trees, has resulted from recent studies by Chapman, Liebig and Parker.[4]

For many years investigators[5] in California have applied manganese to soil and to leaves of citrus trees in an effort to diagnose the cause of subnormal tree behavior. Results of concluded trials are not definite. Recently, however, responses to manganese treatments have been obtained on citrus trees in the Santa Clara River valley in southern California. Certain vigorous lemon trees in that area normally produce young leaves which are pale green in color with sharply defined green midrib and veins. Very pale green blotches, about 3mm in diameter, appear at random in interveinal spaces. Occasional terminal leaves are devoid of green color. Old leaves are frequently affected with a faint mottling. Spraying of the leaves of one limb of one of these trees with a concentrated solution of $MnSO_4$ in August, 1937, resulted in severe injury and repression of new growth. Some new growth appeared on this limb during 1939, however, and in August was considered normal. No improvement was noticed elsewhere. Liberal applications of $MnSO_4$ in 1937 to the soil about other trees in this area have not yet caused improvement.

In July, 1939, we inspected a lemon orchard in the same valley, in which the trees are subject to premature "decline." Recent foliage of trees in the beginning stages of decline showed typical symptoms of manganese deficiency, as recently determined.[4] Analysis of such leaves indicated a low manganese content. Treatment of several trees on August 2, 1939, by injection with C.P. $MnCl_2 \cdot 4\,H_2O$ solutions and crystals (3–8 gm per 3-inch limb), as well as by spraying with 1.25–1.5 per cent. solutions of this material, resulted in greening of leaves within 15 days. The sprays caused slight burning of tender leaves.

Subsequently, several hundred trees were treated by spraying and injection with corresponding effects. The results indicate a deficiency of manganese for citrus trees in this area. The relations of this condition to premature decline of fruit trees may be of importance.

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THE PRODUCTION AND UTILIZATION OF ALCOHOL BY PLANT TISSUES

SOME recent work in this laboratory on the metabolism of legume nodules and of legume and non-legume roots has led to results which apply to the general problem of the role of ethyl alcohol in plant respiration. Since these results have a broader application than merely to nodule metabolism, in connection with which they will be published in full, it seems worth while to give a brief résumé of them here.

The investigations were carried out partly by the Warburg manometric technique and partly by chemical analysis. The following results were secured concerning the production and disappearance of alcohol in the tissues: (1) Under anaerobic conditions alcohol and carbon dioxide were produced by both nodules and roots in proportions which indicate that alcohol was the chief unoxidized product. (2) Under aerobic conditions increasing amounts of alcohol in the medium reduced the respiratory quotient (R.Q.) of both tissues from about 1.00 (lower for roots) without alcohol almost to 0.67 (the theoretical R.Q. for complete oxidation of alcohol) with only a slight increase in oxygen consumption; and, as shown by chemical analyses, part of the alcohol disappeared. Added glucose increased oxygen consumption slightly. Under complete aerobiosis it did not affect the R.Q. if the latter was already approximately 1.00 but increased it under oxygen deficit or if it was already below 1.00. The effect of each substance occurred in the presence as well as in the absence of the other. (3) In nodules, either without added carbon source or with glucose, increasing the oxygen concentration led to increased carbohydrate breakdown, as shown by the CO_2 evolved and O_2 consumed; while in roots, without added carbon source or with alcohol, and in nodules with alcohol increasing the oxygen concentration led to decreased carbohydrate breakdown.

The following seems to be the most reasonable interpretation of these facts: In the absence of oxygen sugar is fermented to alcohol (chiefly) and carbon dioxide, and in the presence of oxygen both sugar and alcohol (if present) are oxidized competitively to carbon dioxide and water. Any oxidative resynthesis of the alcohol to fermentable compounds as the cause of its disappearance seems to be ruled out because of the apparent lack of any decrease in carbohydrate breakdown in tissues respiring carbohydrate chiefly, and because the R.Q. following the addition of increasing amounts of alcohol to the medium did not fall below that (6.67) characteristic of the complete oxidation of alcohol. (The R.Q. of an oxidative resynthesis is smaller than this.) In these tissues the presence of a sparing action of oxygen on carbohydrate consumed seems therefore to depend on the presence of enough alcohol (or similar fermentation product) to serve as a substitute for a considerable fraction of the carbohydrate.

[4] H. D. Chapman, Geo. F. Liebig and E. R. Parker, *California Citrograph*, 24: 454; 25: 11–15.

[5] L. D. Batchelor, E. R. Parker, G. Surr and R. W. Southwick, unpublished data.

Several investigators[1,2,3,4] have furnished evidence that alcohol can be oxidized by higher plants. However, the previously expressed opinion,[5] based on analogy with animal tissues, that alcohol is less quickly oxidized than sugar and the finding by Kostytschew[3] that it is not always oxidized seem to have dominated much of the later thought on the subject.[6,7,8,9] Partly as a result of this impression, the idea that alcohol is an intermediate in normal plant respiration has been largely abandoned. While the results discussed here clearly do not prove the hypothesis, they do seem to remove one weighty objection to it.

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THE EFFECTS OF A BEEF LIVER FRACTION UPON FAT SYNTHESIS IN RATS

WE have previously shown[1,2] that thiamin causes a synthesis of fat in rats and in pigeons. If a diet low in choline is used to study this effect of thiamin the amount of fat in the liver is increased markedly. Choline, subsequently supplied, exercises its well-known lipotropic action. In the course of an investigation of the effects of other members of the vitamin B complex upon fat metabolism we have employed a fraction of beef liver which contains several B vitamins, including pantothenic acid and factor W. Rats fed a fat-free diet devoid of the B vitamins for a period of three weeks lose considerable weight, and the amount of the fat in the body is greatly reduced. If the animals are then given small amounts of the liver fraction the body fat is increased, for example, from 3 to 7 per cent. and the liver fat from 3 to 17 per cent. The administration of choline, even in large dosage, will not cause the liver fat to be normal in amount, but this effect is secured in a few days by feeding a pancreatic extract, "lipocaic" (kindly supplied by Eli Lilly and Company). A reduction in liver fat may also be produced by feeding a concentrate from rice polish or by giving yeast; neither of these supplements is as effective as "lipocaic" when equal weights are used.

The liver fraction is prepared in the course of the manufacture of an anti-anemic extract from beef liver. The fraction effective in causing fat synthesis is secured by removing the alcohol from the 92 per cent. alcohol solution from which the anti-anemic fraction had been precipitated previously. Information is not available to indicate which constituent of the liver fraction is responsible for the increase in fat synthesis.

The effect of this liver fraction is not a toxic one, as might be produced by chloroform or carbon tetrachloride, since there is a coincident increase in body fat and a rapid gain in weight of the rats. Young animals double their weight in seven days and are active and healthy. We regard these observations as confirmation of the conclusions of Blatherwick and associates[3] that liver contains a water-soluble, alcohol-soluble substance which causes fatty livers when fed to rats. However, in their experiments there was no demonstration of fat synthesis such as we have secured.

Preliminary results show that the liver fraction causes a definite increase in cholesterol. The basal diet and the liver fraction are both free of cholesterol, and it seems likely that the increase in cholesterol may have been due to synthesis. "Lipocaic" not only reduces the total amount of fat in the liver but also diminishes the cholesterol. It is suggested that choline may be concerned with the reduction of neutral fat in the liver, while "lipocaic" possibly exerts its effect upon cholesterol esters. If this assumption is correct, "lipocaic" would not be expected to affect the fatty liver produced by thiamin other than would be anticipated from its choline content, as has been found by Best and Ridout.[4]

These results demonstrate that beef liver contains a substance which will markedly stimulate fat synthesis when fed to rats. The total amount of cholesterol in the liver is also increased in amount. The fatty liver thus produced is highly resistant to the lipotropic action of choline but readily responds to "lipocaic." A rapid means of assaying the potency of preparations of "lipocaic" is thus available.

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