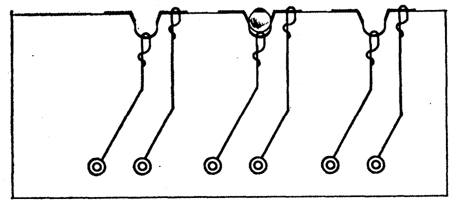

Fig. 2. Schematic view of sockets of a Lucite chamber with associated electrodes. The terminals were connected to the sockets with braided, stainless steel, surgical suture, running in milled channels (subsequently filled with epoxy plastic) in the bottom of the chamber. The silver electrodes were composed of repeated layers of silver printed-circuit ink.

electrical stimulation or after a 30-minute period of drug exposure to the control diameter.

Stimulation of the preparation produced constriction of the pupil. The maximum response was obtained with 8 volts or more, measured between the electrodes. Low frequency stimulation, 1 to 3 pulses per second, produced twitch-like contractions of the sphincter. Frequencies above 3 per second produced a sustained contraction, the degree of which was proportional to the frequency up to a maximum response at 20 pulses per second. In the maximum response, the diameter of the pupil was constricted to 50 percent of that in the control; such a response was achieved in 3 to 5 seconds, and was maintained for the duration of stimulation. Repeated periods of stimulation every 15 minutes for 7 hours decreased the average diameter of the control by 14 percent, and increased the average response by 28 percent. These changes were approximately linear with time over the 7-hour period, indicating excellent stability. Periods of stimulation could be repeated every 4 to 5 minutes, producing similar responses and allowing ample time between each period for return to the initial pupillary diameter of the control. Neither varying the pulse duration from 0.1 msec to 5 msec, nor reversing polarity of the electrodes, had any effect on the response. The routine stimulus parameters adopted were: duration of stimulation, 5 seconds; pulse duration, 0.1 msec; frequency, 20 pulses per second; voltage measured at electrodes, 12 volts.

The effects of various prototypic drugs were as would be expected in a parasympathetic neuro-effector preparation stimulated through the postganglionic nerves. Atropine sulfate ($1.28 \times 10^{-6}M$) not only blocked the constrictor response, but many experiments showed a slight but significant dilation on electrical stimulation. Presumably, this was due to an unmasking of the effect of concomitant sympathetic stimulation which was normally concealed by the preponderance of constrictor strength in the mouse iris. The adrenergic blocking agent phenoxybenzamine hydrochloride ($6.4 \times 10^{-7}M$) abolished this dilation after atropine, and in the absence of atropine, intensified the stimulus-induced constriction by blocking the physiological antagonism of the pupillary dilator muscles.

Physostigmine, in a dose ($1.28 \times 10^{-6}M$) which had no effect on the resting diameter of the pupil, caused the iris to be sensitive to stimulation of the parasympathetic nerves, increasing the constrictor response from 80 to 53 percent of the diameter in the control. Cocaine hydrochloride ($1.28 \times 10^{-3}M$) blocked the pupillary constriction produced by nerve stimulation, but not that produced by the action of the parasympathomimetic drug carbamylcholine. The ganglionic-blocking drugs hexamethonium ($6.4 \times 10^{-3}M$) and tetraethylammonium ($1 \times 10^{-2}M$) had no effect on the preparation (*2*; *4*).

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6 June 1963

Cortisol from Human Nerve

Abstract. *Cortisol was found in myelinated nerve fibers of the lumbosacral plexus (2.0 to 6.0 μg per gram of tissue) and in the sympathetic chain with dorsal root ganglia (0.2 to 0.4 μg per gram of tissue).*

Peripheral nerve tissue, spinal cord, and brain are known to contain large quantities of cholesterol (*1*). Little is known of other possible steroids in these tissues. We have now found that nerve tissues contain relatively large amounts of cortisol (17-hydroxycorticosterone).

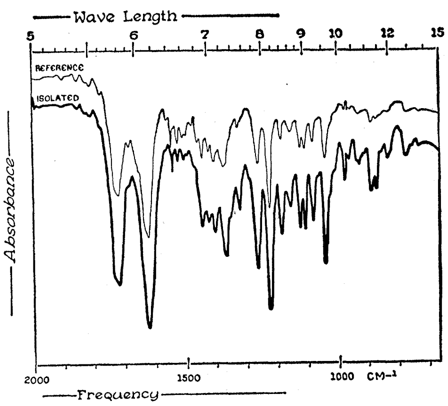

Fig. 1. Infrared spectrum of cortisol isolated from nerve. The acetate was incorporated into a 1.5-mm disk of potassium bromide.

Myelinated nerve fibers of the lumbosacral plexus and the thoracolumbar sympathetic chain which included unmyelinated fibers and dorsal root ganglia were obtained at autopsy. After an average post-mortem period of 10 hours, the tissues were homogenized in a blender with water. After the protein was precipitated with four volumes of acetone, in a manner described for adrenal tissue (*2*), and filtered off, the acetone solution was concentrated by evaporation and the aqueous residue was extracted with a mixture of chloroform and ethyl acetate (1:1); the extract was washed with 5-percent sodium bicarbonate and water. The organic solvents were removed by evaporation and the residue was partitioned between 70-percent methanol and heptane for removal of fats. The 70-percent methanol solution was concentrated by evaporation and the residue was dissolved in chloroform; this chloroform solution was dried over sodium sulfate and the chloroform was evaporated. The residue was subjected to paper chromatography in a toluene–propylene glycol system for three days. The product eluted from the paper chromatogram showed properties identical to those of reference cortisol as follows: an ultraviolet light absorption maximum at 240 mμ; a positive reaction with the blue tetrazolium reagent, specific for the alpha ketol group; absorption spectrum of the Porter-Silber phenylhydrazine reaction product showing a maximum at 410 mμ; the characteristic mobility of the acetate on the paper chromatograms developed in the toluene–propylene glycol system; and the absorption spectrum in sulfuric acid showing maxima at 240, 284, 390, and 475 mμ.

The infrared spectrum of the acetate of the isolated cortisol after chromatography on a microcolumn of silica gel was identical with cortisol acetate formed under the same conditions (Fig. 1). The acetate was made by treatment of the cortisol with pyridine and acetic anhydride at room temperature for 18 hours.

Preliminary data indicate that the amount of cortisol in myelinated nerve fibers of the lumbosacral plexus was of the order of 2.0 to 6.0 μg per gram of tissue, while the sympathetic chain with dorsal root ganglia showed 0.2 to 0.4 μg per gram of tissue. These figures contrast with amounts of 5 to 15 μg per gram in pathological human adrenal tissues (*2; 3*).

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17 July 1963

Exponential Values for the Species-Area Relation

Abstract. *Data on vegetation types in six 900-m² plots in the Midwest reveal that the species-area curves are not logarithmic but more nearly approach an exponential equation of the form* $y = kx^z$ *for areas less than 100 m². For larger areas the curve appears to be sigmoid.*

The relation between the increase in number of plant species as the area sampled increases, termed the species-area relation, has been considered logarithmic (*1*), except for small areas (under about 1 m²). These studies indicate that new plant species are found in proportion to the logarithm of the area sampled. Recently, the logarithmic relation over large areas (greater than 100 m²) has been questioned, and it has been suggested that an exponential equation, based on avian data, best describes the species-area relation (*2*). This report gives results obtained from a study designed to clarify this relation more precisely in plant communities.

Data were obtained in the summers of 1960 and 1961 during field work in six undisturbed stands of vegetation in the Midwest, from Illinois to Minnesota. The stands consisted of jack pine woodland, hill prairie, and oak forest. An area of 900 m² was sampled in each stand. All vascular plants, bryophytes, and lichens in these plots were recorded and collected. Each plot was subdivided into quadrats of the following sizes, with the number of samples of each size indicated in parentheses: point (900), 1 cm² (900), 1 dm² (900), 1 m² (900), 4 m² (225), 25 m² (36), 100 m² (9), 900 m² (1). The large number of samples of quadrats 1 m² and smaller, and the similarity of species-number per quadrat, resulted in a standard error of less than 0.2 for quadrats of these sizes in all six stands, and less than 0.9 for the 4-m² and 25-m² quadrats.

The relationship found among all six stands is similar to that shown in Fig. 1 for jack pine in the plot in Lower Michigan known as Michjap. Inspection reveals that a close fit for the lower and most reliable values is given by the generalized equation

$$y = ke^{m \log x} = kx^z ,$$

where y is the number of species, x is the area, k is a constant equal to the number of species in a 1-m² quadrat, and z is a constant equal to 0.43 m. In the plot illustrated this equation becomes

$$y = 7.5x^{0.30}$$

This exponential fit is far superior to a logarithmic one (for example, $y = k \log x$), particularly for the lower values. Construction of equations for the other five stands reveals the following values for y: jack pine in Upper Michigan, $10\ x^{0.28}$; jack pine in Wisconsin, $16\ x^{0.32}$; jack pine in Minnesota, $15.5\ x^{0.33}$; hill prairie in Illinois (in the plot known as Prairie), $12\ x^{0.26}$; and oak forest in Illinois (Oak), $13\ x^{0.43}$. In these equations the size of the exponent is related to the number of species recorded in the plot—the greater the number of species, the larger the exponent. In Oak, described by the equation with the largest exponent, 113 species were noted, while only 66 were recorded in Prairie, the equation represented by the smallest exponent.

It can be seen that the actual y values from Michjap drop below those of the equation at the 900-m² point. This tendency occurs in the other five samples as well and indicates that further sampling would reveal the relation to be sigmoid, as suggested earlier (*3*). This is further corroborated by comparing data on the number of vascular plant species recorded from the two-county region where the plot Michjap is located (*4*). This area, 3.07×10^9m², inserted into the equation gives a value of 5700. This value far exceeds our best estimates of plant species in this area, which contains 1180 vascular species (*4*), 250 to 350 mosses, and 300 to 350 lichens (*5*); a total of 1750 to 1900 species. Similar evidence from other stands makes it clear that the above equation, though very accurate for smaller areas, is not valid for large ones, and that the actual species-log area relation is sigmoid.

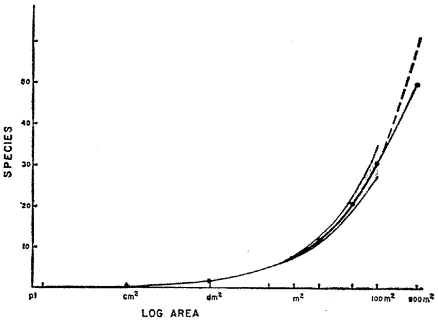

Fig. 1. Graph of the species-log area relation of a jack pine stand (Michjap) in Michigan compared with the equation $y = 7.5\ x^{0.30}$, shown by the dashed line. Lines coincide below 4-m² point. The lightly shaded area between the curves is double the standard error either side of the actual curve.

The data presented support the view put forth by Preston (*2*) that the species-area relation is exponential, not logarithmic, and appropriate equations have been presented. Elucidation of the species-area relation in larger areas than those sampled in this study and in different types of vegetation requires further analysis (*6*).

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1 August 1963