the saliva of two of the calves, but after 24 hours none of the antibiotic was found in any of the calves.

Since the Aureomycin was found in the saliva, some of the antibiotic would necessarily pass into the rumen and consequently might have some effect on the rumen microflora. The effects of antibiotics on rumen microflora and on efficiency of digestion *in vitro* and *in vivo* have been studied, and reports are in preparation. In the continuation of the work of Rusoff *et al.* (*2*), it was reported by Hester *et al.* (*7*) that Aureomycin could not be detected in the rumen following intramuscular injection of Aureomycin. It is possible that the failure of the latter authors to detect Aureomycin in the rumen contents of slaughtered animals might be ascribed to the considerable dilution of saliva after it passes into the rumen, to the smaller amounts of Aureomycin injected, to a possibly less sensitive assay technique than that described in the saliva analyses reported here, or to a combination of all three factors.

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14 March 1955

Methylpentynol (Oblivon) in the Treatment of Epilepsy

In 1952 R. W. Schaffarzick and B. J. Brown [*Science* **116**, 663 (1952)] published reports of experimental and clinical trials that suggested that methylpentynol might be of use in the treatment of epilepsy. They also noticed that two of the six patients under treatment developed strongly positive cephalin flocculation tests, which rapidly became negative when the drug was discontinued, suggesting that this drug might have toxic effects on the liver. As a result of this report the effect of methylpentynol on patients with epilepsy was further investigated and the effect of the drug on liver function tests was observed.

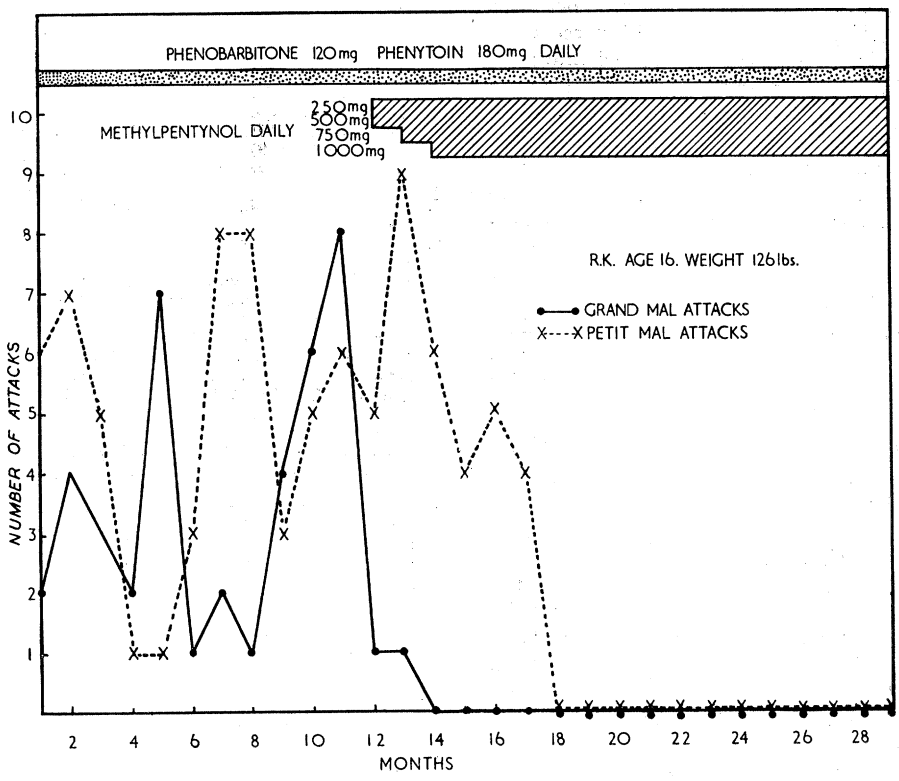

Fig. 1. Effect on epileptic patients of treatment with methylpentynol.

Six children of ages between 11 and 16 years and 18 adult males were treated. All but one of the patients had both grand and petit mal; five had, in addition, psychomotor attacks; the remaining one had psychomotor epilepsy only. Methylpentynol was given by mouth, the dosage being increased up to 1000 mg daily, and treatment was continued for 3 months. In only one patient, a boy of 16, did methylpentynol appear to control the epilepsy; the number of attacks and effect of treatment are shown in Fig. 1. He has now been under treatment with methylpentynol for a further 9 months as an outpatient and has had no further fits. Full empirical liver function tests were performed on all the children at regular intervals up to 3 months and on 10 of the adults at the end of 3 months. No abnormality was found.

Two of the children became sleepy and depressed while they were under treatment; they were inclined to stagger and fall easily. When the drug was omitted they rapidly regained their former wakefulness and spirits.

It is concluded that methylpentynol is unlikely to be useful in controlling epilepsy in patients who have proved resistant to the more usual form of treatment, but that it has no toxic effects on the liver, as far as these tests have shown.

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γ-Sitosteryl Glycoside in Tobacco

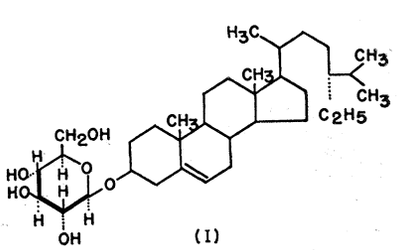
The Indian Cancer Research Centre has been interested for the last 10 years in determining the role of tobacco in the production of oral cancer (*1*). With this object in view, a detailed study of the chemistry of tobaccos that are used for chewing purposes by the people of India has been in progress for the last 2 years. One of the varieties of tobacco extensively used for chewing by the people living in Malabar (southwest coast of India) is known as "Vadakkan" (*Nicotiana tabacum*).

We have now found that it contains a sterol glycoside (I) not so far isolated from tobacco. The glycoside (I) is insoluble in water, sparingly soluble in nonhydroxylic solvents, and crystallizes from alcohol in colorless plates, mp 215°–235°C (found: C, 72.1; H, 10.4; calcd. for $C_{35}H_{60}O_6$: C, 72.8; H, 10.5 percent). Yield 0.01 to 0.02 percent. It yields tetraacetyl derivative, crystallizing from dilute alcohol in lustrous plates, mp 149°C

(found: C, 69.2; H, 8.8; calcd. for $C_{43}H_{68}O_{10}$: C, 69.3; H, 9.1 percent).

The glycoside is hydrolyzed into a reducing sugar and an aglycone (II) on boiling with 20-percent hydrochloric acid for a few minutes or on standing overnight with cold concentrated hydrochloric acid. The sugar formed an osazone mp 208°C, which on mixing with glucosazone (*2*), prepared from D-glucose, showed no depression in melting point and was identified as D-glucose. The aglycone (II) crystallized from dilute alcohol in shining plates, mp 148°C (found: C, 83.9; H, 12.1; calcd. for $C_{29}H_{50}O$: C, 83.97; H, 12.2 percent). α_D^{29}, −43.8 (109 mg in 5 ml chloroform). It gives a green coloration in Liebermann-Burchard test and yields an acetyl derivative, mp 140°C (found: C, 81.6; H, 11.3; calcd. for $C_{31}H_{52}O_2$: C, 81.6; H, 11.4 percent). These properties of (II) suggested that it might be γ-sitosterol (*3*). Phytosterols (i) mp 62°–63°C in tobacco leaves (*4*), (ii) mp 140°–141°C in tobacco seeds (*5*), and (iii) mp 135°C in tobacco tar (*6*), differing in melting points from the one now obtained by us were detected by earlier workers; however, the constitution of these products had not been determined by them.

On the basis of the experimental evidence presented here, it followed that (I) is γ-sitosteryl-D-glucoside and, assuming the suggested constitution (*7*) of γ-sitosterol as correct, (I) could be represented as follows:



(I)

The formation of the tetraacetyl derivative by (I) is evidently the result of acylation of the hydroxyl groups in the sugar radical. The sterol residue with the fatty chain on carbon atom 17 appears to have suppressed the solubilizing character of the hydrophyllic sugar residue on the carbon atom 3 and rendered (I) insoluble in water. The occurrence of "γ"-sitosteryl glycoside does not seem to be as common in plant products as that of the "β"-sitosteryl glycoside (*8*).

Description of the isolation of (I) and details of the foregoing work are in preparation (*9*).

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9. We are indebted to the National Cancer Institute of the U.S. Public Health Service and to the Indian Council of Medical Research for their financial support in this work.

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Diffusion Constant and Diffusion Coefficient

Jacob Verduin shows correctly how Krogh's diffusion constant differs from the diffusion coefficient (*1*). However, he states, incorrectly, that Krogh failed to distinguish between his diffusion constant and the diffusion coefficient, and he complains that the "unfortunate use of tension units . . . in Krogh's diffusion constant" led many biologists to believe that in aquatic media CO_2 has a higher diffusivity than O_2.

With the present note I wish to demonstrate that Krogh very clearly distinguished between his diffusion constant and the diffusion coefficient of Hüfner—which is now often called diffusivity—and that Krogh's choice of his diffusion constant was carefully considered and justified.

In his original paper, "The rate of diffusion of gases through animal tissues, with some remarks on the coefficient of invasion" (*2*), Krogh discussed Exner's work (1875). Exner found that the rates of diffusion of different gases in the same fluid are proportional to the absorption coefficients of the gases in the fluid and inversely proportional to the square roots of their molecular weights. Krogh cited Hüfner's definition of diffusion coefficient, namely, "the quantity diffusing through 1 cm² and 1 cm thickness in 24 hours when the pressure difference is 1 atmosphere, divided by the absorption coefficient for the gas in question."

Krogh gave a major reason why he regarded Hüfner's diffusion coefficient as impractical for physiological work, namely, "the absorption coefficients for gases in tissues are generally unknown and their accurate determination [is] very difficult." Krogh then showed how, *for water,* his diffusion constants can be calculated from Hüfner's diffusion coefficients. Evidently, Krogh not only clearly distinguished between his diffusion constant and the diffusion coefficient of Hüfner, but he also gave, for water, the quantitative relationship between the two units.

Krogh's statement that the differences in CO_2 pressure in animal tissue and blood must be an absolutely negligible quantity is taken by Verduin as a proof that Krogh failed to distinguish between his diffusion constant and the diffusion coefficient, because, argues Verduin, the concentration gradient required to achieve a given CO_2 transport must be higher, not lower, than for the same O_2 transport.

The latter sentence is true enough but when Verduin used it to prove Krogh's failure, he apparently had forgotten a major point of his own article, namely, that Krogh dealt with pressure gradients, not concentration gradients. Since the solubility in water of CO_2 is 28 times as great (at 20°C) as that of O_2, a given difference in concentration of CO_2 in water is achieved for CO_2 with only one-twenty-eighth of the difference in pressure required for the same difference in concentration of O_2 in water; and this may be absolutely—that is, in terms of atmospheres—negligible.

Krogh (*2*, p. 401) discussed Hüfner's idea that diffusion rates of gases in water should be smaller at higher temperature because increase of temperature decreases the solubility of the gases in water. Krogh stated that the effect of decreasing solubility with higher temperature might be offset by a "decrease in the internal friction of the water"—which would mean an increase in diffusivity. With the peritoneal membrane from small dogs, Krogh measured the effect of temperature on his constant for oxygen diffusion in animal tissues. Taking the diffusion constant at 20°C as unity, the constant at 0.2° to 0.5°C was 0.79 ± 0.02 and that at 36° was 1.16 ± 0.05. Based on these measurements Krogh, and later Prosser *et al.* (*3*), could conclude that the diffusion constant of oxygen in tissue increases about 1 percent per degree increase of temperature above 20°C. Verduin in his recent communication (*1*, p. 216) writes "This statement is false."

What led Verduin to this devastating verdict? It looks as if he did not realize that Krogh *measured* the temperature effect on his diffusion constant; it looks as if he presumed that Krogh had calculated his results on animal tissue from data of the temperature effect on diffusivity in water and that in this calculation Krogh had failed to account for temperature effect on solubility. Whatever