their cellularity. In the face of the admitted fundamental agreements in the structures of protozoan individuals and metazoan cells, the arguments advanced by Dobell and by Hyman become irrelevant and of no vital consequence. The cell theory stands as one of the valid generalizations about the protoplasmic systems of animals.

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31 October 1956

Reticular Activating System of Brain Stem and "Animal Hypnosis"

During the evolution of animals and man, certain basic types of reactions to stimuli from the external environment are to be found, the manifestations of which may differ at various evolutionary stages, but whose mechanism is principally identical. These basic types of reactions are called "biological radicals" by Kretschmer (*1*), and their mechanism is considered by this author to be "phylogenetically preformed." Such a biological radical is the so-called "panic reaction" (*Bewegungssturm*); another is the *Totstellreflex,* which is also called "animal hypnosis." The latter phenomenon has a number of analogs in clinical pathology in the form of various manifestations of the stupor-hypnoid syndrome of Kretschmer (*2*).

The onset and the dynamics of "animal hypnosis" as an experimental model of some psychiatric and neurological syndromes have been reported in a number of papers (*3*). In this report, a part of the electroencephalographic analysis of animal hypnosis is brought forward.

Animal hypnosis in a rabbit was experimentally elicited by standard rotation of the animal about its vertebral axis in a special apparatus. After this phenomenon had been evoked, changes characteristic of the onset of sleep and later electric activity of deep sleep appeared in the electroencephalographic record.

When the animal, in a state of animal hypnosis, is exposed to arousing stimuli, then there are changes present in the EEG record that are identical with those produced by arousing stimuli during normal, natural sleep. It is demonstrated in Fig. 1, where the first part of each record represents the wakeful EEG rhythm and the second part represents the rhythm during animal hypnosis, that the applied stimuli (indicated by arrows) lead to a change in the EEG record from the electric activity of sleep to a rhythm of greater frequency and of lower amplitude (record *A,* nociceptive stimulus; *B,* clapping of the hands three times in quick succession; *C,* labyrinth mechanical stimulus; *D,* labyrinth galvanic stimulus). This change can be seen simultaneously in all the electrodes, even though the depression of sleeping activity is not as marked in every electrode. The significance of the arousing stimuli in animal hypnosis is different. Labyrinth stimulation was found to be most effective, with nociceptive, olfactory, acoustic, and optic stimuli following in succession.

The simultaneous appearance of the EEG arousing reaction in animal hypnosis in all the cortical regions at the same time indicates that Magoun's brainstem reticular activating system is capable of function during this inhibitory state. This system represents—in contrast to the classical sensory and sensitive tracts, leading to the primary cortical receptor regions—a secondary afferent tract with a diffuse cortical projection via the thalamic and extrathalamic tract (*4*). The presence of the EEG arousing reaction from animal hypnosis shows that in the course of this form of generalized central inhibition, this system, which is important to the animal's existence and which insures the waking up from sleep, remains functionally active. It is known that, during central inhibition that is evoked, for example, by narcosis, this system is functionally eliminated (*5*).

It is perhaps possible to assume that this observation of the function of one of the most important brain systems during animal hypnosis can contribute toward the elucidation of the mechanism of those human pathological syndromes that appear during regressive forms of human behavior and of which the animal hypnosis represents an experimental model.

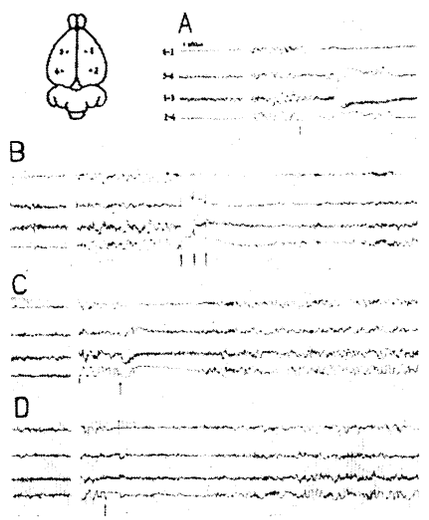

Fig. 1. Electroencephalographic rhythms of animals awake and during "animal hypnosis."

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11 September 1956

Antileukemic Action of Reserpine

During the course of studies in our laboratory on the effect of lysergic acid diethylamide and *d*-amphetamine on the toxicity and marked depression of animals that were administered large (25 mg/kg) doses of reserpine (*1*), we have directed our attention to the metabolic alterations produced by reserpine and reserpine derivatives. Since large doses of reserpine produce marked changes in the normal metabolic patterns (*2*), it was thought possible that reserpine might alter the metabolism of tumor cells more extensively than it did that of normal cells and thereby prove detrimental to the tumor. The data presented here show that reserpine can exert an antileukemic action (*3*).

Hybrid male mice [(BALB/cAn × DBA/2J)F_1] (8 to 10 weeks old and of weight 20 to 25 g) were inoculated in the right hind leg with 0.1 ml of a suspension of leukemic (L1210) cells (*4, 5*). The animals were allowed to develop leukemia until the local tumor had reached a diameter of approximately 9 to 12 mm (estimated by palpation) at which time the disease is generally systemic as well as local. When the disease had reached this preterminal stage, the mice were randomized and the designated groups were treated with a single injection of reserpine. The animals were weighed daily and observed for mortality. The size of the local tumor at the site of leukemic inoculation was obtained by palpation.

The results of a typical experiment are summarized in Fig. 1. A single treatment with reserpine produced an almost threefold increase in the remaining lifetime of mice with advanced leukemia. The mean survival time was an increasing function

of the dose of reserpine administered over the dose range employed (Fig. 1, *A*). Inhibition of the growth of the local tumor was observed consistently in the reserpine-treated mice. Two days after reserpine treatment, the change in the mean tumor diameter was practically nil, even though the tumors of the control (untreated) mice continued to increase in diameter (Fig. 1, *B*). Five days after the administration of reserpine, when all control animals were dead, the change in the mean tumor diameter was an inverse function of the dose of reserpine employed.

Treatment with reserpine frequently appeared to result in complete disappearance of the local tumor at the site of leukemic inoculation. However, transplantation of spleen from several such mice resulted in leukemic growth, indicating that systemic infiltration had not been wholly suppressed. Concomitant with the inhibition of local tumor growth, reserpine also appeared to retard the usual terminal leukocytosis in the peripheral blood.

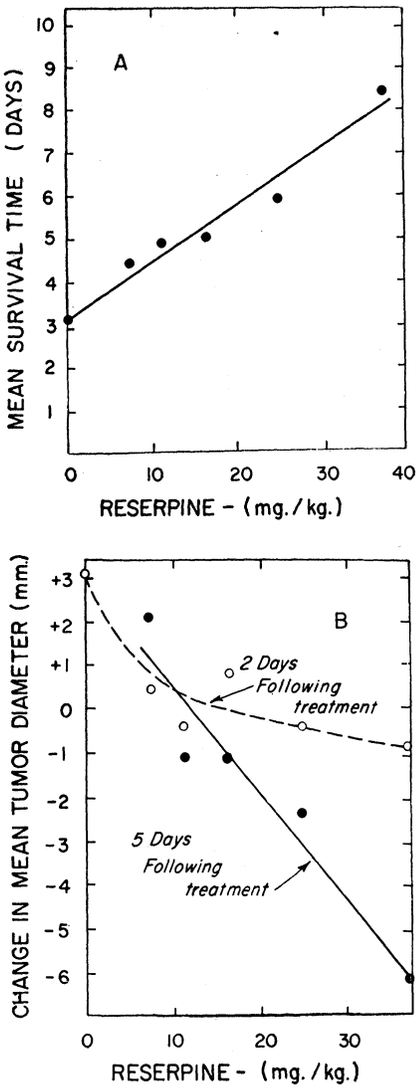

Fig. 1. Dose-response curves for the antileukemic action of reserpine. Reserpine was administered to the mice on the seventh day following inoculation with L1210. The mean survival time shown is the average time, in days, that mice survived after the day of treatment. Each mouse was inoculated with 3.2 million leukemic cells. Groups of ten mice, all with well-developed local tumors (mean tumor sizes ranged from 9.1 to 10.7 mm in diameter) were used for each dose level.

Preliminary experiments indicate that a regime of daily treatment with small doses of reserpine may be superior to treatment with a single large dose of the drug. Other experiments (*6*) have shown that several derivatives of reserpine also possess some antileukemic activity—for example, rescinnamine, deserpedine, and isoreserpine. The vehicle (*7*) used to dissolve the reserpine alkaloids was itself ineffective in inhibiting leukemic growth or in increasing survival time.

The mechanism by which reserpine exerts its antileukemic action is not known. Whether the antileukemic effect is direct or mediated through the host is not clear. Serotonin administration alone did not appreciably influence the course of the leukemia. Overcoming the depression caused by reserpine by administration of *d*-amphetamine did not significantly alter the antileukemic action of reserpine.

At the higher dose levels employed, reserpine-treated animals, both normal and those with leukemia, were severely depressed by the drug and failed to eat or drink for a period of 1 week or longer, and even nonleukemic mice frequently died (of starvation and dehydration?). Drastic loss of weight was invariably observed. Typical data frequently show an average loss of about 30 percent of body weight in 8 days following reserpine administration. Comparable food and water restriction in control animals with advanced leukemia failed to diminish the growth of the local tumors or to increase the survival-time of the mice as compared with untreated controls which received food and water *ad libitum*.

It is generally difficult to increase the survival time of mice with advanced leukemia L1210. Only a few drugs, such as amethopterin (*5*) and 6-mercaptopurine (*8*) have been successful in this respect. Even though reserpine has not been as effective an antileukemic agent as amethopterin, the response of mouse leukemia to treatment with reserpine and several of its derivatives has made available for laboratory study a new group of active antileukemic agents.

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26 November 1956

Infection of Chick Embryos by Crithidia from a Phytophagous Hemipteron

The possibility of a nonvertebrate parasite's infecting vertebrates offers a means of gaining some understanding of the evolutionary processes that are involved in the development of parasitism in vertebrate hosts and as such has attracted the attention of investigators for a number of years. Both negative (*1*) and positive results (*2*) have been reported, and considerable controversy has arisen concerning the correctness of the various results. The present paper (*3*) reports the successful transmission of flagellated protozoans of the genus *Crithidia* from the phytophagous bug *Euryophthalmus davisi* (Barber) to the chick embryo.

Crithidial parasites [possibly *C. euryophthalmi,* McCulloch (*4*)] were obtained from the insect, freed of bacteria by the use of antibiotics, and cultured on N.N.N. media. A luxuriant growth ensued. After a few passages on this medium, the flagellates were grown successfully on a medium consisting of nutrient agar (2 parts) and heparinized duck blood (1 part). On either medium, the crithidial morphology was lost and succeeded by that of a leptomonad.

After the parasites had been 5 days on the modified medium, 1 ml of sterile saline was added to the tube, and several drops of the mixture were placed on the exposed chorioallantoic membrane of 9-day chick embryos. Embryos so treated were placed in an incubator in which a temperature of 30°C ± 2° was maintained. This temperature still supported life in the embryo and more closely approximated the temperature in the hemipteran host.

Five days later, material was withdrawn from the inoculated embryos and examined under a phase microscope. Numerous flagellates were present in the