Hebbel E. Hoff, assistant professor of physiology, and John F. Fulton, Sterling professor of physiology, for a study of the primate motor unit.

Hebbel E. Hoff, assistant professor of physiology, and Louis H. Nahum, research assistant in physiology, for studies of cardiac arrhythmias.

Marion E. Howard, instructor in medicine, for a continuation of (1) studies on lymphogranuloma inguinale, and (2) study of the virus of lymphocytic chorio-meningitis.

Carlyle F. Jacobsen, assistant professor of psychobiology, for a continuation of studies of relations of behavior to neural functions.

Ralph H. Jenkins, assistant clinical professor of urology, for a study of the germinal epithelium of the testicle in relation to the endocrines.

Margaret A. Kennard, assistant professor of physiology, and Delafield DuBois, research assistant in physiology, for a study of skin resistance after cortical lesion.

Margaret A. Kennard, assistant professor of physiology, for study of frontal lobe functions in monkeys.

John H. Lawrence, instructor in medicine, to continue study of the comparative effects of x-ray and neutrons on tumors in animals.

Gustaf E. Lindskog, assistant professor of surgery, for the following studies: (1) The effect of environmental temperatures on the subtidal lung volume; (2) the effect of lipoidal on the normal pericardium; (3) the effect of Roentgen rays on the normal lung and pleura (pathological and physiological changes).

Cyril N. H. Long, professor of physiological chemistry, for a continuation of investigations on the relation of pituitary and adrenal glands to carbohydrate metabolism.

Donald G. Marquis, assistant professor of psychology, for studies of the visual functions of the cortex, and for an investigation of the neurophysiological mechanism of the sympathetic nervous response in emotional situations.

Clyde S. Marshall, assistant professor of anatomy, for studies of the pyramidal system and of accessory motor pathways.

Ralph G. Meader, assistant professor of anatomy, for studies of the evolution of the optic system, together with an experimental analysis of the evolution of retinal projection.

Arthur H. Morse, professor of obstetrics and gynecology, and Gertrude van Wagenen, research assistant in obstetrics and gynecology, for studies relating to the processes of reproduction.

Chris H. Neuswanger, assistant clinical professor of urology, to continue study of methods of treating various diseases of the ureter.

Ashley W. Oughterson, associate professor of surgery, to continue experiments on the effect of tobacco on the vascular system, and for investigations relating to tumors.

John P. Peters, John Slade Ely professor of medicine, and assistants, for studies of metabolism.

Theodore C. Ruch, instructor in physiology, for a comparative study of the sensory tracts of the spinal cord in relation to the process of ''corticalization'' of sensory function.

Elizabeth R. B. Smith, honorary research fellow, and Paul K. Smith, research assistant in pharmacology and toxicology, to continue the study of thermodynamic properties of amino acids and related substances.

Leon S. Stone, associate professor of anatomy, to continue and extend investigations dealing with studies of living nervous tissues in amphibian embryos.

Robert Tennant, assistant professor of pathology and surgery, and Averill A. Liebow, research assistant in pathology, for a study of growth characteristics of mammalian neoplasms by tissue.

Herbert Thoms, associate professor of obstetrics and gynecology, for a continuation of the study of physical constitution in relation to labor and pelvic types.

James D. Trask, associate professor of pediatrics, (1) to continue hemolytic streptococci studies; and (2) for clinical application of Goodpasture's method for the cultivation of virus on the chorio-allantoic membrane of the chicken egg.

George Valley, assistant professor of bacteriology, to continue the study of *Clostridium histolyticum.*

Abraham White, assistant professor of physiological chemistry, for studies on (1) the constitution of insulin and (2) sulfur metabolism.

Arthur M. Yudkin, clinical professor of ophthalmology, to continue studies on cataracts.

Harry M. Zimmerman, associate professor of pathology, for studies on convulsive disorders.

SPECIAL ARTICLES

A PROLONGED AFTER EFFECT FROM ELECTRICAL STIMULATION OF THE CEREBELLAR CORTEX IN UNANESTHETIZED CATS[1]

HAVING developed a technique which allowed permanent electrodes to be implanted on the cerebral cortex,[2] it was decided to try the effect of stimulation with this type of electrode on the cerebellum of unanesthetized animals with intact brains. The results obtained were so significant in the light of previous reports[3, 4, 5] that it was thought worth while to describe the main phenomena in a preliminary account.

The electrode consists of a stainless steel tube, containing an insulated silver wire, which is screwed into

[1] This work has been aided by a grant to Vanderbilt University School of Medicine from the Division of Medical Sciences of the Rockefeller Foundation.

[2] Sam L. Clark and James W. Ward, *Arch. Neur. and Psychiat.* (to be published).

[3] A. T. Mussen, *Brain,* 50: 313, 1927; *Arch. Neur. and Psychiat.,* 23: 411, 1930 and 25: 702, 1931.

[4] W. K. Hare, H. W. Magoun and S. W. Ranson, *Am. Jour. Physiol.,* 117: 261, 1936.

[5] H. W. Magoun, W. K. Hare and S. W. Ranson, *Arch. Neur. and Psychiat.,* 37: 1237, 1937.

a trephine hole in the skull. The end of the silver wire (embedded in a glass rod) which is in contact with the brain through a hole in the dura mater is the stigmatic electrode, the steel tube acting as the indifferent electrode. The voltage of the stimulating current is controlled through a volt-meter and the length of stimulus is determined by a timing device. The cats are unrestrained throughout the experiment.

The results of stimulation depend upon the strength of the stimulus and the location of the electrode, but when a sufficiently strong stimulus is used (2 to 5 volts 60-cycle sine wave current for 2 to 10 seconds) provided the animal has not been subjected to strong stimulation too recently, a series of movements lasting several minutes occurs which involves the various portions of the animal in a definite order. Stimuli of less strength produce briefer and less extensive effects.

The attack is quite different from the epileptic fits provoked by stimulation of the cerebral cortex; the movements are not so spasmodic and resemble somewhat the movements observed in "slow motion" pictures.

The results can best be presented by describing a single attack. In cat 59, for example, on May 5, an electrode was planted on the left side of the cerebellum at the margin of the vermis on the second folium back of the fissura prima. On May 10 a stimulus of 3.1 volts for 5 seconds produced the following results:

During the stimulus the cat suddenly drew back its head, leaving both fore feet extended in front. As the stimulus ceased, the cat lifted its left forefoot and held it up for 30 seconds. At 1 minute after the stimulus the left forefoot was again lifted gradually until it was held high in flexion. The foot and leg seemed sensitive to touch. The foot was gradually returned to the table but remained tense. At 1 minute 50 seconds after the stimulus the cat turned its head to the right and the right forefoot began lifting and appeared hypersensitive. After a short while this foot was replaced and the cat remained inactive until 4½ minutes after the stimulus, when the left hind limb began lifting and was held up. At 5 minutes the tail curved around to the right side and was held in this position while the cat turned in a small circle to the left. At 5 minutes 15 seconds the right hind limb began lifting and was held up awhile and at 5 minutes 30 seconds the tail curved tonically to the left, where it was held until 6 minutes 50 seconds. By 7 minutes after the stimulus the cat had apparently recovered.

While this attack is typical in that it shows the sequence of limb involvement, *i.e.,* homolateral then contralateral fore limb, followed by hind limbs in the same order, it does not present all the effects seen on head and trunk. When the cerebellum is stimulated on the right side (as was done in this animal with another electrode planted later) the same sequence occurs, beginning with the right (homolateral) fore limb. When the electrode is planted as near the mid-line as possible, on stimulation both fore limbs are involved at once and the cat may sit on its haunches with both fore limbs in the air; then both hind limbs become affected, so that the cat may try to raise both at once and teeter on its fore limbs as on a fulcrum, the tail meanwhile being held straight up. Since it is difficult to plant an electrode exactly in the mid-line, the limbs on one side may lead slightly in the parade of events.

The response usually begins with phases similar to those described by Hare, Magoun and Ranson[4]; at first, movements coincidental with the stimulus, followed by movements of opposite nature, appearing as a rebound. This may resemble the "tegmental reaction." Then, appearing over the space of 5 to 10 or more minutes, the series of effects on the head, trunk, limbs and tail described above occur. In a few instances, near the termination of the first series, a new one has started with lifting of the homolateral fore leg, but as yet a second series has not continued through.

The pattern of the response varies with the location of the electrode on the cerebellum and while some 16 electrodes have been planted, much of the surface of the cerebellum is relatively inaccessible and has not yet been explored with this method. While the response begins according to the particular spot stimulated, the events occurring throughout the long after effect seem to suggest that other parts (perhaps all) of the cerebellar cortex, are gradually involved.

The pattern of response is a constant one for each point under controlled conditions, just as was the case with the cerebrum. Similar factors seem to influence responses from the cerebellum and cerebrum, since anesthesia and previous strong stimuli diminish the response to stimuli in both; weaker stimuli produce the beginning of the pattern or a proportional amount of it. Various operative procedures have been carried out to determine the effect on these attacks, and these will be discussed later in detail, but it is interesting that the long sequence of events resulting from stimulation was not abolished by removal of the contralateral motor area of the cerebrum in 2 cats nor by splitting sagitally the cerebellum in one cat. (At autopsy it was found that the cerebellum was successfully split except in the periphery of the vermis both anteriorly and posteriorly.) In two cats when a lateral lobe of the cerebellum was removed and the intact half stimulated, the long after effect involved only the homolateral fore limb.

The results of this work promise support in general for a localization of cerebellar cortical areas as described by Mussen,[3] though details are yet to be worked out.

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CRYSTALLINE PAPAIN[1]

Crystals showing proteolytic activity have been ioslated from the undried latex of green papaya fruit. The crystals show the usual properties of proteins. The substance contains nitrogen precipitated from aqueous solution by trichloracetic acid and has been isolated by methods commonly employed in the purification of protein.

The crystalline material clots milk, digests casein and splits hippurylamide in the presence of added cysteine under the conditions usually employed for demonstrating the activity of papain. The activity of the crystalline preparation per mg of protein nitrogen as measured by milk clotting or by casein digestion is from 25 to 50 per cent. higher than that of any of the amorphous preparations made in this laboratory, and is about twice as great as that of the best commercial preparations.

No essential difference in activity was observed between thrice and five times crystallized material, and the ratio of the milk-clotting, casein-digesting and hippurylamide-splitting properties is approximately the same as found in dried latex and in amorphous precipitates prepared from fresh latex.

Without added activator, the activity of the crystals varies, apparently depending upon the treatment during preparation. Determinations made without added activator are obviously not as accurate as those run in the presence of cysteine, because of oxidation during the time of digestion. Accordingly the values obtained in short-time intervals (milk clotting data) are probably the most accurate. On this basis some of the crystals were nearly inactive, others showed originally about half the maximum activity. All these preparations reached the same level of activity when pre-treated with cysteine. A small sample of thrice crystallized material which was half active was incubated with dilute hydrogen peroxide and then crystallized three times more to remove the reagent. The final crystals were between 94 to 97 per cent. inactive.

Due to lack of raw materials, the quantity of crystals available thus far has been extremely small, and many desirable experiments have had to be postponed until more material is available. Recently crystals have also been obtained from commercial papain but have not yet been freed from amorphous material.

Improvements in the method for preparing the crystals will also be studied. Those used presently were prepared in outline as follows:

Coagulated papaya latex, preserved with toluene, was suspended in some cases in about four times its weight of water, in other cases in about two volumes of 0.25 saturated ammonium sulfate. After about an hour the material was filtered and the clear filtrate was made 0.6 to 0.7 saturated with ammonium sulfate and filtered. The semi-dry filter cake was suspended in about an equal weight of water. The pH was adjusted to light green to brom thymol blue and the solution was cooled slowly from 20° to 5° C. (24 hours). The solution containing about 15 mg protein nitrogen per cc became turbid on cooling and in a few days developed a sheen due to the formation of small needle crystals. Particularly after crystal formation, slow addition of a saturated solution of ammonium sulfate may increase the yield. Recrystallization was carried out by essentially the same technique.

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[1] Food Research Division contribution No. 343.

THE MECHANISM OF BACTERIOPHAGE PRODUCTION[1]

When bacteriophage is added to a culture of susceptible bacteria growing in an appropriate medium two phenomena occur. First, during the period of contact there is produced a considerable additional amount of phage and, second, as a terminal event the bacteria quite suddenly break up, leaving the medium clear.

d'Herelle[2] and Burnet[3] have stressed the importance of cellular lysis in the production of phage. According to them phage particles penetrate into the bacterium, multiply, but remain under spatial constraint until set free when the cell undergoes dissolution. Sufficient experimental evidence has accumulated to prove that bacterial lysis is not causally related to the phage-producing mechanism.[4] In the place of lysis, bacterial growth has come to be considered a *sine qua non* for phage production. Krueger and Northrop[5] found that factors such as reduced temperature or limitation of nutrients, which interfere with bacterial growth, likewise reduce phage formation. They de-

[1] The experimental work cited in this paper was supported by grants-in-aid from the National Research Council, the American Medical Association and the Board of Research, University of California.

[2] F. d'Herelle, "The Bacteriophage and Its Behavior." Williams and Wilkins Co., 1926.

[3] F. M. Burnet, *Brit. Jour. Exp. Path.*, 10: 109, 1929.

[4] A. P. Krueger, *Physiol. Reviews*, 16: 1, 1936.

[5] A. P. Krueger and J. H. Northrop, *Jour. Gen. Physiol.*, 14: 223, 1930.