nervous system disease, so that complete evaluation of folic acid in that respect cannot be made at this time. However, Doan and Moore[8] independently have recently reported the development and progression of central nervous system disease in pernicious anemia patients while receiving folic acid therapy. Folic acid appears to be equally as effective as liver extract therapy in the treatment of uncomplicated cases of Addisonian pernicious anemia. While folic acid is effective in producing a favorable hematologic remission in cases of pernicious anemia, it is certain that liver extracts contain active material other than this compound, since the potency of such extracts is greater than can be accounted for on the basis of their folic acid content.

Conclusion. 1. Folic acid (pteroylglutamic acid) is as effective as liver extract in correcting the hematologic deficiency in Addisonian pernicious anemia. 2. Further experience is needed before one can evaluate the effectiveness of this factor in preventing development and progression of central nervous system disease.

[8] Doan, C. A., and Moore, C. V., personal communication to R. R. K.

15740

Effect of Frequency of Hypothalamic Stimulation upon Bladder Response.

BÖRJE UVNAS. (Introduced by H. W. Magoun.)

From the Departments of Anatomy and Physiology, Northwestern University, Chicago, Ill.

Bladder contraction has been observed by many investigators as the result of the stimulation of the hypothalamus (Karplus and Kreidl,[1] Hess[2] and others). From their experiments on cats Kabat, Magoun and Ranson[3] and Magoun[4] concluded that a center for the contraction of the bladder is localized in a region just rostral to the hypothalamus and that fibres from this region run caudalward through the lateral part of the hypothalamus. Relaxation of the bladder was only infrequently observed but points giving inhibitory responses were found in different regions of the diencephalon (Kabat *et al.*[3]) Beattie and Kerr[5] claimed the existence of centers for the increase of bladder tonus in the anterior hypothalamus and for inhibition in the posterior hypothalamus and upper midbrain.

The blood pressure and respiratory responses to hypothalamic stimulation have been shown to be strongly influenced by the frequency of the stimulus (Hare and Geohegan,[6] Bronk, Pitts and Larrabee,[7] Berry, McKinley and Hodes[8]). In the present experiments the effect of frequency of hypothalamic stimulation upon bladder response was observed.

Methods. Twenty-four cats under intravenous chloralose anesthesia (50 mg per kg body weight) were used. The hypothalamus was stimulated by a bipolar electrode, made by cementing together 2 lengths of enameled nichrome wire, and oriented with the Horsley-Clarke technic. A thyratron stimulator (Rahm), delivering impulses within a frequency range of 3.5 to 1000 per second, was

[1] Karplus, J. P., and Kreidl, A., *Arch. ges. Physiol.*, 1909, **129**, 138.

[2] Hess, W. R., *Arch. f. Psychiatr.*, 1936, **104**, 548.

[3] Kabat, H., Magoun, H. W., and Ranson, S. W., *J. Comp. Neurol.*, 1936, **63**, 211.

[4] Magoun, H. W., *Am. J. Physiol.*, 1938, **122**, 530.

[5] Beattie, J., and Kerr, A. S., *Brain*, 1936, **59**, 302.

[6] Hare, K., and Geohegan, W. A., *J. Neurophysiol.*, 1941, **4**, 266.

[7] Bronk, O., Pitts, R. F., and Larrabee, G., *Res. Publ. Nerv. Ment. Dis.*, 1940, **20**, 323.

[8] Berry, C., McKinley, W., and Hodes, R., *Am. J. Physiol.*, 1942, **135**, 338.

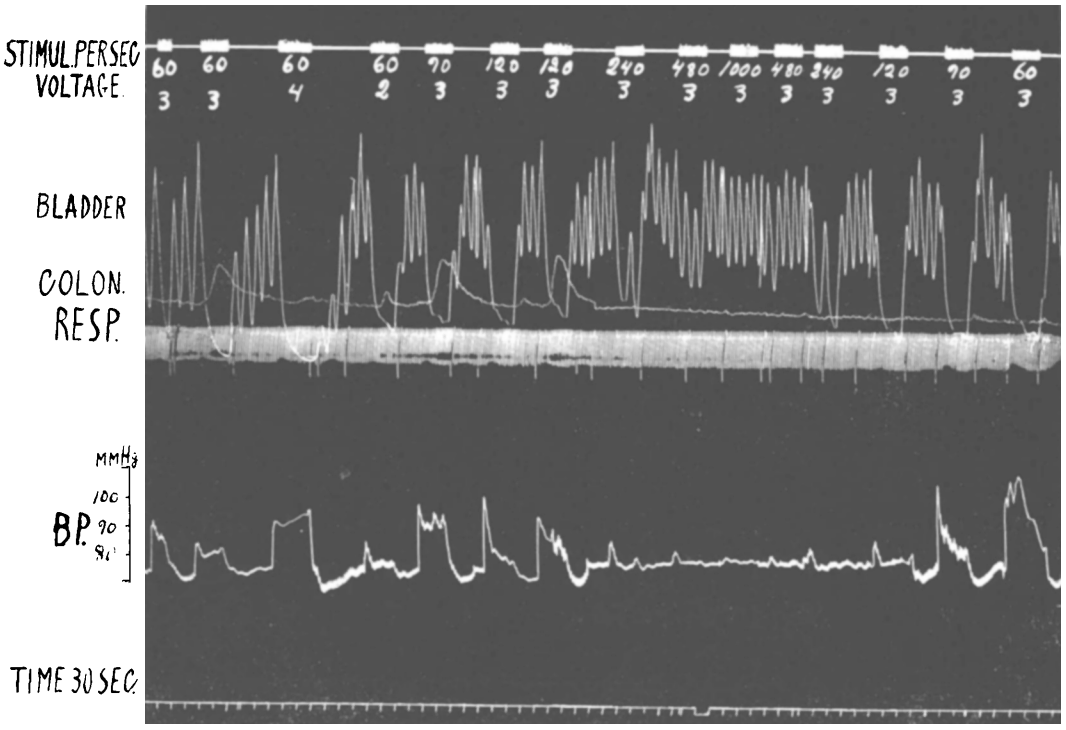

FIG. 1.
Alterations in bladder, colon, blood pressure, and respiration on stimulation of a point in the posterior part of the supraoptic region. Frequency varied.

employed. The pressure of the bladder was recorded by a water manometer connected to a cannula inserted into the bladder through the urethra. In about half the cases the brain was prepared for microscopical examination.

Results. Stimulation of numerous points in the hypothalamus and the preoptic region yielded changes in the intravesical pressure and the peristaltic activity of the bladder. The effects could be either excitatory or inhibitory, the response obtained being strongly influenced by the frequency of the stimulus used. It was an almost regular finding that high frequency stimuli (60 sec. or more), if effective, yielded inhibitory and low frequency stimuli (3.5/sec.), if effective, excitatory responses. The ranges of frequency usually giving inhibitory or excitatory responses are shown in Fig. 1 and 2. As seen, stimuli of a frequency from 15 to 240/sec. yielded a significant inhibition of the bladder. The intravesical pressure as well as the peristaltic activity was depressed. The frequency range giving a pronounced fall of the intravesical pressure and a complete inhibition of the peristalsis in this experiment was observed to be between 30 and 120/sec. At 15/sec. a small initial rise was observed to precede the fall of intravesical pressure and the pressure started to rise before the end of the stimulation. A similar tendency to "escape" was seen at the stimulation with 240 impulses per second. Stimuli of higher frequency were ineffective. The inhibition of the bladder caused by stimuli within the most effective range usually outlasted the stimulation for a considerable time, sometimes for as much as 1-2 minutes or more. Lowering the frequency of the stimuli to 3.5/sec. caused a reversal of the response. A contraction occurred as seen at No. 9 and 10 in Fig. 2. The contractions induced did not persist during the whole period of stimulation, the pressure tending to decline to the initial level after the peak of the contraction was reached. The latent period for the excitatory as well as the inhibitory responses

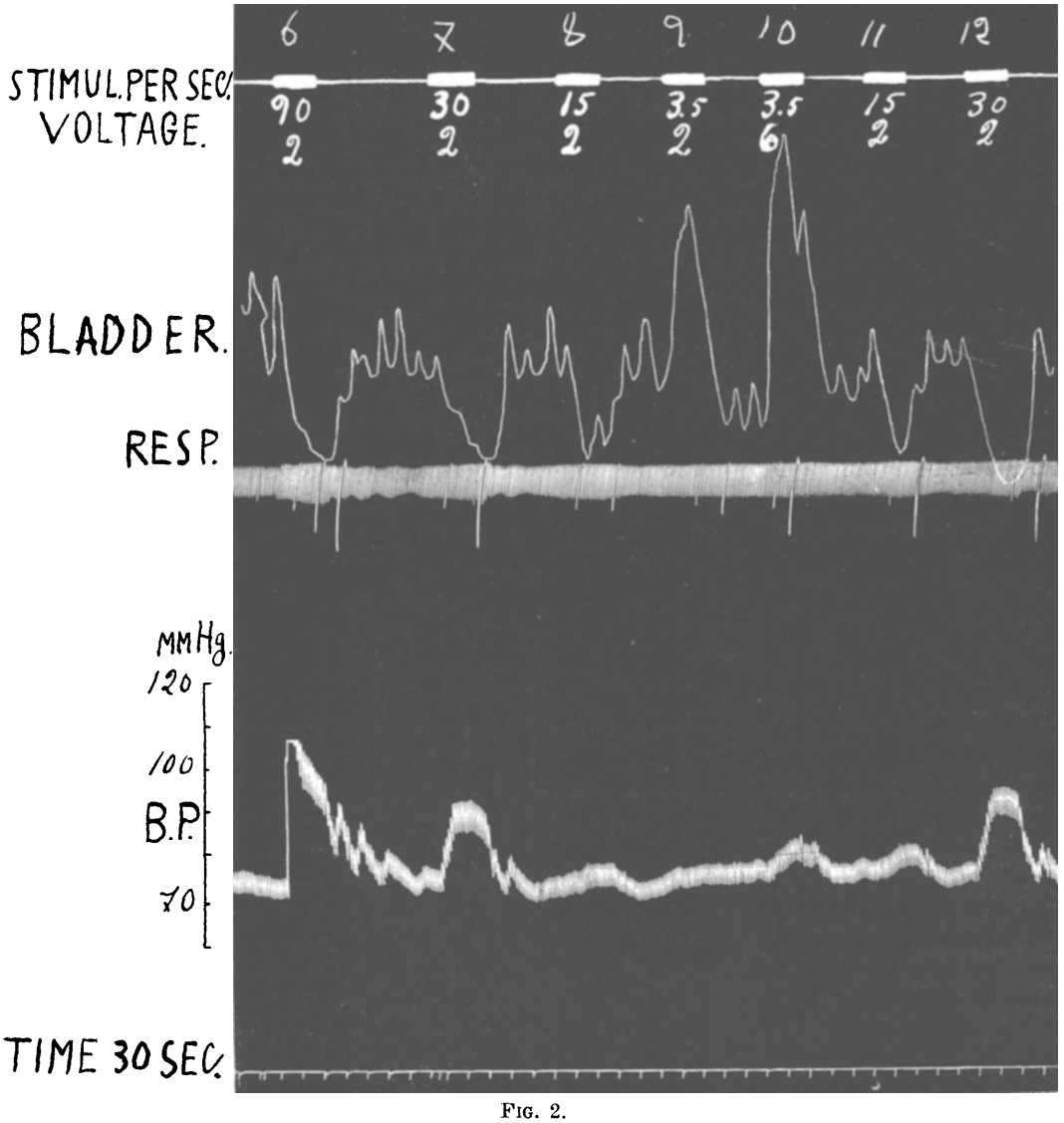

FIG. 2.
Alterations in bladder, blood pressure and respiration on stimulation of a point in the supraoptic region. Frequency varied.

was short, responses being regularly seen to start in a few seconds after the beginning of the stimulus. Fig. 3 illustrates the fact that points yielding bladder contractions will be missed unless stimuli of low frequency are used. As observed by other investigators no correlation was observed between the bladder, blood pressure and respiratory responses.

No definite conclusions can be drawn about the strict localization of the points concerned with bladder activity until a more complete microscopical investigation is made. From the material at hand points giving bladder contractions were found in the preoptic region as well as in different regions of the hypothalamus. Most of them however were concentrated in the anterior part of the hypothalamus and the posterior part of the preoptic region. Most of the excitatory responses could be reversed to inhibitory ones by increasing the frequency of the stimulus. In addition, numerous points were found

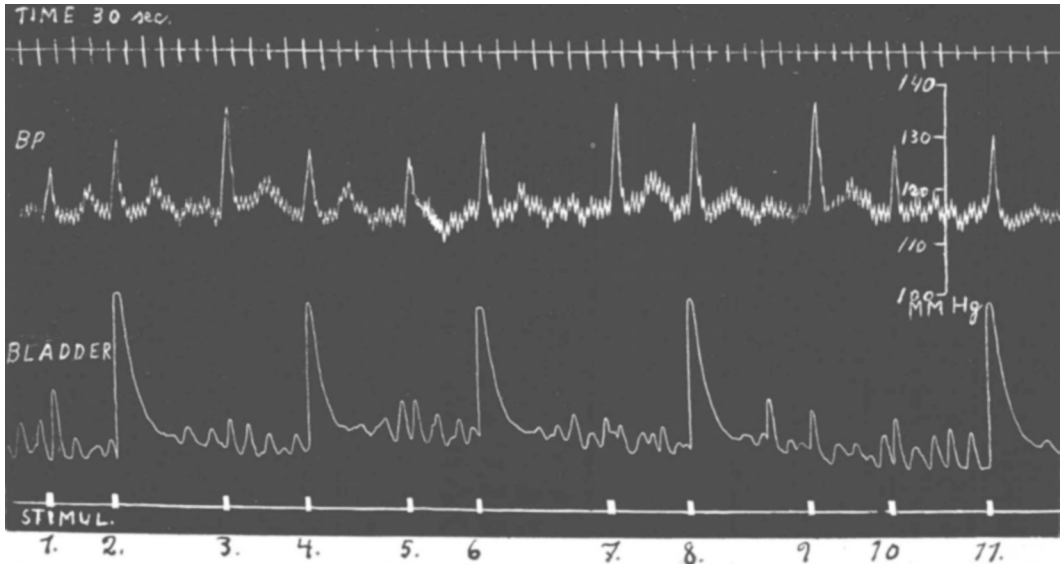

FIG. 3.

Alterations in bladder and blood pressure on stimulation of a point in the supraoptic region. Frequency and intensity of stimulus varied.

No.	Stimul./sec.	Voltage (V)	No.	Stimul./sec.	Voltage (V)
1.	7.5	0.1	3.	60	1.0
2.	"	1.0	5.	60	0.8
4.	"	0.5	7.	60	1.2
6.	"	0.8	9.	120	0.8
8.	"	1.2	10	60	0.8
11.	"	0.8			

scattered throughout the preoptic region, hypothalamus and upper mid-brain from which only inhibition could be elicited.

Discussion. The experiments have shown that the bladder response to hypothalamic stimulation is strongly influenced by the frequency of the stimulus. High frequency stimuli usually give inhibitory, low frequency stimuli usually give excitatory bladder responses. Most of the excitatory responses could be reversed to inhibitory ones by increasing the frequency of the stimulus. Whether the reversal effects are the result of the activation of different nervous structures or whether the same nervous elements are influenced in a different way by stimuli of different frequency or whether some other explanation is to be found, awaits further determination.

In the study of Kabat *et al.*,[3] Magoun[4] and Wang and Harrison[9] points giving inhibition of the bladder were only rarely seen, a fact probably due to the absence of bladder activity in their animals under barbiturate anesthesia. The inability to observe inhibition under these conditions together with the fixed frequency of the stimulus employed (Harvard inductorium), makes it apparent that these investigations did not give a complete picture of the localization of nervous structures concerned with bladder activity. It would seem necessary to reinvestigate this problem using stimuli of varying characteristics.

In their experiments on chloralosed cats Beattie and Kerr[5] reported that points yielding inhibition of the bladder were only found in the posterior part of the hypothalamus and the upper mid-brain. The present results, showing points giving inhibitory bladder responses scattered throughout the whole hypothalamic area, do not favor the view held by these authors that the hypothalamus can be divided in an anterior excitatory and a posterior inhibitory part.

Summary. The bladder response to hy-

[9] Wang, S. C., and Harrison, F., *Am. J. Physiol.*, 1939, **125**, 2.

pothalamic stimulation is strongly influenced by the frequency of the stimulus used. High frequency stimuli (60/sec. or more) usually cause bladder relaxation, low frequency stimuli (3.5/sec.) usually bladder contraction. A reversal of the bladder response is frequently obtained by changing the frequency of the stimulus.

Points yielding excitation and inhibition of the bladder are found scattered throughout the whole preoptic and hypothalamic area but points giving bladder contraction seem to be concentrated in the rostral parts of the diencephalon.

15741 P

Mitotic Activity in Hypophysis of Pregnant Rat after Injections of Estrogen.

THOMAS E. HUNT.

From the Department of Anatomy, Medical College of Alabama, Birmingham, Alabama.

Division of cells by mitosis increases in the hypophysis of young mature rats in the late estrous phase of the sexual cycle.[1] That this is due to the increase in the level of estrogen is shown by the fact that mitoses can be increased by injections of estrogenic hormones.[2] Thus, 25 μg of estradiol benzoate (Progynon-B)* injected into 3-month-old ovariectomized rats 72 and 48 hours before death will result in an average mitotic activity of 22.09 mitoses per square mm of section. During pregnancy, however, even though there is a continued production of estrogenic hormone, mitoses are rare in the hypophysis after the 3rd day.[3] Just what level of estrogen occurs during pregnancy is not known, and it seems possible that either the level is not sufficiently high to cause an increased mitotic activity or that there is some substance that inhibits the mitosis-stimulating effect of the estrogen that is produced.

In order to test this point, a group of 22 pregnant animals approximately 3 months of age were injected with 25 μg of estradiol benzoate, 72 and 48 hours before death. In addition to these, 3 others that did not receive injections were killed on the 13th day of pregnancy to serve as controls. Of the pregnant animals that received injections of estradiol benzoate, 9 were killed on the 6th, 7th or 8th day of pregnancy and 13 on the 12th, 13th, 14th or 15th day. Of those killed early in pregnancy, 3 animals showed evidence that implantation had occurred but in 2 of these abortion had apparently occurred. Those killed later in pregnancy all had living fetuses.

To determine the mitotic activity, all mitotic figures were counted in a 3-micra coronal section of known area from each hypophysis.

The results are summarized in Table I. Animals receiving injections before implantation of ova (Group 1) have an average of 17.33 mitoses per sq mm. This is not significantly different ($P. > .4$) from the results obtained by injecting ovariectomized animals (Group 4),[2] and, as with the latter group, there is a considerable range in mitotic activity. The animal having the lowest count (3.3 mitoses per sq mm) was the only one that showed normal implantation sites.

Animals injected later in pregnancy and killed from the 12th to the 15th day have an average mitotic activity of 4.35 per sq mm (Group 2). Nine of the 13 animals have consistently low counts (average 1.5), the other 4 falling within the range found for

[1] Hunt, T. E., *Anat. Rec.*, 1942, **82**, 263; *Endocrinology*, 1943, **32**, 334.

[2] Hunt, T. E., *Anat. Rec.*, in press.

* The estradiol benzoate (Progynon-B) was kindly furnished by the Schering Corporation, Bloomfield, N.J.

[3] Hunt, T. E., *Anat. Rec.*, 1943, **85**, 32.