drogenated with 0.5 mole hydrogen per mole ester. Triene, diene, and monoene are determined on the hydrogenated esters by gas chromatography or other analytical procedures.[4]. Since triene (A_x) should equal monoene (C_x), any failure to absorb exactly 0.5 mole will be reflected in a high amount of triene and a low amount of monoene, or vice versa, but with little change in diene. The average value for monoene and triene is therefore used to indicate ($\bar{A}_x$) which equals $\frac{A_x + C_x}{2}$. The value of K corresponding to $\bar{A}_x$ is read from Figure 2.

Discussion

Since a variety of equipment may be used to carry out the hydrogenation under many conditions of temperature, pressure, stirring rates, and hydrogen dispersion and to determine when 0.5 mole of hydrogen has been absorbed, no attempt is made here to specify experimental procedures. It is believed that the general experimental design and method of handling data proposed should be applicable to a variety of hydrogenation techniques. Other analytical determinations undoubtedly will be desired such as *trans* acids by infrared spectrophotometry, conjugated dienes by ultraviolet spectrophotometry, position of bond by oxidative cleavage or mass spectroscopy.

Cognizance has not been taken in this discussion of the inevitable traces of saturates present. Their formation in appreciable amounts by any catalyst would, of course, vitiate its usefulness for hydrogenation purposes, as well as introduce errors, or invalidate the procedure outlined. Traces of saturates, when they occur, are corrected by adding their percentage to that of the oleate in the following manner: $\frac{A_x + C_x + S_x}{2} = \bar{A}_x$. The formation of diene geometric and positional isomers and their effect on relative rates of hydrogenation also have not been considered in the present attempt to develop a simple equation. Some justification for this omission may be found in the observation that during the absorption of 0.5 mole of hydrogen per mole of ester by the 50:50 linolenate-linoleate mixture, the maximum proportion of isomeric dienes in the mixture only reaches 10%.

This procedure has been applied routinely in this laboratory for evaluating the selectivity of hydrogenation catalysts, and subsequent papers will describe its use.

[4] Because residual triene is essentially unaltered during hydrogenation, the spectrophotometric determination of linolenic acid following alkali isomerization appears valid.

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The Effect of Some Amino Acids on the Oxidation of Linoleic Acid and Its Methyl Ester[1]

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Manometric studies of the effect of certain amino acids on oxidation (measured as oxygen consumption) of linoleic acid, as well as the methyl esters of linoleic acid and linolenic acid dispersed in water or phosphate buffers at pH 7 to pH 5 have shown that

1. The amino acids tested (except cysteine) have a potential antioxidative effect.
2. The antioxidative capacity of different amino acids may be rather different (it is especially pronounced in the case of histidine and tryptophane).
3. Under suitable conditions extremely low amino acid concentrations may have rather strong effect.
4. The antioxidative efficiency is less pronounced than in earlier experiments with linoleate at pH>7, and decreases with decreasing pH.
5. There may be a tendency towards a prooxidative inversion with relatively high amino acid concentrations, or at low pH.
6. The antioxidative effect is enhanced and a prooxidative effect may be lowered or inverted into an antioxidative one by an addition of phosphate, or an emulsifier like Tween.
7. A strong inhibitory effect is obtained by combined addition of phosphate and emulsifier like Tween, together with the amino acid.
8. The antioxidative tendency was stronger in the case of methyl linoleate than with linoleic acid, and was also stronger with methyl linoleate than with methyl linolenate.

THERE IS an increasing interest in antioxidative substances occurring in biological material and in synergistic relationships with regard to protection against fat oxidation *in vivo* and in food. As the content of so-called primary antioxidants of phenolic type (e.g. the tocopherols) capable of breaking oxidative chain reactions often is very low, the interest is directed towards substances of other types, which may occur in biological material and may participate, e.g. as synergists, in preventing rancidification. These substances may react in different ways: by rendering prooxidative substances innocuous, or by regenerating oxidized primary antioxidants.

Amino acids have been mentioned earlier in the literature as antioxidants (3,15,17,18,34) and as components of patented antioxidant mixtures (8,19,38). But there is relatively little exact knowledge as to their effects. Amino acids are also mentioned as prooxidants (5,15,40).

Their antioxidative effect is generally suggested to be of synergistic nature, i.e., to require the presence of a primary antioxidant. Amino acids are usually supposed to function by chelating prooxidative metal traces. The prooxidative effect of copper traces is mainly due to copper in free state in the fat phase (18). In the presence of amino acids copper complexes are formed and transported into the water phase. In this way traces of copper may be rendered innocuous as catalysts. It has however also been shown (39) that copper bound to amino acids, peptides, and proteins has a strong catalyzing effect on the oxidation of ascorbic acid and that linoleate oxidation is accelerated by copper protein complexes (46). But also an inhibiting action of proteins on the prooxidative effect of metal traces in fat emulsions

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has been found (1). Some amino acids were tested in lard to which metal chlorides were added; none showed any appreciable effect as metal deactivator under these conditions (29).

On the other hand it has been shown (9) that α-alanine, also in the absence of enzymes, may be oxidized by deamination; it was suggested that amino acids in this way are capable of protecting or regenerating primary antioxidants. Nonenzymatic oxidative deamination of amino acids by hydroperoxide has been reported (22).

The existence of synergism of free amino acids and tocopherol in herring has been shown (16). At a later stage the effect of amino acids on herring oil oxidation has been studied in model systems by measuring oxygen consumption in oxidizing herring oil emulsions (24,25).

The results can be summarized as follows: 11 amino acids which were tested (principally the free amino acids identified in the water phase of herring) decreased oxidation in herring oil. Histidine was most effective. The effect of cysteine however was prooxidative. The antioxidative effect of the amino acids was enhanced by the addition of phosphate. Phosphate decreased the prooxidative effect of cysteine.

Because of variation in composition, state of oxidation and, in consequence, rate of oxidation, and especially with regard to the tocopherol content of herring oil, the experiments were continued with pure linoleate at pH about 7.5 and 9.5 as substrate (pH 7.5, heterogeneous; pH 9.5, homogeneous solution of linoleate). Principally the same amino acids were tested as in the experiments with herring oil emulsions, especially alanine, histidine, and cysteine (26).

In this system the amino acids proved to be at least potential antioxidants. They may have antioxidative effect within a certain range of concentration. With increasing concentration the antioxidative effect may pass a maximum (i.e. minimum oxidation rate) and may, at further increasing concentrations, be inverted into a prooxidative effect. Again histidine proved relatively effective (Fig. 7) while cysteine was also prooxidative under these conditions. The antioxidative effect was enhanced by the addition of phosphate. In the presence of tocopherol the effect of tocopherol and amino acids could be synergistically enforced. At pH 9.5 the antioxidative effect was considerably stronger than at pH 7.5 (Fig. 7). At this pH (9.5), cysteine also had a strong antioxidative effect.

Experimental

The effect of amino acids on fat oxidation has now been studied at pH ≤ 7 (pH 7–5) with linoleic acid and methyl linoleate and linolenate as substrates, with these substances (0.05 ml.) dispersed in a water phase (2.95 ml).

The substrates were obtained in small ampules (1–10 ml.); linoleic acid from Hoffmann-La Roche, Basel; the esters from Hoffmann-La Roche, Basel, and from Mann Research Laboratories, New York. After being opened the ampules were stored several days in a desiccator which was twice exhausted and filled with nitrogen at about 2°C. in the dark. No considerable error caused by this storage has been observed. The water phase was either water or 0.1 mol. phosphate buffer (pH 5, 6, and 7).

Unbuffered aqueous emulsions of linoleic acid prepared by sonic vibrations have shown (21,41) a pH of 4.7–5.1 which agrees with the results of our measurements. The reported water solubility (6.7°C.) is 16 mg./100 ml.; in phosphate (0.1 mol.) at pH 5, 5 mg.; at pH 6, 25.5 mg.; at pH 7, 60 mg./100 ml. The pH change of the buffer solutions by addition of linoleic acid is at pH 5, −0.09; at pH 6, −0.12; at pH 7, −0.32 pH units.

Oxidation was followed in a Warburg-apparatus at 40°C., 100 or 120 strokes of 3–4 cm./min., during ca. 8–24 hr., depending on the rate of oxidation.

The course of oxidation is accelerated in the beginning and may slow down eventually (Fig. 1). Generally the experiments cover only the initial stage.

The experiments were evaluated by comparing the oxygen consumption at certain time intervals. The relation between oxygen consumption with a certain amino acid addition, and the oxygen consumption without such addition, was calculated. A relative value of >1 indicates a prooxidative; a value of <1 an antioxidative effect. Because of the more or less S-shaped course of oxidation, such a comparison might not be exact as different samples measured at the same time may be in different stages of oxidation. The error due to this is however negligible, considering the inevitable variation of the results in these experiments, which are influenced by known as well as unknown factors. The rate of oxidation could vary considerably between experiments, but was in general satisfactorily constant within the single experiment series.

The dispersion achieved by shaking in the Warburg apparatus was sufficient to obtain good agreement of duplicates without addition of an emulsifier. Nevertheless the effect on amino acids caused by an addition of an emulsifier has been studied. The emulsifier generally used was Tween 20 (polyoxyethylene sorbitan monolaurate). Some other emulsifiers were also tested, and are reported on later.

Although generally good agreement of duplicates or replicates could be obtained, the agreement was sometimes less than satisfactory and the experiments had therefore to be repeated several times, especially in the case of the methyl linoleate. With phosphate added, the rate of oxygen consumption was higher than without phosphate.

Experiments on Oxidation of Linoleic Acid.

As to the influence of pH, the rate of oxidation increases (20) with increasing pH until it reaches a peak at pH 5.5. It then decreases again to a minimum at pH 8.

The rate of linoleic acid oxidation ($mm.^3$ O_2/hr., calculated on about 8–10 hr. oxidation) was:

in water	
pH 5	35 ± 9 ($n = 23$)
in 0.1 mol. phosphate	
pH 7	44 ± 8 ($n = 20$)
pH 6	65 ± 8 ($n = 14$)
pH 5	41 ± 8 ($n = 15$)

The experiments discussed refer principally to histidine, glycine, and tryptophane. Some further experiments have been carried out with cysteine, alanine, serine, lysine, and proline. The concentration of the amino acid added was varied logarithmically and covered the range $2 \times 10^{-6} - 2 \times 10^{-1}$ mol. Within this frame the concentrations tested were chosen with regard to efficiency and solubility.

Suitable amino acid solutions were obtained by dilution of standard solutions with distilled water.

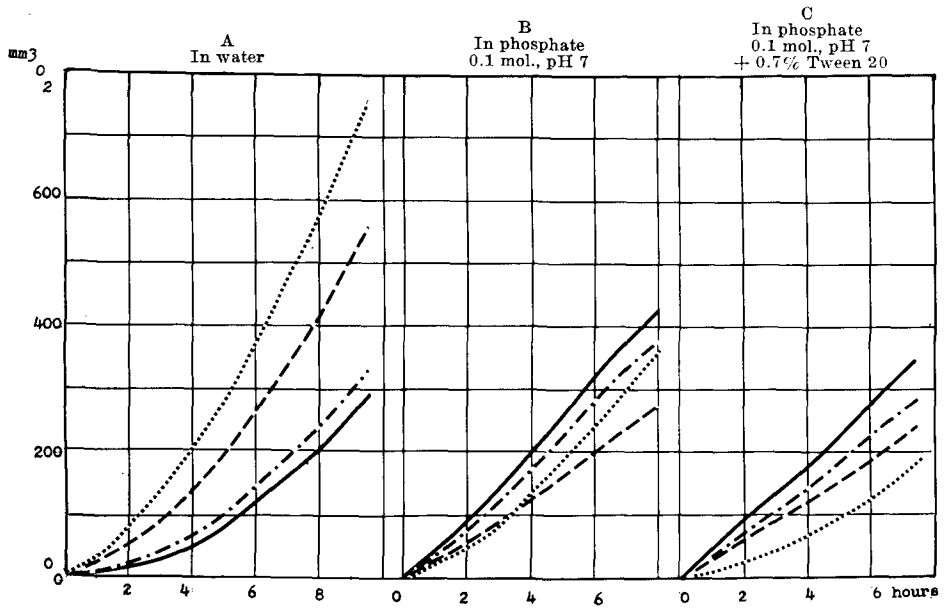

FIG. 1. Oxygen consumption (mm.^{3}) in linoleic acid emulsions (0.05 ml./3 ml.) without and with addition of histidine. ——— = 0, —·—·— = 2×10^{-4}, — — — = 2×10^{-3}, ····· = 2×10^{-2} mol. histidine.

The pH of these solutions was if necessary brought to the pH of the experiment.

The results of the experiments with glycine, histidine, and tryptophane on linoleic acid oxidation (without and with an addition of phosphate at pH 7, 6, and 5) are shown in Fig. 1A and 1B (absolute values), and Fig. 2 (relative values).

Without an addition of phosphate, the effect of these amino acids was exclusively prooxidative; and this prooxidative effect increased with increasing concentration. In the presence of phosphate however the effect may be antioxidative, especially at pH 7 and 6; less at pH 5. In the case of histidine, the maximum of antioxidative efficiency was obtained at pH 7 at a lower concentration (2×10^{-3} mol.) than at pH 6 and the antioxidative effect at a higher concentration level (2×10^{-2} mol.) could be somewhat stronger at pH 6 than at pH 7. The strongest antioxidative effect was reached with 2×10^{-2} mol. tryptophane at pH 7.

Experiments with alanine, serine, lysine, and proline showed only small effects similar to glycine. In some cases a somewhat greater effect was obtained at pH 6 than at pH 7 and pH 5, similar to the observation in the case of histidine mentioned above. The effect of cysteine was prooxidative, without and with phosphate.

Effect of Emulsifiers

As to the experiments with the addition of an emulsifier (usually Tween 20) different concentrations, varying between 0.17 and 2.0%, were used, mainly 0.7%. The effect of 0.7% Tween 20 on the rate of oxidation is slightly retarding without phosphate, as well as with phosphate. At higher concentrations, Tween accelerates oxidation in the presence of phosphate at pH 7 and retards it at pH 6 and 5 (Table I).

The effects of glycine, histidine, and tryptophane on the oxidation of linoleic acid in the presence of 0.7% Tween 20 (both without and with phosphate at pH 7, 6, 5) are shown in Fig. 1C (absolute values) and Fig. 3 (relative values). When comparing Fig. 3 with Fig. 2, it becomes apparent that prooxidative

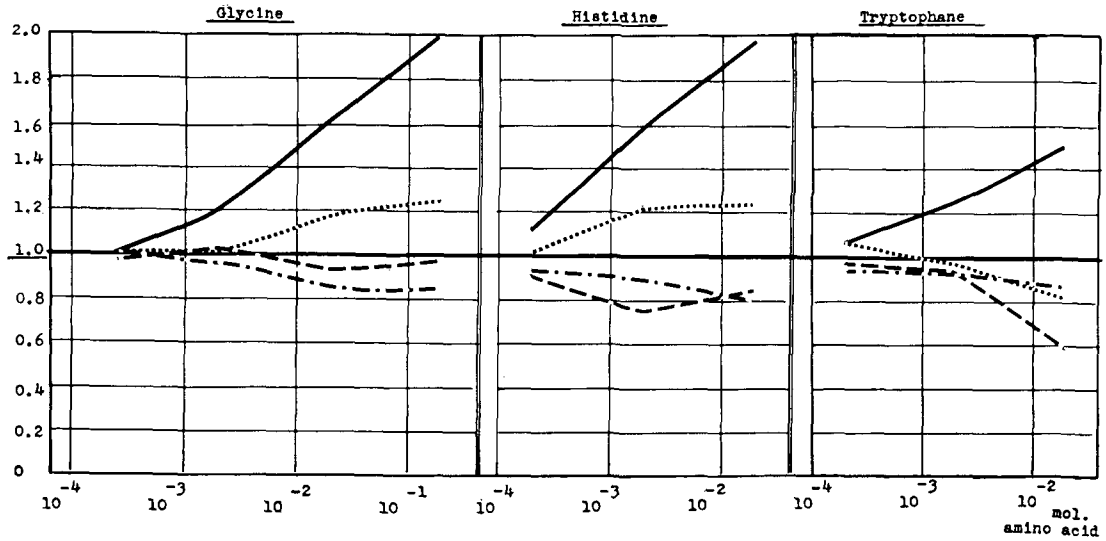

FIG. 2. Effect of glycine, histidine, and tryptophane on oxygen consumption in linoleic acid emulsions (0.05 ml./3 ml.) in water (———) and phosphate 0.1 mol., pH 7 (— — —, pH 6 (·—·—), pH 5 (·····). Relative values calculated on corresponding oxygen consumption without addition of amino acid.

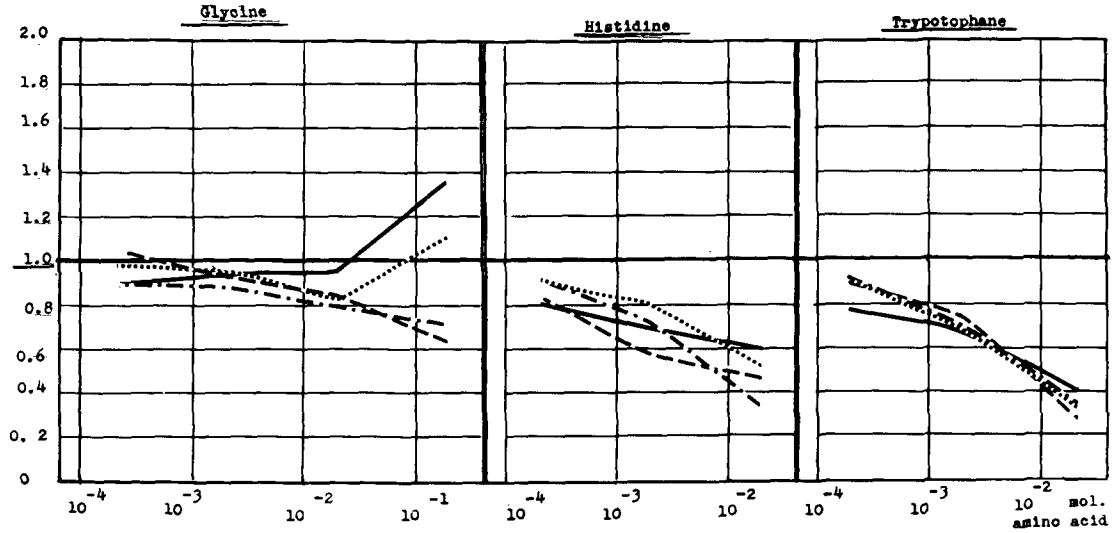

FIG. 3. Effect of glycine, histidine, and tryptophane (relative values) on oxygen consumption in linoleic acid emulsions (0.05 ml./3 ml.), in water (————) and phosphate 0.1 mol., pH 7 (— — —), pH 6 (· — ·—), pH 5 (· · · · · · ·) with addition of 0.7% Tween 20 (40°C.).

effects may become less prooxidative or may be inverted into antioxidative ones, and that antioxidative effects may be enhanced by addition of Tween 20. This phenomenon is more apparent at pH 7, less at pH 6 and 5. Again the antioxidative effect of a high concentration of histidine (2×10^{-2} mol.) is stronger at pH 6 than at pH 7.

The very pronounced dependency on the effect of the Tween 20 concentration is shown in Fig. 4, in the case of histidine without and with addition of 0.1 mol. phosphate pH 7.

In view of the remarkable effect of Tween 20, the question arises if it depends on the emulsifying action or, less probable, on some chemical interaction of Tween 20. Several other emulsifiers have therefore also been tested without and with an addition of phosphate. These experiments are summarized in Table II for 2% additions of the various emulsifiers, without and with 2×10^{-2} mol. histidine, which itself is prooxidative in the absence of phosphate, and only insignificantly weakly antioxidative in the presence of phosphate at pH 7.

In the absence of phosphate the hydrophylic emulsifiers (No. 1–6) themselves had an antioxidant action. The relative histidine effect, instead of being strongly prooxidative without emulsifier, was antioxidative (or only weakly prooxidative in one case). In the presence of phosphate (pH 7) the hydrophylic emulsifiers No. 1–4, greatly accelerated oxidation; but after combined addition of emulsifier and histidine, oxidation was nearly completely inhibited.

The lipophylic emulsifiers (No. 7 and 8) retarded oxidation considerably. Nevertheless, there was also in this case a significant further retardation of oxidation after addition of histidine.

The results seem to indicate that the enforcing effect of the emulsifiers tested is due to the emulsifying action.

TABLE I

Tween 20	0.17%	0.7%	2.0%
	Relative Effect		
In water	0.85	0.82	0.85
In 0.1 mol. phosphate			
pH 7	0.94	0.83	1.47
pH 6	1.00	0.86	0.52
pH 5	0.76	0.60	0.43

Effect of Tween 20 on oxygen consumption of linoleic acid at 40°C. (0.05 ml./3 ml.) in water, and in 0.1 mol. phosphate, pH 7, pH 6, and pH 5, relative to oxygen consumption without Tween 20.

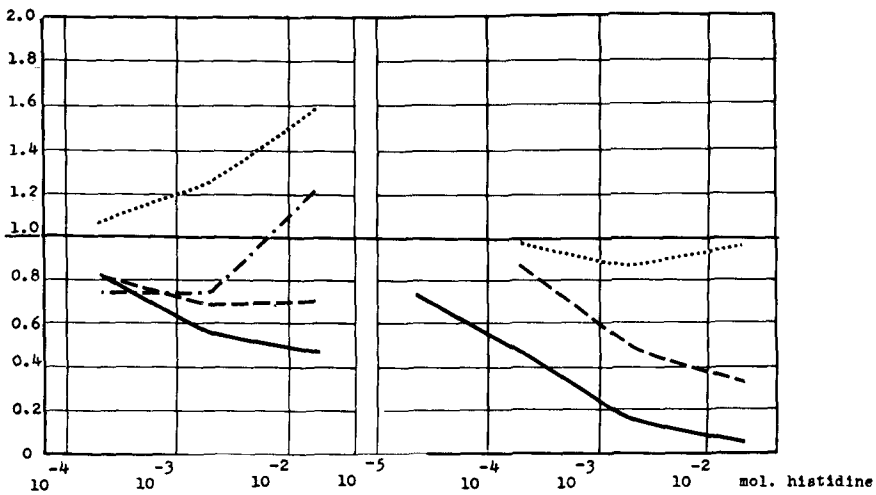

FIG. 4. Effect of histidine (relative values) on oxygen consumption in linoleic acid emulsions (0.05 ml./3 ml.), in water (left) and phosphate 0.1 mol., pH 7 (right), with addition of different concentrations of Tween 20: · · · · · · · = 0%, — · — · — = 0.17%, — — — = 0.7%, ———— = 2.0%.

TABLE II

No.	Emulsifier 2%		Relative Effect: In water, Emulsifier[a]	In water, Histidine[b]	In phosphate 0.1 mol., pH 7, Emulsifier[a]	In phosphate 0.1 mol., pH 7, Histidine[b]
	Without addition of emulsifier:			ca 2.0		ca 0.95
1	Tween 20	Polyoxyethylene sorbitan monolaurate	0.82	0.85	1.48	0.05
2	Tween 40	Polyoxyethylene sorbitan monopalmitate	0.75	0.80	1.91	0.08
3	Tween 60	Polyoxyethylene sorbitan monostearate	0.84	0.65	1.88	0.07
4	Tween 80	Polyoxyethylene sorbitan monooleate	0.98	0.65	2.16	0.05
5	G7596J	Polyoxyethylene sorbitan monolaurate	0.59	1.21	0.94	0.06
6	Bry 35	Polyoxyethylene lauryl alcohol	0.60	0.87	1.01	0.12
7	Span 20	Sorbitan monolaurate	0.08	0.19	0.23	0.10
8	G 672	Glycerol sorbitan laurate	0.03	0.29	0.17	0.10

Effect of 2×10^{-2} mol. histidine, on oxidation of linoleic acid at 40°C. (0.05 ml./3 ml.) in the presence of various emulsifiers (2%) in water, and in 0.1 mol. phosphate, pH 7.0.

[a] The values for the emulsifier effect are relative to the corresponding value without emulsifier.

[b] The values for the histidine effect are relative to the corresponding value with emulsifier, without histidine.

The values have been calculated for an oxidation of about 8 hr. except for Span + histidine and G 672 + histidine (19 hr.).

Experiments on Oxidation of Fatty Acid Esters

The next study was to examine the effect of amino acids on the oxidation of fatty acid esters (methyl linoleate and methyl linolenate). These experiments were carried out in the same way as the experiments with linoleic acid described above.

The amino acids tested were histidine, alanine, glycine, and tryptophane. Rate of oxidation of methyl linoleate (mm^3 O_2/hr., calculated on about 18–22 hr. oxidation) was

in water	8 ± 2 ($n = 30$)
in 0.1 mol. phosphate pH 7	10 ± 3 ($n = 37$)

Fig. 5 summarizes some results obtained with methyl linoleate and histidine without and with phosphate at pH 7, and without and with 0.7% Tween 20. Results show generally a stronger antioxidative (and less prooxidative) effect of histidine than in the case of linoleic acid. Histidine had an antioxidative effect also in the absence of phosphate, with a relatively weak tendency to invert at higher histidine concentrations. With phosphate at pH 7 the antioxidative effect of histidine was considerably enforced; meanwhile there was no significant antioxidative effect at pH 5 (not shown in Figure 5). After addition of 0.7% Tween 20 the antioxidative effect of histidine was generally greatly enhanced. Without phosphate it was enforced at lower concentrations, but inverted into a prooxidative effect at a concentration of 2×10^{-2} mol. histidine; with phosphate, however, it was exclusively enforced both at pH 7 and at pH 5. At pH 7 a strong antioxidative effect was still observed at a concentration of only 2×10^{-6}–2×10^{-5} mol. histidine. The other amino acids tested showed similar tendencies in accordance with their individual antioxidative capacity mentioned above.

Only a few experiments have hitherto been carried out with methyl linolenate. In this case the rate of oxidation (mm^3 O_2/hr.) naturally is much higher. Calculated on about 8–10 hr. oxidation, it was:

in water	27 ± 8 ($n = 6$)
in 0.1 mol. phosphate pH 7	42 ± 3 ($n = 6$)

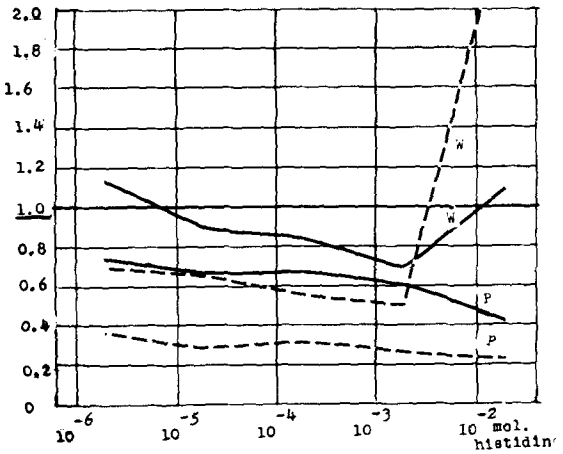

FIG. 5. Effect of histidine (relative values) on oxygen consumption in methyl linoleate emulsions (0.05 ml./3 ml.), in water (W) and phosphate 0.1 mol., pH 7 (P), without (————), and with addition of 0.7% Tween 20 (- - - - - - -).

The results indicate that the amino acid effect is weaker than with methyl linoleate (Fig. 6). Without phosphate, histidine influenced the oxidation in a prooxidative sense; after addition of phosphate pH 7, and still more after further addition of Tween 20 the histidine effect was strongly antioxidative.

Again the results obtained by addition of the other amino acids which were tested (glycine and alanine) corresponded to this tendency of histidine and individual antioxidative capacity of glycine and alanine.

Discussion

The purpose of these experiments was to determine if there is a specific antioxidative behavior of α-amino acids as a group and, if so, how the antioxidative behavior of various amino acids may differ and how it is influenced by environmental factors. Because of the different behavior of the single amino acids, and the manifold dependencies of various factors (controlled as well as uncontrolled), this study has grown to an unexpected extent and about 500 experimental series have now been carried out.

The results indicate the comments which follow. All amino acids tested have been shown to possess potential antioxidative capacity. This antioxidative capacity is not dependent on the presence of a so-called primary antioxidant-like tocopherol. Consequently the

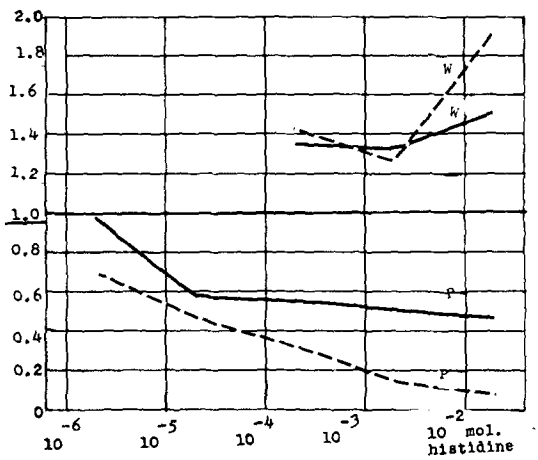

FIG. 6. Effect of histidine (relative values) on oxygen consumption in methyl linolenate emulsions (0.05 ml./3 ml.), in water (W) and phosphate 0.1 mol., pH 7 (P), without (————), and with addition of 0.7% Tween 20 (- - - - - - -).

antioxidative effect of amino acids is not, as is usually suggested, of synergistic character according to the definition of synergism given by Mattill (28). This does not exclude that amino acids may be able to behave as synergists in the presence of a primary antioxidant. Actually it has also been shown in earlier experiments cited (26) that this is the case.

The antioxidative effect is only a "potential" one, i.e., amino acids may be prooxidative, more or less ineffective, or antioxidative, depending upon the circumstances. This agrees with the above cited statements in the literature, where amino acids are mentioned both as prooxidants and as antioxidants. It is often possible to invert prooxidative effects of amino acids into antioxidative ones and to enhance weak antioxidative effects, e.g., by an addition of phosphate or an emulsifier like Tween 20. Also cysteine, normally prooxidative, has been shown to have antioxidative capacity in suitable conditions, e.g., at pH 9.5. These results show the dependency of the "potential" amino acid effect on the circumstances, which will be discussed later.

The Effect of Different Amino Acids

The various amino acids tested had varying antioxidative capacity. To grade this capacity care must be taken both to the relative retardation of oxygen consumption, and the amount of amino acid required. Histidine (β-imidazolylalanine) and tryptophane (β-indolylalanine) have been shown to have a relatively strong antioxidative tendency. The antioxidative effect may in suitable conditions be nearly completely inhibitory, and extremely small concentrations of histidine are rather effective. Histidine has however also a relatively strong tendency to invert to a prooxidative effect at higher concentrations. In the case of tryptophane the tendency to invert is less.

Different Systems and Substrates

The results of the experiments in different systems and with different substrates show partly agreement, partly deviation. It seems however that the differences are not contradictory, but may be regarded as a shifting of the anti- and prooxidative tendencies influenced by environmental factors (discussed later). Great differences may also be expected in dry fat compared with these aqueous fat systems (44,47).

The catalytic property was more pronouncedly antioxidative in the case of the methyl linoleate than with linoleic acid (Fig. 7) and exclusively antioxidative (except with cysteine) in the case of triglycerides (herring oil and other fats). It was less antioxidative with methyl linolenate than with methyl linoleate. The observed tendency of greater antioxidative efficiency with esters, especially triglycerides, agrees with Holtz (13) who could not confirm the prooxidative effect of amino acids, observed with linseed oil fatty acids, in the case of linseed oil. Others (35) could not find any prooxidative effect of amino acids on phosphatides. These early experiments refer to relatively high amino acid concentrations. The interesting catalytic effect of amino acids at that time was the prooxidative one.

It must be observed that the results with different substrates might be influenced by trace impurities, as well as products of oxidation which are difficult to control.

Positive and Negative Catalysis. Inversion.

The phenomenon of substances being prooxidative as well as antioxidative, depending on the circumstances, is well known (10,11,12,30,33,45). The sense of catalysis, positive or negative, is dependent on the conditions of the experiment and not on the nature of the catalyst (30). Such substances may be regarded as catalysts of either pro- or antioxidative reactions. They may, for instance, inactivate hydroperoxide radicals thereby breaking chain reactions, but may also decompose hydroperoxides to form new chain-promoting radicals (37). According to Heimann (10,11,12) three reactions may be responsible for the final result: the normal oxidation of the substrate; the antioxidative reaction; and the prooxidative reaction. The result depends on which of these reactions is prevailing. In the system examined, lard and tocopherol, the prooxidative reaction needs a higher activation energy and therefore only prevails if a larger amount of the catalyst is present of which only a small part can be used by the antioxidative reaction. Consequently the effect may be antioxidative at low concentrations and prooxidative at higher ones.

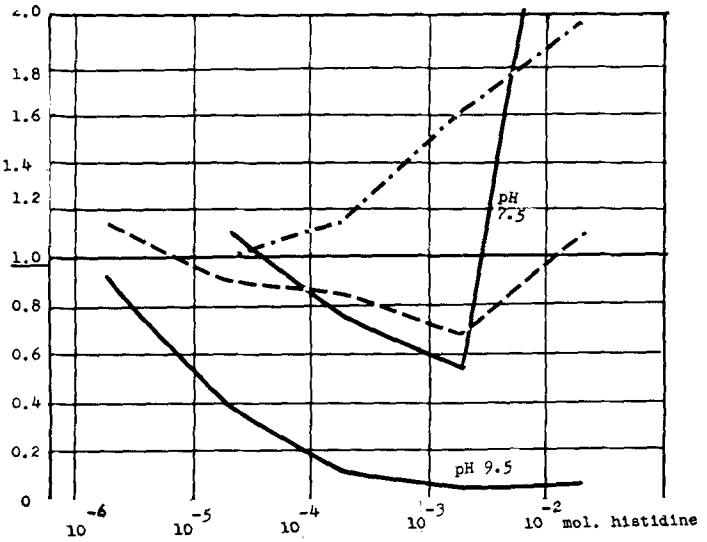

FIG. 7. Effect of histidine (relative values) on oxygen consumption in:
——— linoleate solution, pH 9.5 and 7.5 (0.05 mol., 3 ml., 40°C.);
— · — · — linoleic acid emulsion (0.05 ml./3 ml., in water, 40°C.);
— — — methyl linoleate emulsion (0.05 ml./3 ml., in water, 40°C.).

Such inversion has hitherto been observed mainly in the case of phenolic primary antioxidants and ascorbic acid (23, 48, 49). The same explanation as that given by Heimann for the case of lard as substrate and tocopherol as catalyst might, however, also be valid in the case discussed here. It is clear that the product of the three reactions: autoxidation, antioxidation, and prooxidation, may not only be influenced by amino acid concentration but also by pH, nature of substrate, and other properties of the system. The manifold influences which different types of substrate and other properties of the system may have on the course of oxidation, have been illustrated by the experiments described above as well as by others (42). It has been shown how various substances, which might be present in food, can influence oxidation in methyl linoleate emulsions (44).

The Influence of pH

The final effect of a certain amino acid addition may consequently be regarded as the product of several influences on the three reactions mentioned above.

One of them is pH. On the whole the antioxidative tendency was very pronounced in earlier experiments at about pH 9.5 and moderate at about pH 7.5 (Fig. 7), and decreases with decreasing pH from pH 7 to pH 5. With decreasing antioxidative effect, the prooxidative tendency may increase (inversion). This is however not the case with all amino acids to the same degree.

The effect of 2×10^{-3} mol. histidine was less antioxidative at pH 6 than at pH 7. The effect of 2×10^{-2} mol. histidine was however somewhat less antioxidative at pH 7 than at pH 6. I.e., the maximal antioxidative effect was obtained at pH 7 at a lower concentration than at pH 6 and the effect of 2×10^{-2} mol. histidine at pH 7 was already considerably influenced by the prooxidative reaction.

The Effect of Phosphate

The synergistic effect of phosphate on fat oxidation has been illustrated and discussed (2,6,7,14,29,31,32, 36,45). It is ascribed either to metal binding, to regeneration of primary antioxidants, or sparing action on them (i.e., by retardation of their oxidation). Phosphate may regenerate tocopherol by moving the equilibrium *quinol* $\rightleftharpoons$ *quinone* to the left (6) or a dismutation may take place in the presence of phosphate (14).

In the case discussed here the addition of phosphate alone increases the rate of oxidation. Phosphoric acid can accelerate decomposition of fat peroxides (37). In the presence of amino acids, phosphate has a synergistic effect, enhancing an antioxidative effect, lowering a prooxidative effect, or inverting it into an antioxidative one. It may be possible that an addition of phosphate moves the equilibrium of the three reactions mentioned above in favor of antioxidation. The antioxidative reaction is limited by the rate of production of peroxidradicals in the oxidative reaction (12). The stronger effect of amino acids in the presence of phosphate may be due in part to the greater rate of oxidation which produces more radicals.

The Effect of Emulsifiers

Studies of the effect of various emulsifiers on fat oxidation (4) have shown that admixture of 10% of the hydrophylic emulsifiers Tween (polyoxyethylene sorbitan fatty acid ester), Myrj (polyoxyethylene stearate, and Brij (polyoxyethylene laurylether) shortens the induction period of lard and olive oil, whereas admixture of the lipophylic emulsifier Span (sorbitan fatty acid ester) prolongs it. The difference between the two groups is in certain agreement with the observations reported above. The synergistic effect of emulsifiers and amino acids may be explained in a quantitative manner, emulsifiers decreasing the size of particles and increasing the contact surface of the phases. It has however not been possible to characterize the physical state of the dispersions, especially with regard to the size of the particles as (at least in the case of lower emulsifier concentrations) the emulsions obtained were not stable when not shaken. It may also be possible that by addition of emulsifiers the conditions at the globule surface are qualitatively changed and a more suitable molecular orientation brought about, resulting in the described enhancing of the antioxidative efficiency of amino acids. The effect of some emulsifying agents on oxidation of methyl linoleate emulsions, and the delaying effect of surface-active agents in the presence of primary antioxidants has been ascribed to the Cu-binding capacity of these substances (43,44).

The results obtained show that although it is necessary to study such problems in model systems, there may be a risk of obtaining results which in practical conditions are irrelevant. They further show the risk of drawing conclusions of "one-dimensional" experiments, i.e., one certain concentration in one certain system. Our experiments are not perfect in this regard. As not all amino acids could be studied in all the interesting conditions, an "incomplete block design" has been applied. Experiments are being continued.

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