of *Rhizobium* with stages in the life-cycle of the organism.

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Separation of Anthocyanin and Leuco-anthocyanin in Flowers of *Camellia japonica*

BATE-SMITH[1] observed that a methanol extract of white flowers of *Camellia japonica* turned red after heating with dilute hydrochloric acid. This suggests that leuco-anthocyanin is present in the white flowers. By the same procedure I found that red flowers of several horticultural varieties of *C. japonica* contained leuco-anthocyanin yielding cyanidin, as well as a glycoside of cyanidin.

A methanol-hydrochloric acid extract of red flowers of one of the varieties, Shikainami, was paper-chromatographed with the solvents butanol/concentrated hydrochloric acid/water (5 : 1 : 4, v/v) and 80 per cent formic acid/concentrated hydrochloric acid/water (5 : 1 : 4, v/v)[2]. As controls, chrysanthemin and cyanin were chromatographed on the same paper. R_F values were estimated as 0·24, 0·27 and 0·09, respectively, with the former solvent; and 0·53, 0·58 and 0·75 with the latter. The hydrolysate of the extract and cyanidin were then co-chromatographed using the second solvent. R_F values of the samples were identical, that is 0·20. No other spots of anthocyanin or anthocyanidin could be detected on the chromatograms.

The separation of leuco-anthocyanin from anthocyanin was carried out as follows. The red flowers (20 gm. fresh weight) were macerated with 150 ml. methanol and filtered. The filtrate was concentrated *in vacuo* at 30° C. until 15 ml. of a dark purplish-red syrup remained. The syrup was passed through a resin column (1·3 × 12 cm.) packed with 'Amberlite' *IRC*–120 (H form). The anthocyanin was found to be adsorbed quantitatively on the resin and the filtrate from the column was slightly orange. The filtrate was neutralized with 0·1 *N* sodium hydroxide and saturated with sodium chloride. Then it was exhaustively shaken with 30 ml. portions of ethyl acetate until the aqueous layer remained orange-brown after heating with propanol-hydrochloric acid[3]. The combined colourless ethyl acetate extracts were dried with sodium sulphate and evaporated *in vacuo* until a slightly pink-coloured residue remained. The residue was dissolved in 7 per cent hydrochloric acid and heated in a water-bath until a dark red colour appeared. The red matter was transferred by partition to a small amount of amyl alcohol, and the alcoholic layer was paper-chromatographed, in parallel with cyanidin, with formic acid/hydrochloric acid as solvent. The R_F value of the former was 0·19 (accompanied by brownish trail), and that of the latter (cyanidin) 0·20. This indicates that the leuco-pigment is sugar-free and should be regarded as leuco-cyanidin[4]. Extract of red flowers of the other variety, Somekawa, showed almost the same results.

Thus, it appears that flowers of some varieties of *C. japonica* contain leuco-anthocyanin, irrespective of their flower colours.

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Incorporation of Orotic Acid-^{14}C and Lysine-^{14}C in Regenerating Single Nerve Cells

EXPERIMENTS on the cytoscale and also, by inference, on the tissue and macroscale have demonstrated that proteins, lipids and ribonucleic acid in the central nervous system are in a dynamic state[1–3]. The production and metabolism of these substances are apparently promoted by increased functional demands.

The process of peripheral nerve regeneration can be repeated several times during the life-cycle of the organism. The production of proteins, lipids and ribonucleic acid per nerve cell has been studied by quantitative cytochemical methods during regeneration in hypoglossal cells[4]; the latent out-growth and maturation period could clearly be distinguished cytochemically. The results reported here demonstrate the incorporation of lysine-^{14}C into the nerve-cell proteins, and orotic acid-^{14}C into the ribonucleic acid of nerve cells during regeneration.

Nerve cells belonging to the hypoglossal nucleus in rabbits were used. The nerve was crushed with cooled forceps and 0·01 mc. L-lysine-^{14}C or orotic acid-^{14}C was injected intracisternally. The rabbits treated with lysine were killed 30 min. after the injection, and the others 24 hr. after the administration of orotic acid. The nerve cells were sampled by dissecting by hand under a preparation microscope. Fig. 1 shows some fresh hypoglossal cells, dissected out and photographed in the phase-contrast microscope. Five to ten cells were used for each measurement. The cells were transferred rapidly from the fresh tissue to 0·25 *M* sucrose, freed from surrounding glial cells, placed on a holder and treated with 1 *N* cold perchloric acid for 30 sec., and carefully washed and dried. The hypoglossal control cells then weigh on average 2,200 μμgm., that is 2,000 μμgm. protein and about 10 per cent ribonucleic acid. The activity was measured with a special Geiger microtube with a window thickness of 1·5 mgm./cm.^{2}, provided