chloretone, a mid-dorsal incision is made in the post-clitellar portion of the body and the septa carefully cut away so that the specimen may be pinned out flat on the wax pad of a dissecting dish. The digestive tract is then removed, care being taken to cut the septa close to the under-surface of the intestine, in order that the nephridia may remain undisturbed. The preparation is then wet with several drops of methylene-blue saline solution. Methylene blue, lot NA3, certified by the Commission for the Standardization of Biological Stains, has proved entirely satisfactory for this purpose. For anatomical study one part of dye in 2,000 parts of 0.6 per cent. NaCl solution is a suitable concentration; for the study of cilia in motion a concentration of 1: 20,000 is preferable. After the stain has acted for ten minutes it should be washed off with saline solution (0.6 per cent. NaCl in distilled water), and the preparation covered with this solution.

Two regions of the nephridium stain heavily with the dye—the nephrostome and the ampulla. Within wide limits the more dilute the stain the greater the contrast between the amount of dye absorbed by these regions and that absorbed by the remainder of the nephridium. Since the stained nephrostome is clearly visible, it is not difficult to remove the entire nephridium for study under the microscope. In the more dilute solutions the cytoplasm of the central cell, as well as the cytoplasm of the marginal ciliated cells, is strongly stained. The nature of the ciliary action and the direction of the effective beat can be clearly made out. In more concentrated solutions ciliary action is likely to cease, concurrent with the staining of nuclei of the cells of the nephrostome.

The ampulla is stained distinctly in solutions of the concentrations mentioned above, due to the accumulation of the dye within the cells.[2] The outer portion of the ampulla does not betray its cellular nature in dilute solutions, but in strong concentrations cell nuclei are clearly delineated. The inner portion of the ampulla stains strongly in any case. This area, said by Maziarski[3] to consist of rod-shaped bacteria packed closely together, is so strongly stained that when examined macroscopically it may be mistaken for the nephrostome. Its position at the distal part of the long loop of the nephridium serves to distinguish it from the nephrostome, which lies much nearer the median ventral axis.

Complete nephridia, strongly stained, may readily be removed, dehydrated rapidly in absolute alcohol, cleared in xylene, and mounted in balsam. If desired, to retain the dye fully, such preparations may be fixed in ammonium molybdate and washed before dehydration. Such mounts usually show fine detail, together with unusual translucency, and are therefore well adapted for careful study.

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SPECIAL ARTICLES

ALTERNATING CURRENT CONDUCTANCE AND DIRECT CURRENT EXCITATION OF NERVE

THE Fourier integral has proved to be a powerful and useful tool in many branches of science. In the Heaviside operational calculus form it has been particularly valuable in studying the transient behavior of electric circuits. When certain simplifying assumptions and approximations are made, this type of analysis points out a relation between the alternating current conductance and the direct current excitation of irritable biological tissues.

Alternating current resistance and capacity measurements over a wide frequency range show that biological materials may be considered electrically equivalent to a circuit containing two fixed resistances and a polarization element having an infinite impedance at zero frequency and a zero impedance at infinite frequency. This element may be considered as a resistance and a capacity in series, both of which decrease with increasing frequency, n. When $r(\omega)$ is the resistance and $x(\omega)$ is the reactance $(1/C\omega)$ of the capacity, it is often found that $r(\omega) = r_1\omega^{-\alpha}$, $x(\omega) = x_1\omega^{-\alpha}$, where $\omega = 2\pi n$, r_1 and x_1 are the resistance and reactance for $\omega = 1$, and α is a constant between zero and one. The impedance of the element $z(\omega) = z_1 p^{-\alpha}$, where $z_1 = \sqrt{r_1^2 + x_1^2}$, $p = j\omega$, and $j = \sqrt{-1}$, and the phase angle is constant, $\phi = \tan^{-1} x_1/r_1 = \alpha\pi/2$,[1] (1).

When a constant current i is started through this element at time $t = 0$, the potential difference across the element may be found by either the Fourier integral[2] or the operational[3] method to be $e(t) = z_1 i t^{\alpha}/\Gamma(1+\alpha)$, where $\Gamma(1+\alpha)$ is the gamma function. This means that when the equivalent polarization element of a biological tissue has a constant phase angle and an impedance which is a power func-

[2] R. Chambers, "Some Changes in Dyeing Cells," *Proc. Soc. Exper. Biol. and Med.*, 20: 367–368, 1923.

[3] S. Maziarski, "Sur la structure des néphridies des Vers de terre," *C. R. Soc. Biol.*, Paris, Vol. 53, 1901.

[1] H. Fricke, *Phil. Mag.* (7) 14: 310, 1932.

[2] G. A. Campbell and R. M. Foster, "Fourier Integrals for Practical Applications." Bell Telephone System Monograph B–584, New York, 1931. Pair No. 516.

[3] V. Bush, "Operational Circuit Analysis," p. 197, New York, 1929.

tion of the frequency for alternating current, then the potential difference across this element should build up as a power function of the time after a constant current is started through it. The strength of current necessary to change the potential by a fixed value e_0 in the time t is then given by $i = i_1 t^{-\alpha}(2)$, where $i_1 = e_0 \Gamma (1+\alpha)/z_1$.

It is reasonable to assume that this polarization element is to be identified with the cell membrane and that a threshold change of potential across the membrane will stimulate an excitable tissue. When it is further assumed that the membrane parameters are constant up to a threshold potential change, then the strength-duration relation for constant current excitation should be given for short times by equation (2).

The fixed resistances of the equivalent tissue circuit may be neglected for stimuli of short duration, but they are important for the longer stimuli and determine the rheobase. In the general case, the superposition[4] formulation and the Heaviside operational method lead to the same asymptotic expansion for e(t). For the simple condenser hypothesis, $\alpha = 1.0$ and the solution is well known. The Nernst diffusion hypothesis corresponds to $\alpha = 0.5$ and the solution may be given in terms of the error function.[5] This theoretical membrane potential change has the general form of the subthreshold direct current excitability curve[6] up to its maximum, but does not give the subsequent decrease, which is found experimentally.

Both alternating current conductance and direct current excitation data on the same preparation are not yet available, but equation (2) has at least qualitative support. Conductance measurements show that the polarization elements often have an approximately constant phase angle for the complete frequency range or at least a major portion of it.[7] Equation (1) gives a value of α from 0.62 to 0.71 for frog sciatic nerve,[8] and 0.73,[7] 0.79[9] for mammalian muscles. Excitation data for short times give a value of α from 0.53 to 0.86 for frog and toad sciatic,[10] and 0.75 for toad sartorius muscle.[11] These data suggest that conductance and excitation phenomena involve the same polarization element and that this element is not represented by either the condenser or Nernst hypotheses in their simple forms.

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[4] The author is very much indebted to Professor H. T. Davis, of Indiana University, for the inversion and expansion of the Volterra integral equation encountered.

[5] Campbell and Foster, *loc. cit.*, Pair No. 551.

[6] G. H. Bishop, *Am. Jour. Physiol.*, 85: 417, 1928; J. Erlanger and E. A. Blair, *ibid.*, 99: 108, 1931.

[7] K. S. Cole, *Jour. Gen. Physiol.*, 15: 641, 1932.

[8] H. Lullies, *Arch. ges. Physiol.*, 221: 296, 1928; R. Labes, *Arch. exp. Path. u. Pharm.*, 168: 521, 1932.

[9] H. Fricke, *Physics*, 1: 106, 1931.

[10] K. Lucas, *Jour. Physiol.*, 35: 105, 1906; L. Lapicque, "L'Excitabilité en Fonction du Temps," pp. 92, 95, 96, Paris, 1926; W. A. H. Rushton, *Jour. Physiol.*, 74: 424, 1932.

[11] K. Lucas, *loc. cit.*, p. 104.

THE EFFECTS OF CIGARETTE SMOKING UPON THE BLOOD SUGAR[1]

THE gratification derived from smoking has always been rather a mystery. Exactly what elements in the smoke exert the pleasurable physiological effects has never been determined, nor precisely what these effects are. Numerous theories have been advanced. But these theories merely show how little is known.

Tobacco differs from other leafy vegetables in its characteristic alkaloid. That alkaloid, nicotine, is named for Jean Nicot, who introduced tobacco chewing to Catherine de Medici. Nicotine is a powerful drug. It paralyzes nerve ganglia when applied directly to them. But it has not been shown—and it is on the whole improbable—that this property of nicotine accounts for the effects of tobacco smoking.

Chemists have pointed to the carbon monoxide in tobacco smoke and have suggested that it is a cause of the ill effects, if not the pleasure, of smoking. But in fact a heavy smoker accumulates less carbon monoxide than does the non-smoker who takes a walk on Fifth Avenue, New York, during the hours of heavy automobile traffic.

Other products of combustion, notably pyridine, have likewise been suggested; but they occur, not only in tobacco smoke, but also in the smoke from other vegetable matter, such as corn silk, maple leaves and coffee beans. That these substances do not contribute appreciably to the gratification of smoking is conclusively demonstrated by the fact that few smokers adhere to the juvenile substitutes for tobacco. Such substitutes are cheap, yet tobacco maintains its popularity. Why tobacco?

The answer we believe is nicotine. Smoking, we find, produces a definite, although temporary, increase in the concentration of blood sugar, and a corresponding increase in the rate of sugar combustion in the body. These effects certainly are due to the nicotine of the tobacco and they arise from the action of this alkaloid upon the adrenal glands. There can be little doubt that this is the source of at least a considerable part of the gratification from smoking.

Our observation of the hyperglycemia from smoking occurred by chance. We had been investigating the question of the optimum mealtime interval—how often should children, college students and industrial workers be fed. To this end we determined the respiratory quotients, at hourly intervals during the day, on several hundred subjects. In a number of cases

[1] From the Laboratory of Applied Physiology, Yale University.