

solution containing 0.25 per cent borax delayed and decreased cracking, with the result that some 50 per cent of the fruits were still sound after 48 hours.

Immersion trials were conducted with Bing cherries early in July 1946. In water, 80 per cent cracked in 16 hours and 100 per cent in 64 hours; with stems or only the leaves dipped in water, none cracked. Therefore, water absorption is through the skin. This caused gain in volume and weight. At the red stage of ripening, 100 per cent cracked in 16 hours. When fully ripe, 80 per cent of the nearly black fruits were cracked by immersion for 64 hours.

Solutions of 0.25–0.01 per cent anhydrous copper sulfate completely prevented cracking for four days, while those containing 0.25 per cent fructose, table sugar, sodium chloride, sodium oxalate, zinc sulfate, aerosol, or pretreatment with aerosol or calcium propionate, had little effect.

Pretreatment for 30 minutes with sulfur or calcium hydroxide, followed by immersion in water, decreased cracking somewhat. In two days immersion after the pretreatment with sulfur, 88 per cent were cracked. Pretreatment for 30 minutes with 0.1 per cent calcium hydrate reduced cracking to 16 per cent; that with 0.1 per cent copper sulfate, to 2 per cent. Continuous submergence of ripe Royal Ann cherries in the lime solution resulted in cracking of 16 per cent of the fruit in three days and 24 per cent in four days, while in the same strength of copper sulfate there were no cracked fruits at the end of four days.

Microorganisms in relation to cherry crack. Cracked cherries were removed from the solution after 24 hours; sound fruit and additional cherries developing cracks were withdrawn at 48 hours. Bacteria, yeasts, and molds were determined on 1 per cent dextrose agar. In the case of cracked fruit the cracks and adjacent skin were swabbed with a loop and streaked on poured plates. Sound fruit was rolled directly on the agar surface. In most cases the number of colonies was sufficiently restricted to permit counting, but with the Royal Anns confluent growth in many cases permitted only a rough estimate. Colony characteristics and confirmatory microscopic examination were used to differentiate spore formers (*Bacillus* sp.), micrococci, flavobacteria, and yeast. While the results are not rigidly quantitative, they present an index of the relative abundance of the various groups determined.

Numbers and kinds of microorganisms apparently are not related to cracking; flora of cracked fruit was found to be quantitatively and qualitatively similar to that of the sound cherries.

No molds developed on cracked fruit after removal from the solutions and maintenance at room temperature (25° C.) until shriveled (four or five days); neither was there any macroscopic development of bacteria or yeast.

After three to five days mold and bacterial growth appeared on all solutions except CuSO₄ above 0.01 per cent concentration. Bacteria but no molds developed on the 0.01 per cent CuSO₄ solution. Sound cherries left in the solutions after 48 hours remained sound at the close of the five-day observation period.

Trials with protective sprays. Pretreatment of very ripe Royal Ann cherries with 0.1 per cent copper sulfate spray and then repeated spraying caused only 1 per cent cracking in fruit; the water spray without pretreatment caused 6 per cent. Further copper tests with prunes and related pressure

tests indicate bearing strength increased. It appears probable that 0.1 or 0.05 per cent anhydrous copper sulfate can be included in the cherry fruit fly spray or 3 per cent with the sulfur-containing dust for prunes and applied early in the morning.

Microscopic examination. Examination of skins of fruits after 48 hours submergence indicates that water increased turgidity of cells compared to that from lime, while plasmolysis was more in evidence after the copper treatment. In all cases there was a gain in weight during immersion.

Discussion and conclusion. Copper sulfate has given control of cracking of fruit in immersion tests, and preliminary spraying tests indicate that its use as a spray or dust will check cracking of fruit due to rains. The benefit reported from use of Bordeaux spray (3) appears to be due more to the copper than the calcium contained. Bordeaux reportedly increases transpiration (1) and may decrease fruit size.

How copper sulfate solution functions to prevent cracking of fruit is not fully determined. Cracking after rain has been related to osmotic concentration of the fruit juice, turgor of fruit, temperature, and skin permeability (4). Possibly the fruit cells are affected so less water is absorbed or held. The copper sulfate is effective at too low a concentration to have any appreciable osmotic effect. No specific fungi or other causative organism was found. It now appears more probable that the copper sulfate has a toughening effect (2) on the fruit skin comparable to that of tannin on leather.

It appears that the solution or spray, to be effective and noninjurious, should have a concentration of 0.1 to .01 per cent. Perhaps this material can be included in the cherry fruit fly spray or early morning dusting trials. Dilution of anhydrous copper sulfate with diatomite or other fine inert powder is suggested.

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Methionine Metabolism and A-Aminobutyric Acid

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A-amino-n-butyric acid has been identified by paper chromatography in the urine of a case of Fanconi syndrome (1).

It also occurs in appreciable amounts in normal blood and urine and has been found in a dilute acetic acid extract of yeast. It would appear to be very generally distributed in tissue nonprotein nitrogen.

On giving methionine (10 grams) by mouth, an increased output of α -aminobutyric acid and of methionine sulfoxide, as well as of methionine, can be detected in the urine, the overflow of all three following a similar course. This has been seen in a normal subject but was much more obvious in a case of Fanconi syndrome, in which "renal" aminoaciduria occurs.

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The conclusion is drawn that the methionine can be metabolized to α -aminobutyric acid and that it is probably the main source of the latter, since it does not, of course, occur in the protein of the diet. The fate of the carbon chain of the former now seems clear. A reinvestigation of the possible role of α -aminobutyric acid in the body is indicated. If its presence is essential, methionine may have to be wasted in order to produce it, and, in that case, giving it to animals on a low methionine diet may exert a methionine-sparing action.

Methionine sulfoxide may be of significance in oxidation-reduction potential. It can oxidize cysteine to cystine *in vitro* (2). Methionine, on standing under various conditions, readily changes largely into the sulfoxide and may therefore act as an oxygen carrier. In view of this easy oxidizability, however, its presence in the urine after methionine feeding needs more careful checking. It may have been formed by aerial oxidation. The validity of procedures for the determination of methionine in urine by oxidation reactions (H_2O_2 , etc.) also arises.

Further details of these findings, which arose out of an investigation into the aminoaciduria in Fanconi syndrome, have been submitted to the *Biochemical Journal*.

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Failure to Produce Neoplasms in Rats by Feeding Heated Wheat-Germ Oil

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The announcement by Rowntree (2) in 1937 of the occurrence of neoplasms in Wistar rats following the ingestion of crude wheat-germ oil created considerable interest. The suggested relationship to diet as well as the production in experimental animals of malignant lesions of the gastrointestinal tract were factors of scientific importance. Attempts to duplicate these results were made in many laboratories, unfortunately without success (1). Since one of us (G. D.) had prepared some of the original wheat-germ oil used by Rowntree in his successful experiments, an attempt was made to duplicate his work, using oil prepared by the same operator and under the same laboratory conditions. Rowntree's diet was followed in detail, and Wistar rats were fed for almost a year. No neoplasms were produced (unreported work).

Some recent evidence has suggested that heated fats may contain carcinogenic factors possibly due to the conversion of sterols to carcinogens by heat (3). Wheat-germ oil is rich in sterols, and Rowntree, in the preparation of his diet, actually might have changed some of the sterols in the wheat-germ oil to carcinogens, since the oil was usually heated again before use to insure that no trace of ether smell remained.

In order to test this possibility, crude wheat-germ oil was prepared exactly as it had been previously. It was then

heated at 275° C. for two hours. Three liters of heated oil were mixed with 10 kg. of basic diet in accordance with Rowntree's directions. Thirteen female Wistar rats with an average weight of 64 grams and 14 male Wistar rats with an average weight of 65.5 grams were started on the diet in November 1945. The animals grew somewhat slowly, compared to colony rats, but the growth rate was steady and the animals appeared healthy. There were a number of deaths due to colds and pneumonia. The surviving animals were killed and autopsied in July 1946. Seven males averaging 253 grams and 5 females averaging 213 grams survived. The condition of all animals was excellent, with no suggestion of any malignant lesions.

This evidence would seem to indicate that the sterols in wheat-germ oil are not converted to carcinogens by heat. The heat conversion of sterols to carcinogens was apparently not a factor in Rowntree's production of gastrointestinal lesions in the rat.

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The Occurrence of Two Fertile Florets in the Spikelets of *Chloris*

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During the writer's experience in seed analysis Rhodes grass (*Chloris Gayana* Kunth) was frequently received for purity analysis and for germination test. Early examination of spikelets of this species disclosed that many contained a fertile pedicellate floret in addition to the fertile basal and sessile floret. The presence of caryopses was the criterion employed.

Thus far no confirmation of this observation has been found in the available grass and general floras examined. The seed analyst is perhaps the person best situated to make such an observation, since each of his many routine analyses requires the examination of thousands of individual seeds or seed units, but his usually elementary knowledge of taxonomy might lead him to overlook the significance of such a fact.

Descriptions of the genus *Chloris*, as far as the writer has been able to determine, appear to be unanimously in agreement on the occurrence of one fertile floret in the spikelet, and this the basal, sessile floret. For example, Hitchcock (1) states: "Spikelets with 1 perfect floret, sessile, . . . the rachilla disarticulating above the glumes, produced beyond the perfect floret and bearing 1 to several reduced florets consisting of empty lemmas. . . ." In no instance was there more than one floret in the spikelet described as fertile in the literature available. Silveus' description (3) is worded identically, and those in other floras are either essentially similar or identical.

As the average purity, or seed set of *C. Gayana* by weight, was found to be approximately 25 per cent (37 lots) (2), it may be assumed that the number of pedicellate florets matur-