

Quality often depends upon harvesting the vegetables at the proper stage of development. All crops that need punctual harvesting fall within groups 3 and 4. During periods of inadequate labor or unfavorable weather, this is a further disadvantage of groups 3 and 4.

In the past, the importance of root, stem and leafy crops from a dietetic standpoint has been pointed out largely on the basis of individual crops. As Table 1 shows, high food value and efficient use of labor and land areas is a general characteristic of this group of vegetables. An average diet contains 739 pounds of plants out of a total consumption of 1,420 pounds of food. About a third of our plant food is vegetative

portions of vegetables; the remainder is vegetable, grain and tree fruits. Unfortunately, data are available to test this hypothesis only in our vegetable plants. Vegetables, despite their low energy and protein content, have praiseworthy qualities for improving our diet, whether they are produced commercially or in a home garden. The high efficiency of these plant parts as food calls attention to the fact that only in the case of vegetables do the American people consume a vegetative part of the plant.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE DESTRUCTION OF PYROGENS BY HYDROGEN PEROXIDE

PYROGENS are toxic, non-dialyzable substances formed by various micro-organisms. They are relatively stable in boiling water and cause prompt temperature rises in animals when injected in microgram doses. In man, Co Tui¹ estimates that an intravenous

for removing pyrogens are therefore of utility, and several such methods have been described. For example, Co Tui and Wright² have recommended adsorptive filtration with Seitz filters, although Francke and Rees³ found preliminary treatment with powdered charcoal before Seitz filtration to be required for complete removal of pyrogens from solutions of

TABLE 1
EFFECT OF HYDROGEN PEROXIDE TREATMENT UPON PYROGEN ACTIVITY

Pyrogen preparation	Source	Concentration of H ₂ O ₂	Heating		pH		Rectal temp. rises (°C.)			Average rise (°C.) ⁴
			Time (min.)	Temp. (°C.)	Before heating	After heating				
A ¹	<i>Pseudomonas aeruginosa</i>	0	30	100	6.58	6.66	1.25, 1.25, 1.60			1.37
		0.001 M	30	100			1.50, 1.55, 2.25			1.77
		0.01 M	30	100	6.82	6.75	0.80, 1.05, 1.10			0.98
		0.1 M	60	100	6.21	6.64	0.00, 0.20, 0.40			0.20
B ²	<i>Pseudomonas aeruginosa</i>	0	20	100	7.26	6.88	0.95, 1.40, 2.25			1.53
		0	120	100	7.28	7.02	0.70, 1.05, 1.40			1.05
		0.01 M	120	100	7.27	6.99	-0.10, -0.10, 0.15			0.05
		0.1 M	120	100	7.33	6.88	-0.15, -0.05, 0.35			0.12
C ³	Gelatin	0	120	100	7.20	7.19	0.95, 1.10, 1.10			1.05
		0.1 M	120	100	7.03	6.51	0.25, 0.60, 0.80			0.55
		0.1 M	120	100	7.16	5.12	-0.25, -0.10, 0.00			0.00
D ³	Gelatin	0	20	116	...	...	0.60, 0.75, 0.85			0.73
		0.04 M	20	116	...	...	0.00, 0.15, 0.20			0.12

¹ Pyrogen solution prepared by U. S. Food and Drug Administration for First Collaborative Assay (H. Welch, H. O. Calvery, W. T. and McClosky and C. W. Price, *Jour. Am. Pharm. Assoc.*, 32: 65, 1943), diluted 1:10 with 0.9 per cent. NaCl solution.

² Dried concentrated pyrogen from *Pseudomonas aeruginosa*, kindly supplied by Dr. Henry Welch, U. S. Food and Drug Administration dissolved in 1:100,000 dilution in 0.9 per cent. NaCl solution buffered with sodium phosphate.

³ Commercial gelatin, in approximately 5 per cent. solution in distilled water.

⁴ Negative values considered as zero temperature rise, in calculating averages.

dose of 0.02 micrograms of typhoid pyrogen per kilogram of body weight will provoke a rise in body temperature of 0.5–0.6° C.

The necessity for the complete absence of pyrogens from solutions intended for parenteral administration is well recognized,² but many substances injected parenterally, especially in investigative work, are not available in pyrogen-free form. Practicable methods

for removing pyrogens are therefore of utility, and several such methods have been described. For example, Co Tui and Wright² have recommended adsorptive filtration with Seitz filters, although Francke and Rees³ found preliminary treatment with powdered charcoal before Seitz filtration to be required for complete removal of pyrogens from solutions of

inulin. Seitz filtration was also found unsatisfactory for removing pyrogens from enzymatic hydrolysates of protein by Zittle *et al.*,⁴ who resorted to heating with acid to destroy the pyrogens.

Since no satisfactory general method for removing pyrogens from all solutions appears to have been found, our observation that pyrogens can be destroyed by heating with dilute hydrogen peroxide may prove

³ D. E. Franke and V. L. Rees, *Jour. Am. Pharm. Assoc. (Pract. Pharm. Ed.)*, 4: 158, 1943.

⁴ C. A. Zittle, H. B. Devlin, G. Rodney and M. Welcke, *Jour. Lab. Clin. Med.*, 30: 75, 1945.

¹ Co Tui, D. Hope, M. H. Schrifft and J. Powers, *Jour. Lab. Clin. Med.*, 29: 58, 1944.

² Co Tui and A. M. Wright, *Ann. Surg.*, 116: 412, 1942.

useful for specific applications. The action of hydrogen peroxide might also be utilized in the study of the chemical nature of pyrogens.

Pure pyrogens have not yet been isolated and their chemical structure has therefore not been fully established, but the properties of partly purified preparations have received considerable study.^{1,5-7} The evidence indicates that pyrogens are neither proteins nor protein split-products and that highly purified preparations may contain no nitrogen whatsoever.⁷ Purified preparations exhibit the properties of polysaccharides which can be hydrolyzed to reducing sugars.

The effect of oxidizing agents upon pyrogens appears not yet to have been studied, although in 1930 Carter⁸ proposed heating with dilute permanganate solution as a test for the presence of pyrogens in water, on the theory that pyrogens are oxidizable bacterial products. Although the permanganate test has since been proved totally inadequate, because it is not specific and is insufficiently sensitive, Carter's surmise that pyrogens can be readily oxidized may be correct. In 1912, Hort and Penfold⁹ observed that if centrifuged cells of *B. typhosus* were washed with hydrogen peroxide, their injection no longer produced the fever which followed injection of similar cells washed with water. Presumably the pyrogens in the cells were destroyed by the peroxide, but the concentration employed was not stated.

In the course of an investigation of plasma substitutes, one of us (D. H. C.) observed that pyrogenic solutions of gelatin were rendered non-pyrogenic by heating with potassium permanganate or hydrogen peroxide. This effect was studied further using two different preparations of partially purified pyrogens in addition to two lots of pyrogenic gelatin. The pyrogen content was estimated by intravenous injection, into each of three rabbits, of 10 cc of solution per kilogram of body weight. Control rectal temperatures were measured within 30 minutes prior to the injection and the temperatures were again measured 1, 2 and 3 hours after the injection. A rise of 0.6° C. or more is regarded as positive indication of the presence of pyrogens.^{1,4}

The results summarized in Table 1 show that no significant rise in temperature followed injection of pyrogenic solutions which had been heated at 100° C. for 60-120 minutes in the presence of 0.1 M-hydrogen peroxide, whereas control solutions heated without peroxide caused rises of 0.60-2.25° C. Even at 0.01 M

concentration, the peroxide caused a decrease in the temperature response. That the change effected by the peroxide was not due to alteration of pH is shown by the results for Preparation B, the solutions of which were all at essentially the same pH.

On the basis of these results, it is suggested that treatment with hydrogen peroxide might be of practical use in rendering solutions non-pyrogenic, where the peroxide does not adversely affect other constituents of the solution and where the amount used is sufficient to destroy the pyrogens present without leaving a deleterious excess.

Further work on the chemistry of the oxidation reaction is being planned for as soon as reasonably pure pyrogenic material is available.

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STIRRER BEARING FROM BROKEN HYPODERMIC

MOST chemists have felt the need of a device to supplant the clumsy and frequently inadequate mercury seal stirrer. It has been found possible in these laboratories to eliminate almost completely the use of such seals by the use of a bearing constructed from a hypodermic syringe. The method of utilization is to cut off both ends of the plunger and the closed end of the barrel. The barrel is then inserted in the rubber stopper and acts as the outer bearing. The stirrer shaft is then passed through the converted plunger and sealed into it either by means of rubber tubing or a small stopper, depending on the relative size of the syringe and stirrer shaft. A light lubricant such as vaseline or glycerine should be used. Under these conditions, the bearing may be used with a relatively high-speed stirrer and has proved adequate for pressures as low as 6 mm.

Even where it is necessary to purchase a new syringe for this purpose, it will be found to be a worthwhile investment, as the syringes are usually stoutly constructed and quite durable. However, by arrangement with a hospital a more than adequate supply of damaged or defective syringes suitable for this purpose usually may be obtained.

No credit is claimed here for originating this device as it appears to have been used for some time in other laboratories. Inquiry has shown, however, that knowledge of it is extremely limited, and there appears to be no mention of it in the literature. Hence we feel that the publication of its description should serve a useful purpose.

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¹ H. M. Banks, *Am. Jour. Clin. Path.*, 4: 260, 1934.

⁵ E. Centanni, *Deut. Wochschr.*, 66: 263, 1940.

⁷ E. S. Robinson and B. A. Flusser, *Jour. Biol. Chem.*, 153: 529, 1944.

⁸ E. B. Carter, *Jour. Lab. Clin. Med.*, 16: 289, 1930.

⁹ E. C. Hort and W. J. Penfold, *Jour. Hygiene*, 12: 361, 1912.