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.

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NATIONAL BUREAU OF STANDARDS
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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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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^+ ⇌ acetoacetyl-S-CoA + DPNH + H^+

(2) Acetoacetyl-S-CoA + CoA-SH ⇌ 2 acetyl-S-CoA

(3) 2 Acetyl-S-CoA + 2 oxalacetate ⇌ 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 ⇌ β-hydroxybutyryl-S-CoA

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