In order to obtain the total amount of solution B discharged, we subject equation (2) to the same treatment:

$$\int dQ_B = \frac{\Pi P R^4}{8 \eta L} \int_{t_{L,0}} dt - \frac{2 \Pi \eta L^3}{P} \int_{t_{L,0}}^{t} \frac{dt}{t^2}.$$

Carrying out the integration and simplifying:

$$Q_B = \frac{\Pi P R^4 t}{8 \eta L} + \frac{2 \Pi \eta L^3}{P t} - \Pi R^2 L. \qquad (7)$$

Equation (7) is obviously valid only for $t \geq t_{L,0}$.

Let us finally consider the special case in which the amount of solution B introduced into the tube is equal to the capacity of the tube. Then, adding equation (6) to equation (7),

$$Q_A + Q_B = \frac{\Pi P R^4 t}{8 \eta L} = \Pi R^2 L; \qquad t = \frac{8 \eta L^2}{P R^2}.$$

Substituting t in equation (7) and dividing both sides by $\Pi R^2 L$,

$$Q_B = \frac{\Pi R^2 L}{4}. \qquad (8)$$

Under these circumstances, the volume of solution B discharged is one quarter the volume of solution B injected.

Résumé. On offre un ensemble d'équations qui s'appliquent à l'écoulement laminaire de deux solutions A et B, de même viscosité, lorsqu'on introduit B dans un tube préalablement rempli de solution A.

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The Selective Esterification of the 5'-Hydroxyl of Thymidine

The usual procedure for the esterification of nucleosides[1-4] involves the use of protective groups for achieving selectivity. We now wish to report that the 5'-hydroxyl of thymidine can be selectively esterified by controlling the conditions of acylation. For this purpose, the use of a relatively large volume of solvent is necessary. The bulk and quantity of acylating agent and the amount of base present in the reaction mixture are also of significance, as selectivity is based on the emphasis of the greater reactivity of the primary hydroxyl in comparison with the secondary hydroxyl.

Thymidine-5'-phosphate was obtained as follows: Thymidine[5] (48.4 mg, 0.2 mM) was dissolved in acetonitrile (50 ml) containing pyridine (0.04 ml, 0.5 mM) by refluxing for 15 min. The solution was cooled to $-10°$ and stirred while a solution of dibenzyl phosphorochloridate (104 mg, 0.35 mM) in acetonitrile (25 ml) was added dropwise over 4 h. After standing at room temperature for 12 h, the solution was concentrated and examined by paper chromatography. A single UV-absorbing zone (Rf 0.38 (A))[6] was discernible, and no thymidine (Rf 0.70 (A)) was present[7]. The product was isolated by evaporating the solution to dryness, dissolving the residue in chloroform (20 ml), washing the chloroform solution with water, drying and removing the solvent. Its identity as thymidine-5'-dibenzyl phosphate was established by its hydrolysis with crude snake venom in glycine buffer pH 9.0 to thymidine[8]. Hydrogenolysis[1] in the presence of Pd/C yielded thymidine-5'-phosphate (Rf 0.30 (A)).

For the preparation of *thymidine-5'-sulphate*, a solution of chlorosulphonic acid (100 mg, 0.85 mM) was added over a period of 4 h to a stirred solution of thymidine (200 mg, 0.82 mM) in acetonitrile (100 ml) containing pyridine (0.1 ml, 1.26 mM), cooled to $-5°$. After the reaction mixture had stood overnight at room temperature, it was evaporated to dryness and the residue dissolved in water (0.5 ml). Paper chromatography in solvent A revealed the presence of three UV-absorbing zones, Rf 0.34, 0.40, and 0.70; the fast-moving zone being due to thymidine. The optical densities of the eluates of these zones were in the ratio of 7.5:1.5:1.0 at 260 mμ. Fractionation of these products was done by chromatography on Whatman 3MM paper employing the same solvent system. The zones were separately eluated with water and lyophilized. The minor product (Rf 0.40 (A), 0.61 (B)) was identified as *thymidine-3'-sulphate* by comparison with material obtained unambiguously. The major product was therefore thymidine-5'-sulphate (Rf 0.34 (A), 0.53 (B)) and was isolated as the pyridinium salt. Found: C 44.28, H 5.07. $C_{10}H_{14}O_8N_2S \cdot C_5H_5N$ requires: C 44.87, H 4.74%.

Thymidine-3'-sulphate was obtained unambiguously by treating 5'-o-trityl thymidine (121 mg, 0.25 mM) in acetonitrile (75 ml) containing pyridine (0.1 ml) with chlorosulphonic acid (0.1 ml) at $-5°$. After allowing the reaction mixture to stand overnight, solvent was removed and the product detritylated by treating a solution in acetone-water (4:1) with 4N HCl (2 ml) at 15° for 1 h. Neutralization with triethyl amine evaporation of acetone and removal of triphenylcarbinol by extraction with ether yielded an aqueous solution containing thymidine-3'-sulphate. Pure thymidine-3'-sulphate could then be

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[2] G. M. TENNER, J. Am. chem. Soc. *83*, 164 (1964).
[3] J. ARNOLD and T. D. PRICE, J. Am. chem. Soc. *84*, 1402 (1962).
[4] P. W. WIGLER and H. U. CHOI, J. Am. chem. Soc. *86*, 1636 (1964).
[5] All acylations carried out under strictly anhydrous conditions and carefully dried materials were used. Thymidine was recrystallized from CH_3CN. Acylating agents were freshly distilled.
[6] Descending paper chromatography. Solvent systems: A, *n*-butanol-acetic acid-water (4:1:5); B, isopropanol-water (4:1).
[7] Unreacted thymidine was present when lesser amounts of pyridine were used. Some diesterification occurred when pyridine content was increased to 0.1 mM.
[8] W. E. RAZZELL and H. G. KHORANA, J. biol. Chem. *234*, 2105 (1959).

obtained by preparative paper chromatography on Whatman 3MM paper.

The selective acylation of the thymidine-5'-hydroxyl is also possible with organic acid chlorides provided these have bulky substituents. Thus *5'-trichloroacetyl thymidine'* was obtained as follows: A solution of thymidine (250 mg, 1.033 m*M*) in acetonitrile (40 ml) containing pyridine (0.2 ml) was cooled to 10° and stirred while a solution of trichloroacetyl chloride (188 mg, 1.03 m*M*) in acetonitrile (10 ml) was added over 45 min, stirring was continued for a further 2 h after which the solution was filtered from a small amount of thymidine, evaporated to dryness and taken up in methylene chloride (20 ml). On standing overnight, 40 mg of thymidine crystallized out. The solution after filtration of thymidine was evaporated to dryness and the residue chromatographed over silicic acid (20 g). 5'-Trichloroacetyl thymidine was eluated with $CHCl_3$-EtOH (9:1) and crystallized from chloroform (300 mg), m.p. 167–168°, λ_{max} 5.65 μ (CH_2Cl_2), 264.5 mμ (EtOH) (ε 12,220). Found: C 36.68, H 3.18, Cl 27.72. $C_{12}H_{13}O_6N_2Cl_3$ requires: C 37.16, H 3.50, Cl 27.48%.

In a similar manner, using the respective acid chlorides, *5'-D,L-α-chloro phenylacetyl thymidine* (m.p. 135-145°), *5'-bromoacetyl thymidine* (m.p. 159–160°), and *5'-chloroacetyl thymidine* (m.p. 160°) have been obtained[9].

Zusammenfassung. Ein einfacher Weg zur selektiven Esterbildung mit der 5'-Hydroxylgruppe von Thymidin wird beschrieben. Beispiele: Thymidin-5'-phosphat, Thymidin-5'-sulfat und Thymidin-5'-trichloracetat.

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[9] Gifts of chemicals obtained through the Wellcome Trust are gratefully acknowledged.

In vitro Production of Indole Acetic Acid by *Fusarium vasinfectum* Atk.

The importance of indole acetic acid (IAA) and other growth regulators in plant diseases has been recently reviewed[1,2]. The cotton wilt pathogen *Fusarium vasinfectum* Atk. has been reported to produce growth-promoting substances in various culture media[3–5]. The presence of indole compounds was not explored by HIRATA[3] or by KALYANASUNDARAM and LAKSHMINARAYANAN[4], while VENKATARAM[5] discounted the production of IAA in culture filtrates.

Erlenmeyer flasks of 250 ml capacity with 50 ml Czapek's medium as well as flasks with 50 ml Czapek's medium plus 0.1% DL-tryptophan were sterilized and inoculated with 8 mm culture discs of the actively growing fungus, grown on Czapek's agar medium and incubated at laboratory temperature (26–29°C) in the dark. After incubating for 6 and 9 weeks, the fungal mats were separated and the filtrates adjusted to pH 4.0, with 2*N* HCl. The filtrates were shaken with equal volumes of peroxide-free ether and extracted in a refrigerator at 4° ± 1°C for a period of 24 h with solvent changes at 8 and 16 h. At the end of 24 h, all the ether phases were pooled and evaporated to dryness under reduced pressure between 35° and 40°C. The residue was taken in a small volume of distilled methanol, spotted on Whatman No. 1 paper and developed ascendingly for 17 h in isopropanol water (80:20), isopropanol-ammonia-water (10:1:1) and in *n*-butanol-ammonia-water (10:1:1)[6]. The dried chromatograms thus developed were sprayed either with Salkowski or Ehrlich reagents[7]. The Rf values of the fungal IAA and of the known IAA samples were identical in all systems tested. There was no secretion of IAA in the extracts from the fungal cultures grown in Czapek's medium. The IAA-like spots were observed only with tryptophan cultures.

The IAA-like compound was further purified chromatographically, employing water as the solvent, and eluted in distilled methanol. The concentration of the compound was estimated by GORDON and PALEG's method[8], employing 4 ml Salper reagent (50 ml of 35% perchloric acid and 1 ml of 0.5*M* ferric chloride) and 2 ml of the eluate and determining the absorbancy at 535 mμ in a Hilgar Spectrophotometer, after allowing 1 h for colour stability. There were indications to show that the fungus synthesized 6.02 mg IAA during the 6th week of incubation, while the compound increased by 25% by the end of the 9th week.

The results show that *F. vasinfectum* is capable of synthesizing indole acetic acid from tryptophan and the concentration of the growth promoter increased with the age of the cultures. Further studies are required to investigate the IAA metabolism in *Fusarium* infected cotton plants[9].

Résumé. Fusarium vasinfectum Atk., le champignon qui flétrit le coton synthétise l'acide indol-3-acétique (IAA) de tryptophan et la concentration augmente avec l'âge de la culture.

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