Fleischer[2] on the gaseous heat capacity of CF_2Cl_2. While the graph in question was not intended as a direct comparison of experimental data, it seems to imply a consistent disagreement of about +1% between the Buffington and Fleischer experiments and the more recent precise values being reported. The actual comparison of experimental C_p at one atmosphere gives the deviation of the older work from that of this paper as −0.16, −0.05 and +0.78%, respectively, at 0, 25.8 and 49.9°, the temperatures of Buffington and Fleischer's measurements.

(2) R. M. Buffington and J. Fleischer, *Ind. Eng. Chem.*, **23**, 254 (1931).

NATIONAL BUREAU OF STANDARDS
WASHINGTON, D. C. JOSEPH F. MASI

RECEIVED APRIL 1, 1953

ON A PROBABLE ENZYMATIC CONVERSION OF HYDROXYCHALCONE GLYCOSIDE INTO HYDROXYBENZALCOUMARANONE GLYCOSIDE

Sir:

The co-existence of glycosides[1] of hydroxychalcones and hydroxybenzalcoumaranones in species of *Cosmos* and *Coreopsis* suggested that there may be enzymatic interconversion. Some preliminary evidence for a "Chalconase" was obtained by macerating fresh rays of *Cosmos sulphureus* or *Coreopsis lanceolata* in a glass mortar with an equal quantity of water, $^2/_5$ of McIlvaine's buffer solutions of various *p*H, and $^1/_{10}$ to $^1/_{25}$ of *M*/20 potassium cyanide. The latter was used in order to inhibit the activity of polyphenoloxidase. A hydroxybenzalcoumaranone gives a purple coloration and a hydroxychalcone gives a red one with 1 *N* sodium hydroxide solution, but the former color is apt to be obscured by the red color produced by the chalcones present. When left at *p*H 3–4 the color of the solution hardly changed; it only changed to red on the addition of sodium hydroxide solution. At *p*H 7–8 the color changed eventually to brown, owing to autoxidation in alkaline medium. The conversion of chalcone into benzalcoumaranone did not take place to any extent in these cases. However, after the mixtures were allowed to stand at *p*H 5–6, the color given by the addition of sodium hydroxide solution was strongly purple accompanied by no reddish tint, showing the complete disappearance of chalcone compound. In good accord with these observations, the brown spot of the chalcone, which was clearly visible on paper chromatograms under ultraviolet light, completely disappeared after standing at *p*H 5–6, and the golden yellow spot of the corresponding benzalcoumaranone made its appearance quite strongly. The chromatograms usually were run with *n*-butanol–acetic acid–water (4:1:1) as solvents. The time required for complete reaction was 10–15 minutes under the optimum *p*H of 5–6. This comparatively rapid conversion was effectively prevented by heating at 100° for about ten minutes.

These observations may be effected by an enzyme in the tissue of the rays. This enzyme unfortunately has not yet been extracted from the rays, owing to its insolubility in water. It is, however, at least evident that this enzyme has little to do with the usual metal-bearing oxidases which suffer severe inhibition by cyanide, although the enzyme concerned effects dehydration in the presence of oxygen.

The powder, prepared from rays after extracting several times with cold alcohol at room temperature until the anthochlor pigments were completely removed, proved to be effective in bringing about this reaction. It is very interesting to note that, when the powder thus prepared from the rays of one species was added to any chalcone glycoside isolated from other plant species, the enzymatic conversion occurred readily and apparently at the same rate and to the same degree. For example, the ray powder of *Cosmos sulphureus* proved to be active in forming benzalcoumaranone when added to the extract of the rays of *Coreopsis lanceolata*, *C. tinctoria*, *Bidens laevis* and *Dahlia variabilis*.

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BOTANICAL INSTITUTE
FACULTY OF SCIENCE MASAMI SHIMOKORIYAMA
UNIVERSITY OF TOKYO SHIZUO HATTORI
HONGO, TOKYO, JAPAN

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ENZYMATIC REACTION OF CROTONYL COENZYME A[1]

Sir:

Evidence from various sources indicates that reactions (1),[2,3] (2)[2,4,5,6] and (3)[7] are catalyzed by soluble enzyme preparations from liver and heart.

(1) β-Hydroxybutyral-S-CoA + $DPN^+ \rightleftharpoons$ acetoacetyl-S-CoA + DPNH + H^+

(2) Acetoacetyl-S-CoA + CoA-SH $\rightleftharpoons$ 2 acetyl-S-CoA

(3) 2 Acetyl-S-CoA + 2 oxalacetate $\rightleftharpoons$ 2 citrate + 2 CoA-SH

Recent results strongly suggest the occurrence in liver and heart of an enzyme catalyzing reaction (4). The name crotonase is suggested for this enzyme.

(4) Crotonyl-S-CoA + $H_2O \rightleftharpoons$ β-hydroxybutyryl-S-CoA

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(2) F. Lynen, L. Wessely, O. Wieland and L. Rueff, *Angew. Chem.*, **64**, 687 (1952).

(3) J. R. Stern, M. J. Coon and A. del Campillo, THIS JOURNAL, **75**, 1517 (1953).

(4) E. R. Stadtman, M. Doudoroff, and F. Lipmann, *J. Biol. Chem.*, **191**, 377 (1951).

(5) J. R. Stern, M. J. Coon and A. del Campillo, *Nature*, **171**, 28 (1953).

(6) D. Goldman, *Federation Proc.*, **12**, 209 (1953).

(7) S. Ochoa, J. R. Stern and M. C. Schneider, *J. Biol. Chem.*, **193**, 691 (1951).

(1) M. Shimokoriyama, and S. Hattori. THIS JOURNAL, **75**, 1900 (1953).

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

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

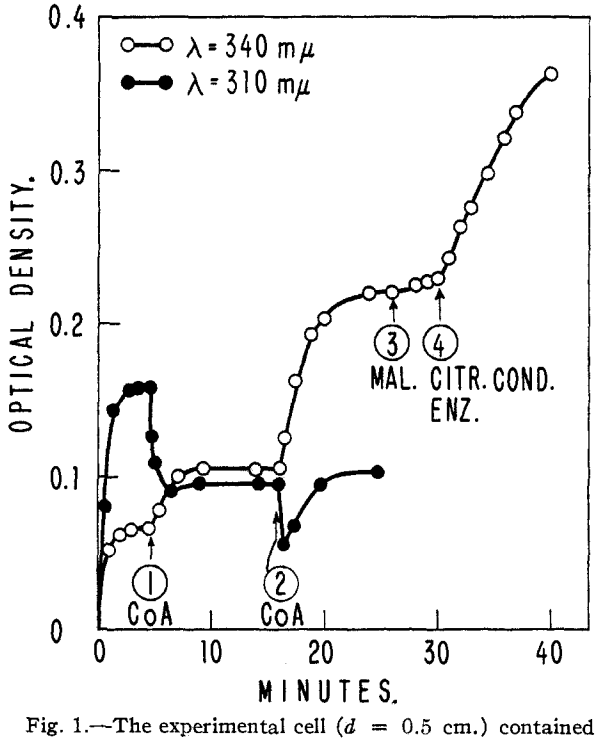

Fig. 1.—The experimental cell (d = 0.5 cm.) contained initially 100 μM. TRIS–HCl buffer, *p*H 8.1, 8 μM. $MgCl_2$, about 0.24 μM. crotonyl-S-CoA, and 0.27 μM. DPN^+. Crotonyl-S-CoA and DPN^+ were omitted from the control cell. The reaction was started by adding ox liver fraction (0.49 mg. protein). This 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.

(8) E. J. Simon and D. Shemin, THIS JOURNAL, in press.

(9) J. R. Stern, I. Raw and A. del Campillo, unpublished.

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

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

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.

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.

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

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

DEPARTMENT OF PHARMACOLOGY JOSEPH R. STERN
NEW YORK UNIVERSITY COLLEGE OF MEDICINE
NEW YORK, N. Y. ALICE DEL CAMPILLO

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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

(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).