Hippocampal Lesions Following Administration of 3-Acetylpyridine. (24152)

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In testing hypotheses regarding the role of the hippocampus in emotion, memory, and learning, it would be desirable to observe the effects of complete removal of this structure (1). With usual technics, this is impossible to accomplish without causing extensive damage to other structures. In reporting acute experiments with 3-acetylpyridine (3AP), Hicks stated that cerebral changes were limited to destruction of cells in the hippocampus and supraoptic nuclei(2). Although the extent of damage in the hippocampus was not specified, this suggested to us the possibility of obtaining hippocampal lesions in chronic preparations which could be used for behavioral and neuro-anatomical studies. The findings in the mouse reported here have encouraged an extension of this investigation to cats and monkeys.

Materials and methods. Adult, male, non-inbred albino mice and rats, maintained on stock diet, were used. The LD_{50} of 3AP was 300-350 mg/kg for mice and 80 mg/kg for rats. A single intraperitoneal injection of LD_{50} dose was given in water. Animals were sacrificed with ether and perfused through the heart with 10% formalin. The brains were mounted in celloidin and sectioned at 25 μ. Every tenth section was stained with cresyl-violet for cells, and, in most instances, facing sections were stained by the method of Weil or Woelcke for myelin. For control, brains of 2 normal mice were prepared in the same manner. In a few instances the frozen section technic was used. The brains of 20 mice and 10 rats were examined.

Results. About 6 hours after injection of 3AP, rats developed an apparent weakness of extremities and rolled from side to side upon attempting to move. Surviving animals manifested inspiratory rhonchi, urinary incontinence, and weakness of hind legs. Gross pathological examination of organs and microscopic examination of brains were not remarkable.

Mice. Surviving mice lost weight and developed an unkempt appearance. Inspiratory rhonchi were characteristically present. About a third of the animals developed urinary incontinence and a high-stepping gait. Fiery red tongue was not noted. The animals were sacrificed at intervals from 4 hours to 2 months after treatment. The gross pathological examination was not remarkable. Histological examination was confined to the brain. Of the 20 mice examined, 15 showed slight to complete loss of neurons in areas of the hippocampus designated *CA3* and *CA4* (Fig. 1A) by Lorente de Nó(3) (area H3 of the Vogts). When the damage was slight, it was most conspicuous in the region of *CA3a*. In cases of moderate damage, there was an extension of cell loss, usually complete, to the rest of area *CA3* (Fig. 1C). With severe damage, there was a complete loss of neurons in *CA4* as well (Fig. 1D). There was no neuronophagia, and no increase in glia was evident. Of the 12 mice allowed to survive at least one month, 5 showed moderate to severe damage of the foregoing areas. In some cases, damage was irregularly present in other areas of the hippocampus and in the dentate gyrus, but in no other parts of the brain.

Discussion. The present study adds to the growing evidence that the metabolism of hippocampus is distinctive from that of other types of cortex. It is possible that our findings will eventually be found to have some correlation with the observations: (i) 3AP, which is an analogue of nicotinic acid, may act as a competitive antagonist of this substance(4); (ii) chronic nutritional deficiency in man, characterized by lack of niacin (pellagra), may be accompanied by psychotic manifestations; (iii) dithizone, a chelating agent which gives a reddish color when combined with zinc, stains and sharply demarcates the same area of the hippocampus destroyed by 3AP(5,6); (iv) *in vitro* studies suggest that the active site of DPN dependent

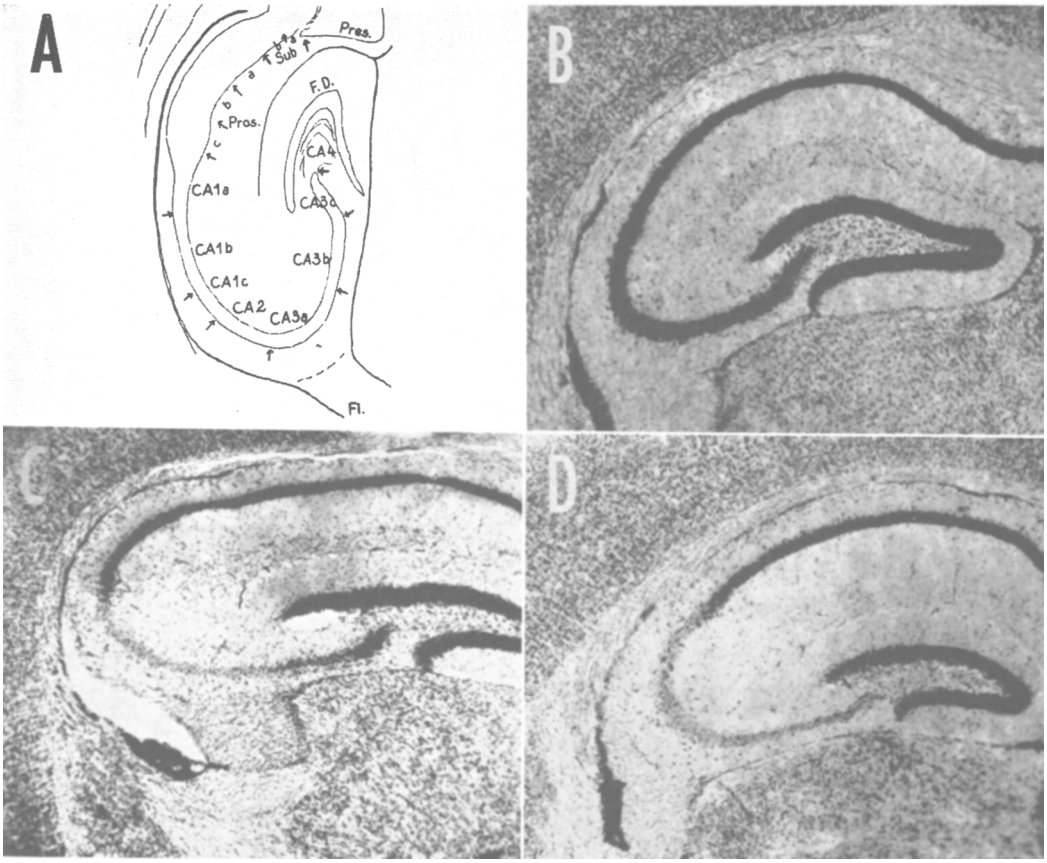

FIG. 1. A: Lorente de Nó's areal subdivision of hippocampus in mouse (Lorente de Nó, 1934); CA, cornu ammonis; F.D., fascia dentata; Pros., prosubiculum; Sub, subiculum; Pres., presubiculum. B: Nissl stain of section through "normal" hippocampus of mouse. C: Loss of neurons in region corresponding to *CA3* in 3AP-treated mouse. D: Section from another experiment showing, in addition, destruction in region of *CA4*.

systems may involve an interaction involving sulfhydryl groups and zinc(7); (v) administration of C^{14}-labeled isoniazid leads to high radioactivity in the hippocampus(8); (vi) isoniazid is related to iproniazid which inhibits one of the enzymes that participate in destruction of serotonin, noradrenalin, and other amines; (vii) serotonin is found in high concentration in the hippocampus(9); and (viii) radioautographic studies employing S^{35}-labeled l-methionine suggest a high protein turnover in the hippocampus(10).

Summary. Administration of 3-acetylpyridine (3AP) to non-inbred, adult mice results in cerebral changes restricted almost exclusively to areas *CA3* and *CA4* of the hippocampus. Ability to induce such lesions in chronic preparations has encouraged extension of this investigation to cats and monkeys with the eventual purpose of performing behavioral studies concerned with elucidating the functions of the hippocampus.

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Properties of Myxoma Virus Transforming Agent. (24153)

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Investigations of the phenomenon described by Berry and Dedrick(1) have developed currently in several stages, the first having been to transform fibroma into myxoma virus in a predictable fashion. This has been accomplished in tissue culture as previously described(2,3). The present report concerns the nature of the heat-inactivated transforming agent (TAM) used in above experiments and of conditions under which it operates in tissue culture. The effect of enzymes, organic solvents, and the photodynamic action of light on this preparation were studied (Table I). Information gained by such means has proved of value in continuing studies to determine the relation of the nucleic acids of myxoma virus to the Berry-Dedrick phenomenon. The results are presented here.

Materials and methods. Transforming agent myxoma (TAM) was prepared by concentrating myxoma virus in an ultracentrifuge at 30,000 to 40,000 rpm in a No. 40 rotor, then heating the resuspended sediment at 65°C for 40 minutes. TAM mixed with live fibroma virus was inoculated into cultures of trypsinized rabbit kidney cells. Supernatant fluids harvested from these cultures 3 times a week, were tested for presence or absence of transformation by intracutaneous inoculation of rabbits. Since fibroma is a benign and myxoma a fatal disease of rabbits, these tests had a sharp end point. More complete details are given in previous publications(2,3). Additional methods are presented under various headings below.

Results. Effect of organic solvents. Infectivity of myxoma virus was destroyed when

TABLE I. Effect of Various Treatments on Myxoma Virus and on TAM (Transforming Agent Myxoma).

Treatment	Activity of TAM	Infectivity of myxoma virus
Heat 65°C (3 to 45 min.)	Retained	Destroyed
Anesthetic ether	"	"
Chloroform	Destroyed	"
DNAse	Retained	ND*
Trypsin	"	ND
Visible light + toluidine blue	Destroyed	Not totally destroyed
Ultraviolet light	"	*Idem*

* ND = Not done.

1:5 suspensions were extracted with equal volumes of anaesthetic ether for 30 minutes to 2½ hours at room temperature. This was shown by separation and testing of the aqueous layer. No live virus was recovered from 7 lots of myxoma treated in this manner, and tested by intracutaneous inoculation of rabbits, as well as by passage in tissue culture. Two lots, 18 and 19, were further tested in rabbit skin in dilutions of 10^{-1} to 10^{-8}. No live virus was revealed. The 7 lots of myxoma were now designated as ETHER-TAMS and when mixed with live fibroma virus, were tested for transforming activity in a series of tissue culture experiments. Table II shows that 4 of these ETHER-TAMS (Lots 18, 19, 28, and 39) were active in transformation, a finding indicated by appearance of live myxoma virus in 16 of 21 experiments. Aliquots from 2 of these ETHER-TAMS, were heated at 65° for 12 to 40 minutes. These exposures were well beyond those needed to inactivate live myxoma, as elsewhere described(3). The ETHER-TAMS subjected to both treatments in this manner appeared as effective as when ether was the only method used to inactivate.

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