of determining the toxicity of antiseptics to cells. The antiseptics tested differed markedly in their cytocidal activity, sodium sulfathiazole being the least and mercuric chloride the most toxic. Tinctures of merthiolate and zephiran were more toxic than the corresponding solutions. The polymorphonuclear leukocyte was more resistant to most of the reagents tested than the lymphocyte.

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Direct Stimulation of the Hypoglossal Nucleus by Acetylcholine in Extreme Dilutions.

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Acetylcholine (ACh.), when applied in minute amounts to the eserinized cerebral cortex, induces marked excitatory effects;[1] it was inferred that the ACh. stimulates or facilitates various cortical synapses. The experiments here reported indicate that ACh., on direct application in extreme dilutions, stimulates a motor nucleus, namely the hypoglossal nucleus; this nucleus is readily accessible, as the *trigonum hypoglossi,* immediately beneath the floor of the fourth ventricle.[2]

Methods. Decerebration under ether was by the Sherrington decerebrator. Both common carotids were tied or, in other experiments, both internal carotids were tied and also the branches of external carotids, but leaving lingual arteries intact; essentially similar results were obtained in both preparations. The skull was held in a special clamp, so that the entire tongue could be observed. The floor of fourth ventricle was exposed by removing median parts of cerebellum. Acetylcholine chloride (ACh.Cl) (Merck) was dissolved in buffered Ringer-Locke solution and was applied in small rectangles 2 mm x 6 mm of spot test paper to the XII nuclei of both sides.

Results. Preliminary localization of effects from the XII nucleus was by unipolar faradization with a fine silver wire, ending in a minute bulb; the diffuse electrode was a saline-soaked pad on animal's back. Secondary distances varied from 35 to 50 cm, using a large Palmer inductorium (Fig. 1).

The following effects of ACh. were obtained in a decerebrate cat (3100 g), having lingual arteries intact. Preliminary eserinization was by 0.25 mg eserine sulphate (Merck) intravenously. A rectangle of spot test paper soaked in ACh.Cl. 1:50 millions was applied to both XII nuclei: in 4 sec. a slight median furrowing appeared at base of tongue between circumvallate papillæ (Fig. 2); the furrowing, which coincided with inspiration, increased and was accompanied by simultaneous retraction of tongue base, elevation of soft palate and dilatation of remainder of mouth cavity. The ACh. increased respirations from 22 to 32/min.; these observations on respiratory stimulation agree with those of Worzniak and Gesell[3] and of Hansen, Worzniak and Gesell.[4]

Application of another rectangle of ACh.Cl. 1:10 millions intensified above effects within 5 sec.; in consequence of a further application of a rectangle of ACh.Cl. 1:10 millions and 2 successive rectangles of ACh.Cl. 1:1 million, with 0.25 mg eserine intravenously, the following symptoms developed (Fig. 2): a marked furrowing at base of tongue, coin-

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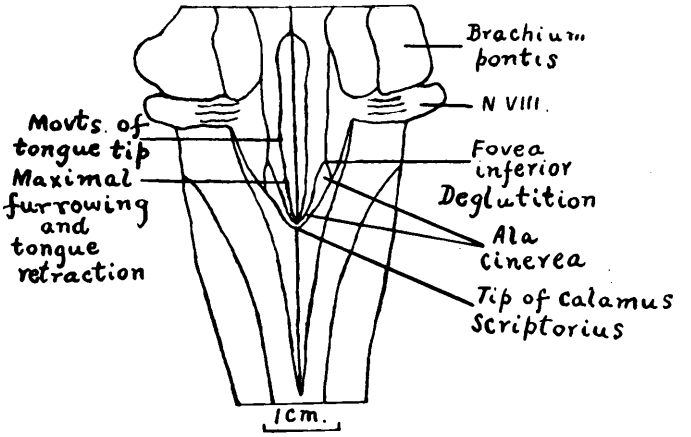

FIG. 1.

Floor of fourth ventricle of cat. × 2. (Wt of cat 3700g.) Effects of unipolar faradization: XII nucleus yielded movements of tongue tip at point shown; caudally to near tip of calamus scriptorius it yielded median longitudinal furrowing and general retraction of tongue; maximal furrowing at point shown. Inferior fovea yielded deglutition.

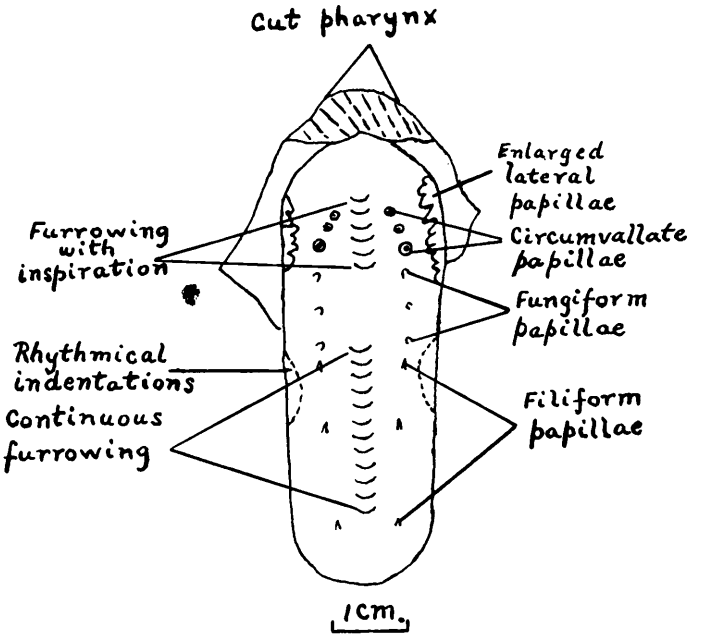

FIG. 2.

Tongue of cat; actual size. Effects of ACh. applications to XII nuclei. Rectangles of spot test paper 2 mm × 6 mm soaked in ACh. were applied from point for tongue tip back to calamus (Fig. 1). Dilutions of ACh. 1:50 millions, later 1:10 millions and 1:1 million. Cat eserinized.

cident with inspiration, also simultaneous elevation of soft palate and dilatation of mouth cavity, as at first, but all more intense; a continuous furrowing farther forward, with bilateral rhythmical indentations; all these movements were very violent; at the same time respirations were accelerated to 40/min.

Four mg atropine sulphate (Merck) were then given intravenously, whereupon, within 15 to 20 sec., all the above tongue and other movements ceased completely, while respirations fell to 22/min. Repetition of intravenous eserine and application to the XII nuclei of ACh.Cl. 1:1 million now yielded

none of the previous effects on tongue, palate or mouth cavity, while the respiratory rate remained unaltered. Thus atropinization abolishes and precludes the action of ACh.

In another experiment a rectangle of ACh.Cl. 1:50 millions to the XII nuclei, followed by one of ACh.Cl. 1:10 millions augmented tremors in the tongue, initiated by intravenous eserine, and also evoked furrowing, tongue retraction and later violent generalized tongue movements; applications also evoked repeated deglutitions.

In a *non-eserinized* decerebrate preparation ACh.Cl. 1:50 millions on both XII nuclei yielded, in 35 sec. (longer than after eserinization), movements of tip of tongue, retractions and repeated deglutitions; thus, while responding to ACh., the nucleus was definitely less sensitive than after eserinization; also its responsiveness declined to further applications.

Discussion. The view is expressed here that ACh. exerts a local stimulating or facilitating action on synaptic terminations in the XII nucleus; support for this view is afforded by the following considerations: the general similarity between effects of ACh. application and faradization of nucleus; the slight depth of nucleus below floor of fourth ventricle, permitting rapid diffusion to it of ACh , the extreme dilutions (1:50 millions) of effective solutions of ACh.; the brief latency for effects of ACh. (4 to 15 sec. in some cases). The neurones of the XII nucleus are surrounded by numerous synapses derived from an extensive interneuronal plexus.[5] Thus it is inferred that ACh. diffuses and directly stimulates or facilitates these synaptic terminations and that this action is much intensified by eserinization. The specific nature of the action is further emphasized through its being annulled by atropinization.

Deglutition may be explained by the action of ACh. near the inferior fovea; faradization of this point yields reflex deglutition[6] and it was supposed that the currents impinged on afferent fibers in the *tractus solitarius,* which lies sufficiently close to the inferior fovea. Similarly the ACh. may be supposed to diffuse to, and stimulate or facilitate, synaptic afferent terminations (probably glossopharyngeal and vagal) within the nucleus of the *tractus solitarius;* fibers from this nucleus probably pass to the XII nucleus, the nucleus ambiguus and dorsal vagus nucleus, through which pathways deglutition would be evoked.

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14406

Production of Diabetes Mellitus in Rats with Alloxan.*

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Jacobs[1] observed that alloxan causes hyperglycemia followed by hypoglycemia in rabbits. Shaw Dunn and associates[2,3] have shown that the pathologic substrate of this response is necrosis of the pancreatic islets. Further study of the animals was impossible because they all died within a few days. Brunschwig, Allen, Goldner, and Gomori[4] succeeded in keeping rabbits alive for longer periods after

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