tionally high values in content of vitamin C when the latter was estimated by the method of Tillmans[1] and as perfected by others.[2, 3, 4, 5]

Leaves of trees found in the wild run equally high in vitamin C as those from trees of named varieties.

TABLE 1

Variety	Milligrams of Vitamin C per kilogram of leaves		Milligrams of Vitamin C per kilogram of fruit	
	Fresh green leaves	Leaves recently dried in a Bussler oven	Green fruit	Ripe fruit
Early Golden.	32,500	40,700	3,000	1,050
Silkeline ...	20,300		3,800	950
Lucinda	22,700			
Miller	26,900	28,500	2,500	
Ruby	30,600	40,900	3,700	
Wild	32,800	38,000	2,100	
Wild	25,000	41,500	2,500	
Wild	27,100	25,500		

The fresh leaves seem to have about ten times the vitamin C concentration of the fruit. Leaves picked and held in the dried condition since October 9, 1940, still retained about one tenth of the original titratable material.

A tea made from green leaves was very acceptable, after the addition of a little sugar, as was also that made from leaves dried in a Bussler oven at 140° F. for 18 hours, with the fan on the entire time. In drying the leaves lost 58 per cent. of their weight and were quite brittle when removed from the oven.

The tea was made in the orthodox way by steeping the finely divided leaves in a cheese-cloth bag or ball for five minutes in water, after the latter had been brought to a boil. The flavor of the tea was similar to sassafras tea, and in color and general appearance it was much like a light-colored tea from tea leaves. About 60 per cent. of the titratable material in the original dried persimmon leaf was in the tea. There was about one third as much titratable material in the tea from green leaves as that from dried leaves. The titratable material in *tea* from *tea* leaves was about one per cent. of that in tea from the same weight of dried persimmon leaves.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SEROLOGICALLY ACTIVE POLYSACCHARIDE FROM TRICHINELLA SPIRALIS

A POLYSACCHARIDE, which gives immunologically specific reactions when tested on Trichinella-infected rabbits has been obtained in a reasonably pure chemical condition. The methods used were similar to those employed for the studies of polysaccharides from Ascaris[1] and other parasitic helminths.[2, 3]

The worms were liberated from the host tissue (hog) by peptic digestion of the infected muscle and washed with sterile saline until free of debris. The cleaned material was then rapidly frozen in a dry-ice bath and lyophiled. The dehydrated material was finely ground and suspended in 20 volumes of pH 8.0 buffer, placed in a boiling water bath and stirred vigorously with a mechanical stirrer. After 30 minutes the mixture was removed, cooled and centrifuged. The residue was washed once and the solution added to the original extract. The polysaccharide along with other soluble worm materials was then precipitated by the addition of 5 volumes of chilled 95 per cent. ethanol. In order to facilitate precipitation enough NaCl was added to give a concentration of approximately 0.5 per cent. before the addition of ethanol. After 4 hours at 4° C. a white gummy precipitate formed which was removed by centrifugation and carefully resuspended in approximately 20 volumes of pH 4.6 acetate buffer. The material which failed to redissolve was removed and discarded and the solution, which contained mostly polysaccharide, adjusted to pH 8.0 by the addition of 1.0 N sodium carbonate solution. The polysaccharide was again precipitated by the addition of sodium chloride solution and 2 volumes of 95 per cent. ethanol. Reprecipitation at pH 8.0 and redissolving at pH 4.6 was repeated until a preparation was obtained which failed to give the usual tests for protein and was completely soluble at pH 4.6 after alcohol precipitation. Five to eight treatments were required to obtain such a product. The polysaccharide was finally precipitated with ethanol and dried with several changes of absolute ethanol and ether. Approximately 0.3 gram of polysaccharide was obtained from 3.0 grams of whole worm material.

The resulting product was a fine white powder which readily dissolved in water giving an opalescent

[1] J. Tillmans and P. Hirsch, *Biochem. Zeits.*, 250: 312, 1932.
[2] O. A. Bessey and C. G. King, *Jour. Biol. Chem.*, 103: 687, 1933.
[3] H. Dick, Dissertation, Frankfurt, 1932.
[4] C. H. Knight, R. A. Dutcher and N. B. Guerrant, SCIENCE, 89: 183, 1939.
[5] N. C. Thornton, *Contrib. Boyce Thompson Inst.*, 9: 273, 1938.
[1] D. H. Campbell, *Jour. Infect. Dis.*, 59: 266, 1936.
[2] *Ibid.*, 65: 12, 1939.
[3] *Ibid.*, *Jour. Parasitol.*, 23: 348, 1937.

solution in low dilutions and failed to diffuse through Cellophane membranes. It gave a Molisch reaction in extremely high dilutions but no protein tests in relatively high concentrations. Test for nitrogen by Nessler's method on a 10 mgm sample was negative. No reducing property was observed before acid hydrolysis but rapidly appeared on treatment with 1.0 N HCl solution. Its precipitating and skin reactive properties were not affected by autoclaving for 15 minutes at 15 pounds pressure at pH 7.0.

Serological studies indicated that the polysaccharide was a good precipitating antigen. It gave typical polysaccharide plaque-like precipitates in dilutions of 1: 200,000 when tested against sera from infected rabbits. Cross precipitin reactions did not occur with antisera against other roundworms such as *Ascaris suum, Nippostrongylus muris* or the larval tapeworm, *Cysticercus taeniaeformis*. Specificity studies with respect to the more closely allied forms such as *Trichuris* are being made at present. Although positive skin reactions were obtained in infected rabbits, the preliminary studies indicate that approximately 0.1 to 1.0 mgm of material is required to give a good reaction.

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A SPORULATION STOCK MEDIUM FOR YEASTS AND OTHER FUNGI

MOST yeasts do not sporulate freely on the commonly used stock media such as wort-agar or grape juice agar. Carrot, beet, cucumber and potato wedges, gypsum blocks, Gorodkowa slants and other media are used to induce ascospore formation. Many yeasts sporulate on carrot wedges, but some do so only on one of the other media indicated above. During the past eight months it has been observed that agar slants made from a water extract of carrots, beets, cucumbers and potatoes will induce sporulation and at the same time serve as an excellent stock culture medium. The medium is prepared by grinding equal weights of washed, but unpeeled carrots, beets, cucumbers and potatoes and then mixing with a quantity of water equal to the total weight of the vegetables used. The mixture is autoclaved at 10 pounds pressure for 10 minutes, after which the extract is separated from the solid material by use of cheese-cloth and pressure. The pH value of the extract is approximately 5.7 and the Balling degree about 4. Two per cent. of agar is added to the extract and slants are prepared. The sterilization recommended is 15 pounds for 15 minutes.

Good sporulation has been obtained on this medium within 7 days or less with several hundred yeast cultures representing species of *Schizosaccharomyces, Endomycopsis, Saccharomyces, Zygosaccharomyces, Pichia, Zygopichia, Hansenula, Zygohansenula, Debaryomyces, Schwanniomyces, Saccharomycodes, Hanseniaspora, Nadsonia* and *Nemataspora*. By using vegetable agar Roberts[1] has been able to confirm some of the observations made by Windisch[2] concerning the sporulation of *Torulopsis pulcherrima*, the type species of a non-sporulating genus. Vegetable agar contains no added nutrients. Sufficient carbohydrates, nitrogenous substances, minerals and accessory factors are present, however, to support an excellent growth and good sporulation. When used as a stock culture medium, it offers the advantage of always having available sporulating yeasts. It is reasonable to believe that the use of vegetable agar for stock cultures should retard or eliminate the loss of sporulating ability which occurs commonly when yeasts are held in culture for long periods of time.

A limited number of trials indicate that other fungi also grow well on this medium and some show a strong tendency toward increased conidium production.

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[1] Roberts, *Phytopath.* (Abs. in press).
[2] Windisch, *Archiv. f. Mikrobiol.*, 9: 551, 1938; *ibid.*, 11: 368, 1940.

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