LETTERS TO THE EDITOR

Indole Compounds and Growth

SIR,—The possible evolutionary relationship of 5-hydroxytryptamine (5–HT) in animals to auxin (3-indoleacetic acid, IAA) in plants has recently been discussed by Woolley[1]. The fact that one is a base which has been hydroxylated whereas the other is the corresponding acid suggests that in the course of evolution the usefulness of the indolic nucleus for specific purposes has long been recognized by Nature. In animals where the tissue fluids are usually alkaline, the indolic base has been evolved, whereas in plants where the cell sap is usually acidic the corresponding acid has proved to be more useful. In each situation, the compound probably exists predominantly in the unionized form.

Auxin causes plant cells to grow, probably by changing the permeability of the cell wall and so allowing an increased uptake of water and other metallic ions. If 5–HT is the counterpart in the animal kingdom of IAA in the plant world, then it is reasonable to expect some similarity in basic biochemical roles. This is in fact true since both 5–HT and IAA increase the permeability of plant cells[2,3] and animal cells[3]. We have also found that one precursor of 5–HT, namely 5-hydroxytryptophan, increases the permeability of plant cells whereas tryptamine does not, and the same is true for animal cells. This latter finding may explain why higher vertebrates utilise 5–HT and not tryptamine, and why only traces of tryptamine have been detected in mammalian tissues. The insertion of the 5-hydroxy group in the molecule of tryptamine may also be a device which has been evolved to retard the passage of 5–HT from the blood to the brain, since injected 5–HT does not pass in measurable amount into the brain. What 5–HT the brain needs it makes for itself from 5-hydroxytryptophan, a substance which readily penetrates the blood-brain barrier.

During a systematic examination by paper chromatography of the presence of indole compounds in animals and plants, the following results were obtained with pure compounds and these may be linked with some of the above considerations. Hydroxylated indolic compounds always migrated up the paper at a slower rate than did the corresponding unsubstituted ones; indolic amino-acids always migrated up the paper at a slower rate than did the corresponding amines; and 5–HT migrated at a faster rate than did IAA when an alkaline solvent was used, and the reverse was true in acid systems. For this study, the compounds used were 5-hydroxytryptamine creatinine sulphate, tryptamine hydrochloride, 5-hydroxytryptophan, tryptophan, 5-hydroxyindoleacetic acid and indoleacetic acid. They were detected as coloured spots after treatment with Ehrlich's reagent. The solvent systems were butanol : acetic acid : water (4 : 1 : 5), butanol : acetic acid (100 : 1), butanol saturated with N HCl, isopropanol : acetic acid : water (20 : 1 : 2 and 53 : 1 : 46), sodium chloride solution (8 per cent w/v) : acetic acid (100 : 1), n-propanol : water (3 : 1), isopropanol : water (3 : 1), sodium chloride solution (8 per cent w/v), n-propanol : ammonia : water (20 : 1 : 2), and isopropanol : ammonia : water (20 : 1 : 2 and 53 : 1 : 46). Further work is in progress to determine whether 5–HT plays a role in the growth of cells in higher vertebrates.

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The Excretion of Scillaren A by Rats

SIR,—As part of a survey of the metabolism and excretion of cardiac glycosides being carried out in this department[1-4] we have examined the bile and urine of rats after doses of scillaren A. The excretory products have been separated by paper chromatography and estimated colorimetrically. The squill glycosides (bufadienolides) do not give colours with the reagents normally used to detect the unsaturated lactone ring in the cardenolides and we have therefore used the pink colour given by these glycosides with 80 per cent sulphuric acid to detect them on paper and to estimate them colorimetrically after elution of the glycoside areas from paper chromatograms.

The bile of male albino rats (150–300 g.) was collected for 6 hours after intravenous injection of doses of 1 μg./g. of body weight of scillaren A.[3] After dilution to about 15 ml. with water the bile from each rat was extracted with chloroform in a liquid-liquid extractor for 4 hours and the extracts streaked across strips of Whatman Paper No. 1 (1¼ in. × 18 in.) and the chromatograms developed with the solvent mixture chloroform : methanol : water (10 : 4 : 5) by the descending method. After 5 hours the papers were dried at 100° for 10 minutes and a longitudinal strip ¼ in. wide cut from the chromatogram and treated with 65 per cent v/v sulphuric acid. Under these conditions scillaren A gave a pink colour on the paper strip. The corresponding area on the remainder of the strip was cut and eluted with methanol. The methanol extracts were evaporated to dryness, 1·75 ml. of 65 per cent v/v sulphuric acid added and the optical density of the pink colour produced was measured in an EEL Colorimeter (green filter 624). The colour obtained reached a maximum intensity in 10 minutes and was stable for at least 25 minutes (5 μg. of scillaren A could be readily detected and with the quantities assayed an error of ± 2 per cent was possible).

Using this procedure, extraction of known quantities of scillaren A from bile gave mean recoveries of 82 per cent (79–85 per cent, P = 0·95) and this figure was used as a correction factor to obtain a close approximation of the scillaren A content of the bile collected in the excretion experiments.

Only one band was detected in the chromatograms of the bile extracts obtained after doses of 1 μg./g. This was eluted and identified as scillaren A by rechromatography on paper with the original glycoside using four different solvent systems for development: chloroform : methanol : water (10 : 8 : 5), toluene : butanol (8 : 2) saturated with water, ethyl acetate : butanol : chloroform (16 : 16 : 68) saturated with formamide, and chloroform : benzene : butanol (70 : 10 : 10) saturated with formamide.

Quantitative determination of the amount of glycoside present in bile showed that 84 per cent (79–90 per cent, P = 0·95) of the dose was excreted in 5 hours.

Chloroform extracts of urine collected for 12 hours after intraperitoneal doses of 1 μg. and 2 μg./g. of scillaren A were chromatographed but no glycosides or metabolites could be detected on the papers indicating urinary excretion was very low during this period.

It appears from these results that scillaren A is similar to the polar digitalis glycosides lanatoside A and lanatoside C[3] and to ouabain[4] in being excreted mainly in the bile and without chemical modification.