Enzyme fractions from liver and heart, which contain the enzymes catalyzing reactions (1) and (2), form citrate from crotonyl-S-CoA when supplemented with DPN$^+$, CoA-SH, oxalacetate, and crystalline citrate condensing enzyme. In a typical experiment with an ox liver fraction (0.49 mg. protein) and about 0.45 μM., crotonyl-S-CoA, 0.55 μM. citrate were formed in 40 minutes at 25° by the complete system. No citrate was formed in the absence of DPN$^+$ or crotonyl-S-CoA, or when crotonate replaced crotonyl-S-CoA. Crotonyl-S-CoA was synthesized by reaction of crotonic anhydride and CoA-SH after the procedure of Simon and Shemin[8] for the synthesis of succinyl-S-CoA.

Pigeon liver extracts also contain an enzyme(s) capable of forming β-hydroxybutyryl-S-CoA (or crotonyl-S-CoA) from *d,l*-β-hydroxybutyrate (or crotonate), ATP, and CoA-SH[9] and, with the above conditions, form citrate when the acetyl donor is either crotonyl-S-CoA or a mixture of *d,l,*β-hydroxy butyrate (or crotonate), ATP, and CoA-SH.

The coupling of reaction (4) with reaction (1) to give reaction (5) can be followed spectrophotometrically at *p*H 8.1 (Fig. 1) either by the increase in light absorption at λ = 310 mμ, due to the formation of acetoacetyl-S-CoA,[2] or by the increase in absorption at λ = 340 mμ, due to the formation of DPNH. On addition of CoA-SH (arrows 1 and 2), the absorption at 310 mμ decreases while that at 340 mμ increases due to the additional occurrence of reaction (2) which shifts the equilibrium of the system to the right. On addition of L-malate and citrate condensing enzyme (arrows 3 and 4) there is further reduction of DPN$^+$ because of the occurrence of reaction (6) catalyzed by malic dehydrogenase together with the citrate condensing enzyme.[10] Sufficient malic dehydrogenase is present in the ox liver fraction. The equilibrium of reaction (6) favors citrate formation so that the equilibrium of the system as a whole is shifted farther to the right.

$$(5)\quad \text{Crotonyl-S-CoA} + H_2O + \text{DPN}^+ \rightleftharpoons \text{acetoacetyl-S-CoA} + \text{DPNH} + H^+$$

$$(6)\quad \text{Acetyl-S-CoA} + \text{L-malate} + \text{DPN}^+ \rightleftharpoons \text{citrate} + \text{CoA-SH} + \text{DPNH} + H^+$$

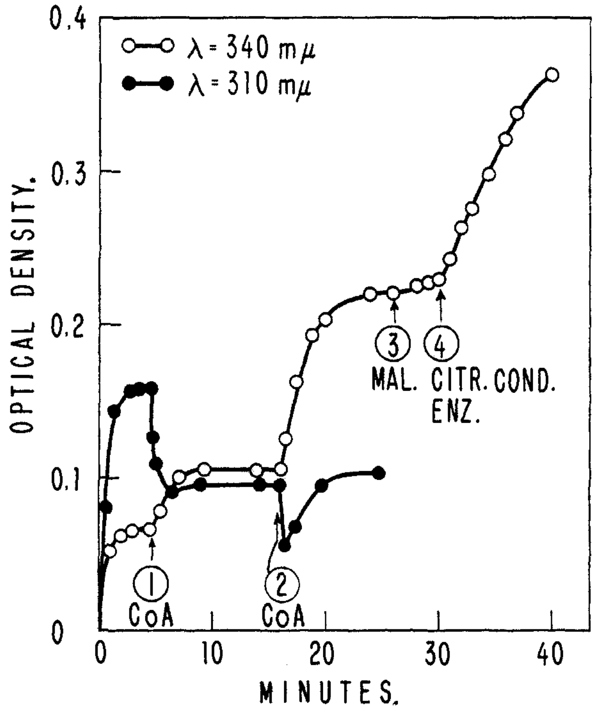

Fig. 1.—The experimental cell (d = 0.5 cm.) contained initially 100 μM. TRIS–HCl buffer, *p*H 8.1, 8 μM. MgCl$_2$, about 0.24 μM. crotonyl-S-CoA, and 0.27 μM. DPN$^+$. Crotonyl-S-CoA and DPN$^+$ were omitted from the control cell. The reaction was started by adding ox liver fraction (0.49 mg. protein). This was followed by CoA-SH (0.03 and 0.08 μM.), potassium L-malate (5 μM.) and crystalline citrate condensing enzyme (30 μg.) as indicated. Volume was 1.5 ml.; temp., 25°. The increase in light absorption at 310 mμ after the second addition of CoA–SH is due to the formation of DPNH, which absorbs also at this wave length.

When crotonyl-S-CoA (but not crotonate) is incubated with ox liver fractions (free of crotonyl-S-CoA deacylase) there occurs a decrease in light absorption at λ = 240 mμ due to the hydration of the —C=C— bond.[11] Crotonyl glutathione and crotonyl thioglycolate are not hydrated when so tested.

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[8] E. J. Simon and D. Shemin, THIS JOURNAL, in press.

[9] J. R. Stern, I. Raw and A. del Campillo, unpublished.

[10] J. R. Stern, S. Ochoa and F. Lynen, *J. Biol. Chem.*, **198**, 313 (1952).

[11] E. Racker, *Biochim. Biophys. Acta*, **4**, 211 (1950).

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COPOLYMERIZATION OF BENZENE WITH VINYL ACETATE

Sir:

Polymerization of vinyl acetate is retarded in all aromatic solvents, including those without alkyl side-groups such as chlorobenzene[1], ethyl benzoate[2] and benzene itself.[1–4] In view of the known high reactivity of phenyl radicals,[5] an explanation based on the conventional type of transfer reaction

$$R^\cdot + C_6H_6 \longrightarrow RH + C_6H_5^\cdot \qquad (1)$$

is unattractive, but a hydrogen-atom transfer in the reverse direction

$$RCH_2CH(OAc)^\cdot + C_6H_6 \longrightarrow RCH{=}CHOAc + C_6H_7^\cdot \qquad (2)$$

would yield a cyclohexadienyl radical considerably less reactive than a vinyl acetate radical.

However, even reaction (2) fails to account for the observations, since inconsistencies are encountered in the kinetic analysis. Briefly, the difficulty is that the apparent transfer constant, as obtained from molecular-weight measurements, is far too low to account for the observed retardation and at the same time to keep the order of the

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[1] G. M. Burnett and H. W. Melville, *Disc. Faraday Soc.*, **2**, 322 (1947).

[2] J. T. Clarke, unpublished measurements in this laboratory.

[3] A. C. Cuthbertson, G. Gee and E. K. Rideal, *Proc. Roy. Soc. (London)*, **A170**, 300 (1939).

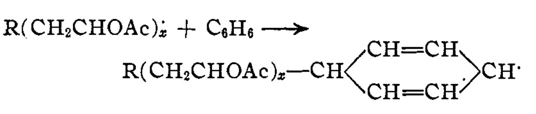
[4] S. Kamenskaya and S. Medvedev, *Acta Physicochimica U.R.S.S.*, **13**, 565 (1940).

[5] D. H. Hey and W. A. Waters, *Chem. Revs.*, **21**, 169 (1937).

reaction with respect to initiator at its observed value, which is[1,4] scarcely greater than 0.5.

Using great care in purification of materials, we have again measured rates of vinyl acetate polymerization in benzene at 60° and the molecular weights of the polymers produced, with the same anomalous results. To fit the observed rates, we require the transfer constant to benzene to have a value $C = 2.5\ (\pm 0.5) \times 10^{-3}$, while the molecular weights lead to $C = 1.2 \times 10^{-4}$. It would of course still be formally possible to attribute the effect to an unusual type of impurity, but this would merely shift rather than relieve the burden of explanation.

These results require the existence of a reaction which can retard the polymerization without decreasing the molecular weight, namely, the *addition* of a radical to the aromatic nucleus



$$R(CH_2CHOAc)_x^{\cdot} + C_6H_6 \longrightarrow R(CH_2CHOAc)_x{-}CH\langle {}^{CH=CH}_{CH=CH} \rangle CH^{\cdot} \quad (3)$$

This reaction accounts for the retardation as well as does reaction (2), and if the resulting radical can add to monomer

$$R(CH_2CHOAc)_xC_6H_6^{\cdot} + CH_2{=}CHOAc \longrightarrow R(CH_2CHOAc)_xC_6H_6CH_2CHOAc^{\cdot} \quad (4)$$

a self-consistent explanation of all our results can be achieved.

We have shown that reaction (3) actually takes place with about the required frequency, by preparing a sample of low-conversion polyvinyl acetate at 60° in the presence of C^{14}-labelled benzene,[6] using a mole ratio 10.9 of benzene to vinyl acetate. This polymer contained radioactive carbon which could not be removed by repeated precipitations, nor by dilutions with ordinary benzene followed by evaporation; the mole ratio of benzene to vinyl acetate in the polymer remained at 0.029 ± 0.005. This figure gives a ratio of $2.7\ (\pm 0.5) \times 10^{-3}$ for the specific rate of reaction (3) to that of normal propagation, in remarkable agreement with the kinetic value. The polymerization degree $\bar{P}_n$ of the radioactive polymer sample (estimated viscometrically) was about 700, so that there were about 20 benzene residues per average molecule. The reactions above postulated would require these residues first to enter the chains as disubstituted ortho- or para-cyclohexadiene units, but we have been unable to detect any significant unsaturation in our benzene-containing samples. Presumably the cyclohexadiene units are rapidly oxidized to aromatic form, possibly by atmospheric oxygen during dissolution and precipitation of the polymer.

Addition of a free radical to an aromatic nucleus is scarcely a novel reaction.[5] Within the field of polymerization mechanism, it has been postulated as a possible step in retardation by aromatic nitrocompounds,[7] in the polymerization of styrene in aromatic solvents,[8] and indeed in the polymerization of vinyl benzoate,[9] in order to account for the ready gelation of the latter. We believe, however, that ours is the first direct proof of its occurrence in a polymerizing system. From a general comparison of transfer activity in styrene and vinyl acetate systems,[2] we estimate the reactivity-ratio (to normal propagation) of reaction (3) for a styrene radical with benzene or with styrene monomer to be not less than 10^{-5} at 60°.

We thank the American Chicle Company for a grant in aid of this investigation, and Clare M. Regan for the radioactive assay.

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[6] Obtained from Tracerlab, Inc., on allocation from the United States Atomic Energy Commission.

[7] C. C. Price and D. A. Durham, THIS JOURNAL, **65**, 757 (1943).

[8] F. R. Mayo, *ibid.*, **65**, 2324 (1943).

[9] G. E. Ham and E. L. Ringwald, *J. Polymer Sci.*, **8**, 91 (1952).

THE RELATIONSHIP BETWEEN METALLIC RADII IN BODY-CENTERED CUBIC AND CLOSE-PACKED STRUCTURES

Sir:

In the first of two recent papers dealing with the resonance-bond theory of metals Pauling[1] develops an empirical correction for the conversion of the atomic radius corresponding to the shorter of the two bond lengths in the body-centered cubic structure to that appropriate to coördination number 12. This correction is derived from a comparison of the body-centered cubic radii with the face-centered cubic or hexagonal close-packed radii of iron, titanium, zirconium and thallium, respectively.

In every instance except that of iron, however, Pauling has compared two radii the values of which are measured at different temperatures and has not allowed for the differences which arise on account of thermal expansion. In the case of iron he appears to have used an unreliable extrapolated room temperature value for γ-Fe obtained by Jette and Foote[2] from a series of Ni–Fe alloys. This value has, therefore, been rejected by the present author and the high temperature values of the cell dimensions have been used in all cases. When allowance is made for thermal expansion, it becomes apparent that the correction, ΔR, given by Pauling's equation

$$R(1) - R(n) = 0.300 \log n$$

needs little or no modification. There is, in each instance, a small difference between the calculated and observed values of ΔR but the differences are not systematic and in any case may well arise from the uncertainties associated with the temperature corrections involved.

The cell dimensions on which the calculations are based, the values of the thermal expansion coefficients used and the resulting values of the radii for C.N. 12 at 20° are given in Table I. The cell dimensions are those given by Barrett[3] but are quoted in kX. units to conform with Pauling's usage.[4] The calculated and observed values of the

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[1] L. Pauling, THIS JOURNAL, **69**, 542 (1947).

[2] E. R. Jette and F. Foote, *Metals Technology*, **3**, 1 (1936).

[3] C. S. Barrett, "Structure of Metals," 2nd Edition, McGraw-Hill Book Co., New York, N. Y., 1953.

[4] In the case of iron there is yet another reported value available for the cell dimension of γ-Fe; Wyckoff, "Crystal Structures," Vol. I, Interscience Publishers, New York, N. Y., (1948) gives a_0 = 3.63 kX. at 1100°. This makes iron an unfortunate choice for the present purpose.