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18 September 1961

On the Site of Action of Amethopterin

Abstract. In the liver of the intact mouse, the conversion of exogenous folic acid to compounds with citrovorum-factor activity is inhibited completely by an amount of amethopterin similar to that bound to the enzyme folic acid reductase in vitro. Because this amount of amethopterin is several thousand times smaller than the LD_{50}, the toxic effects produced by the larger doses must be mediated via some additional mechanism.

Both folic and folinic (5-formyl-5,6,7,8-tetrahydrofolic) acids can protect mice from aminopterin toxicity (*1*). While folinic acid is effective when given simultaneously or even after the drug, folic acid must be given about 1 hour before aminopterin in order to provide any protection. During the period of 1 hour after the administration of folic acid, the folic acid is converted to compounds with citrovorum-factor activity, which can then serve to protect against aminopterin (*2*). A priming dose (nontoxic) of aminopterin abolishes the protection afforded by folic acid by preventing its reduction to more active materials. Folinic acid, because it is already reduced, is unaffected by prior administration of aminopterin and can substitute for the biologically active derivatives of folic acid. The measurement of liver citrovorum factor after the administration of folic acid provides an in vivo assay of the enzymes responsible for this conversion.

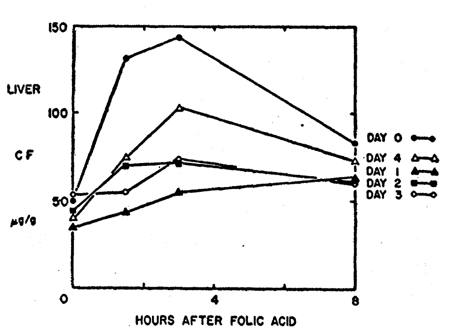

Fig. 1. The effect of amethopterin (0.05 mg/kg) on liver citrovorum factor derived from folic acid (25 mg/kg). Folic acid was given without amethopterin (day 0) and after amethopterin (days 1, 2, 3, and 4). Each point represents the mean for two mice. Amethopterin and folic acid were given subcutaneously.

The citrovorum-factor content of the livers of (C57 × DBA)F_1 male mice was determined by incubation of acetone powders with ascorbate and histidine and subsequent microbiological assay with *Pediococcus cerevisiae* (ATCC No. 8081), as described elsewhere (*2*). The influence of amethopterin (0.05 mg/kg) on the liver citrovorum factor after administration of folic acid (25 mg/kg) on the days indicated is presented in Fig. 1. In the animals that received no amethopterin (day 0), liver citrovorum factor increased from 50 to 140 μg/g in the first 3 hours after folic acid was given. On subsequent days, after administration of amethopterin, this response was abolished and had not been completely re-established by day 4, the last day of observation. Thus, in this experiment, the conversion of folic acid to citrovorum factor was inhibited completely by a very small dose of amethopterin.

The degree of inhibition of the conversion of folic acid can also be determined by observing the protective effect of previously administered folic acid on the toxicity of amethopterin. The data summarized in Table 1 show that administration of folic acid (25 mg/kg) 1 hour before administration of amethopterin increased the LD_{50} from 200 to 350 mg/kg. The administration of amethopterin (0.1 mg/kg) 24 hours before the LD_{50} injections abolished this protective effect.

Inhibition of the conversion of folic acid to liver citrovorum factor was produced by administration of 0.05 mg of amethopterin per kilogram of mouse, or 1 μg for a 20-g mouse. If all of the drug were localized in the liver, the concentration would be 2 mμmole/g of liver. Since the amount of folic acid reductase in 1 g of mouse liver can bind 0.8 mμmole of amethopterin in vitro (*3*), these results suggest that at this low dose of amethopterin most of the drug was bound to this enzyme. The disappearance of the protective action of previously administered folic acid after such a small dose of amethopterin further demonstrates the effectiveness of amethopterin in inhibiting the action of this enzyme.

Table 1. Protective effect of previously administered folic acid on the toxicity of amethopterin.

Prior treatment	Time before amethopterin administration (hr)	Amethopterin LD_{50} (mg/kg)
None		200
Folic acid (25 mg/kg)	1	350
Amethopterin (0.1 mg/kg) and	24	
folic acid (25 mg/kg)	1	180

If doses of amethopterin several thousand times smaller than the LD_{50} completely inhibit the conversion of folic acid to citrovorum factor in the intact mouse, larger doses cannot increase the degree of inhibition and therefore must produce toxicity via some additional mechanism. It is not possible to account for all the effects of the folic acid antagonists solely on the basis of inhibition of this conversion process. Because all these effects can be reversed by administration of folinic acid, the additional sites of action may involve the further metabolism of tetrahydrofolic acid and its derivatives (*4, 5*).

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13 June 1961

An Overview of Sleep as an Experimental Variable (1940–1959)

Abstract. Less than one half of 1 percent of the psychological literature relates to sleep. Although there has been a relative decline in such research, the central nervous system and pathological aspects have recently received increased attention. The United States is producing less than 17 percent of the research on sleep.

In a recent review of the research literature on sleep, some statistics of interest regarding this research area were assembled. The review covered the primarily psychological research since 1941, since Kleitman's book, published in 1939 (*1*), presents comprehensive coverage of the research to that date.

The most obvious fact to emerge was the relative neglect of this important behavioral variable. *Psychological Abstracts*, published by the American Psychological Association, covers the psychological literature published in the United States as well as that published in a number of foreign journals. The average percentage of the abstracts on the topic of sleep (exclusive of dreams) between 1940 and 1959 was 0.267, or slightly over 2½ articles per 1000. This percentage ranged from a high of 0.45 in 1945 to a low of 0.12 in 1954. In the last 10 years the relative amount of literature on sleep has been below this average, and such studies appear to be decreasing relative to other research.

The *Current List of Medical Literature* (now *Index Medicus*), published by the National Library of Medicine, has a broad coverage of journals in both a disciplinary and a geographic sense. In addition to articles from strictly medical journals, many psychological, physiological, biological, pharmacological, anatomical, and other related journal articles are listed by title from countries throughout the world. In a recent 2-year period (1958 and 1959), 221,256 titles were listed. Of these, 235 were concerned with sleep—or approximately 1 article per 1000.

An added confirmation of this neglect was found in ten of the most recently published introductory psychology texts. Of these texts, four contained no reference to sleep at all. In the remaining six, such references varied from 2 paragraphs in 443 pages to 14 paragraphs in 648 pages. In all, a total of about 7½ pages (approximately 0.1 percent) of these texts were devoted to describing one-third of man's activity.

In considering the kind of research, however limited in amount it may be, the research studies reported since 1940 in *Psychological Abstracts* were classified into those in which sleep was a dependent variable (studies of effects of experimental variables on sleep) and those in which it was an independent variable (studies of the effects of sleep on other experimental variables).

Figure 1 presents data on studies of sleep as a dependent variable. The category "environmental factors" included studies of the effects of submarines, day nurseries, air raids, night

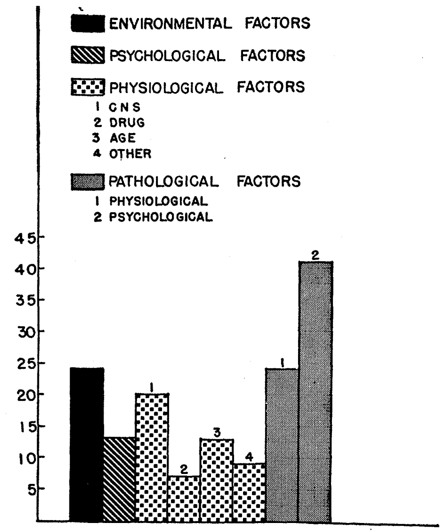

Fig. 1. Sleep as a dependent variable.

shifts, and light and sound. "Psychological factors" included studies of the effects of attitudes, activities prior to sleep, hypnosis, and similar conditions. Most of the "age" studies were observational studies of sleep cycles in children and in the aged. The "physiological" studies revealed an increasing interest in the role of the reticular formation and in central-nervous-system stimulation. Other physiological studies included studies of the effects of hypothalamic extracts, cerebrospinal-fluid conditions, and comparative studies of hibernation. Relations between sleep and a wide variety of pathological conditions received considerable attention: epilepsy, encephalitis, tumor, and others, among the physiologically centered conditions, and neuroticism, homosexuality, anxiety states, and others, among the psychological conditions. In only one category, the

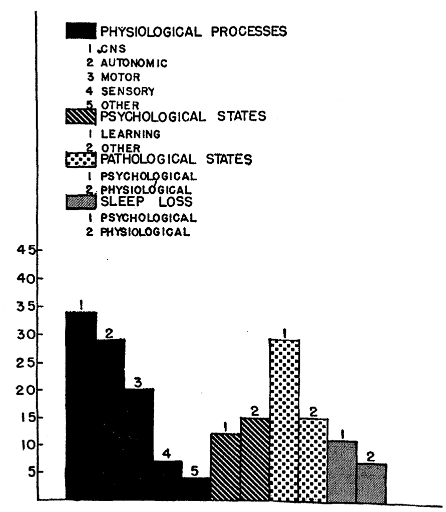

Fig. 2. Sleep as an independent variable.

effects of drugs, has there been a general increase in research in the last 10 years.

Figure 2 presents data on studies of sleep as an independent variable. By far the most widely studied central-nervous-system response variable is the electroencephalographic response. Such studies have shown a marked increase over the last 10 years. Blood pressure, basal metabolism, and the psychogalvanic skin response were the most widely studied of the autonomic responses. Nearly all of the observations on motor response were on general motility and eye movements. Threshold studies, primarily of auditory stimuli, were categorized under "sensory" processes. Studies of blood anoxia are the most common type under the "other" category. Most of the studies categorized under "psychological states" were concerned with the question of learning during sleep. Other studies included studies of the effects of such variables as "feeling rested" hypnogogic reverie, and near-waking states.

There has been an increasing amount of research on the influence of sleep on pathological states over the last 10 years, which undoubtedly stems from a general increase in interest in psychopathology among psychologists and is related to the significant increase in the use of sleep as a therapeutic by the Russians and the French. There has been a steady interest in sleep-loss studies—studies of the effects of sleep privation on various psychological and physiological variables—over the years.

As is shown in Figs. 1 and 2, in more than half the studies sleep was the independent variable, and more than half were physiologically oriented. In addition to the studies represented in Figs. 1 and 2, some 19 reviews of certain aspects of sleep have been abstracted.

Who is doing the research on sleep? Because of its extensive coverage of the literature in general and of foreign literature in particular, the *Current List of Medical Literature* was used as a source of information on this question. The percentages of articles, by country, for the years 1958–59 for the 235 articles related to sleep were as follows: United States, 17 percent; U.S.S.R., 20 percent; Germany, 18 percent; France, 13 percent; Great Britain, Canada, and Australia, 12 percent; Italy, 5 percent; Czechoslovakia, 4 percent; other countries, 9 percent. Since this source undoubtedly favors coverage of American articles, the 17 percent of current research attributed to the United States by this survey is undoubtedly spuriously high.

The Russian journals display a strong interest in the therapeutic aspects of sleep. Most of the German reports center on the effectiveness of various drugs in dealing with insomnia and more exaggerated sleep disturbances.

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Miniature Subcutaneous Frequency-Modulated Transmitter for Brain Potentials

Abstract. A device for broadcasting electrical signals from the brain of an animal is described. It is small enough to be implanted under the animal's skin. That signals are broadcast without distortion is shown by the comparison of a broadcast recording of an electrocorticogram of a cat with a simultaneous recording made directly with wire leads.

In recent years physiologists have been concerned with the study of the relationship between the electrical activity of the brain of an animal and its behavior. It is common practice to implant electrodes subcortically and connect them with wire leads to a sensitive recording apparatus. This technique has the disadvantage of artificially restraining the animal and impeding normal behavior. Radio telemetry can be used to eliminate the wires (*1*). A transmitter small enough to be implanted under the skin of the animal would be ideal.

Some requirements of a transmitter for this application follow:

1) The input impedance should be several times the source impedance in order to record essentially the open-circuit voltage. Measured source impedances were of the order of 15,000 ohms.

2) The frequency response should be nearly perfect in the range from 1 to 1000 cy/sec.

3) Transmitted signals with peak-to-peak magnitude as small as 50 μv should be intelligible to a good antenna-receiver system located within 100 feet of the animal.

4) The circuit should contain the fewest possible number of components consistent with the foregoing requirements in order to lend itself to miniaturization and implantation.

The circuit diagram of a transmitter which meets these requirements is shown in Fig. 1. The second stage is an amplifier-modulator-oscillator, essentially as developed by Thomas and Klein but with fewer components and a different type of transistor (*2*).

Sufficient circuit amplification is provided in the second stage; however, the input impedance of the second stage is about 25,000 ohms, which is considerably less than the required value. The first stage is needed to increase the input impedance. It is an emitter-follower which uses the direct-coupled input impedance of the second stage as its load (*3*).

A photograph of the transmitter and its battery is also shown in Fig. 1. Assembly was accomplished by mounting standard miniature components upon a substrate. Conventional soldering techniques were used. Before implantation, the transmitter was encapsulated with a polyethylene resin.

Measured characteristics of the transmitter are as follows: carrier frequency, 94 Mcy/sec; input impedance, 250,000 ohms; frequency response, 3 db down at 0.1 cy and 16 kcy/sec; deviation sensitivity, 8 mv (r.m.s.) for ± 100 kcy/sec; equivalent noise input, 5 μv (r.m.s.) with 20,000-ohm source resistance and 16-kcy/sec bandwidth; weight with battery (encapsulated), 7.3 g; volume (encapsulated), 5 cm^3; battery drain, 500 μa. Battery life is approximately 48 hours when the smallest commercially available battery is used (two Mallory cells, type RM-312T2, in series).

Two electrocorticogram tracings taken from a single pair of electrodes implanted in the cortex of a cat are also shown in Fig. 1. The upper trace is the signal which was broadcast to an FM receiver before it was recorded. The lower trace was recorded simultaneously with wire leads from the same pair of electrodes. An electroencephalograph was used as the recorder in both cases.

Satisfactory recordings of other brain potentials have been made with all leads and the transmitter implanted subcutaneously.

With the advent of more efficient transistors of smaller size, a transmitter of about one-third the size of the one described here has been fabricated. A system whereby the battery may be recharged by induction while remaining in the animal is being investigated. Also under investigation are methods of providing for several channels of information (*4*).

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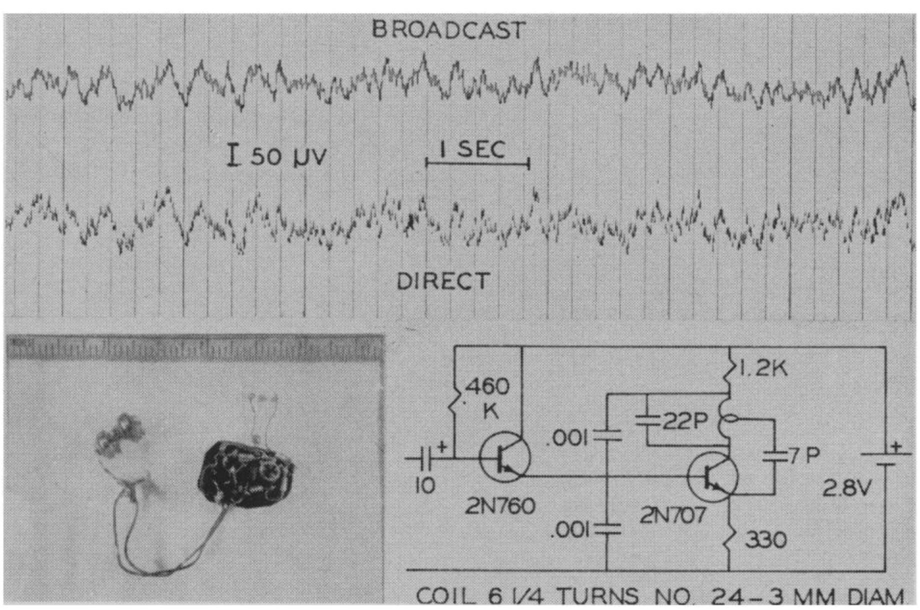

Fig. 1. (Top) Comparison of broadcast (upper trace) and direct-wire (lower trace) electrocorticogram of a cat. (Bottom, left) Photograph of the FM transmitter before encapsulation. (Bottom, right) Circuit diagram of the FM transmitter.