consisting of yellow corn 76, wheat gluten 20, $CaCO_3$ 3, NaCl 1. Severe rickets was observed after 3–4 weeks on this ration. Fifty USP units of vitamin D was fed by mouth daily for 4 days before the administration of gallium. Healing of rickets was proceeding rapidly in the rats fed vitamin D, as evidenced by increased

TABLE 1

RADIOGALLIUM (Ga^{72}) CONTENT OF LONG BONES OF RATS 18–24 HR FOLLOWING SUBCUTANEOUS INJECTION OF GALLIUM (Ga^{72}) CITRATE

Treatment	Av body wt	No. of animals	Dose (mc/kg)	μc Ga^{72}/g dried bone		E/S† ratio
				Shaft	Ends	
Series A*						
Immature	108	5	.62	2.9	7.9	2.7
Adult	237	5	.56	2.6	7.1	2.7
Series B						
Adults	205	5	.46	1.4	3.8	2.7
Adults fed 100 units vitamin D	205	5	.46	1.4	3.6	2.6
Series C						
Rachitic rats	67	6	.56	3.7	5.3	1.4
Rachitic rats fed 200 units vitamin D	67	6	.56	3.9	7.5	1.9

* Albino rats. Series A of a different stock colony from series B and C.

† E/S ratio calculated from

$$\frac{Ga^{72} \text{ content of dried bone ends}}{Ga^{72} \text{ content of dried bone of shaft}}$$

bone ash and by a line test performed (*6*) on a part of the group. Those not receiving vitamin D remained severely rachitic.

Table 1 gives a résumé of the results of the studies outlined above. In series A, in which the animals were maintained on a good commercial stock diet, the gallium deposition in immature and adult rats was found to be essentially the same. The small difference observed is readily accounted for by the difference in dosage. These results indicate that neither age nor body weight of rats is a significant factor influencing the amount of gallium deposited in the long bones of the rat.

The animals used in series B were maintained on a stock diet compounded to contain a minimal amount of vitamin D necessary for normal calcification and growth. Normal calcification was indicated in the bone ash data (not shown here). In these rats, the administration of a large supplemental dose of vitamin D over a 4-day period prior to the injection of gallium failed to influence the deposition of this element in bone.

The data presented for series C demonstrate that subcutaneously injected gallium is readily deposited in the bones of severely rachitic rats. It is noteworthy that this deposition of gallium has occurred in bones where there was little or no concurrent deposition of calcium salts as indicated by bone ash determinations.

A marked difference in the distribution of gallium laid down in rachitic bone compared with its deposition in normal bone is clear, first from the amount of gallium in the shaft of the rachitic bone and, second, from the ratio of the gallium in the ends of the bone to that in the shaft (E/S ratio). In experiments not detailed here, it was invariably observed that the gallium content of rachitic shafts was higher than that of the shafts of comparable normal control animals. In normal animals the E/S ratio is 2.6 to 2.7, whereas in the rachitic femur it falls to 1.4.

When vitamin D was administered to rachitic rats for 4 days prior to the gallium injection, calcification was observed both by line test and by increased bone ash. The increase in gallium content of the shafts of these bones, over that of the untreated controls, was small and of questionable significance, but the increase in the ends of the bones was greater than 40%. This resulted in an increased E/S ratio, approaching that of the normal animals.

Other studies were designed to test the usefulness of Ga^{72} as a tool in the bioassay of vitamin D. The results indicate that the quantitative estimation of radiogallium in the bones of rachitic rats is not a reliable or sensitive indication of vitamin D dosage.

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On the Mechanism of Formation of Higher Alcohols During Alcoholic Fermentation

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It has long been accepted that the higher aliphatic alcohol fraction, so-called fusel oil, present in yeast-fermented media is formed by deamination and decarboxylation of amino acids (*1, 2*). This has been referred to as the Ehrlich mechanism by Thorne (*3*). It was further pointed out by Ehrlich (*1*) and Neuberg and Hildescheimer (*4*) that a requirement for higher alcohol formation from amino acids by yeasts was the simultaneous conversion of appreciable amounts of sugar to ethanol. However, fusel oil is not formed during fermentation of sugar by cell-free yeast preparations (*5, 6*). Although the mechanism of the conversion of carbohydrates to ethanol has been intensively studied, the nature of the relationship between it, the Ehrlich mechanism, and other biochemical processes during alcoholic fermentation is not clear.

In the course of studies on the influence of various factors on fusel oil formation in alcoholic fermentation of grape juice, data were obtained on the relationship between the course of fusel oil formation, multiplication of yeast cells, amino acid utilization, and conversion of sugar to ethanol. These data shed some additional light on the mechanism of the formation of fusel oil. The results reported here were obtained during the course of fermentation of a 60-gal batch of grape juice heavily inoculated with a pure yeast culture. The juice was obtained from freshly picked, crushed, and pressed French Colombard grapes, a "white" variety of *Vitis vinifera*. The juice contained 24% reducing sugar, and had a pH of 3.5 when inoculated with starter yeast. The yeast employed was *Saccharomyces cerevisiae* var. *ellipsoidus* (Montrachet strain; No. 522 of the Enology Laboratory Collection). The juice was permitted to ferment at room temperature (approx 20° C), with mechanical stirring only at moments of sampling. During the fermentation the temperature rose from 19.5° to a maximum of 29.5° C, reached between the 60th and 70th hr after inoculation, 15–20 hr after yeast multiplication ceased, then fell slowly to 19.5° C, as conversion of sugar to ethanol was completed.

Samples were taken at intervals, after stirring sufficient to uniformly suspend the yeast cells. Fusel oil was determined by a modification of the method of Penniman *et al.* (*7*). Ethanol was determined according to the A.O.A.C. method for alcohols in wines (*8*). Yeast cell count was done by the direct haemacytometer method, using a Levy-Hausser counting chamber. Isoleucine, leucine, and valine naturally present in the juice were determined by microbiological assay.

The data are shown in Fig. 1. They are typical of other pilot-scale experimental fermentations, in which the same yeast strain and juice of the same grape variety were used, the results of which will be reported in detail elsewhere. The points to be noted in connection with Fig. 1 are as follows:

1) The amounts of the amino acids, isoleucine, leucine, and valine diminished rapidly to low levels within 18–37 hr after starting the fermentation and thereafter there were only slight changes, in general a slow, steady increase.

2) Yeast cell multiplication ceased between 45 and 50 hr after the fermentation was started, whereas formation of ethanol and fusel oil continued up to about 170 hr.

3) The course of fusel oil formation was concurrent with that of ethanol production, rather than with amino acid disappearance or yeast cell multiplication. Fusel oil formation was not noticeably interrupted by cessation of either amino acid disappearance or yeast cell multiplication.

4) The greater part of the total amount of fusel oil was formed after both rapid yeast cell multiplication and rapid loss of certain amino acids had ceased.

Calculations based on the data of Fig. 1 indicate discrepancies between the utilization of the three

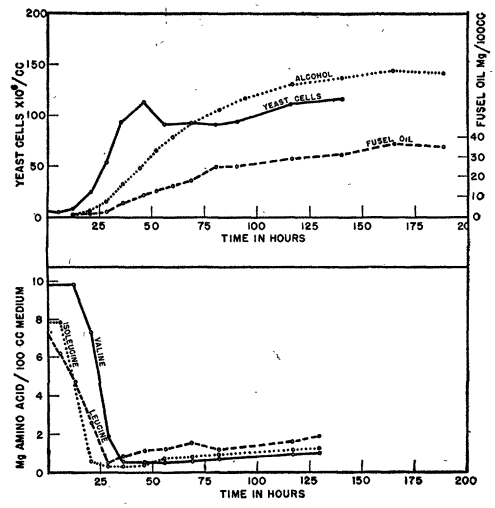

FIG. 1. The course of amino acid disappearance, yeast cell multiplication, and formation of ethanol and fusel oil during alcoholic fermentation of grape juice.

amino acids involved and the course of formation and final yield of fusel oil. At the point when the rapid decrease of valine, the last of the three amino acids to reach a low level, had ceased (37th hr), only about 34% of the theoretical yield of higher alcohols to be expected from them, if they had been completely transformed by the Ehrlich mechanism, had been formed. However, in the final stages of the fermentation, the amount of the higher alcohols found greatly exceeded the theoretical yield based upon the disappearance of the amino acids concerned. At the 168th hr the actual yield amounted to 255% of the theoretical. The calculations included only those higher alcohol components of fusel oil which could be formed from isoleucine, leucine, and valine by the Ehrlich mechanism. Analyses of the fusel oil fraction separated from grape wine by commercial distillation indicated that the major portion consists of these higher alcohols. Webb and Kepner (*9*) found recently that active amyl, isoamyl, and isobutyl alcohols account for about 88% of the higher alcohols of such a fusel oil sample.

It was previously assumed, on the basis of Ehrlich's work, and the subsequent interpretations of Neubauer and Fromherz (*2*), that deamination of amino acids furnished ammonia nitrogen for the synthesis of protein during yeast cell multiplication. In our experiments the failure of approximately equivalent amounts of the higher alcohol residues to appear concurrently with disappearance of their specific amino acid precursors might, at first sight, suggest that the Ehrlich mechanism was directly involved to only a slight extent in amino acid assimilation during yeast multiplication. The idea of direct assimilation by yeast of intact amino acids, earlier advanced by Thorne (*3*) on the basis of evidence not concerned with fusel oil formation could explain our findings. However, the later formation of large amounts of fusel oil is consistent with the idea of Ehrlich mechanism activity. Left un-

explained is the lag between apparent early deamination, incident to the rapid production of yeast protein, and the later appearance of the higher alcohols. This suggests that some intermediate steps between amino acid deamination and formation of the corresponding alcohols proceed more slowly than the earliest steps in assimilation. Neubauer and Fromherz (*2*) demonstrated that the most likely early intermediates were ketonic acids, and not hydroxy acids as previously supposed by Ehrlich (*1, 10*). These ketonic acids are later decarboxylated, according to Neubauer and Fromherz, to form aldehydes which are subsequently reduced to alcohols. These facts, together with the lag between amino acid assimilation and the appearance of fusel oil, the concurrence between ethanol and fusel oil formation, and the simultaneous cessation of both processes found in our experiments, suggest a simple explanation of the relationship between conversion of sugar to ethanol and the formation of fusel oil. It is likely that the final steps of both these metabolic processes make use of one or more identical enzyme systems. The slow appearance of the higher alcohols could be explained by competition between large amounts of sugar fermentation intermediates and small amounts of fusel oil intermediates, for the carboxylase and the hydrogen-transferring alcohol dehydrogenase-Coenzyme I systems of the zymase complex. When the supply of sugar fermentation intermediates which yield hydrogen to Coenzyme I failed because of the disappearance of sugar, the hydrogen transfer system could no longer reduce the aldehyde precursors of fusel oil components. Thus the formation of both ethanol and higher alcohols would simultaneously be terminated.

The appearance of the excess yield of fusel oil, which in our experiments greatly exceeded the theoretical yield based on the actual amount of leucine, isoleucine, and valine that disappeared, raised a question as to the source of precursors. A good possibility lies in the statements of Ehrlich (*1*) and Harden (*11*), that yeast can form fusel oil at the expense of its own protein when the extracellular supply of suitable organic nitrogenous nutrients is negligible. Freshly cultured yeast cells, when prevented from rapidly multiplying under conditions which still permit vigorous enzymatic activity, may be considered to be undergoing autolysis. Among the products of autolytic breakdown of yeast protein under such conditions there should be amounts of the specific amino acid precursors of fusel oil. There is some evidence for this in our experiments—namely, a measurable increase in the residual amounts of isoleucine, leucine, and valine in the medium during the stage of alcoholic fermentation subsequent to the cessation of rapid yeast multiplication (Fig. 1).

Other possibilities of accounting for the formation of fusel oil in excess of the theoretical yield have been considered, but such possibilities involve the abandonment of the Erhlich mechanism as a major factor in fusel oil formation. The writers prefer for the present to accept the Ehrlich mechanism, with the modifications suggested by Neubauer and Fromherz, as the source of fusel oil precursors, and the idea that the final steps are mediated by the carboxylase and hydrogen transfer systems of the zymase complex.

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Prevention of Postharvest Decay of Stone Fruits by Volatile Chemicals

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In-transit loss of peaches from disease is the limiting factor in the marketing of this crop. In spite of recommended field practices, brown rot losses in transit caused by *Monilinia fructicola* (Wint.) Honey[1] frequently exceed 10% of the crop value and greatly limit distribution.

In 1950, preliminary experiments were undertaken to investigate the possibility of using volatile chemicals to reduce the losses. Peaches and plums were inoculated artificially with a mixed spore suspension of *M. fructicola* and *Rhizopus* spp. These fruits were then incubated at 80° F and above 90% relative humidity for a 24-hr period prior to treatment with volatile chemicals. Replicated fruit samples were then placed in closed glass cylinders of 40-l capacity, inside of which the chemicals under test were introduced in open glass containers, permitting complete volatilization within the closed treatment chamber. After a 24-hr treatment period, the fruits were removed from the chemical atmosphere and were stored at laboratory temperature at a relatively high humidity for an additional period of 72 hr.

Trichloroethylene prevented all breakdown of the inoculated fruit and was effective at a concentration as low as 1:10,000 (1 vol nonvolatilized chemical in 10,000 vol atmos). No injury resulted to peaches and plums when exposed for 24 hr to concentrations of 1:4,000 and 1:10,000 of the chemical. Untreated con-

[1] In a recent publication (*Mycologia*, **37**, 648 [1945]), Whetzel recognizes the genus *Monilinia* Honey and thus treats this species as *Monilinia fructicola* (Wint.) Honey.