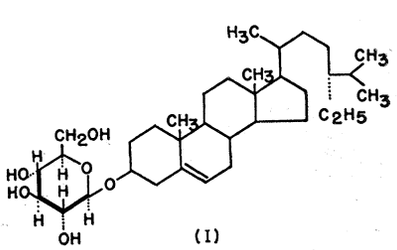
(found: C, 69.2; H, 8.8; calcd. for $C_{43}H_{68}O_{10}$: C, 69.3; H, 9.1 percent).

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.

22 November 1954

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

the reason, Verduin's pronouncement "This statement is false" is as erroneous and unfortunate as his allegation that Krogh failed to distinguish between his diffusion constant and diffusivity.

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28 April 1955

In a communication "Diffusion constant and diffusion coefficient" [*Science* **121**, 215 (1955)] J. Verduin criticizes the use of diffusion constants for gases in tissues based on partial pressures (tensions).

Diffusion constants based on partial pressures were introduced by A. Krogh [J. Physiol. **52**, 391 (1919)], who fully understood the difference between a constant so defined and one based on concentration gradients. He choose partial pressures because the absorption coefficients of gases in tissues were—and still are—generally unknown and difficult to determine, which makes the use of concentration-based coefficients impractical in physiological reasoning. Moreover, one of the most important of the diffusion problems of Krogh's time—respiration—necessitates comparison between air and blood, which can hardly be done except on the basis of partial pressure.

Verduin also criticizes Krogh's statement that the diffusion constant as defined by him increases about 1 percent for each centigrade degree increase in temperature. Using the diffusivity and the solubility of oxygen, Verduin calculates the diffusion constant for oxygen in water at 20°C and 30°C as defined by Krogh and finds the values to be 0.346 and 0.338, respectively, which is in contrast with Krogh's statement.

Krogh's statement on the influence of temperature refers, however, to animal tissues and is based on actual determinations that he undertook because the influence of temperature on the solubility of gases in tissues and on the internal friction of the tissues themselves is impossible to calculate.

Krogh was, of course, fully aware that the diffusion rates for the different gases are inversely proportional to the square roots of their molecular weights and of other differences between the constants as defined by him and by the physicists.

Verduin is correct in his statement that the gradient in molecular concentration necessary for the transport of carbon dioxide from the tissues to the capillaries is somewhat greater for carbon dioxide than for oxygen, but translated into partial pressure this concentration gradient is, as Krogh said, absolutely negligible.

It would, of course, be possible to introduce diffusion constants for gases based on concentrations into physiology, but I doubt that it would be practical to give up the use of partial pressures, which for the understanding of the diffusion of gases between alveoli and blood is unavoidable and which we use also in other problems (O_2 dissociation curve of hemoglobin).

However, in order to avoid confusion it would perhaps be useful in every case to state definitely which of the two diffusion constants is used, for example, by denoting the old diffusion coefficient of the physicists—"the Fick diffusion constant" defined as the quantity diffusing through an area of 1 cm² and a thickness of 1 cm in unit time, when the concentration difference is unity—and the one used by the physiologists in the case of gases—"the Krogh diffusion constant" defined as the quantity diffusing through an area of 1 cm² and a thickness of 1 μ/min, when the partial pressure difference is 1 atm.

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15 March 1955

Ultrasonic Lesions in the Mammalian Central Nervous System

Early histological studies of nerve tissue of animals irradiated with intense focused ultrasound at this laboratory indicated that nerve cell bodies were more susceptible than nerve fibers to changes by the ultrasound (*1*). These preliminary histological results have not been substantiated in subsequent studies. Rather, it has been found, as was previously reported (*2*), that white matter is more readily affected by the sound and that higher ultrasonic dosages are required for producing changes in gray matter. It can be readily seen that this selectivity provides a unique tool for basic neurological studies. Recent publications of this laboratory present results on the production and time sequence of changes in relatively large white-matter lesions of controlled shape (*2, 3*). This paper, however, is concerned primarily with small ultrasonic lesions in both gray and white matter (*4*).

Selective, accurately positioned lesions as small as 2 to 3 mm in maximum diameter can be produced. The lesions, which can be localized at any desired depth in the brain without affecting intervening tissue, are quantitatively reproducible from one animal to another, so that dosage studies made on a series of animals can be used as a guide in choosing the conditions of irradiation for neuroanatomical or functional studies. The blood vessels are most resistant to the action of the sound. It is, therefore, possible to interrupt fiber tracts without destroying neighboring gray matter and without breaking blood vessels even within the site of the lesion. It is also possible, by appropriate choice of the ultrasonic dosage, to affect irreversibly the nerve tissue (fibers and cell bodies) in gray matter without causing hemorrhage.

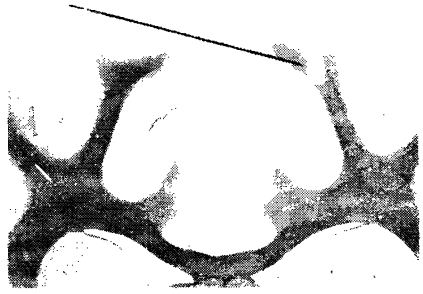

Fig. 1. Small ultrasonic lesion in the subcortical white matter of the brain of a cat. Dosage used selectively affects the fiber tracts, and no damage is produced in the neighboring gray matter. (PTAH stain)

The results reported here were obtained from histological studies of ultrasonically irradiated cats and monkeys. Extensive dosage studies have been completed, and the time course of development of the lesions has been followed in animals sacrificed from immediately after irradiation (5 min) up to 30 days. The preparation of the animal and the technique of irradiation are described in previous papers (*2, 3*). Results of investigations concerned with the physical mechanism of the action of the sound on the nerve tissue have been published (*5*).

When a region of the white matter of the central nervous system is irradiated at one spot with a single exposure of ultrasound at a dosage just above the minimum required to produce an effect, a small lesion about 2 to 3 mm in maximum diameter is produced. Figure 1 illustrates such a lesion in the subcortical white matter 12 days after irradiation (dose 51 atm acoustic pressure and $4.8(10)^3$ cm/sec acoustic particle velocity for 1.00 sec). It shows a sharp boundary between the affected white matter (lower end) and the neighboring unaffected gray matter.

A lesion such as that shown in Fig. 1 is first seen 10 to 15 min following irradiation in tissue sections prepared with Weil's myelin stain. The lesion area is first recognized as a light-staining matrix as compared with normal tissue. One hour after irradiation the myelin sheaths appear beaded. The perivascu-