light in such a way that its virulence may be destroyed without complete loss of its immunizing power.

Thirty cc of clear rabies culture virus,[2] containing 33,000 mouse intracerebral lethal doses per cc, were placed in a quartz flask 12.5 cm from a quartz mercury vapor lamp. The intensity of the ultra-violet light was measured by means of a photronic cell and filter. During the period of irradiation the temperature of the virus was kept at 23° C.

The virus was irradiated 45 to 60 minutes and then tested for virulence by injecting 0.03 cc intracerebrally into each of eight or ten mice. The immunizing potency of the irradiated virus was determined[3, 4] by vaccinating mice each with 0.25 cc intraperitoneally. In all, six vaccinating doses were given, one every other day. About 3 weeks later the immunity of the vaccinated mice was tested by injecting each of them intracerebrally with 0.03 cc of mouse-brain rabies fatal dose of test virus, as contrasted with only one of 19 vaccinated mice; 12 of 12 unvaccinated mice died after inoculation with 10 fatal doses, as contrasted with 3 of 11 vaccinated mice; and 8 of 8 unvaccinated mice succumbed to 100 fatal doses, as contrasted with 2 of 8 vaccinated animals. These differences are significant according to the χ^2 test, $P = < .01$.

Further experiments showed that culture virus irradiated only 30 minutes immunized well but remained virulent to a slight degree, while virus irradiated 2 hours became inert both as to immunizing potency and virulence. Finally, culture virus in a glass flask, wrapped with tinfoil, which is impervious to ultra-violet light, and exposed to the mercury vapor lamp for 2 hours, showed no loss of virulence.

It is concluded, therefore, that rabies culture virus, exposed to a proper dose of ultra-violet light, becomes avirulent and yet retains enough of its immunizing power to protect mice against 10 intracerebral lethal doses of test virus.

TABLE I
IMMUNIZING POTENCY OF IRRADIATED, NON-VIRULENT RABIES CULTURE VIRUS

Treatment of culture virus	Virulence following irradiation	Fate of mice receiving test virus					Protection afforded by vaccination in minimal lethal doses
		Mice	Dilutions of virus				
			10^{-4}	10^{-5}	10^{-6}	10^{-7}	
Test 3							
Irradiated		Controls	...	4/4†	2/4	1/4	
60 minutes	None 0/8*	Vaccinated	...	0/3	0/3	...	10
Test 4							
Irradiated		Controls	4/4	7/13	1/4	...	
45 minutes	None 0/8	Vaccinated	0/4	1/8	...	...	10
Test 5							
Irradiated		Controls	4/4	4/4	3/4	1/4	
45 minutes	None 0/10	Vaccinated	1/4	2/4	0/4	0/2	10
50 minutes	None 0/10	Vaccinated	1/4	1/4	0/4	0/4	100

* 0 of 8 injected mice succumbed.
† 4 of 4 injected mice succumbed.

virus. Some received 1,000 lethal doses, some 100, some 10 and some one lethal dose of the test virus. Unvaccinated mice of the same age received like amounts of the virus, respectively.

These experiments (Table I) show that the irradiated, avirulent culture virus immunizes against 10 or more intracerebral lethal doses of test virus. In each experiment, taking one fatal dose as the greatest dilution killing 50 per cent. or more of the unvaccinated mice, it appears that 10 to 100 times this dose was withstood by 50 per cent. or more of the vaccinated mice. Combining results of the three tests, it is noted that 12 of 21 unvaccinated mice succumbed to one

[2] L. T. Webster and A. D. Clow, *Jour. Exp. Med.*, 66: 125, 1937.

[3] L. T. Webster, *Amer. Jour. Pub. Health*, 26: 1207, 1936.

[4] L. T. Webster, *Amer. Jour. Pub. Health*, 1937 (in press).

The technique described above is being further developed and applied.

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CHANGES IN HUMAN BRAIN POTENTIALS DURING THE ONSET OF SLEEP

WHEN a person goes to sleep, the pattern of his brain potentials alters systematically. Five clearly defined stages have already been described[1] as follows:

A—*alpha:* the normal waking 10-per-second rhythm
B—*low voltage:* the alpha rhythm is lost
C—*spindles:* short groups of 14-per-second waves ap-

[1] A. L. Loomis, E. N. Harvey and G. Hobart, *Jour. Exp. Psychol.*, 21: 127, 1937.

pear and also random "delta" waves 0.2 second or more in length

D—*spindles plus random:* both types of wave increase and the delta waves become longer

E—*random:* the spindles become inconspicuous, but delta waves continue to increase in voltage and wave-length

We have now investigated the finer details of the *A* and *B* stages, and are able to relate alterations of the pattern to changes in the subject's state of consciousness.

The subject lies down to sleep with a rubber bulb in one hand, and is instructed to squeeze it once whenever he feels that he has just "drifted or floated off" for a moment and twice if he feels that he has awakened from "real sleep." As the subject becomes drowsy, the alpha waves (if he has them normally) diminish in voltage, and interruptions of their rhythm are more and more frequent. The interruptions become longer and more complete, and when one of them has lasted for 5 seconds or thereabouts, with the low-voltage record characteristic of the *B* state, the subject usually signals "have floated." The alpha waves usually return a second or two before the signal. With the first long break in the sequence of the alpha waves, we often see a measurable decrease in delta activity (see Fig. 1). The delta waves are best recorded from the top of the head. In persons who have very little alpha rhythm when awake, the appearance of these relatively short (0.2 to 0.25 second) low-voltage (75 microvolts) delta waves may be the first clear change in the record.

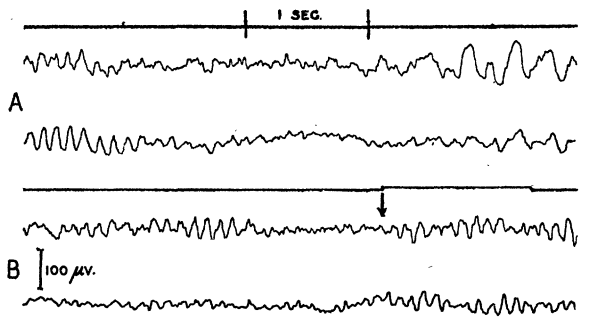

FIG. 1. A—Subject falling asleep. Upper line, vertex to ear; lower line, occiput to ear. B—A brief "float," signalled at arrow.

If the delta waves have reached 150 microvolts and have persisted for half a minute or more, the subject may signal "real sleep" when he next awakes. The still deeper *C* stage, with its spindles, is unquestionably "real sleep." The transition from the intermediate "floating" stage to "real sleep" apparently occurs somewhere in the *B* stage.

The transition from *A* (waking) to *B* ("floating" or sleep) is not simultaneous in all parts of the brain. The alpha waves may be suppressed and the delta waves appear in one part while the alpha rhythm continues normally in another region (see Fig. 1). On such occasions the subject may report that he has been fully conscious. This condition merits further study, for apparently different parts of the brain may "go to sleep" separately and to different degrees. It makes quite meaningless any question as to the exact moment at which a person falls asleep.

The data of Table 1, based on two subjects, are broadly typical of the whole group, particularly as to the prolongation of the "floats" as sleep approaches. The interval between the return of alpha waves and the signal varies and may sometimes be as much as 5 seconds. It is interesting, however, that occasionally the signal is given just *before* the alpha waves return.

TABLE 1

Subject*	Signals	Average time between signals	Average duration of depression of alpha waves before signals	Average time from return of alpha waves to signal
A	1–5	16.9 sec.	2.9 sec.	0.2 sec.
	6–10	12.1 sec.	4.1 sec.	0.2 sec.
	11–15	17.8 sec.	7.8 sec.	0.4 sec.
	16–20	20.5 sec.	13.5 sec.	0.3 sec.
	(sleep)			
B	1–3	30.2 sec.	6.2 sec.	2.4 sec.
	4–6	37.8 sec.	10.4 sec.	2.6 sec.
	7–9	36.2 sec.	22.7 sec.	1.1 sec.
	(sleep)			

* Subject A fell asleep after his 20th signal, subject B after his 9th. The grouping of the signals by five's and three's for obtaining running averages is entirely arbitrary.

The accuracy of the signalling is remarkable, considering how unfavorable drowsiness is for introspection and signalling. In 9 experiments 6 subjects who have strong alpha rhythm when awake signalled "have floated" or "have slept" 165 times. All but 6 of these signals were preceded by definite depressions of the alpha waves. The records show only 39 similar depressions which were not signalled.

Two subjects who have very few alpha waves when awake showed partial depressions of their own characteristic quick waves and often increases of delta activity which correlated very well with their signals.

Only one subject gave signals which showed no constant relation to alterations in his brain potentials. The results of his tests are not included in the figures above, and we have also omitted three preliminary experiments and two in which the subjects were "asleep" most of the time and often failed to signal when a few seconds of alpha waves appeared on the record. In this condition the subject apparently does not rouse himself sufficiently to give the signal.

The common denominator in the subjective reports of the experience of "floating" is a depression of sensory perception. Some identify the state by suddenly realizing that they have ceased to hear noises or that they have lost their awareness of the bed clothes or the position of their body. Others stress the appearance of visual fantasies or interruptions in the train of logical thought, but in all cases there is loss of awareness, particularly for immediate external stimuli. This transient depression of consciousness appears to be correlated with definite objective alterations in the electrical activity of the brain.

We may summarize the initial stages of sleep as follows:

A —*alpha:* at rest but awake.
B_1—*low voltage, alpha rhythm lost:* intermediate drowsy or "floating" state.
B_2—*low voltage, delta waves appearing:* intermediate, merging into sleep.
C —*spindles and moderate delta waves:* real sleep.

Two practical points are important for clinical electroencephalography. First, the drowsy state must be strictly avoided when determining the amount of alpha or of delta activity which is characteristic of a given subject. Second, the electrical patterns of early sleep strikingly resemble those which we have seen in some patients who are psychotic or otherwise abnormal. We must not be misled in diagnosis by an unsuspected dozing or "floating off" of the patient during a test. On the other hand, many abnormal conditions may prove to depend upon general modifications of function which are fundamentally similar to those of normal sleep. We are now investigating this possibility.

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TREATMENT OF THE R39 RAT SARCOMA

DURING the past year, a study has been made of some new methods of treating animal tumors. These experiments are not completed. However, they have led to another approach, the use of which has a definite influence on the growth of the tumors and which will be described in this communication. All the work was done with rats which had been inoculated with the R 39 sarcoma.

Iron, in various forms, had been employed in the other experiments. Because of the peculiar carbohydrate metabolizing qualities of malignant tumors, it was decided to administer a combination of iron with sugar. For this reason ferric gluconate was made. It was discovered that ferric gluconate alone had some power to impede the growth of tumors. However, only 12 of 36 animals so treated showed this response.

The principle of double injections which, as far as we know, has not been used before, was therefore tried. In view of the fact that some animal tumors take up injected dyes readily, it was planned to administer either neutral red or azo blue intraperitoneally, and to follow this injection with an intravenous one of ferric gluconate. The hypothesis was that the dye might make up a chemical bed in the tumor, by virtue of which the ferric gluconate might either be held in increased quantity, or be made more effective, in the tumor. Various dosages and changes in the intervals between the two injections were used. The results were striking. Of 64 animals so treated 47 (73 per cent.) showed that the growth of the tumor had been sharply influenced. In all 47 instances the tumor stopped growing; in roughly half of the cases it receded. Cessation of growth always occurred in close association with the administration of the ferric gluconate. For example, in 31 instances the dye was given daily for two or three days, the first ferric gluconate administration not being given until a day or two after the last dye injection. In the 23 cases of this group in which there was an effect, stoppage of growth occurred regularly within 24 hours of the giving of the ferric gluconate. Ferric gluconate has proved to be either non-toxic or only slightly so. There have been some deaths, but the facts indicate that these are not from the drug, which is ordinarily tolerated very well, but probably from the absorption of dead tissue products.

It should be emphasized that influence on growth, and not cure, is being discussed. In only a few instances did the tumor recede completely. Evidently, as indicated by histological study, most of the tumor cells were killed, but a few, especially around the large blood vessels, remained alive and grew again later. Microscopical sections showed profound and widespread changes in the tumors, with only occasional normal looking nests of cells. While histological changes in experimental tumors alone may not be very significant, it is felt that they have a decided significance in these experiments, not only because of the predictable and high frequency with which they occur massively in large tumors, but also because of their linkage with cessation of growth of the tumors.

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