The requirements for sterols seem, therefore, to be qualitatively the same for the six insect species under investigation, but there are obvious quantitative differences. From a comparison of the growth on three diets relatively deficient in sterols (summarized in Table 2) it can be concluded that *Sitodrepa*, *Ptinus* and *Ephestia kuehniella* require larger quantities of sterols than *Tribolium*, *Lasioderma* and *Silvanus*.

SUMMARY

The sterol requirements of six different insect species have been investigated; all the insects re-

quire a sterol in the diet for growth. The quantitative requirements of sterols by different insects are different, but the qualitative requirements of the insects under investigation are the same. The specificity of the sterol requirements was studied.

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Apparent Vitamin C in Foods

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Titration with 2:6-dichlorophenolindophenol is used to estimate vitamin C in a wide variety of foodstuffs, though the dye may react with substances besides ascorbic acid. Harris [1933] showed that dye-reducing substances can be produced by heating certain sugars with alkali. Johnson [1933] found with germinated peas that dye titrations indicated higher vitamin C contents than was shown by biological tests. In acid extracts part of the dye-reducing power was due to a substance which reduced the dye more slowly than ascorbic acid. Bacharach, Cook & Smith [1934] found an increase in the dye-reducing power of malt extract heated to 100° for $\frac{1}{2}$ –2 hr. McHenry & Graham [1935] obtained increases of 50–170% by heating certain vegetables for a few minutes at 58° or by standing these for a few hours at room temperature in 1% HCl. Ahmad [1935] found that boiling cabbage for 10 min. with 20% CCl_3COOH increased the apparent vitamin C content by nearly 200%. Reedman & McHenry [1938] found that the apparent vitamin C in heated potatoes resembled true vitamin C in both physiological and many chemical properties. Their suggestion that ascorbic acid might exist in combination with protein from which it was set free by heating was discredited by Harris & Olliver [1942], who suggested three possible causes for the

results: (1) incomplete extraction from unheated tissues, (2) variations in sampling, (3) inhibition by heat of the ascorbic acid oxidase in the foods examined. The possible occurrence of non-specific dye-reducing substances in vegetable tissues was considered of little or no practical importance, although it was admitted that germinated seedlings, malt extract, yeast and fermented juices might repay further study.

During the last three years we have been accumulating evidence to show that the apparent vitamin C content of foods can become considerably higher than the true vitamin C value under manufacturing and storage conditions which may occur in practice. Preliminary reference to these findings was made by Wokes, Johnson, Duncan, Organ & Jacoby [1942] and by Wokes, Organ, Duncan & Jacoby [1943a]. The provisional term 'apparent vitamin C' was proposed to describe substances occurring in natural and processed foods which, although not possessing antiscorbutic activity, simulate vitamin C very closely in their chemical and physical properties, so that they are not distinguished from it by the indophenol titration as usually applied, and may therefore cause results to be too high. Methods of estimating true vitamin C in the presence of this apparent vitamin C have

been described by Mapson [1943*a,b*] and by Wokes, Organ & Jacoby [1943*b*]. In this paper a brief account is given of the production and occurrence of apparent vitamin C in foodstuffs, and of some of its properties.

EXPERIMENTAL

Indophenol titrations

In our earlier experiments we used only the visual dye titration method essentially as described by Harris & Olliver [1942]. Later we also used the potentiometric method of Harris, Mapson & Wang [1942] with modifications. This proved invaluable both for deeply coloured products and for following dye reactions more precisely than is possible visually, and we are greatly indebted to these workers for details of the method prior to publication. Satisfactory agreement was obtained between visual and potentiometric results, provided that timing and rate of dye additions were carefully controlled as described by Wokes *et al.* [1943*b*].

Estimation of apparent vitamin C

This was carried out by a modification [Wokes *et al.* 1943*b*] of the method of Lugg [1942]. Lugg divided the various dye-reducing substances into three groups according to their rate of reaction with formaldehyde: (a) react readily at pH 1.5 and 3.5, (b) react readily at pH 3.5, slowly at pH 1.5, (c) do not react readily at either pH. Ascorbic acid was the only substance he had found in group (b); reductone occurred in group (c). Hence a basis was provided for differentiating ascorbic acid from reductone.

It has been shown by Mapson [1943*b*] and ourselves [Wokes *et al.* 1943*b*] that reductone and other components of apparent vitamin C react too rapidly with formaldehyde at pH 3.5 for satisfactory differentiation. We have obtained satisfactory results by treating mixtures of true and apparent vitamin C with formaldehyde at pH 4.5 for 6 min. This destroys the whole of the true vitamin C but little, if any, of the apparent vitamin C. Recovery experiments are made to correct for any apparent vitamin C destroyed in certain materials. The action of the formaldehyde is checked at the end of 6 min. by adding excess HPO_3 to bring the pH to about 1.0. To calculate true vitamin C the apparent vitamin C thus determined is subtracted from the total vitamin C estimated with similar timing and rate of dye additions.

Production of apparent vitamin C by heating with mineral acid

Potatoes, cabbages, carrots, germinated grains (wheat, barley and oats), grass and lucerne were treated with various concentrations of HCl, H_2SO_4 , HPO_3 or CCl_3COOH in a water-bath at 90–95°, or occasionally on a sand-bath, water being added at intervals to make good any loss. With some materials (e.g. grass), heating on a sand-bath proved more effective. With HCl or H_2SO_4 , 2% (v/v) of the concentrated acid gave the best results, producing as much apparent vitamin C as higher (up to 8%) concentrations of acid, but when less than 2% of concentrated acid was used the rate of production of apparent vitamin C fell rapidly with decreasing acid concentration. 1% HPO_3 was more effective than lower concentrations, but the rate

of production of apparent vitamin C was still much slower than with 2% conc. HCl. The apparent vitamin C produced with 1% HPO_3 or with 0.25% conc. HCl was unstable to prolonged heating. When heating cabbage extract in 25% CCl_3COOH we were not able to obtain any increase in the dye titration values such as Ahmad [1935] described, but, on the contrary, as heating continued the titration value became less. Our cabbage extracts were prepared by grinding with quartz sand, with precautions to avoid destruction of ascorbic acid by oxidizing enzymes [vide Wokes & Organ, 1943]. Hence our findings indicate that the increase in the dye titration value observed by Ahmad when heating cabbage with 25% CCl_3COOH was due to extraction difficulties and not to production of apparent vitamin C. We were able to find only slight traces of apparent vitamin C in our cabbage extracts when examining these by the method of Wokes *et al.* [1943*b*]. On the other hand, when we heated germinated grains, grass and lucerne with acid, the dye titration figures steadily increased during several hours' heating (in contrast with the increase in 10 min. observed by Ahmad). The presence of apparent vitamin C was confirmed by the above method, although the amount found was sometimes less than the increase observed, and the possibility of formation of hydroxytetrone acid was not precluded. Nevertheless, ample evidence was obtained that the increase in the dye titration values was not due to any of the three possible causes mentioned by Harris & Olliver, viz. incomplete extraction, variation in sampling, or inhibition of ascorbic acid oxidase. A few representative results are given in Fig. 1.

Fig. 1. Production of apparent vitamin C by heating with 2% conc. HCl or of conc. H_2SO_4 at 100°. Germinated grains, ○—○. Grass, ×—×. Lucerne, *—*—*.

Production of apparent vitamin C by heating without acid

In certain foods and food materials apparent vitamin C can be produced by heating to about 100° without addition of acid. This effect is strongly marked with malt extract, in which it is possible to increase the apparent vitamin C content as much as 10 times, but may also be observed with fruit syrups and other products. If true vitamin C be present, its destruction by heat may be masked by the simultaneous production of apparent vitamin C. This production of apparent vitamin C can take place at pH 4–5 as compared with a pH below 1 in the experiments with acid. A few typical results are given in Table 1.

Table 1. *Apparent and true vitamin C contents of foods, etc.*

Material	pH of material	Vitamin C content			True as percentage of total
		(mg./100 g.)		True	
		Total	Apparent (a)		
Fresh fruit and fruit preparations:					
Black currants, fresh	3.2	202	4	198	99
Black currant juice	3.2	140	5	135	96
Black currant syrup, freshly made	—	103	2	101	98
Black currant syrup after 8 weeks at room temp. in dark	—	96	4	92	96
Black currant syrup after 13 weeks at 27° in dark	—	58	3	55	94
Black currant syrup after 10 weeks at 37° in dark	—	68	8	60	88
Rose hip syrup after 12 months at 0-5°	3.7	156	20	136	88
Rose hip syrup after 12 months	3.6	101	69	32	31
Cherry juice, conc., after 5 years at 0-5°	3.8	47	45	2	4
Lemon juice, conc., after 18 months at 0-5°	1.7	193	42	151	78
Lemon juice, conc., after 4 years at 0-5°	1.7	27	21	6	22
Walnuts, unripe (<i>Juglans regia</i>), 2-3 g.	—	2619	1310	1309	50
Walnuts, unripe, 6-7 g.	5.2	2961	987	1974	67
Walnuts, unripe, 15-20 g.	3.9	2028	408	1620	80
Walnuts, unripe, after precipitation of tannin (b)	3.9	1909	400	1509	79
Dried fruits and vegetables:					
Dried bananas (c)	5.0	6	1	5	83
Dried carrots (c)	4.8	90	49	41	45
Dried potatoes (d)	4.5	27	4	23	85
Dried rose hip extract (mean of five samples) (e)	4.1	1300	50	1250	96
Dried spinach (c)	7.0	2	0.5	1.5	75
Dried tomatoes (c)	4.7	89	55	34	38
Malt extracts:					
Liquid malt extract, low diastatic power (D.P.)	4.7	3	(f)	0	0
Liquid malt extract, normal D.P.	4.7	12	(f)	0	0
Liquid malt extract, normal D.P.	4.8	5	(f)	0	0
Liquid malt extract after ½ hr. at 100°	4.8	12	(f)	0	0
Liquid malt extract after 2 hr. at 100°	4.8	20	(f)	0	0
Liquid malt extract after 9 months at 37°	3.6	103	(f)	0	0
Dried malt extract, low D.P. (g)	4.7	3	(f)	0	0
Dried malt extract, normal D.P. (g)	4.7	12	(f)	0	0
Sugars and molasses:					
Sugar, white (mean of three samples)	—	0	0	0	0
Sugar, brown (mean of four samples)	—	1	1	0	0
Golden syrup	—	27	27	0	0
Molasses, beet	—	18	18	0	0
Molasses, cane	—	11	11	0	0
Molasses, before charcoal treatment (h)	5.6	46	46	0	0
Molasses, after charcoal treatment	6.2	19	19	0	0
Molasses, beet concentrated preparation	5.4	222	(d)	0	0
Molasses, beet concentrated preparation	—	304	(d)	0	0
Molasses, cane concentrated preparation	—	196	(d)	0	0
Cocoa, chocolate:					
Cocoa, defatted, mixed sample	7.0	65	65	0	0
Cocoa, defatted, after precipitation of tannin	7.0	12	(d)	0	0
Chocolate, vitaminized	6.1	44	8	36	82
Chocolate, vitaminized	5.7	36	4	32	89
Miscellaneous:					
Beer, light draught	4.6	0.3	0.3	0	0
Beer, dark draught	4.4	1	1	0	0
Parsley (i)	7.0	220	40	180	82

Notes(a) Apparent vitamin C determined by method of Wokes *et al.* [1943b] checked occasionally by Mapson's method [1943b].

(b) By method of Mirimanoff & Mori [1940].

(c) After about 4 years' storage in air-filled containers at room temperature.

(d) After 6 weeks' storage in air-filled containers at room temperature.

(e) pH range 4.0-4.2.

(f) Some of the dye reductant in these preparations reacted with HCHO under the given conditions, but since it had been produced by heating was considered not to be ascorbic acid.

(g) Prepared by evaporation *in vacuo* of the corresponding liquid malt extract.

(h) Treatment normally used in refining sugar.

(i) During extraction cyanide used to inhibit action of oxidizing enzymes and stabilize apparent and true vitamin C.

Development of apparent vitamin C during storage

Apparent vitamin C may be gradually formed during storage under normal conditions. The rate of formation is affected by temperature, moisture content and exposure to air and enzymic action.

(a) *Effect of storage temperature.* Fig. 2 summarizes some results obtained on malt extract, which show that the rate of formation of apparent vitamin C is affected by the

Fig. 2. Effect of storage temperature on rate of production of apparent vitamin C in malt extract. Increase at 37°, *—*—*. Increase at room temperature, x—x—x. Increase at 0-5°, ○—○—○.

Fig. 3. Rate of production of apparent vitamin C in malt extract stored in air-filled containers at 37°C, showing effect of enzyme and moisture content. Results on dried extract of low diastatic power (12 Lintner), □—□—□. Dried extract of normal diastatic power (112 Lintner), ○—○—○. Liquid extract from which low d.p. dry extract prepared, x—x—x. Liquid extract from which normal d.p. dried ext. prepared, *—*—*.

storage temperature. At 37° increases of 12-103 mg./100 g. were observed during 268 days' storage. In the refrigerator at about 5° an increase of 46% was observed in 150 days.

(b) *Effect of moisture content.* Removal of all but 1-2% of the moisture greatly retards the rate of production of apparent vitamin C. This is clearly shown by results of experiments on malt extract before and after drying. Rather less conclusive evidence was obtained, indicating that destruction of the malt enzymes also diminished the rate of production of apparent vitamin C (see Fig. 3).

In dried extracts of vegetables and fruits stored for 4 years at room temperature in air-filled containers there was marked development of apparent vitamin C, masking

Fig. 4. Total and apparent vitamin C in dried fruit and vegetables stored in air at room temperature. A, total vitamin C in dried tomatoes. B, true vitamin C in dried tomatoes. C, total and true vitamin C in dried spinach. D, total vitamin C in dried bananas.

the gradual destruction of true vitamin C (see results with tomatoes in Fig. 4). Storage in nitrogen-filled containers reduced the rate of formation of apparent vitamin C but did not entirely prevent it.

Occurrence of apparent vitamin C in miscellaneous materials

(a) *Cocoa and chocolate.* Apparent vitamin C has been found in considerable amounts in cocoa, and may therefore occur in chocolate used as a carrier of the vitamin (Table 1). When the true vitamin C gradually disappears during storage, the apparent vitamin C remains, and if no correction is made for its presence the error increases in magnitude. The apparent vitamin C is not entirely tannin, since it is not completely precipitated by the method of Mirimanoff & Mori [1940].

(b) *Fruits and fruit syrups.* Fresh fruits appear on the whole to contain only small amounts of apparent vitamin C, which does not usually exceed 10% of the total vitamin C found. Unripe walnuts are an exception; in some species the apparent vitamin C may be 80% of the total [Melville, Wokes & Organ, 1943].

When fruit juices are heated or subjected to prolonged storage more apparent vitamin C may be produced, so that it may eventually form the greater part of the total vitamin C found by the usual dye titration (Table 1). Some of this may be reductic acid formed by the action of the fruit acids on pectin or other constituents.

(c) *Sugar, syrup and molasses.* Demerara and other coloured sugars contain traces of apparent vitamin C, which may produce an appreciable effect on the total vitamin C content of fruit syrups. Some samples of table syrup contain more apparent vitamin C. This is also found in molasses, from which very concentrated preparations

can be obtained. In the process of sugar refining the apparent vitamin C is presumably produced by the treatment with mineral acid, and partially removed by subsequent treatment with charcoal.

(d) *Vegetables.* Significant amounts of apparent vitamin C have not been found in common raw vegetables, but may be produced by cooking at a high temperature (e.g. roasting or frying), and also occur in some herbs such as sorrel and parsley.

(e) *Beer.* Traces of apparent vitamin C, comparable with the total vitamin C contents of South African beers reported by Levy & Fox [1935], have been found in English draught beer, especially in the dark variety.

Properties of apparent vitamin C

(a) *Temperature coefficient.* The rate of production of apparent vitamin C increases with rise in temperature. Between 0 and 37° the temperature coefficient for 10° rise is of the order of 2. At 100° a sharp rise in the rate occurs.

(b) *Effect of pH on rate of formation and stability.* Apparent vitamin C develops as rapidly at pH 4-5 as at pH 1, and in part at least is unstable in 5% HPO_3 solution (pH below 1) as well as in less acid aqueous solutions. Its decomposition may be retarded by bubbling nitrogen through the solution to expel air, and by storage in the dark in nitrogen-filled containers; also by the use of cyanide.

(c) *Effect of oxidizing enzymes.* These, including ascorbic acid oxidase, were extracted from outer cabbage leaves by the method of Szent-Györgyi [1931], from cauliflower inner florets by the method of Hopkins & Morgan [1936] and from unripe tomato skins by the method of Wokes & Organ [1943], and tested on apparent vitamin C obtained either at a pH of about 1 (e.g. beet and cane molasses, acid-heated extracts of various foods) or at a pH of about 5 (e.g. dehydrated foods, malt extract). Freshly prepared solutions in buffers of pH 2-6 were mixed with the enzyme extracts at room temperature and apparent vitamin C determined at intervals. The enzymes destroyed apparent vitamin C under the same conditions as they destroyed real vitamin C.

(d) *Effect of oxidizing agents.* Practically the whole of the apparent vitamin C in different foods can be destroyed within 2-3 min. by treatment with 0.1% hydrogen peroxide or sodium percarbonate at 37°. This treatment also destroys true vitamin C. With lower concentrations less of the true or apparent vitamin C was destroyed. Even 1:10⁸ dilution of H_2O_2 produced an appreciable effect. The rate of destruction was most rapid in the first minute. It did not appear possible to differentiate between true and apparent vitamin C by such means.

(e) *Effect of ultra-violet light.* Griffiths [1943] suggested that reductones might be formed by the action of ultra-violet light on substances in the atmosphere. Solutions of dehydrated foods, in which it was known that apparent vitamin C could be developed during prolonged storage, were irradiated (mercury vapour lamp) at pH 4-6 in covered Petri dishes for several hours, screens being used to cut out short wave-length rays not present in sunlight, without development of apparent vitamin C. Solutions of foods in which the apparent vitamin C was already present were irradiated under similar conditions, and the apparent vitamin C was gradually destroyed, having almost entirely disappeared after 3-4 hr. irradiation [Wokes, 1943]. Whilst ultra-violet light did not produce any apparent vitamin C

in these dehydrated foods, the position may be quite different in natural foods, such as fruits, which are exposed to sunshine and contain various pigments and enzyme systems.

(f) *Spectroscopic evidence.* Dr R. A. Morton kindly examined spectroscopically three of our most concentrated sources of apparent vitamin C obtained from malt extract by prolonged incubation, and from beet and cane molasses respectively. The treatment which these had undergone precluded the presence of true vitamin C, and the absence of antiscorbutic properties was also shown by physiological tests. The incubated malt extract showed a very distinct maximum at about 280 $\text{m}\mu$ (Fig. 5). Maxima in the beet

Fig. 5. Absorption spectrograms of concentrated preparations of apparent vitamin C obtained from: malt extract, ----; beet molasses, x-----x; cane molasses, ○—○.

molasses at about 276 and 264 $\text{m}\mu$, and in the cane molasses at about 275 and 256 $\text{m}\mu$, were obscured by general absorption, due to the dark colour. When this dark colour was removed by treatment with charcoal, the apparent vitamin C was not recovered. Attempts to obtain colourless extracts suitable for spectroscopic examination by shaking or Soxhlet extraction with methanol or ethanol or by prolonged shaking with ether proved unsuccessful.

DISCUSSION

The production of non-specific dye reductants by heating different foodstuffs with or without acid has been mentioned by a number of previous workers. Very little was known of the nature and properties of these reductants and they were generally assumed not to affect the vitamin C value of foods as determined by dye titrations, either because they were not present in significant amounts or because they reacted more slowly with the dye so that their interference was obviated by carrying out the titration very rapidly, the end-point being reached in a

minute or less. However, some doubt has been cast on this assumption by the recent findings of Hochberg, Melnick & Oser [1943] and Taylor [1943] that, when the end-point is reached still more rapidly by extrapolation of photoelectric data, the results may be appreciably lower than those given by the ordinary visual methods. Taylor [1943] found the total vitamin C in unripe walnuts to be about 13% less by the more rapid method, and similar discrepancies occurred with other foods.

Wokes *et al.* [1943*b*], using a potentiometric method, have shown that the vitamin C value obtained for samples of dehydrated carrots, in which much apparent vitamin C had developed during 4 years' storage, was increased as longer intervals were allowed between the dye additions, thus allowing more time for the apparent vitamin C to react. With additions every 15 sec. the result was about 40 mg./100 g.; with additions every 8 min. the result was over 100 mg./100 g. It was suggested that this was due in part to enol-keto tautomerism. Apparent vitamin C was thought to exist in the solutions undergoing titration as a mixture of two isomers. Only one of these reacts with the dye. As the dye is added it removes the active isomer, and more of this is formed from the inactive isomer to restore the equilibrium. Thus the time effect is due to the rate of enol-keto tautomerism as well as to the rate of reaction of the active form with the dye.

In the present investigation it has been found that apparent vitamin C can be formed under much less drastic conditions, and occurs more widely and in greater proportions than was previously supposed. Moreover, its components resemble true vitamin C closely (1) in their susceptibility to oxidation by very low concentrations of inorganic or organic peroxides—an oxidation which is accelerated by enzyme extracts containing ascorbic acid oxidase, (2) in their removal by activated charcoal and general behaviour towards adsorbents, and (3) in their reduction of iodine, ammoniacal silver nitrate or alkaline copper solutions (Benedict's, Fehling's) in the cold. Some of the reductants in apparent vitamin C are gradually destroyed on standing in HPO_3 solution at room temperature, but may be stabilized by nitrogen or cyanide; others are stable in HPO_3 solution. They also differ from vitamin C in possessing no antiscorbutic activity and in being more stable towards heat and towards formaldehyde at pH 4–5 [Wokes *et al.* 1943*b*]. They should resemble ascorbic acid closely in chemical constitution and contain the active dienol and carbonyl groups. These are present in reductone, reductic acid, dihydroxymaleic acid and hydroxytetronic acid, four compounds closely related to ascorbic acid, as shown by comparison of their formulae.

Reductone is formed, amongst other products, when carbohydrates are treated with alkali. Such treatment may produce a variety of enolic compounds, hence the 'reductones' which Mapson prepared may have contained other dye reductants besides pure reductone, especially as their properties differed in certain respects (e.g. stability towards HPO_3) from those of our apparent vitamin C. Martius & Euler [1934] showed that reductone and dihydroxymaleic acid react much more slowly with the dye when the pH is >5 , and suggested that reductone could dissociate into two tautomeric ionized forms

Snow & Zilva [1938], when describing their experiments with ascorbic acid oxidase on reductone, suggested that their findings might be explained by the high dye-reducing power of one of these isomers, the concentration of which would be controlled by the velocity of the tautomeric conversion. A similar explanation is suggested for the variations we have observed in the reaction velocity of the dye with apparent vitamin C in different foodstuffs. Reductone has an absorption maximum at $287\text{m}\mu$ above pH 5.7 and at $268\text{m}\mu$ at pH 4.0 or lower, indicating a change in the proportion of the two isomers in this pH range which may be related to the difference in properties of apparent vitamin C in foods of pH above or below this range. The maximum at $268\text{m}\mu$ would be in good agreement with the maximum of $264\text{m}\mu$ found in our beet molasses at a similar pH, but the evidence for the presence of reductone is not conclusive. Euler & Martius [1933] stated that reductone has no antiscorbutic activity.

Reductic acid was obtained by Reichstein & Oppenauer [1933] by heating beet sugar pectin or various carbohydrate derivatives with mineral acid, treatment like that which produced apparent vitamin C in our molasses. Reductic acid has an ab-

sorption maximum at 280m μ at pH 6 and at 264m μ at pH 3 [Mayer, 1937]. The spectroscopic data on our materials do not preclude the presence of reductic acid, but the fact that the apparent vitamin C was not completely destroyed on standing in HPO₃ [Wokes *et al.* 1943b] suggested that the latter contained at least two different substances.

Dihydroxymaleic acid is formed when tartaric acid is oxidized by H₂O₂ in presence of small quantities of ferrous salts in sunlight. Szent-Györgyi [1939] states that it is quite common in plants, where it probably serves as a hydrogen transmitter analogous to ascorbic acid, being reversibly oxidized by an oxidase similar to ascorbic acid oxidase. Dihydroxymaleic acid, like ascorbic acid, is rapidly oxidized by traces of peroxides, but unlike ascorbic acid is unstable in acid solutions, in which it resembles part of the apparent vitamin C in our materials. Solutions of dihydroxymaleic acid in HPO₃ are not stabilized by nitrogen or cyanide. Such stabilization might be effected in the presence of certain food extracts, but our experiments in this direction have so far proved unsuccessful. Potentiometric studies of its rate of reaction with the dye confirm Szent-Györgyi's suggestion that in aqueous solution it is subject to enol-keto tautomerism. The maximum light-absorption for dihydroxymaleic acid in water is at 290m μ . This differs appreciably from the maximum at 280m μ in our incubated malt extract, and the maximum at 275–276m μ in the molasses, but the possibility must be considered that the acid in the molasses may have shifted the dihydroxymaleic acid maximum from 290m μ to a lower wave-length. The presence of dihydroxymaleic acid in our materials is therefore not excluded. The destruction of the non-specific reductant in these materials by our enzyme extracts may have been due to their containing dihydroxymaleic acid oxidase, which Banga & Philipot [1938] found in many plants. Dihydroxymaleic acid is not antiscorbutic [Dalmer, 1934].

Hydroxytetronic acid is chemically of interest as being the parent substance from which ascorbic acid is probably formed. It resembles ascorbic acid closely in its rate of reaction with HCHO and with the indophenol dye. Its maximum absorption at 246m μ is appreciably lower than the nearest maximum of 256m μ in any of our materials, and would presumably tend to become still lower in presence of acid. Its occurrence in our materials seems less likely than that of reductic or dihydroxymaleic acids. Hydroxytetronic acid is not antiscorbutic [Micheel & Jung, 1934].

Other possible constituents of apparent vitamin C. It appears from spectroscopic evidence, and from the effects of HPO₃ and HCHO at different pH [Wokes *et al.* 1943b], that apparent vitamin C frequently contains more than one non-specific re-

ductant. In addition to the reductants mentioned above, enolic substances may be present which reduce the dye fairly rapidly but which are less closely related to ascorbic acid. Certain German workers [cf. Enders, 1938; Luers, 1937] have described the occurrence and gradual development, in malted barley extracts and beer during storage, of dye reductants which they state are formed by condensation of sugars and amino-acids with evolution of CO₂. These reductants are stated not to reduce Fehling's solution, which indicates that their chemical nature differs more widely from that of ascorbic acid than does that of the above four reductants.

SUMMARY

1. The provisional term 'apparent vitamin C' is proposed to describe a group of substances occurring in foods and closely resembling ascorbic acid in chemical and physical properties (as well as in behaviour towards oxidizing enzymes), so that they are not distinguished from vitamin C by the dye titration generally employed.

2. These substances probably all contain the dienol group, and may include reductone, reductic acid, dihydroxymaleic acid and hydroxytetronic acid. They undergo enol-keto tautomerism, which causes their rate of reaction with the dye, and other properties, to be affected by the pH, the range 4–6 appearing to be critical.

3. They differ from ascorbic acid in being produced by the action of heat on certain constituents (e.g. pectins, carbohydrates) in foods and also (except hydroxytetronic acid) in their rate of reaction with formaldehyde under given conditions [Wokes *et al.* 1943b]. These differences in behaviour have formed the basis of methods of estimating apparent vitamin C in foodstuffs.

4. Apparent vitamin C has been found in germinated grains, malt extract, cocoa, chocolate, dehydrated fruits and vegetables, beer, and in certain fruits and herbs such as walnuts, parsley and sorrel. Particularly high contents have been found in unripe walnuts, in concentrated preparations from beet and cane molasses, and in fruit juices and dried foods stored for several years under normal conditions.

The apparent vitamin C content of processed and dehydrated foods may gradually increase during storage under normal conditions, and thus obscure loss of true vitamin C in storage experiments.

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The Osmotic Pressure of 'Defatted' Human Serum

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McCarthy showed [1938] that foetal sheep serum exerts a significantly higher osmotic pressure per gram of protein than does the maternal serum. This phenomenon could not be explained by the different albumin-globulin ratios of the two sera. Molecular weight determinations by McCarthy [1942] did not reveal any striking difference between foetal and maternal crystalline horse-serum albumins. Assuming that maternal and foetal serum albumins in the sheep are also identical, as is probable in the horse, we sought another explanation for the phenomenon observed in the foetal sheep serum. Since the lipid content of foetal blood is considerably less than that of the maternal blood [data for human: Slemmons & Stander, 1923], and since Macheboeuf [1929], Macheboeuf & Sandor [1931] have stated that the lipid constituents of serum protein exert a considerable influence on osmotic pressure, it seemed desirable to investigate further the effect of lipids on the osmotic pressure of the serum proteins. Experiments were therefore undertaken to compare the osmotic pressure of serum before and after lipid

extraction. The chief problem regarding the extraction was to find a suitable method which would remove the lipids without denaturing the serum proteins.

The method of McFarlane [1942] seemed the most convenient for our purpose, the method being also of especial interest since it is used in preparing pooled sera for transfusion. The method consists of shaking the serum with ether and freezing the mixture by cooling it to below -25° , then thawing it. The ethereal layer which separates during thawing contains a large proportion of serum lipids, and by repeated extractions approximately three-quarters of the lipids can be removed. McFarlane showed by electrophoretic measurements that the only change that takes place in the serum proteins is the diminution of the β -globulins.

METHODS

We extracted fresh pooled human serum by McFarlane's method. The serum was shaken with ether, frozen and thawed five times. Since the method employed by us may