

intensity of sound. In the first experiment the thresholds of a 1,200 c./s. tone and of noise in the octave 800–1,600 c./s. were compared. If it can be assumed that the published figures for critical bandwidths¹ are valid at threshold, then the cochlear 'resonator' at 1,200 c./s. has an equivalent bandwidth of some 50 c./s., and the ratio of threshold power for noise to threshold for a 1,200 c./s. tone should be 840/50 or 16.8, which is more than 12 decibels, whereas our results indicate a mean ratio of only 1 db., with a quartile of 2 db.

In the second experiment we attempted to produce a band of noise covering several octaves; but owing to imperfections of the speaker the equivalent width of the response, corrected according to the acuity curve given by Fletcher², was only 1,800 c./s. centred at 2,450 c./s. The threshold of this noise was compared with that of a 2,000 c./s. tone, which is good enough because the sensitivities of the ear at 2,000 and 2,400 c./s. are nearly equal². The equivalent band-width of the 'resonator' at 2,400 c./s. is given in Beranek¹ as 80 c./s., so that ratio of threshold for the noise to threshold for the tone would be 1,800/80 or 22.5, which is 13.5 db., whereas our results gave a ratio of 2–3 db. with a quartile of 3–4 db., and less than 4 per cent of the results exceeding 13 db. Although the discrepancy between calculation and observation is not as telling as in the first experiment, it remains true that in both cases the calculated ratio is about 20 whereas the observed ratio is close to unity, at variance with a resonance theory of hearing in which thermal noise of the 'resonator' determines threshold. This conclusion would appear to be confirmed by some headphone measurements on the masking of pure tones by noise which have been carried out by Hawkins and Stevens³. We feel that the work should be repeated under better acoustic conditions, with more subjects, and with wide bands of noise of spectra adjusted to match the hearing curves so as to avoid preponderance of a narrow region of frequencies in fixing the threshold of the noise.

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¹ Beranek, L., "Acoustic Measurements" (John Wiley, New York; Chapman and Hall, London, 1949).

² Fletcher, H., "Speech and Hearing in Communication" (Van Nostrand, New York and Toronto; Macmillan, London, 1953).

³ Hawkins, J. E., and Stevens, S. S., *J. Acoust. Soc. Amer.*, **22**, 9, Fig. 3 (1950).

Role of Formate in the Biosynthesis of Chlorophyll *a*

WHEN cultures of *Chlorella vulgaris* were incubated with formate-¹⁴C it could be demonstrated that the carbon atom of formate may serve as a precursor of the methyl ester carbon atom of chlorophyll *a*, without contributing extensively to the biosynthesis of the dihydroporphyrin ring. The procedure for growing *Chlorella vulgaris* as well as the preparation of methyl-phæophorbide *a* has been described¹. However, in the place of inert acetate and glycine added

Table 1. THE INCORPORATION OF FORMATE-¹⁴C INTO CHLOROPHYLL *a* DERIVATIVES

	¹⁴ C-activity*	Dilution factor†
Sodium formate- ¹⁴ C	68.0	
Methyl phæophorbide <i>a</i>	1.6	42.0
Chlorin <i>e₆</i>	0.15‡	452.0

* The ¹⁴C-activity is expressed in units of c.p.m. × 10⁴ per mgm. carbon.

† Dilution factor = $C_0/C - 1$, where C_0 = ¹⁴C-activity of formate and C = ¹⁴C-activity of the chlorophyll derivative isolated.

‡ The stated specific activity of chlorin *e₆* is less precise than the comparable value for methyl phæophorbide *a* due to the low isotope concentration and other methodological factors.

previously, inert sodium formate ($2.44 \times 10^{-3} M$) as well as 8.6 mgm. (33 μ c.) of sodium formate-¹⁴C (2.1×10^7 c./m.) were added to the growing medium. Methyl phæophorbide *a* was converted to chlorin *e₆* according to the method of Willstätter², and both degradation products of chlorophyll *a* were subjected to paper chromatography. Methyl phæophorbide *a* was chromatographed twice in decane/4-methyl-2-pentanone, 2:1 (v/v) and once in *n*-decane/1-propanol, 6:1 (v/v). Repeated chromatography did not significantly alter the specific activity. Chlorin *e₆* was chromatographed once in the lutidine/water/ammonia system of Falk *et al.*³. The separated pigments were cut out and eluted with acetone and acidified acetone, respectively. Details of this chromatographic procedure will be published elsewhere. The concentration of methyl phæophorbide *a* or chlorin *e₆* in the respective eluate was determined spectrophotometrically, applying the absorption coefficients reported by Holt and Jacobs⁴ and Stern and Wenderlein⁵. The solvents of the eluates were evaporated and the solid material deposited as an infinitely thin layer on glass planchettes. The carbon-14 activity was then determined with a windowless methane gas-flow counter operated in the proportional region. The experimental results are shown in Table 1.

These results indicate that approximately 90 per cent of the total carbon-14 activity incorporated into methyl phæophorbide *a* is localized in the methyl-ester carbon atom of the carboxyl group linked to the cyclopentanone ring. The dilution of the isotope concentration in this carbon atom is only about forty-fold as compared with the formate added. Since no degradation of chlorin *e₆* was carried out, the distribution of the carbon-14 activity among the carbon atoms of this substance remains unknown. The results (Table 1) show that formate makes only a minor contribution to the biosynthesis of the dihydroporphyrin ring of chlorophyll.

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⁴ Holt, A. S., and Jacobs, E. E., *Amer. J. Bot.*, **41**, 710 (1954).

⁵ Stern, A., and Wenderlein, H., *Z. Phys. Chem.*, **174**, 81 (1935).