melting point with benzaldi-β-naphthol prepared according to the method of Hewitt and Turner[9] did not vary.

The dimethyl, diethyl and piperidyl derivatives are only slightly basic. They are quite stable and are not affected by light or oxidation. Further work on these compounds including attempts at their resolution is contemplated.

Summary

A study has been made of the condensation of secondary amines with benzaldehyde and β-naphthol and intermediate products in the condensation have been isolated.

It has been shown that the use of amines as catalysts in condensations of a carbonyl with an active methylene may yield first an amine or diamine followed by addition to the active methylene with the formation of a substituted amine, which on further treatment yields the free amine and the final condensation product.

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[CONTRIBUTION FROM THE CONVERSE MEMORIAL LABORATORY OF HARVARD UNIVERSITY]

POLYMERIZATION REACTIONS UNDER HIGH PRESSURE. I. SOME EXPERIMENTS WITH ISOPRENE AND BUTYRALDEHYDE

BY J. B. CONANT AND C. O. TONGBERG

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In a preliminary paper[1] on the irreversible transformations of organic compounds under high pressure, some experiments were reported on the polymerization of isoprene and other unsaturated compounds at room temperature by the application of pressures of the order of magnitude of 9000 atmospheres. This work has been continued with the aim of discovering the factors which control the rate of polymerization under high pressures and the nature of the products formed. A great majority of the experiments were performed with isoprene since this substance polymerizes more rapidly than any other substance of this general type which is readily available; furthermore, the early observations showed that very erratic results were obtained with different samples of isoprene and we desired, if possible, to discover the catalytic substances which seemed to be responsible for the peculiar behavior. A few experiments also were performed with some other compounds and they will be discussed at the end of this communication. The pressure apparatus employed was kindly lent us by Professor Bridgman. We wish to express our appreciation of his kind coöperation, and the courtesy extended to us by the Director of the Jefferson Physical Laboratory of Harvard University in which the physical part of the experiments was actually performed. Manipulation of the

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[1] P. W. Bridgman and J. B. Conant, *Proc. Nat. Acad. Sci.*, 15, 680 (1929).

presses was performed by Mr. Zisman and without this valuable assistance the work would not have been possible.

The isoprene used in all of the experiments was prepared by the thermal decomposition of limonene by means of a hot wire, essentially according to the method described by Harries.[2] It was purified by distilling through an efficient column and had a boiling point of 36–38°. The material to be compressed was placed in small glass tubes and pressure transmitted by means of mercury in the apparatus referred to in the previous paper.[1] In most of the experiments the temperature of the press varied between 23 and 25°. The pressure was read by means of the usual device used by Professor Bridgman. The pressures recorded in this paper are usually given in even thousands and are significant within ±100–200 atmospheres. Unfortunately it was not always possible to hold the pressure at exactly the desired point; the actual variations of a few hundred atmospheres probably did not introduce serious errors in the work.

The extent of the polymerization in the case of isoprene was determined by removing the sample from the glass container (if necessary by breaking the container), rapidly weighing, and allowing the material to stand in air until constant weight was obtained. The amount of non-volatile material under these conditions was taken as a measure of the polymerization. This is, of course, only an approximate method but it sufficed for the purposes at hand. The greatest amount of polymerization which we ever observed was 92%. We obtained many figures running between 88 and 90. The fact that 100% was never recorded probably indicates a certain constant error in our method of procedure, although it may correspond to the difficulty of polymerizing the last traces of the isoprene.

It was clear at the outset of our work that the previous history of the isoprene was of great importance in determining the rate of polymerization; for example, some freshly prepared and distilled material was only 30% polymerized in twenty hours at 12,000 atmospheres, whereas some material similarly prepared and distilled which had then been allowed to stand for nine days at 2° in a bottle from which most of the air had been displaced by a stream of nitrogen, was 88% polymerized in the same time and at the same pressure. Oxygen catalysis was, of course, suspected, and the importance of this factor was shown by the fact that it was possible to increase the rate of polymerization by shaking the isoprene with oxygen and then allowing it to stand for some hours. The addition of peroxides and particularly of pinene which had been shaken with oxygen, was very effective in accelerating the rate of polymerization. Hydroquinone, a well-known negative catalyst for reactions subject to oxygen

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[2] C. D. Harries, "Untersuchungen über die natürlichen und künstlichen Kautschukarten," J. Springer, p. 142.

catalysis, had a marked effect on decreasing the rate; in fact, at 9000 atmospheres pressure it was possible to reduce the rate from 40% in twenty hours to less than 3% in the same time by the addition of about 0.1% hydroquinone. In this experiment a sample of isoprene was used which had been allowed to stand for some time in the presence of oxygen.

Although peroxide catalysis was found to play a very important part in the acceleration of the polymerization, it was not essential to the process. This was demonstrated by the fact that a sample of isoprene carefully distilled in an atmosphere of oxygen-free nitrogen and introduced into the apparatus without the admission of oxygen, nevertheless polymerized under a pressure of 12,000 atmospheres to the extent of 37% in forty-eight hours. It is interesting that at 12,000 atmospheres the presence of 0.1% hydroquinone only changed the rate by about 50%.

Our attempts to measure the effect of pressure and temperature on the rate of polymerization were severely handicapped by the difficulty of preparing uniform samples of isoprene. The use of freshly distilled material did not solve the difficulty as evidently varying amounts of peroxides were formed in an irregular fashion. This is illustrated in Table I. Our most consistent results were obtained with samples of isoprene which had been allowed to stand at 2° in nitrogen or air at least seven days and which then only slowly altered their intrinsic tendency to polymerize. For example, a sample from a given lot which had stood for one day after distilling was 34% polymerized in twenty hours; at the end of the third day another sample from the same lot was 64% polymerized in the same time; on the seventh day the rate had increased to 86% in twenty hours. Further standing up to eleven days seemed to be without effect. A sample from the same lot was freshly distilled on the ninth day and the rate of polymerization dropped from 88% in twenty hours to 25% in twenty hours. Another lot of material which was allowed to stand under practically the same conditions came to a fairly constant rate which was, however, different from the rate of the first lot; the new maximum rate was about 61% in twenty hours. Still a third lot gave final values nearer the first.

The most significant data which we have so far been able to obtain are given in Table II; the results were obtained with two different lots of isoprene which had stood for at least eight days. An inspection of Table II shows that duplicate experiments usually give very concordant results. This fact at first misled us into believing that it would be possible to determine the rate over the entire range with an accuracy of a few per cent. The results which we later obtained showed that the uncertainties due to catalytic effects, and also caused to a slight extent by the variations in pressure and temperature, made our probable error much greater than we had at first estimated. Some continuous method of following the reaction with one sample for an extended period will have to be developed in order to obtain really satisfactory results.

TABLE I

POLYMERIZATION OF FRESHLY DISTILLED ISOPRENE

Each experiment recorded below was performed with a separate sample of material distilled within one hour of being subjected to pressure

Pressure, 12,000 atmospheres
Temp., 23–25°

Time of application of pressure, hours	Polym-erized, %	First order reaction constant, $k \times 10^2$
7	19	3.0
15.25	15	1.1
15.25	12	0.8
29	46	2.1
29	40	1.7
45	25	0.6
68	66	1.6
	Av.	1.6

$k = \frac{2.3}{t} \log \frac{1}{1 - Z}$ where t is in hours and Z is the fraction polymerized.

TABLE II

POLYMERIZATION OF ISOPRENE WHICH HAD STOOD IN NITROGEN CONTAINING A FEW PER CENT. OF OXYGEN AT LEAST NINE DAYS AFTER DISTILLATION

Pressure, 12,000 atmospheres
Temp., 23–25°

Time, hours	Polym-erized, % Lot 1	Reaction constant, $k \times 10^2$
5	27	6.3
5	33	8.0
5	35	8.6
16	83	11.2
20	87	10.2
	Av.	8.9
	Lot 2	
5	11	2.3
20	61	4.7
23	66	4.7
23	65	4.6
	Av.	4.1

In order to have some way of comparing the extent of the reaction at different times we have calculated a reaction velocity constant according to the usual first-order equation. The reaction is presumably one of high order but some autocatalytic effect makes the first-order constants actually increase somewhat with time instead of decreasing as would be expected. The result is that they may be used to characterize approximately the rate of the process, and we have used them for this purpose throughout this paper. Some experiments in which indifferent diluents were employed showed that the reaction was actually of an order higher than the first. For example, a sample of isoprene with which it normally took only ten hours for 65% polymerization, required twenty hours for the same extent of reaction when diluted with an equal volume of petroleum ether. The same material when diluted with 5.5 times its volume of petroleum ether or toluene was polymerized to the extent of 5 to 13% in fifteen hours; undiluted, only about one hour would have been required for the same degree of polymerization.

The catalyst which gave the most consistent results was a sample of pinene which had been shaken for four or five hours with oxygen and which was then used from time to time without further treatment. Definite amounts of this pinene were added to freshly distilled isoprene. The results are summarized in Table III, the first order reaction constants being given as a rough measure of the rate of the polymerization. It is evident that the consistency of the results leaves much to be desired but in spite of their variation the effect of the catalyst is evident. The rates obtained with the smallest amount (0.001 per cc. of pinene, a concentration obtained by a dilution procedure) were probably not effective since the average rate of freshly distilled isoprene (Table I) was about the same. The larger amounts of catalyst had a very real effect, although this effect is not directly proportional to the amount; thus an increase of 20-fold in the amount of catalyst only doubled the rate. A few experiments with this same catalyst at pressures of 9000 and 6000 atmospheres indicated the same sort of effect although the results were very erratic at times.

TABLE III

CATALYTIC EFFECT OF OXIDIZED PINENE ON POLYMERIZATION OF FRESHLY DISTILLED ISOPRENE

Pressure, 12,000 atmospheres. Temp., 23–25°

Amount of catalyst, cc. per cc.	Time, hrs.	Percentage polymerized	Reaction constant, $k \times 10^2$
0.1	4.5	83	39.3
.1	4.5	63	22.1
			Av. 30.7
.04	4.5	28	7.3
.04	4.5	41	11.7
.04	4.5	63	22.1
			Av. 13.7
.01	4.5	38	10.6
.01	4.5	12	2.8
.01	16.0	54	4.8
			Av. 6.1
.002	15.75	55	5.1
.002	15.75	55	5.1
			Av. 5.1
.001	4.5	7	1.6
.001	4.5	12	2.8
.001	15.0	24	1.8
			Av. 2.1

We carried out many experiments in an attempt to obtain sufficient information to enable us to give with some accuracy the pressure coefficient of the rate of polymerization; again we have employed the first order constants in comparing the rates. The results are summarized in Table IV. As in all the other work, we were confronted with the difficulty of obtaining reproducible measurements. However, the average value of $k_{12,000}/k_{9000} = 3.5$ is probably of considerable significance. If we assume this value and calculate the ratio of rates for 6000 atm. and 2000 atm. compared to 12,000 from the relationship $\Delta \log k/\Delta P$ = constant, the results are considerably lower than were actually found. Thus, for $k_{12,000}/k_{6000}$ we found 17 and 30 (Table IV, note) while the calculated value is 12. For 2000 atmospheres $k \times 10^2$ for uncatalyzed isoprene is 0.02 (about 2% in five days); this gives a value for $k_{12,000}/k_{2000}$ of about 100 against the calculated 67. As a rough first approximation the effect of pressure seems to be represented from 6000 to 12,000 by the expression $\log k_1 - \log k_2 = 1.8 \times 10^{-4} (P_1 - P_2)$. A very large increase of the pressure coefficient at lower pressures is indicated, however, by the few facts just given and by an extrapolation to atmospheric pressure. Using the equation just given the value of $k_{12,000}/k_1$ is 145 and taking $k \times 10^2$ at 12,000 as 10 (for isoprene after standing), $k \times 10^2$ at 1 atm. = 0.069. This value, which corresponds to 10% polymerization in ten days at 1 atmosphere and 23°, is certainly too large by at least a factor of 100 and probably by 1000 or more. The spontaneous polymerization of isoprene at room temperature and pressure appears to be so slow as to be capable of detection only after a period of years.[3]

TABLE IV

ESTIMATION OF THE PRESSURE COEFFICIENT OF THE RATE OF POLYMERIZATION OF ISOPRENE (TEMP., 23–25°)

Isoprene and catalyst (if any)	Average value of reaction constant		
	12,000 atm., $k \times 10^2$	9000 atm., $k \times 10^2$	$\frac{k_{12,000}}{k_{9000}}$
Freshly distilled	1.7	Less than 0.2	...
Lot 3, 9 days old	9.0	2.9	3.1
Lot 1, 14 days old	8.9	2.7	3.3
0.1 cc. of pinene	30	7.0	4.3
0.04 cc. of pinene	10	3.5	2.9
0.01 cc. of pinene	6	2.8	2.1
0.01 cc. of pinene	2	0.4	5.0
		Av.	3.5

NOTE.—At 6000 atmospheres a value of $k \times 10^2$ for freshly distilled isoprene was found to be about 0.1 (3% in twenty-six hours), giving a ratio of $k_{12,000}/k_{6000} = 17$. With 0.1 cc. of pinene a value of $k \times 10^2 = 1.0$ at 6000 atm. was obtained (20% in twenty-six hours), giving a ratio of $k_{12,000}/k_{6000} = 30$.

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[3] Since this was written we have unexpectedly been in a position to obtain some additional information in regard to the pressure coefficient. Through the kind coöperation of Professor Bridgeman we have determined the rate of polymerization of isoprene at a pressure of approximately 18,000 ± 500 atm. at 23°; the following amounts of polymer were obtained: 10% in twenty minutes; 76% in three hours; $k \times 10^2$ = 52–65. The same material gave the following values at other pressures: 6000 atm., $k \times 10^2 = 1.9$; 12,000 atm., $k \times 10^2 = 8.4$.

Since the publication of the preliminary paper we have discovered the claims of Herman Plauson to the polymerization of butadiene hydrocarbons at normal temperatures in a few days by applying pressures of the order of 500 to 600 atmospheres. An extrapolation of the results given above to those pressures is not at all consistent with Plauson's results.[4] We have not attempted to repeat all of the six examples given in the Plauson patent but have tried the effect of 600 atmospheres' pressure at room temperature on a mixture of isoprene and dimethylbutadiene emulsified in gelatin solutions as specified and have been able to obtain only the merest trace of possible polymer after six days. Samples of dimethylbutadiene behaved similarly. We are thus unable to confirm the statement that the butadiene hydrocarbons may be polymerized at room temperature by the application of 600 atmospheres' pressure for five to six days.

The temperature coefficient of the rate of polymerization may be estimated from the data given in Table V. If these results are plotted in the usual way (log k against the reciprocal of the absolute temperature) the three points at 12,000 atmospheres fall on a straight line within the probable error. The value of the heat of activation (calculated from the usual equation) is of the order of magnitude of that of simple chemical reactions in solution at room temperatures. The difference in the values at the different pressures cannot be considered as definitely significant in view of the errors in our work. Such an effect may exist but many more experiments at the lower pressures will be necessary to prove it.

TABLE V

TEMPERATURE COEFFICIENT OF THE RATE OF POLYMERIZATION OF ISOPRENE UNDER PRESSURE

Isoprene employed	Average value of reaction velocity constant ($k \times 10^2$)			E calcd. from max. temp. range, calories
	(A) 12,000 atmospheres			
Lot 1 (stood for 13 days)	0.8 (0°)	9.0 (23°)	42 (41°)	16,300
	(B) 9000 atmospheres			
Freshly distilled +0.01 cc. of pinene	...	About 3 (23°)	37 (43°)	23,000
	(C) 2000 atmospheres			
Isoprene which had stood for 6 days	0.01 (23°)	1.5[a] (62°)	6.3 (80°)	24,000

[a] Freshly distilled isoprene +0.1 cc. of pinene; value perhaps considerably too high compared to those at 23 and 80°.

A comparison of the temperature and pressure coefficients shows that at the higher pressures and room temperature a change of 15° is about equivalent to 3000 atmospheres' pressure as regards the effect on the reaction rate. Over a wider range we estimate that raising the pressure from

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[4] U. S. patent 1,415,468 (May 9, 1922).

2000 atmospheres to 12,000 atmospheres increases the rate about 100-fold; this increase could also be obtained by raising the temperature from about 20 to 60°. If the temperature and pressure coefficients of the polymerization of isoprene are typical, it is clear that the mere acceleration of the reaction is more readily accomplished by raising the temperature than by increasing the pressure. However, there must be many cases (and the polymerization of isoprene is probably one) where the nature of the products differs according to whether they are formed at high pressures or elevated temperatures. This will evidently be the case when side reactions are possible which have a large temperature coefficient but negligible pressure coefficient. A future field of profitable inquiry into the *acceleration of* reactions by high pressures would seem to be with those complex reactions (such as polymerization) where certain side reactions might be avoided by operating at low temperatures and high pressures.

It has already been emphasized that the polymerization of isoprene under pressure is markedly subject to positive catalysis by oxygen and peroxides and to negative catalysis by hydroquinone. The effect of hydroquinone is more marked at 9000 atmospheres than at 12,000, though the effect of positive catalysts appears to be about the same at the two pressures. The most effective catalyst of all we found to be the isoprene ozonide. For example, if ozonized oxygen (about 5% ozone) was passed into isoprene for twenty minutes and the material then subjected to pressure, the substance was practically completely polymerized (88%) at 12,000 atmospheres in only five hours ($k \times 10^2 = 42$). Although ozone was very effective at this pressure we were disappointed in its action at lower pressures; for example, at 6000 atmospheres it was not so effective as 0.04 cc. of pinene, since in twenty-six hours' time slightly ozonized isoprene was only 5% polymerized. The action of oxygen itself on isoprene is rather peculiar. Freshly distilled material, if shaken with oxygen for a few hours and at once subjected to a pressure of 12,000 atmospheres, polymerizes at about the same rate as though the oxygen treatment had not been given (10–15% in five hours; $k \times 10^2$ about 1). On the other hand, if, after the oxygen treatment, the material is allowed to stand for one to two days, the rate of reaction is greatly accelerated (74–80% in fifteen hours; $k \times 10^2$ about 10). Samples of isoprene which had never been shaken with oxygen but which had stood either in air or nitrogen containing a little oxygen for many days could not be made to polymerize at a faster rate by shaking with oxygen before introducing into the press. From these facts it seems clear that the catalytic action of oxygen is primarily due to a peroxide which is slowly formed from the oxygen dissolved in the isoprene. If an organic peroxide is added directly as in the case of oxidized pinene, or if benzoyl peroxide is used, then there is no indication of an induction period being required. One may imagine that the action of the peroxide is to cause the formation of a series of chain reactions according to the theory which has received such wide acceptance in recent years.

Nature of the Product

In all cases the product obtained at room temperatures was a transparent, colorless, rubbery solid. Its elastic properties, strength and particularly its solubility seemed to be a function primarily of the extent to which the polymerization had been allowed to proceed. Thus the material obtained in runs at 12,000 atm. where the reaction was 80% or more complete was slightly elastic and, though it showed no tendency to crumble, would not stand stretching more than twice its length without breaking. It was extremely insoluble; in fact, we have not found any solvent in which it would dissolve to any appreciable extent. A sample of it was continuously extracted with dry ether in an atmosphere of nitrogen according to the method given by Pummerer;[5] after twelve hours only 1% had been extracted. On the other hand, material from an experiment in which the polymerization was only 30% complete behaved quite differently. After the evaporation of the unpolymerized isoprene the material was very elastic and tough. It could be elongated three to four times without breaking. It was more than 50% extracted by ether in twenty hours and in forty hours was at least 90% soluble. The slight residue was soluble in a benzene–piperidine mixture but not in pure benzene. The density of the polymerized material was about 0.9 as compared with 0.68 for isoprene; thus the volume change in the process is very considerable.

An experiment was performed in order to determine whether the volatile material in an experiment in which the polymerization was incomplete, was wholly isoprene. The excess isoprene (about 50%) was removed by a stream of nitrogen which was then passed through a tube immersed in a carbon dioxide snow–ether mixture. The condensate was examined and found to be practically pure isoprene with only a trace of high-boiling material which had a terpene-like odor. This result, together with all the facts mentioned above, would seem to indicate that the polymerization of the intermediate products must proceed faster than the first step in the process which involves isoprene itself. The extreme insolubility of the completely polymerized material suggests that under these high pressures the reaction proceeds very far even at room temperature. We attempted to see if natural rubber itself would be rendered more insoluble by being subjected to pressure. We employed both crude sheet rubber and the material obtained by extracting this material with ether. In the case of the ether extract there seemed to be some change after subjecting to 12,000 atmospheres for many hours; the resulting material was somewhat less soluble,

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[5] Pummerer, *Ber.*, **61**, 1583 (1928).

but the results cannot be regarded as conclusive. More work along this line is in progress. A solution of guttapercha in toluene subjected to 12,000 atmospheres for some hours was apparently unchanged.

Experiments with Other Materials

The rate of polymerization of vinyl acetate is considerably less than that of isoprene; thus in twenty hours at 12,000 atm. the material was only a few per cent. polymerized and even after forty-eight hours only about 50% was converted to the polymer. The effect of oxygen on the rate of polymerization is much less evident than in the case of isoprene; indeed, it is not certain that this polymerization is subject to a positive catalytic effect of this sort. The oxidized pinene catalyst which we found so effective with isoprene had the reverse effect on vinyl acetate, decreasing the rate very markedly. Another strange effect is that the addition of a small quantity of hydroquinone instead of decreasing the rate increased it nearly 10-fold. Because of the long time required in order to obtain appreciable polymerization, the behavior of vinyl acetate was not studied in as much detail as isoprene since the time at our disposal was relatively short.

The peculiar behavior of *n*-butyraldehyde and isobutyraldehyde was referred to in the preliminary paper.[1] We have now studied the polymerization of *n*-butyraldehyde in a little more detail. The rate of polymerization is markedly increased by passing ozone through the material for about twenty minutes. Material thus treated was polymerized in twenty-five hours at 12,000 atmospheres to a hard solid amorphous mass without strength or elasticity. This material was evidently of high molecular weight as it was very difficultly soluble in alcohol, ether or other organic solvents. A softer material having the consistency of cheese was obtained by the action of 12,000 atmospheres for twenty-five hours on redistilled aldehyde. In this case the polymerization did not seem to have proceeded so far, since the material was soluble in alcohol. When the alcohol solution was poured into water the material was reprecipitated as an amorphous solid. Both the soft and the hard solids changed rather rapidly on standing at 25°, the eventual product being *n*-butyraldehyde. The change is definitely catalyzed by the presence of acids and somewhat retarded by the presence of pyridine. However, it was found impossible to keep the material at room temperature for more than about a day even when pyridine was added to it. This rapid reconversion hindered us greatly in studying the material. Attempts to obtain derivatives of the more soluble form by reactions with such reagents as *p*-nitrophenylhydrazine yielded only derivatives of *n*-butyraldehyde. It seems probable that the products obtained from *n*-butyraldehyde are polymers similar to the well-known polymers of formaldehyde (trioxymethylene). In the case of formaldehyde the energy relations are such that these polymers of high molecular weight are stable substances at room temperature and pressure, whereas with the higher homologs the complex polymer is perhaps only stable at high pressures. The effect of pressure on the aliphatic aldehydes is in many ways the most interesting result of our work, since it is the one case which have discovered where a reaction can be brought about only by the application of high pressures. We seem to be concerned here not only with increasing the rate of a process but with an actual change of the equilibrium conditions.

Summary

1. The rate of polymerization of isoprene under high pressures has been studied. The reaction is subject to positive catalysis by peroxides and negative catalysis by hydroquinone. Although the reaction is of a high order, the rate is approximately in accord with a first order reaction presumably because of an autocatalytic effect. The temperature and pressure coefficients of the rate have been estimated. The solubility and elasticity of the product depend on the extent to which the isoprene has been polymerized; when the polymerization is practically complete at room temperature at 12,000 atm. the product is very insoluble

2. The action of high pressures on *n*-butyraldehyde produces a solid only slightly soluble in organic solvents. It reverts to *n*-butyraldehyde rapidly. It is suggested that this polymer is similar to the well-known polymers of formaldehyde but that the energy relationships are such that the polymer is stable only at high pressures.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF COLUMBIA UNIVERSITY, No. 622]

A QUANTITATIVE STUDY OF THE INFLUENCE OF ACETATE AND OF PHOSPHATE ON THE ACTIVITY OF MALT AMYLASE

BY H. C. SHERMAN, M. L. CALDWELL AND H. H. BOYNTON

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In previous work upon the enzymic activity of malt amylase by different investigators, acetate and phosphate have been used somewhat interchangeably as electrolytes helpful to the activity and conservation of the enzyme. The use of phosphate mixtures, which are not effective buffers at the hydrogen-ion activities employed in work with malt amylase (*P*H of about 4.5), has probably come about largely through the thought that the phosphate ion is a constant accompaniment of this enzyme when acting in its natural environment and that it may have some specific activating effect. Recent work[1] has shown that the optimal hydrogen-ion activity

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[1] (a) Hahn and Michalik, *Z. Biol.*, **73**, 10 (1921); (b) Hahn and Meyer, *ibid.*, **76**, 227 (1922); (c) Myrback, *Z. physiol. Chem.*, **149**, 1 (1926); (d) Luers and Nichimura, *Wochschr. für Brauerei*, **43**, (No. 38) 415 (1926); (e) Sherman, Caldwell and