulation took place within 24 hours they were reported negative. Three sets of tests were made, extending over a period of four days. Table II contains the results.

TABLE II.

Tube No.	Formaldehyde.	Mixtures of Milk and Formaldehyde tested at the end of 4½ hours.	36 hours.	4 days.
1..	1:25	No coagulation.	No coagulation.	No coagulation.
2..	1:100	"	"	"
3..	1:500	"	"	"
4..	1:1000	Prompt coagulation.	Prompt coagulation.	Milk thickened in about two hours and became solid later.
C..	....	"	"	Prompt coagulation.

Experiment IV.—Another series was made, using as before portions of 240 cc. of milk to which formalin had been added in the following proportions:

Flask 1 received 1.2 cc. formalin (40 per cent) = formaldehyde..... ....1:500
" 2 " 0.8 " " 1:750
" 3 " 0.6 " " 1:1000
" 4 " 0.4 " " 1:1500
" C " no formalin and served as a control.

The tests were made in the same way as those just described, using, however, a stronger ferment solution (1-1), which moreover contained formaldehyde in the proportion of 1:500. The same mixture was used for all three series of tests. As will be explained later, the ferment is not affected by very small amounts of formaldehyde, and although the formaldehyde will rapidly alter the casein of the milk, coagulation will result too rapidly for this effect to be of any influence. In the series of tests made on the 4th day the milk was warmed to 40° before use. The results were as follows (Table III):

TABLE III.

Tube.	Formaldehyde.	Mixtures of Milk and Formaldehyde tested at the end of 1 hour.	24 hours.	4 days.
1..	1:500	Thick in 1¼ hours, solid soon after.	No coagulation.	No coagulation.
2..	1:750	Thick in 15 min., solid in 30 min.	Thick in 20 min., solid in 40 min.	No coagulation even after 24 hours.
3..	1:100	Coagulation in about 15 min.	Thick in 20 min., solid in 40 min.	Thick in 1¼ hours, solid soon after.
4..	1:100	Coagulation in 15 min.	Coagulation in 20 min.	Thick in 15 min., solid in 20 min.
C..	....	Coagulation in about 15 min.	Coagulation in 10 min.	Coagulation in 3-4 min.

These results show that milk to which formaldehyde has been added in considerable quantity undergoes alteration. The casein is rendered incapable of being coagulated by rennet ferment. If formalin is added in smaller amount (1:1000 formaldehyde), the casein is acted upon more slowly and will be coagulated slowly or not at all. The action of formaldehyde on rennet ferment will be considered later.

Several tests were made to determine whether the coagula produced by rennet in milk to which formaldehyde had been added would be digested by pepsin-hydrochloric acid. It was found that the casein behaved just as the fibrin had, that is, if very little formaldehyde had been used the process was simply retarded, while if formaldehyde had been added in larger amount digestion would fail to take place.

It is evident from the preceding experiments that a 1 per cent solution of formaldehyde acting on fibrin for 24 hours will render this practically insoluble in pepsin-hydrochloric acid. Furthermore, the addition of formalin to milk alters the casein to such an extent that this will not be precipitated, or but slowly on subsequent addition of rennet ferment.

ACTION OF FORMALDEHYDE ON PEPSIN.

The pepsin employed (Kahlbaum's) was a white powder, very easily and completely soluble in water. One gramme in 1000 cc. of 0.25 per cent hydrochloric acid formed a very active digestive fluid, as will be seen from the rapidity with which fibrin was dissolved. One series of experiments was made in which formaldehyde was added to portions of a solution of pepsin-hydrochloric acid. In another series the formaldehyde was added in the same amounts to like solutions of pepsin in distilled water. In the latter series an equal volume of 0.5 per cent hydrochloric acid was added just before making the tests.

Formaldehyde and Pepsin.

Experiment V.—One gramme of pepsin was dissolved in 1000 cc. of water. 75 cc. of this solution were placed in flasks and additions made as follows:

Flask	1	received	3.75 cc. formalin (20 per cent) =	formaldehyde	.....	1:100
"	2	"	1.50	"	"	.....1:250
"	3	"	0.75	"	"	.....1:500
"	4	"	0.375	"	"	.....1:1000
"	5	"	0.15	"	"	.....1:2500
"	C	"	no formalin and served as control.			

After standing at ordinary temperature for 24 hours, 15 cc. of each mixture were measured into a test-tube and an equal volume of 0.5 per cent hydrochloric acid and a few shreds of fibrin were added. The tubes were then placed in a water-bath which was kept at a temperature of 38°-40°. The fibrin in all of the tubes began swelling immediately and at the end of two or three minutes the liquid became cloudy, indicating that digestion was taking place. At the end of half an hour the fibrin in each tube was almost entirely dissolved, and 15 minutes later it disappeared completely. So far as could be observed there was no difference in the appearance of any of the tubes.

The original mixtures were set aside at ordinary room temperature and at the end of two weeks, and again at the end of four weeks, these were tested as outlined above. The results were exactly the same as in the first, *i. e.* the fibrin swelled immediately and disappeared completely in from one-half to three-quarters of an hour. The mixtures of pepsin-formaldehyde all remained clear and contained no deposits, with the exception of the control solution C. This soon began to give indications of decomposition; within three days the solution was cloudy and had a strong putrid odor; yet it was active, since after standing for a month it was able to digest fibrin as rapidly as when perfectly fresh.

In some other work carried on with solutions of pepsin in ordinary tap-water it was found that the ferment lost its activity within 24 hours and was no longer able to digest fibrin. On referring to the literature, however, it was found that certain inorganic salts, which are commonly found in tap-water, exert more or less effect on pepsin, some destroying it completely. The destruction of the ferment action in these cases is thus explained.

Formaldehyde and Pepsin-Hydrochloric Acid.

Experiment VI.—One gramme of pepsin was dissolved in 1000 cc. of 0.25 per cent hydrochloric acid. 75 cc. of this solution were placed in each of six flasks and additions of formaldehyde were made to these as in Experiment V. These mixtures of formaldehyde and ferment were made at the same time as those in the preceding experiment and the tests were made together.

15 cc. of each solution were measured into a test tube and fibrin added. The tubes were then placed in a water-bath and kept at a temperature of 38°-40°. Tests were made at the end of 24 hours, two weeks and four weeks. The results obtained were exactly the same as in the preceding experiment. In fact, if the tubes had not been labeled, it would have been impossible to distinguish one from another.

These results led us to doubt the statement of Loew that formaldehyde destroys the activity of pepsin. Consequently several sets of tests were made, in which the same amounts of ferment and of formaldehyde were used as were employed by him.

Experiment VII.—One gramme of ferment was dissolved in 10 cc. of water and 5 cc. of a 15 per cent solution of formaldehyde were added. In the first set the Kahlbaum pepsin was used, and in the second, a sample made by Parke, Davis & Co., labeled "Pepsin, Aseptic (Pepsin U. S. P. 1890) 1:3000." This was in the form of yellow scales, and was easily and completely soluble in water, and very active. The mixtures of ferment and formaldehyde were then allowed to stand in corked flasks for 24 hours. Inasmuch as formaldehyde in strong concentration rapidly hardens fibrin and renders it incapable of being digested, especially when allowed to act at an elevated temperature, the mixtures of ferment and formaldehyde were strongly diluted with 0.25 per cent hydrochloric acid just before making the test, and small shreds of fibrin were used. The dilutions made in each case were 1:15, 1:30, 1:60. Fibrin was added to portions of each, and the tubes then placed in a water-bath and kept at 40°. The fibrin in each tube was nearly dissolved in about a quarter of an hour and disappeared completely a few minutes later. At the end of five days, and again after three weeks, new portions of the ferment-formaldehyde solutions were diluted and tested as above. The results were exactly the same as those just given.

From this it would seem that Loew did not dilute his formaldehyde-ferment solution, and if so, his failure to obtain digestion would seem to be due, not to the action of formaldehyde on pepsin, but rather to the action of formaldehyde on the fibrin, altering it before the pepsin could act. This view is confirmed by the following experiments:

Experiment VIII.—Mixtures were made using the same amounts of both samples of pepsin, water, and formaldehyde. These were allowed to stand for 24 hours and were then tested as follows: 10 cc. of each mixture were diluted with an equal volume of 0.5 per cent hydrochloric acid, a few shreds of fresh fibrin were added, and the tubes were then kept at 40°. At the same time control tests were made, with small portions of the same mixtures diluted strongly with 0.25 per cent hydrochloric acid. At the end of half an hour, the fibrin in the tubes containing the very dilute mixture was nearly dissolved, whereas that in the other tubes

was dissolving slowly, although the amount of ferment present was much larger. The formaldehyde was evidently hardening the fibrin so rapidly that the pepsin could not accomplish its work completely. At the end of 3 hours there remained some shreds of fibrin still undissolved, and these gave no further evidence of dissolving during the following 6 hours, but appeared hardened.

The same tests were repeated at the end of three days, the mixtures being kept in the incubator for 24 hours in the meantime. The results obtained were exactly the same as in the preceding.

These results fully confirm the statement made by Simons, that formaldehyde has no apparent effect on peptic digestion. These results, taken in conjunction with those obtained in studying the action of formaldehyde on fibrin also confirm the statement made by Maybery and Goldsmith, that with an increase in the amount of formaldehyde there will be a decrease in the amount of fibrin digested in a given time. This, however, is due solely to the action of formaldehyde on fibrin and not to any alteration of the pepsin. In the experiments outlined above small portions of fibrin were used; if, however, larger amounts were employed some of the fibrin would be hardened by the formaldehyde before digestion could take place, and hence the process would be retarded. At the end of a given time there would still be undigested fibrin in the tubes in which formaldehyde was present.

Benedicenti found that proteids, albumin, fibrin, casein, etc., which had lost their power of being digested by gastric or pancreatic juices through the action of formaldehyde, recovered this power after being heated in a current of steam. The formaldehyde was removed from its combination with the proteid. Inasmuch as ammonia rapidly unites with formaldehyde, thus neutralizing its action, it seemed probable that ammonia would act in a manner similar to that of a current of steam, removing the formaldehyde from the proteid and thus restoring to the latter its property of being dissolved by ferments. Several experiments were made in order to determine whether ammonia would have this action on fibrin and on casein previously treated with formaldehyde.

Experiment IX.—Fresh fibrin was allowed to stand in a solution of formaldehyde 1:100 in a closed flask at ordinary temperature. At the end of 6, 24, and 48 hours portions were transferred to dilute ammonia, about 3.5 per cent in some cases, while in other cases very dilute ammonia was used. After allowing the ammonia to act for some time, the fibrin was washed and subjected to the action of pepsin-hydrochloric acid. At the same time portions of fresh fibrin and of formaldehyde-fibrin which had not been subjected to the action of ammonia were also added to pepsin-hydrochloric acid. Table IV shows the results.

TABLE IV.

Tube No.	Time of action of formaldehyde.	Time of action of ammonia.	Results.
1.	.....	.....	Fibrin nearly dissolved in ½ hour; dissolved completely a few minutes later.
2.	6 hours.	.....	Fibrin swollen slightly in 1 hour; liquid clear; nearly dissolved in 5 hours.
3.	"	3.5% NH_3 15 min. 40°.	Fibrin swollen slightly in 1 hour; liquid clear; partially dissolved in 5 hours.
4.	"	3.5% NH_3 30 min. 40°.	Fibrin swollen slightly in 1 hour; liquid clear; nearly dissolved in 5 hours.
5.	.....	.....	Fibrin nearly dissolved in 1 hour.
6.	24 hours.	.....	No change at the end of 1 hour; fibrin hardened; liquid clear; dissolved in 12 hours.
7.	"	3.5% NH_3 5 hours.	No change at the end of 1 hour; fibrin hardened; liquid clear; dissolved in 12 hours.
8.	"	Trace NH_3 5 hours.	No change at the end of 1 hour; fibrin hardened; liquid clear; dissolved in 12 hours.
9.	.....	.....	Fibrin dissolved in about 1 hour.
10.	48 hours.	.....	No change at the end of 6 hours.
11.	24 hours.	3.5% NH_3 24 hours.	Fibrin very slightly swollen; liquid clear at the end of 3 hours; partially dissolved in 6 hours.
12.	"	Trace NH_3 24 hours.	Fibrin very slightly swollen; liquid clear at the end of 3 hours; partially dissolved in 6 hours.

A test made with papaïn gave very similar results. Fibrin was allowed to stand for about 3 hours in formaldehyde 1:100 at 40°. It was then washed and placed in 3.5 per cent ammonia. After allowing the latter to act for 4 hours, the fibrin was washed and added to a strong solution of papaïn, and the tube kept in the incubator over night. On the following day there was no change in the appearance of the fibrin and the ferment solution was perfectly clear, thus showing that no digestion had taken place.

The results with casein agree with those obtained with fibrin. Formaldehyde was added to milk in the proportion of 1:100 and the mix-

ture kept at 40° for half an hour. At the end of that time a portion was removed and to this was added the calculated amount of a 3.5 per cent solution of ammonia necessary to neutralize the formaldehyde present. This mixture was likewise put at 40° for half an hour, then 10 cc. were removed and 1 cc. of rennet solution (1-1) added. No coagulation took place, even after several hours, but a precipitate of coarse floccules appeared.

These results indicate that ammonia will have but little if any effect on the proteid which has been altered by formaldehyde.

ACTION OF FORMALDEHYDE ON RENNET.

The rennet ferment used was the same as that employed in studying the action of formaldehyde on milk. Two series of experiments were made; one with dilute rennet solution (1-4), and the other with stronger rennet solution (1-1). The results were the same in both cases except that with the more dilute ferment solution coagulation took place more slowly. The conditions were the same as in the previous tests on milk, namely, 1 cc. of the ferment solution was added to 10 cc. of milk, and the tubes were then placed in a water-bath and kept at a temperature of 40°. In the absence of formaldehyde coagulation would occur in about three-quarters of an hour to an hour with the dilute ferment solution (1-4), and in about 10-15 minutes with a stronger solution (1-1). If, however, the milk was previously warmed to about 40° coagulation would result much more quickly, sometimes even in one or two minutes.

Formaldehyde and Dilute Rennet.

Experiment X.—Formalin was added in the following proportions to portions of 40 cc. of rennet solution (1-4) in flasks:

Flask	1	received	4 c.c.	formalin (40 per cent) =	formaldehyde	1:25
"	2	"	2	"	"	1:50
"	3	"	1	"	"	1:100
"	4	"	0.5	"	"	1:200
"	5	"	0.2	"	"	1:500
"	6	"	0.1	"	"	1:1000
"	C	"	no formalin.	This served as control.		

Tests were made after the mixtures had stood 1½, 4, 7 and 14 days at ordinary room temperature. Table V shows the results obtained. In

the tests made on the 4th, 7th and 14th days the milk was previously warmed to 40° and then the rennet-formaldehyde mixture added.

TABLE V.

Tube No.	Form-aldehyde.	Mixtures of Rennet and Formaldehyde tested at the end of 1½ days.	4 days	7 days.	14 days.
1.	1:25	No coagulation even after 24 hours.	No coagulation even after 24 hours.	No coagulation even after 24 hours.	No coagulation even after 24 hours.
2.	1:50	No coagulation even after 24 hours.	No coagulation even after 24 hours.	No coagulation even after 24 hours.	No coagulation even after 24 hours.
3.	1:100	Coagulation in about 2 hours.	Coagulation in about 2 hours.	Thick in 5 hours, solid later.	No coagulation even after 7 hours.
4.	1:200	Coagulation in about 1¼ hours.	Coagulation in about 1¼ hours.	Coagulation in about 1¼ hours.	Coagulation in about 2½ hours.
5.	1:500	Coagulation in about 40 minutes.	Coagulation in about 1 hour.	Coagulation in about 1 hour.	Coagulation in about 1¼ hours.
6.	1:1000	Coagulation in about 40 minutes.	Coagulation in about 1 hour.	Coagulation in about 1 hour.	Coagulation in about 1 hour.
C.	.....	Coagulation in 40 minutes.	Coagulation in about 1 hour.	Coagulation in about 1 hour.	Coagulation in about 1 hour.

It is evident from this experiment that solutions of rennet containing formaldehyde in strengths of 1:25 and 1:50 will apparently completely lose their ferment action in less than 36 hours. Mixtures containing formaldehyde 1:100 apparently slowly lose their power of coagulating milk, and hence retard the coagulation which, however, still takes place even after an exposure of 14 days. Mixtures containing formaldehyde 1:200 or less practically seem to have no effect on rennet. This loss of ferment action is not, however, due to destruction of rennet but rather, as will be shown later, to the action of formaldehyde on the casein of the milk, rendering this non-coagulable. This action corresponds to that of formaldehyde on egg albumen, on blood serum and on fibrin.

The apparent action of formaldehyde on rennet is well shown in the preceding experiment. This is because the weak rennet solution acts slowly on the milk and hence the formaldehyde has time to act on the casein. When a stronger rennet solution is used, as in Experiment XI, the formaldehyde is not given the time to alter the casein. Hence in Experiment XI coagulation takes place, or is but slightly retarded as compared with Experiment X.

Formaldehyde and Strong Rennet.

Experiment XI.—Formalin was added to portions of 20 cc. of the rennet solution (1-1), in flasks, in the following proportions:

Flask	1	received	1 cc. of formalin (40 per cent)=formaldehyde			1:50
"	2	"	0.5	"	"	1:100
"	3	"	0.25	"	"	1:200
"	4	"	0.1	"	"	1:500
"	C	"	no formalin.	Served as control.		

These mixtures were allowed to stand at ordinary room temperature, and were tested at intervals during a period of 5 weeks. The results are given in Table VI.

TABLE VI.

Tube No.	Formaldehyde.	Mixtures of Rennet and Formaldehyde tested at the end of 1 hour.	24 hours.	4 days.	11 days.	35 days.
1.	1:50	Coagulation in about 1¼ hrs.	Thick in 30 min.; solid in 40 min.	Thick in 1 hr.; solid later.	.....	.....
2.	1:100	.....	Coagulation in 10 min.	Thick in 10 min.; solid in 15 min.	Coagulation in about 30 min.	Coagulation in 15 min.
3.	1:200	.....	Coagulation in 10 min.	Thick in 5 min.; solid in 10 min.	Coagulation in about 30 min.	Coagulation in 10 min.
4.	1:500	.....	Coagulation in 7 min.	Coagulation in 5 min.	Coagulation in 20 min.	Coagulation in 7 min.
C.	.....	Coagulation in 10 min.	Coagulation in 3 min.	Coagulation in 5 min.	Coagulation in 15 min.	Coagulation in 7 min.

From these results it will be seen that formaldehyde, when added to a strong solution of rennet ferment, even in the proportion of 1:50, exerts no apparent effect on the ferment. The mixtures of ferment and formaldehyde were as active at the end of five weeks as when fresh. The slight differences in time required for coagulation by the same solution of ferment on different days are most probably due to differences in the composition or in the temperature of the milk. Market milk was used, and is liable to vary slightly from time to time.

From the tables it will be seen that the ferment solutions which contained the most formaldehyde did not produce a coagulation as rapidly as those in which formaldehyde was present in smaller amount or was entirely absent. This is no doubt due to the rapid alteration which the casein undergoes, especially at a temperature of about 40°, rather than to a change in the ferment. The following tests favor this view.

Experiment XII.—0.25 cc. of 40 per cent formalin was added to 100 cc. of fresh milk, which was already warmed to 40°, making the ratio of formaldehyde 1:1000. This was kept at 40° and portions of 10 cc. were removed and tested at intervals with 1 cc. of rennet solution (1-1).

After an exposure to formaldehyde of 15 minutes the milk would coagulate in 10 to 15 minutes. After standing for an hour, it would thicken in about 25 minutes, but would not become solid till some time later. When tested at the end of two hours the same results were obtained.

On comparison with Experiments III and IV, it will be noticed that when formaldehyde is added to milk in the ratio of 1:1000 there is slow action at ordinary room temperature. After standing for a day or so, rennet will give a coagulation almost as promptly as with fresh milk. If, however, the formaldehyde is added to milk and the mixture kept at about 40°, the action is more rapid, as shown above.

In order to render perfectly clear the action of formaldehyde in Experiments X and XI, attention should be called to the fact that the formaldehyde rennet mixtures were added in portions of 1 cc. to 10 cc. of milk. Consequently the resultant mixture of milk, rennet and ferment contained approximately one-tenth the amount of formaldehyde in the original rennet mixture. Thus, the rennet-formaldehyde mixture (1:50) on addition to milk yields a solution containing formaldehyde 1:500. Now, on reference to Experiments III and IV, it will be seen that the addition of formaldehyde (1:500) to milk altered the casein in 4½ hours and 1 hour respectively to such an extent that in the former case (dilute rennet) no coagulation resulted, whereas in the latter case (strong rennet) coagulation was retarded. It required 1¼ hours to coagulate, whereas in the control test the milk coagulated in 15 minutes. This, it will be seen, corresponds exactly to the behavior of formaldehyde-rennet mixture 1:50 in Experiments X and XI.

If, however, formaldehyde is added to warm milk the action is much more rapid. Portions of 10 cc. each of milk were warmed to 40° and 0.25 cc. of formalin (40 per cent) were then added to each, making the proportion 1:100. After these mixtures had stood for 1, 3, 10 and 15 minutes, 1 cc. of the rennet solution (1-1) was added. Coagulation resulted within 1 or 2 minutes in the first and second tubes, and in about 5 minutes in the third. The milk in the fourth tube did not give any indication of coagulating during the first quarter of an hour. Then it began to thicken, gradually became thicker and finally became almost solid in about an hour.

ACTION OF FORMALDEHYDE ON PAPAÏN.

The papaïn used was made by Kahlbaum. It was a very light gray powder, readily soluble in water, forming a clear solution. It was not, however, nearly as active, weight for weight, as the pepsin, and consequently was used in more concentrated solution.

Experiment XIII.—5 grammes of the ferment were dissolved in 400 cc. of distilled water. This solution was divided into portions of 75 cc. each, which were placed in flasks and formaldehyde was added as follows:

Flask	1	received	3.75 cc.	of formalin (20 per cent) =	formaldehyde	1:100
"	2	"	1.50	"	"	1:250
"	3	"	0.75	"	"	1:500
"	4	"	0.375	"	"	1:1000
"	C	"	no formalin and served as control.			

After standing for 24 hours at ordinary room temperature, 10 cc. of each mixture were placed in test-tubes; an equal volume of a 0.4 per cent solution of hydrochloric acid and a few small shreds of fibrin were added to each tube. The tubes were then placed in a water-bath and kept at a temperature of 38°-40° for several hours.

Within a few minutes the fibrin in all of the tubes had become swollen, owing to the action of the acid. There was, however, no sign of digestion in any of the tubes in which formaldehyde was present. Even after being kept at that temperature for 24 hours the liquid was perfectly clear and there was no diminution in the amount of the fibrin. Digestion did proceed, however, in tube C, which served as a control. At the end of two hours the liquid was cloudy and about half of the fibrin had dissolved; two or three hours later the fibrin had entirely disappeared.

It did not seem probable that the failure of papaïn to digest fibrin in the above experiment could be due to any action of the formaldehyde on the fibrin. Fibrin, as seen in Experiment II, which has been allowed to stand for three days in a formaldehyde solution of the same strength as that used in mixtures 3 and 4 is quite readily digested by pepsin and by pancreatin. It was found, however, that fibrin which had been exposed to the action of a formaldehyde solution 1:1000 for half an hour at 40° will be digested by papaïn. Moreover, a solution of papaïn in hydrochloric acid, containing formaldehyde in the proportion of 1:1000, will not digest fresh fibrin.

The following experiments will serve to explain the previous results:

Experiment XIV.—1. Some fresh fibrin was added to a solution of formaldehyde 1:1000 and kept at 40°. At the end of 3 and again at the end of 7 hours small portions were taken out, thoroughly squeezed in order to remove the formaldehyde adhering, and then tested with the control solution C used above. Even after standing over night at a temperature of 37°-40° they showed no signs of digesting. The liquid was perfectly clear and there was no diminution in the amount of the fibrin, which, however, had become swollen.

2. Another test was made in order to determine whether fibrin would be altered in less time than three hours so that it would become non-digestible.

5 grammes of papaïn were dissolved in 500 cc. of 0.15 per cent hydrochloric acid, thus making a ferment solution of nearly double the strength of the one used in the preceding experiment. Fibrin was added to a solution of formaldehyde 1:1000 and kept at 40°. At intervals of one-half hour small portions were removed, washed, and added to portions of 15 cc. of the ferment solution, and the tubes kept at 40°. Three tests were made, in which the fibrin had been exposed to the action of the formaldehyde for one-half, one and one and one-half hours, respectively. In none of these did digestion take place. Even after standing in the incubator for 24 hours the fibrin was unchanged in appearance and the liquid was perfectly clear in each tube. In the control tube, however, the liquid became cloudy in a short time, and the fibrin was almost entirely digested within three hours.

3. 1 gramme of papaïn was dissolved in 50 cc. of water and the solution divided into three portions, *a*, *b* and *c;* to *b* formaldehyde was added in the proportion of 1:1000. The solutions were then set aside at ordinary room temperature for 24 hours. Fresh fibrin was then added to *a* and *b*, whereas *c* received fibrin which had stood for 24 hours in formaldehyde 1:1000. An equal volume of 0.3 per cent hydrochloric acid was added to each and the tubes were then placed in the water-bath at 40°. At the end of an hour the liquid in tube *a* was cloudy; the fibrin slowly dissolved during the afternoon. The fibrin in tubes *b* and *c* gave no indications whatever of digesting after being kept at that temperature for several hours. The fibrin was swollen, but there was no diminution in its amount and the liquid was perfectly clear.

4. 5 grammes of papaïn were dissolved in 250 cc. of water and formaldehyde was added in the proportion of 1:1000. The solution was then allowed to stand for 24 hours at ordinary temperature, then divided into two portions. Ammonia was added to the first in sufficient amount to

exactly neutralize the formaldehyde, while double this quantity of ammonia was added to the second. After standing for three hours at ordinary temperature portions of each of these mixtures were placed in tubes, the free ammonia neutralized with dilute hydrochloric acid, and then an equal volume of 0.3 per cent hydrochloric acid and fibrin were added. The tubes were then placed in the incubator at 38°-40° and examined on the following day. The fibrin in both tubes was swollen, but none had dissolved; both solutions were perfectly clear.

The same experiment was repeated after the ammonia had been allowed to act for eight hours. The results were exactly the same as in the preceding.

It was found, however, that ammonia even in very small amount destroyed the ferment. A few drops of a dilute solution of ammonia added to the above solution of papaïn and allowed to act for half an hour rendered it incapable of digesting fibrin.

It is evident from the above results that formaldehyde interferes with papaïn digestion, both by destroying the ferment as well as by hardening the fibrin.

ACTION OF FORMALDEHYDE ON COMMERCIAL PANCREATIN.

Commercial pancreatin (Parke, Davis & Co.) was employed in the following experiments:

Experiment XV.—5 grammes of the yellow powder were dissolved in 400 cc. of water and formaldehyde was added in the same proportions as in the case of papaïn, Experiment XIII, namely, 1:100, 1:250, 1:500 and 1:1000; another portion was reserved for control tests. 10 cc. of the control solution, to which an equal volume of one or two per cent Na_2CO_3 solution was added would dissolve a few shreds of fibrin in two or three hours. The proteolytic action was therefore quite strong, though not as marked as that of the aqueous extract of pancreatic gland employed in experiments presently to be described. Inasmuch as this solution possessed very weak diastatic power its action on starch paste was not tried.

After the solutions of ferment and formaldehyde had been made, they were allowed to stand at ordinary temperature for 24 hours. Then 10 cc. were taken from each mixture, diluted with an equal volume of one per cent Na_2CO_3 solution, and small shreds of fibrin added. The tubes

were then placed at a temperature of 38°-40°, and examined occasionally during the next 24 hours. The results are given in Table VII.

TABLE VII.

PANCREATIN-FORMALDEHYDE MIXTURE (24 HOURS OLD), TESTED WITH FIBRIN.

Tube No.	Formaldehyde.	Digested at 38°–40° for 2 hours.	4 hours.	6 hours.	24 hours.
1..	1:100	No change, except that the fibrin seems hardened.	Same as before.	Same as before.	Same as before.
2..	1:250	No change, except that the fibrin seems hardened.	"	"	"
3..	1:500	No change.	"	"	Same as before; fibrin slightly hardened.
4..	1:1000	No change.	"	"	Same as before; fibrin slightly hardened.
C..	....	Fibrin nearly dissolved.	Fibrin entirely dissolved.	"	

There was no diminution in the amount of the fibrin in the first four tubes and no indication whatever that digestion was taking place. It appears from these results that formaldehyde, even in very small amount, prevents pancreatic digestion of proteid material, and that this is due to alteration of the ferment rather than to a change in the material acted upon. It has already been stated under Experiment II that fibrin, when exposed to the action of dilute formaldehyde solution (1:1000) for 24 hours is digested, though the process is retarded. This fact is brought out clearly in Test 3 below.

The following experiment was made in order to demonstrate positively the action of formaldehyde on trypsin. The solution of pancreatin used was the same as that employed in the preceding experiment.

Experiment XVI.—Tube 1 contained pancreatin solution and fibrin, and served as a control.

Tube 2 contained pancreatin and formaldehyde 1:1000 24 hours old, and fibrin.

Tube 3 contained pancreatin to which was added fibrin which had stood in formaldehyde 1:1000 for 24 hours at room temperature.

The results are given in Table VIII.

TABLE VIII.

Tube No.	Digesting mixtures kept at 38°–40°, and examined at the end of 2 hours.	3 hours.	6 hours.
1..	Fibrin dissolving.	Fibrin dissolved.	
2..	No change.	No change.	No change; fibrin is slightly hardened.
3..	"	Fibrin digesting slowly.	Fibrin dissolved.

It is evident therefore that fibrin which had been exposed to the action of a very dilute formaldehyde solution is soon rendered very resistant to the action of commercial pancreatin. The latter, however, is quickly altered and rendered incapable of digesting fibrin.

ACTION OF FORMALDEHYDE ON AN EXTRACT OF PANCREATIC GLAND.

The results with commercial pancreatin were confirmed with an aqueous extract of the fresh pancreatic glands. This extract was also used for the purpose of studying the effect of formaldehyde on the diastatic ferment.

Experiment XVII.—Two fresh glands were cut up as finely as possible and a litre of water was added. The mixture was allowed to stand over night in a cool place. It was then filtered and the clear filtrate was divided into portions, to which formaldehyde was added in the same proportions as in the case of the pancreatin solutions, namely, 1:100, 1:250, 1:500 and 1:1000; another portion was reserved for control tests. These mixtures were allowed to stand at ordinary room temperature for 24 hours. Then 10 cc. of each mixture were diluted with an equal volume of two per cent sodium carbonate, a few shreds of fibrin added, and the tubes placed in the water-bath at 38°-40°. The results were as follows (Table IX):

TABLE IX.

PANCREATIC EXTRACT AND FORMALDEHYDE MIXTURES, 24 HOURS OLD

Tube No.	Formaldehyde.	Digested at 38°–40° for 2 hours.	4 hours.	6 hours.	24 hours.
1..	1:100	No change, except that the fibrin seems hardened.	Same as before.	Same as before.	Same as before.
2..	1:250	No change, except that the fibrin seems hardened.	"	"	"
3..	1:500	No change.	"	"	"
4..	1:1000	Fibrin dissolving slowly.	Fibrin about half dissolved.	Fibrin almost entirely dissolved.	
C..	....	Fibrin nearly dissolved.	Fibrin dissolved.		

Only one set of tests was made with these mixtures, since it was found that when formaldehyde had been added to the solution in the proportion of 1:500 or stronger and allowed to act for 24 hours, digestion of fibrin would not take place; and when added in smaller amount, 1:1000, the activity of the ferment was considerably lessened.

Experiment XVIII.—A stronger solution of the trypsin ferment was made than that used in the preceding experiment. Two beef glands, finely divided, were suspended in 500 cc. of water. The mixture was allowed to stand 24 hours and was then filtered. Formaldehyde was added to portions of the clear filtrate in the ratio of 1:500 and 1:1000, while another portion received none and served as a control solution. The solutions were allowed to stand for 24 hours longer. Then 10 cc. of each mixture were diluted with 10 cc. of a two per cent sodium carbonate solution and tested with fresh fibrin, and also with fibrin that had been exposed to the action of formaldehyde 1:500 and 1:1000 for 24 hours. The results are given in Table X.

TABLE X.

MIXTURES OF TRYPSIN AND FIBRIN-FORMALDEHYDE DIGESTED AT 38°-40°.

24 hours' Mixture of trypsin-formaldehyde.	Material used.	Examined at the end of	
		½ hour.	1½ hours.
Formaldehyde, 1:500.	Fibrin.	No change.	Fibrin nearly dissolved.
	Fibrin-formaldehyde, 1:500.	No change.	Fibrin dissolving slowly.
	Fibrin-formaldehyde, 1:1000.	No change.	Fibrin dissolved.
Formaldehyde, 1:1000.	Fibrin.	Fibrin dissolving slowly.	Fibrin dissolved.
	Fibrin-formaldehyde, 1:500.	No change.	Fibrin dissolved.
	Fibrin-formaldehyde, 1:1000.	Fibrin nearly dissolved.	Fibrin dissolved.
Plain trypsin.	Fibrin.	Fibrin nearly dissolved.	Fibrin dissolved.
	Fibrin-formaldehyde, 1:500.	Fibrin dissolving slowly.	Fibrin dissolved.
	Fibrin-formaldehyde, 1:1000.	Fibrin nearly dissolved.	Fibrin dissolved.

At the end of six hours the solutions in the last three tubes had a strong, putrid odor, whereas none of the others possessed any such odor.

When formaldehyde is added in rather strong concentration (1:100 or stronger) to an aqueous extract of the pancreatic gland, rich in ferment and proteid matter, a coarse, voluminous precipitate is thrown down. It was thought that possibly the ferment might be enclosed within this precipitate and prevented from exerting its action through mechanical

interference, although it might still possess its proteolytic property. This was not found to be the case, however, as will be seen from the following experiment.

Experiment XIX.—Two fresh glands were crushed in a mortar with fragments of glass, 250 cc. of water were added, and the mixture allowed to stand over night. It was then filtered, and formaldehyde was added to portions of the clear filtrate in the proportion of 1:25, 1:100, 1:500 and 1:1000; another portion was reserved for control tests. These mixtures were again allowed to stand over night. At the end of that time the first two flasks contained a coarse precipitate, while the other three mixtures were almost perfectly clear. Portions from the first and second flasks were ground finely in a mortar with fragments of glass, an equal volume of one per cent sodium carbonate added, and then small portions of fibrin. The tubes were then placed in an incubator and kept at a temperature of 35°-40° over night. At the end of 24 hours the fibrin in these two tubes gave no indication whatever of dissolving; on the contrary, it appeared hardened. The fibrin in the control tube, however, was completely dissolved within a few hours and the solution had the strong, putrid odor characteristic of tryptic digestion.

Inasmuch as fibrin is rapidly hardened by formaldehyde, especially when the latter is allowed to act at a temperature of 35°-40°, it seemed possible that failure to undergo digestion by a solution of trypsin containing formaldehyde might be made due to this cause. Consequently portions of each of the mixtures of ferment mentioned above were diluted with 20 parts of 0.5 per cent sodium carbonate solution, fibrin was added, and the tubes were placed in the incubator over night. At the end of 24 hours the fibrin in the first tube was slightly hardened, but had not dissolved. The fibrin in the second, third, and fourth tubes had apparently undergone no change whatever; that in the fifth, or control, tube was dissolving slowly. A few hours later the fibrin in the fourth tube was nearly dissolved, and that in the control was completely dissolved.

The following experiment was made in order to determine whether neutralization of the formaldehyde with ammonia would restore the proteolytic property to the ferment.

Experiment XX.—An extract was made as in the above experiments, using two glands and 500 cc. of water. Formaldehyde was added in the proportions of 1:25, 1:100, 1:1000, while another portion was reserved for control experiments. After allowing these mixtures to stand over night, the calculated amounts of ammonia required to neutralize the

formaldehyde were added to portions of each of the mixtures, and they were then allowed to stand for five hours. An equal volume of one per cent sodium carbonate solution and a few shreds of fibrin were added, and the tubes were then placed in the incubator over night. Next morning the fibrin in the first and second tubes had undergone no change; that in the third tube had shown no indications of dissolving during the first seven or eight hours, but later it did dissolve completely. The fibrin in the control tube dissolved completely within about three hours.

A few drops of ammonia were added to another portion of the control solution of ferment, allowed to act for five hours, and then the solution was tested as above. The fibrin in this tube was dissolved with about the same rapidity as that in the control test, showing that a small amount of free ammonia has but very little if any action on trypsin.

The conclusions that are to be drawn from these experiments are that formaldehyde exerts a powerful action on trypsin. When present even in so small an amount as 1:1000, digestion of fibrin is greatly retarded. When added to a strong extract of the ferment in the proportion of 1:500, digestion of fibrin will take place but very slowly. If, however, formaldehyde is added in the same ratio to a weaker solution of the ferment, digestion will fail to take place. That formaldehyde alters the trypsin and renders it inactive is seen from the results in Experiment XIX; for if failure to digest fibrin were due to the hardening action of formaldehyde alone, dilution of the ferment-formaldehyde mixture should make the proportion of formaldehyde so small that its action would be imperceptible, in which case the ferment should dissolve the fibrin. The addition of ammonia will not restore to the ferment its proteolytic property.

ACTION OF FORMALDEHYDE ON AMYLOPSIN.

The influence of formaldehyde on the diastatic ferment of the pancreatic gland was tested in connection with the experiments on trypsin just described. In the first few tests, a starch paste made from corn starch was employed; this was not suited for experiments with diastatic ferments, however, since corn starch is not so easily and rapidly digested as potato starch. Consequently the latter was used instead in the later tests, and only these tests will be given.

Experiment XXI.—The mixtures of extract and formaldehyde employed were those described in Experiment XIX. After these mixtures had stood for about 24 hours, 10 cc. of each were diluted with 10 cc. of a one per cent sodium carbonate solution, and 20 cc. of a freshly prepared one per cent starch paste were added. Two other tubes were prepared, using the formalin-ferment mixtures Nos. 1 and 2 (containing formaldehyde 1:25 and 1:100 respectively), which had previously been ground up finely with fragments of glass. The tubes were kept in the incubator and small portions of each were tested from time to time with iodine solution. The results are given in Table XI.

TABLE XI.

PANCREATIC SUSPENSION AND FORMALDEHYDE (KEPT FOR 24 HOURS AT ORDINARY TEMPERATURE), THEN STARCH ADDED AND RESULTANT MIXTURE KEPT AT 38°–40° AND TESTED WITH IODINE SOLUTION.

No. of Mixture.	Formaldehyde.	Results.
1.	1:25	Deep blue at the end of 24 hours.
1. (ground.)	1:25	" " " "
2.	1:100	Violet in 15 min.; color gradually became lighter, pink at the end of 24 hours.
2. (ground.)	1:100	Deep blue at the end of 24 hours.
3.	1:500	Pink in 15 min.; colorless in 30 min.
4.	1:1000	Colorless in 15 min. or less.
C.	....	" " "

Experiment XXII.—The mixtures of ferment and formaldehyde were those described in Experiment XX. The tests were made in the same way as in the preceding experiment, and the results are given in Table XII.

TABLE XII.

MIXTURES OF FERMENT-FORMALDEHYDE (24 HOURS OLD) AND STARCH TESTED WITH IODINE.

No. of Mixture.	Formaldehyde.	Results.
1.	1:25	Deep blue at the end of 2 hours; deep violet in 3 hours.
2.	1:100	Violet at the end of 15 min.; colorless at the end 30 min.
3.	1:1000	Colorless in 15 min., or less.
C.	.....	" " "

Experiment XXIII.—Portions of the same mixtures as those used in Experiment XXII were treated with the calculated amounts of ammonia necessary to neutralize the formaldehyde present, allowed to stand at ordinary temperature for five hours, and then tested in the same manner as above. A few drops of ammonia were also added to a portion of the control mixture at the same time. The results were quite similar

to those given in Experiment XXII, although the conversion was slower in each case.

TABLE XIII.

MIXTURES OF FERMENT-FORMALDEHYDE-AMMONIA (24 HOURS OLD) AND STARCH TESTED WITH IODINE.

No. of Mixture.	Formaldehyde.	Results.
1.	1:25	Deep blue at the end of 24 hours.
2.	1:100	Deep blue at the end of one hour; color gradually became lighter; pink at the end of 24 hours.
3.	1:1000	Colorless in about 1 hour.
C.	.....	Colorless in about 30 minutes.

These results show that ammonia will not remove the formaldehyde that is held in combination by the ferment, and thus restore to the latter its amylolytic property. On the contrary, the presence of free ammonia seems to hinder the action of the ferment. This view is confirmed by the following test, made with mixture No. 3 (containing formaldehyde 1:1000).

To two portions of 10 cc. each of this solution 1 and 4 cc. respectively of a 3.5 per cent solution of ammonia were added, and the mixtures allowed to stand for about three hours. An equal volume of starch paste was then added to each and the tubes were placed in the incubator over night. Next morning small portions of each were tested with iodine, after neutralizing the free ammonia with dilute acetic acid. In one case a deep wine color was produced, while in another, in which ammonia had been added in larger amount, a deep violet color was produced. A control test made with the same mixture of ferment and formaldehyde gave no color with iodine after 15 minutes at 40°.

From the above results it will be seen that formaldehyde has a depressing action on the diastatic ferment of the pancreas, though this action is not so marked as in the case of trypsin. Formaldehyde added to an active solution of trypsin in the proportion of 1:500 will completely destroy the action of the ferment, unless the solution is an exceedingly active one. Even when present in so small an amount as 1:1000 the action of the ferment is greatly retarded. On the other hand, formaldehyde added to a solution of amylopsin in the proportion of 1:500 exerts but little action on the ferment after being allowed to act for 24 hours. When present in the ratio of 1:100 conversion of starch is greatly retarded, but will still take place.

Like trypsin, amylopsin seems to be destroyed, and not rendered inactive merely by mechanical interference; and like trypsin, papaïn and saliva, its properties are not restored by adding the calculated amount of ammonia required to neutralize the formaldehyde.

ACTION OF FORMALDEHYDE ON PTYALIN.

Several samples of ptyalin were tested, but inasmuch as these possessed little or no action, saliva was employed instead. The experiments were carried on in general in the same manner as those with amylopsin, and the results were very similar to those obtained with that ferment; they agree also with the results obtained with malt diastase to be described.

When formaldehyde was added to saliva in small amount it had very little effect on ptyalin; if added in larger amount, and the mixture kept at ordinary temperature, it had a depressing action, but did not completely destroy the ferment for several days. If such a mixture were kept at about 40° for some time, however, the action of the formaldehyde was more marked, and the ferment was eventually destroyed.

Experiment XXIV.—Fresh saliva was collected, filtered, and divided into portions of 20 cc. each. Formaldehyde was added to these in the proportions of 1:100, 1:250 and 1:1000; another portion was reserved for control tests. These mixtures were allowed to stand in corked flasks for 24 hours at ordinary temperature. At the end of that time 1 cc. of each was added to 25 cc. of freshly prepared one per cent potato starch paste, the tubes placed in the water-bath at 40°, and at intervals portions from each tube were tested with iodine solution. The results are given in Table XIV.

TABLE XIV.

MIXTURES OF SALIVA-FORMALDEHYDE (24 HOURS OLD) AND STARCH TESTED WITH IODINE.

Tube No.	Formaldehyde.	Results.
1.	1:100	Color gradually changed to light wine-red at the end of 5 hours; colorless next morning.
2.	1:250	Light wine-red at the end of ½ hour; colorless in ¾ hour.
3.	1:1000	Nearly colorless in 10 min.; colorless in less than 20 min.
C.	.....	Colorless in less than 5 min.

The tests were repeated on the following day with almost exactly the same results. Conversion, however, took place slightly more slowly in tubes Nos. 1, 2 and 3. At the same time a series of tests was made in like manner, using portions of these same saliva-formaldehyde mixtures which had been kept in the incubator at about 40° for 24 hours. Conversion took place much more slowly in these, showing that the formalin exerts more effect on the ferment if allowed to act on it for some time at an elevated temperature. The results are given in Table XV.

TABLE XV.

MIXTURES OF SALIVA-FORMALDEHYDE (24 HOURS AT 40°) AND STARCH TESTED WITH IODINE.

Tube No.	Formaldehyde.	Results.
1.	1:100	Deep wine-red at end of 7 hours.
2.	1:250	Light " " "
3.	1:1000	Colorless in about 1½ hours.
C.	.....	Colorless in less than 5 min.

These results were confirmed in the following experiment:

Experiment XXV.—Saliva-formaldehyde mixtures were made in the same manner as in the preceding experiment. Each was divided into two portions, one of these being kept at ordinary temperature, and the other at 35°-40° during the time the experiment was carried on. Tests were made at the end of 4 hours, 24 hours, 3 days and 9 days. 25 cc. of freshly prepared starch paste and 1 cc. of the saliva-formaldehyde mixture were used in each case. The results are given in Tables XVI and XVII; the former table is with the mixtures that had been allowed to stand at ordinary temperature, the latter with those kept at 35°-40°.

TABLE XVI.

Mixtures of Saliva–Formaldehyde (ordinary temperature) and Starch tested with Iodine at the end of

Tube No.	Form-aldehyde.	4 hours.	24 hours.	3 days.	9 days.
1.	1:100	Colorless in about ½ hour.	Colorless in about 6 hours.	Light violet at the end of 20 hours.	Deep violet at the end of 24 hours.
2.	1:250	Colorless in about 20 minutes.	Colorless in about ¾ hour.	Nearly colorless at the end of 10 hours.	Light wine-red at the end of 24 hours.
3.	1:1000	Colorless in less than 10 minutes.	Colorless in about 15 minutes.	Colorless in about 4 hours.	Colorless in about 12 hours.
C.	.....	Colorless in less than 10 minutes.	Colorless in less than 10 minutes.	Colorless in less than 10 minutes.	Colorless in less than 10 minutes.

TABLE XVII.

Mixtures of Saliva–Formaldehyde (kept at 35°–40°) and Starch tested with Iodine at the end of

Tube No.	Form-aldehyde.	4 hours.	24 hours.	3 days.	9 days.
1.	1:100	Wine-red at the end of 2 hours. Colorless later.	Deep violet at the end of 20 hours.	Deep blue at the end of 20 hours.	Deep blue at the end of 24 hours.
2.	1:250	Colorless in about ¾ hour.	Nearly colorless at the end of 20 hours.	Violet at the end of 20 hours.	Deep blue at the end of 24 hours.
3.	1:1000	Colorless in less than 10 minutes.	Nearly colorless at the end of 1 hour.	Wine-red at the end of 10 hours; colorless next morning.	Deep violet at the end of 24 hours.
C.	.....	Colorless In less than 10 minutes.	Colorless in less than 10 minutes.	Colorless in less than 10 minutes.	Colorless in less than 10 minutes.

Experiment XXVI.—A third short series was made in the following manner: Formaldehyde was added to each of two portions of 20 cc. of saliva in the proportions of 1:100; one of these mixtures was placed in a corked test-tube and kept in the incubator for five days at a temperature varying from 30°-40°, while the other was kept at ordinary room temperature. At the end of five days tests were made as in the preceding experiments. The saliva-formaldehyde mixture that had stood at ordinary temperature gave slow conversion; when tested at the end of ten hours with iodine solution a slight red color was given. The other mixture gave no conversion at all; at the end of 24 hours a deep blue color was produced with iodine. A control test made at the same time with some of the same saliva gave conversion in less than ten minutes.

Attempts were made to remove the formaldehyde from its combination with the ferment by means of ammonia. As in the case of papaïn, trypsin, and amylopsin, these attempts were unsuccessful. Ammonia was added to a portion of a saliva-formaldehyde mixture (formaldehyde 1:500) several days old and the tube was set aside for three hours. The free ammonia was then carefully neutralized with very dilute acetic acid, and the saliva was then added to starch paste. A control test was also made with the same mixture of saliva and formaldehyde. Conversion took place slowly in both, but with the same rapidity; when tested with iodine solution the same colors were produced by both mixtures.

That the formaldehyde acts on the ferment and not on the starch is proven by the following test: Formaldehyde was added to a fresh one per cent starch paste in the proportion of 1:100, the tube corked, and

kept at 35°–40° for five days. Saliva was then added, as in the preceding tests. Complete conversion resulted in less than ten minutes.

It is evident from these experiments that formaldehyde in very small amount exerts but very little action on ptyalin, unless the mixture is allowed to stand for several days or is kept at a temperature slightly above ordinary room temperature for a few hours. When present in larger amount the action is more marked. The ferment is slowly destroyed at ordinary temperature, though it does not completely lose its power even when exposed to the formaldehyde for several days. If, however, the formaldehyde-saliva mixture is kept at an elevated temperature the ferment is destroyed much more rapidly.

ACTION OF FORMALDEHYDE ON MALT DIASTASE.

A water extract of ground malt was used, and also malt itself. The results were exactly the same in both cases. In Experiments XXVII, XXVIII and XXIX starch paste made from corn starch was used, whereas in the remaining experiments potato starch was used.

Experiment XXVII.—100 grammes of ground malt were added to 1000 cc. of water and the mixture allowed to stand for about three hours with occasional shaking. It was then filtered clear, divided into portions, and formaldehyde added in the proportions of 1:100, 1:250, 1:1000 and 1:2000; another portion was reserved for control tests. These solutions were then tested after standing for two and for eight days. 5 cc. of each mixture were added to 25 cc. of fresh one per cent starch paste (corn), and the mixtures were kept at 58°-60°. From time to time small portions of each mixture were tested with iodine solution. The results are given in Table XVIII.

TABLE XVIII.

Mixtures of Malt Extract and Formaldehyde Tested with Starch and Iodine after standing for

Tube No.	Formaldehyde.	2 days.	8 days.
1.	1:100	Light violet at the end of 2 hours.	Light violet at the end of 4 hours.
2.	1:250	Colorless in about ¾ hour.	Light violet in 30 min.; colorless in about ¾ hour.
3.	1:1000	" " "	Pink in 30 min.; colorless in about ¾ hour.
4.	1:2000	" " "	Pink in 30 min.; colorless in about ¾ hour.
C.	.....	Blue at the end of 2 hours.	Blue at the end of 4 hours.

A duplicate set of solutions made in the same way and at the same time gave exactly the same results.

Experiment XXVIII.—Another set of solutions was made in the same manner as above and formaldehyde was added in the proportions of 1:100, 1:500 and 1:1000. These were tested with two per cent and with one per cent starch paste (corn) after standing for 24 hours. In this series 1 cc. of the ferment solution was added to 20 cc. of the starch.

TABLE XIX.

MIXTURES OF MALT EXTRACT AND FORMALDEHYDE TESTED WITH STARCH PASTE AND IODINE, AFTER STANDING 24 HOURS.

Tube No.	Formaldehyde.	2 per cent starch.	1 per cent starch.
1.	1:100	Deep violet at the end of 4 hours.	Light violet at the end of 3 hours.
2.	1:500	Light violet at the end of 4 hours.	Colorless in 1 hour.
3.	1:1000	Colorless in about 3 hours.	Colorless in 1 hour.
C.	.....	Nearly colorless in 5 hours.	Light violet at the end of 2 hours.

Experiment XXIX.—Ten grammes of malt and 100 cc. of water were placed in each of half a dozen flasks, and formaldehyde was added in the proportions of 1:100, 1:250, 1:500, 1:750 and 1:1000; the sixth mixture was reserved for control tests. These mixtures were tested at the end of 3, 9 and 20 days, using 5 cc. of the clear solution and 25 cc. of fresh one per cent starch paste. The results are given in Table XX.

TABLE XX.

		Mixtures of Malt Extract and Formaldehyde tested with Starch and Iodine at the end of		
Tube No.	Formaldehyde.	3 days.	9 days.	20 days.
1.	1:100	Bluish violet at the end of 2 hours.	Bluish violet at the end of 3 hours.	
2.	1:250	Light violet at the end of 2 hours.	Colorless at the end of 3 hours.	All the same as when tested on the 9th day.
3.	1:500	Nearly colorless at the end of 2 hours.	Colorless at the end of 2 hours.	
4.	1:750	Colorless at the end of 2 hours.	Colorless at the end of 2 hours.	
5.	1:1000	Colorless at the end of 2 hours.	Colorless at the end of 2 hours.	
C.	.....	Deep blue at the end of 2 hours.	Deep blue at the end of 2 hours.	

Experiment XXX.—An extract of malt diastase was prepared as described in Experiment XXVII. Formaldehyde was added to portions

of this in the proportions of 1:100, 1:250, 1:500 and 1:1000; another portion was reserved for control tests. The mixtures were allowed to stand at ordinary temperature for 24 hours. 5 cc. of each were then added to 25 cc. of a freshly prepared one per cent starch paste made from potato starch and the tubes were kept at 60°. When tested with iodine solution at the end of fifteen minutes, all showed complete conversion. These results did not seem to agree with those previously obtained. It was found, however, that formaldehyde in rather strong concentration will destroy malt diastase when allowed to act on the latter for a few hours at 60°. This, taken in conjunction with the fact that corn starch is not so easily digested as potato starch, will explain the apparent differences in the results.

On the following day the above tests were repeated, and at the same time another series was made with portions of the same mixtures of extract and formaldehyde which had been heated to 60° for four hours. The results are given in Tables XXI and XXII.

TABLE XXI.

MIXTURES OF MALT EXTRACT AND FORMALDEHYDE TWO DAYS AT ORDINARY TEMPERATURE), TESTED WITH STARCH AND IODINE.

Tube No.	Formaldehyde.	Results.
1.	1:100	Colorless in about 20 minutes.
2.	1:250	Colorless in less than 15 minutes.
3.	1:500	" " "
4.	1:1000	" " "
C.	.....	Deep red at the end of 2 hours.

TABLE XXII.

MIXTURES OF MALT EXTRACT AND FORMALDEHYDE (KEPT AT 60° FOR FOUR HOURS), TESTED WITH STARCH AND IODINE.

Tube No.	Formaldehyde.	Results.
1.	1:100	Deep violet at the end of 5 hours.
2.	1:250	Light violet at the end of 5 hours.
3.	1:500	Nearly colorless at the end of 2 hours.
4.	1:1000	Colorless in about 15 minutes.
C.	.....	Violet at the end of 5 hours.

It would seem from these results that, as already pointed out by Weigle and Merkel, the action of malt diastase is aided by the presence of formaldehyde. This, however, is due to the inhibition of bacterial growth by the formaldehyde. A glance at the tables shows that a solution of the ferment in water soon undergoes decomposition; after such a solution has stood for two or three days it is able to convert little if any of the starch. If formaldehyde is present in the proportion of

1:500 or 1:1000, the ferment does not decompose; on the other hand, it does not seem to be affected by the formaldehyde, for it is as active at the end of three weeks as when fresh.

CONCLUSIONS.

The following general conclusions may be drawn from the preceding work:

Fibrin is altered by formaldehyde and is then less easily digested by pepsin and by trypsin. Papaïn is apparently unable to digest fibrin even when this is exposed to very weak formaldehyde (1:1000) for a very short time.

The casein of milk, on contact with formaldehyde, undergoes rapid alteration and is as a result not coagulated by rennet, or but very slowly. Such altered casein, like similar fibrin, is not readily digested by the proteolytic ferments. The longer the formaldehyde acts on casein and on fibrin the more marked is the result.

Pepsin is not affected by a one per cent solution of formaldehyde, even when the mixture has stood for four weeks. Even a five per cent solution of formaldehyde acting for three weeks has no effect on pepsin. Contrary results obtained by others are due to an alteration of the fibrin by the formaldehyde. A putrid solution of pepsin in distilled water one month old digests fibrin as readily as a fresh solution.

Rennet is not affected even by a four per cent solution of formaldehyde acting for several weeks. The absence of coagulation at times is due to the action of formaldehyde on the casein of the milk and not on the rennet ferment.

Papaïn is very quickly altered by formaldehyde, even in very dilute solution. Moreover, it is unable to digest fibrin that has been exposed to the action of a very dilute solution of formaldehyde for a short time.

Trypsin is altered by formaldehyde to such an extent that digestion of fibrin will not take place, or but very slowly. The extent to which trypsin is affected by formaldehyde depends largely upon the amount of organic matter present, as well as on the amount of ferment in the solution.

Amylopsin is not destroyed by very dilute solutions of formaldehyde, but stronger solutions decrease the activity of the ferment, and if used in sufficient concentration will destroy it completely.

Ptyalin, like the diastatic ferment of the pancreas, is not destroyed by dilute solutions of formaldehyde. If the latter is used in rather strong concentration and allowed to act for some time it will destroy the ferment. The action of formaldehyde is more rapid and more marked at a slightly elevated temperature than at ordinary room temperature.

Malt diastase, unlike the diastatic ferments of the saliva and pancreatic solution, is not destroyed by formaldehyde when this is used in moderate amount and at ordinary temperature. Unlike pepsin, a solution of malt diastase readily undergoes decomposition on standing even for one or more days. This destruction is undoubtedly due to bacteria since it does not take place when formaldehyde is present. Consequently the favoring action which formaldehyde apparently exerts on diastase really consists in the inhibition of the growth of micro-organisms, and hence the diastase is protected against decomposition.