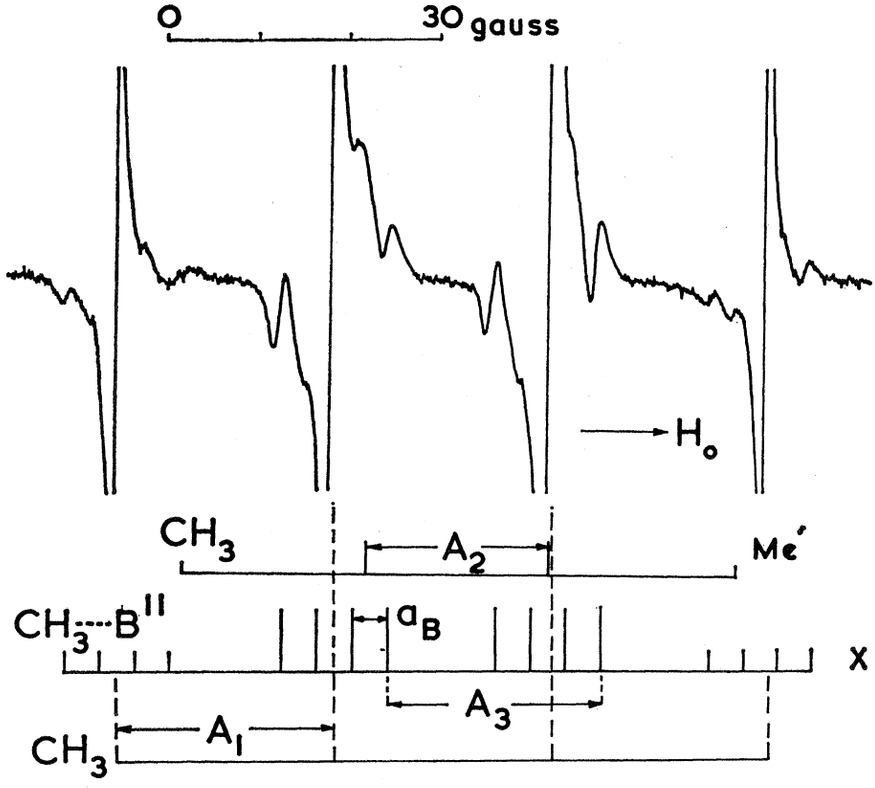

Fig. 1. Electron paramagnetic resonance spectrum of radicals from the photolysis of CH_3I on porous Vycor surfaces, preheated to 450°C for 4 hours. The surface coverage was 2 to 3 percent. Note that there is an indication of another type of CH_3 (Me'), which could be observed more clearly at early stages of irradiation.

an active site, may form a weak one-electron bond with the boron, giving rise to an additional four-line structure on each proton hyperfine line of CH_3. The observed hyperfine constants for such a radical X are $A_3 = 23.1 \pm 0.2$ gauss for the proton coupling, and the $a_B = 2.6 \pm 0.2$ gauss for the boron coupling constant. Note that the proton constant is slightly smaller than A_1, indicating a shift of a small amount of spin density to the B^{11} nucleus.

The above interpretation seems reasonable, judging from the catalytic property of silica-alumina in which aluminum impurities play essential roles. The maximum catalytic power is obtained when the surface of silica-alumina is dehydrated at about 450°C. One may thus conclude that some CH_3 radicals are interacting with such active boron sites.

A separate experiment was also carried out on a porous Vycor surface that was reacted with boron trichloride, and subsequently the B–Cl bonds hydrolyzed to B–OH and then dehydrated. The boron atoms thus introduced onto Vycor surfaces were presumably inactive, and electron paramagnetic resonance spectra of CH_3 showed no indication of boron interaction. It is also noted that there is no spectrum of Me' radicals in this case.

The presence of a boron hyperfine interaction in Vycor glass has already been demonstrated by Muha and Yates (*6*) in their papers on γ-irradiated Vycor. Therefore, our finding of a boron interaction in CH_3 spectra is not surprising.

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1 March 1967

Alkaline Phosphatase and Leucine Aminopeptidase Association in Plasma of the Chicken

Abstract. *Two electrophoretic variants of leucine aminopeptidase show direct association with two genetically controlled forms of alkaline phosphatase. Treatment of plasma with neuraminidase converted the faster-migrating form of both enzymes to slower-moving forms, but plasmas with slower-migrating forms were unaffected by this treatment. The two forms of the enzymes may be due to the presence or absence of a single gene controlling the attachment of sialic acid to the enzyme molecules.*

Alkaline phosphatase in the plasma of the chicken has two genetically controlled variants when examined by starch-gel electrophoresis (*1*). The observation that the gene controlling the faster-moving form (Ap^2) was dominant over the slower-moving form (Ap^1) has been confirmed (*2*). Continuing efforts to elucidate genetically controlled variations in plasma enzymes have shown that different molecular forms of leucine aminopeptidase also exist among plasma samples from chickens of inbred lines. Leucine aminopeptidase is defined as an enzyme capable of hydrolyzing 1-leucyl-β-naphthylamide-HCl (*3*). Zymograms (*4*) show two characteristic zones of activity within a single sample. A leading band appears uniform in all chicken plasmas. A second band is seen in one of two positions, one closer to the origin than the other.

Electrophoresis was carried out for both leucine aminopeptidase and alkaline phosphatase of the blood from 750 chickens from several inbred lines and closed populations. In all samples, the classification of the forms of leucine aminopeptidase corresponded to the classification of mobility of alkaline phosphatase; that is, all samples showing the fast form of leucine aminopeptidase also exhibited the fast form of alkaline phosphatase (Ap^2). To verify that the two staining patterns were the result of independent reactions, some gels were stained consecutively with both substrate mixtures to give a doubly stained zymogram (Fig. 1). These tests clearly showed that the two enzymes had similar but distinctly different locations after electrophoresis. The fast form of alkaline phosphatase was slightly anodal to the fast form of leucine aminopeptidase. As observed in

studies with alkaline phosphatase, the faster-moving form of leucine aminopeptidase appears to be controlled by a dominant gene. When plasmas from known heterozygotes (*1*) were stained for leucine aminopeptidase, they were indistinguishable from the zymogram of known homozygotes of the fast form. The variations in leucine aminopeptidase and alkaline phosphatase are apparently controlled by the same or closely linked genes in view of the fact that there were no exceptions to this observation in all samples tested.

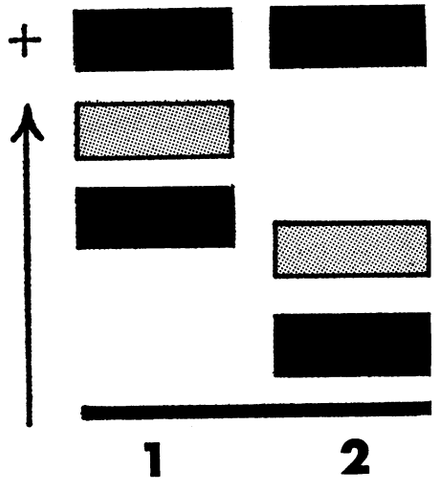

Fig. 1. Zymogram doubly stained for leucine aminopeptidase (solid) and alkaline phosphatase (cross-hatched) showing association of both enzymes in faster-migrating (1) and slower-migrating (2) forms. (Electrophoresis: 0.04*M* tris buffer adjusted to *p*H 8.6 with boric acid, 5 volt cm^{-1} for 16 hours. Staining: 1-leucyl-β-naphthylamide as substrate with fast black salt B as dye coupler, and α-naphthyl phosphate as substrate with fast blue RR as dye coupler in staining mixtures for both enzymes, respectively.)

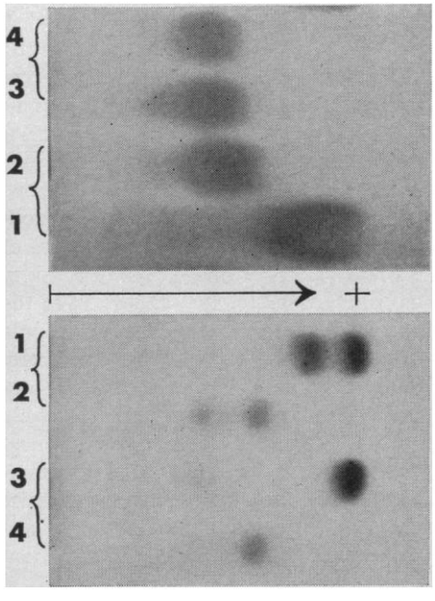

Fig. 2 (left). (Top) Zymogram stained for alkaline phosphatase. (Bottom) Other half of same gel stained for leucine aminopeptidase. Samples 1 and 2 are from the same plasma containing faster-migrating forms of enzymes; samples 3 and 4 are from the same plasma with slow forms. Samples 1 and 3 were untreated; samples 2 and 4 were treated with neuraminidase before electrophoresis (0.5 mg of neuraminidase per milliliter of plasma incubated 1 hour at 37°C).

To study further the similarities of these two enzymes, some samples of plasma were treated with neuraminidase (*5*) before electrophoresis. By visual observation of the staining process after electrophoresis, the rate of reaction between the substrates and the enzymes in the treated plasmas was not appreciably altered. However, the rate of migration of the fast form (Ap^2) of both enzymes was retarded to a rate indistinguishable from the slower-moving forms of both enzymes. Treatment with neuraminidase of a sample known to show the slow forms of both enzymes did not affect the rate of migration of those variants. The leading band of leucine aminopeptidase, which to date has not shown natural variation, was retarded in mobility in all treated samples regardless of whether they were of the faster- or slower-moving variants (Fig. 2).

Neuraminidase is a carbohydrase that splits off terminal sialic acid from mucoproteins and other substances (*6*); it has been used to characterize the different forms of leucine aminopeptidase found in man (*7*). Some electrophoretic forms were resistant to neuraminidase while others were retarded in mobility. Such differences after neuraminidase treatment were ascribed to the different sialic acid content of the various forms of leucine aminopeptidase in man.

In view of the specificity of neuraminidase, the faster-moving forms of these enzymes probably have a greater sialic acid content than the slower-moving forms. The slower-moving forms would lack much or all of the sialic acid. It is necessary to measure the sialic acid content of the enzymes. The genetic mechanism for the observed variations in both alkaline phosphatase and leucine aminopeptidase probably operates through a third enzyme system which is active during the synthesis of these complex enzyme molecules. Dominance can be explained by the presence or absence of a gene controlling the attachment of sialic acid units to both leucine aminopeptidase and alkaline phosphatase.

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3 April 1967

Lack of End-Product Inhibition and Repression of Leucine Synthesis in a Strain of Salmonella typhimurium

Abstract. *Mutants of* Salmonella *have been isolated which lack either end-product inhibition or repression of leucine biosynthesis. In a minimal salts-glucose medium, growth is not impaired by the lack of either control mechanism alone. The loss of both control mechanisms, however, leads to a 43 percent reduction in cell yield and a 1.5-fold reduction in the growth rate.*

The production of leucine by *Salmonella typhimurium* is normally restrained by the concerted functioning of two control mechanisms, end-product inhibition and end-product repression. In this laboratory, we have isolated a number of mutant strains of this organism which can no longer efficiently control the production of leucine (*1*). These mutants were isolated by their resistance to 5,5,5-trifluoro-DL-leucine (*2*), an analog of leucine and a potent inhibitor of the growth of *S. typhimurium*. Inhibition of growth caused by this analog is specifically reversed by small amounts of leucine, and it thus seems likely that the analog acts by interfering with the production or utilization of leucine.

The properties of two fluoroleucine resistant (FLR) mutants, *flr*-19 and *flr*-191, and their parent, LT-2 *ara*-9 are compared in Table 1. In contrast to the parent, these two mutants excrete a small amount of leucine as judged by auxanographic tests. One of the two, *flr*-19, has high constitutive