It is interesting to note that the combinations that were most effective against *E. coli* were often ineffective in the tests against neoplasms, whereas some combinations that were less effective against *E. coli* were effective against these neoplasms. For example, although the value of the sum of the fractional inhibitory concentrations for azaserine plus 6-mercaptopurine is 0.30 and that for 3-(1,2,4-triazolyl)alanine plus 6-mercaptopurine is 0.09, the former combination was effective against leukemia L1210 whereas the latter was ineffective. Other similar examples could be picked from the table. On the other hand, combinations that have been found to be potentiating in tests with animal neoplasms are also potentiating in inhibiting the growth of *E. coli*. Although the correlation between the data for *E. coli* and the indicated neoplasms is not as good as would be desired, the bacterial screen does indicate combinations that should be tested against neoplasms in animals. For example, the potentiating effects of combinations of desoxypyridoxine and acid hydrazides were first detected in tests with bacteria.

It is also possible that some of the combinations that are potentiating against *E. coli* might be of value in studies related to the chemotherapy of infectious diseases caused by bacteria or protozoa.

Summary. Data are presented for the potentiating effect of several combinations of compounds in inhibiting the growth of *E. coli*. Data of this kind can serve as guides for the selection of combinations of agents to be tested in the chemotherapy of cancer and of infectious diseases.

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1. Elion, G. B., Singer, S., and Hitchings, G. H., *J. Biol. Chem.*, 1954, v208, 477.

2. Davis, B. D., and Mingioli, E. S., *J. Bact.*, 1950, v60, 17.

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Tremor Production in Cats Given Chlorpromazine. (22491)

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The production of a Parkinson-like state in humans receiving large daily doses of chlorpromazine has been observed clinically by us and others(1-4). To study further this phenomenon, chlorpromazine in different dosages and for variable periods of time, was injected in cats and the results observed. It was anticipated that a state of rigidity would be most prominent if any effects were obtained, but an unexpected feature was the appearance of an alternating tremor at rest. This has previously been produced in primates by fulgurations in the region of the subthalamus(5,6), ablations of the cerebellar cortex and nuclei (7), fulgurations in the mesencephalic and pontine tegmentum(8), electrical stimulation of the medial reticular formation(9), and by use of Reserpine(10). Folkerts and Spiegel (11) observed an alternating tremor during electrical stimulation of the midbrain tegmentum in the cat.

Methods. Twenty-eight adult cats were observed prior to and following the intramuscular injection of chlorpromazine to determine the quality of placing and hopping reactions, labyrinthine righting reactions, stütz response, both dependent and supine, pupillary reaction, general performance, gait, tendon reflex activity, muscle tonus, and tremor. Drug doses were calculated on the basis of those frequently employed for a 70 kg human which range from 300 to 2500 mg/day and were proportioned according to the cat's weight. Doses were given once daily. They varied in amount as mentioned and were administered as single injections, intermittent injections, or consecutive daily injections for as long as 30 days. Tremors and other drug

effects were recorded by motion picture camera and by this means tremor frequency was analyzed.

General observations. Tremor. Of the 28 animals studied, 13 (46%) developed a tremor of one or more extremities or of the head during some phase of the investigation. The basic response consisted of a coarse, alternating tremor at rest with a frequency of 6-8 sec. as judged by observation and motion pictures. Some common variations of this basic response included: (1) more rapid tremor of smaller amplitude in which the rate was estimated at 16-18/sec. by observation; (2) slower tremor of greater amplitude at about 4-6/sec.; (3) lack of spontaneously occurring tremor, but one which could be precipitated by a light tap on one of the footpads, affecting either the stimulated limb or another extremity without regard to laterality; (4) irregular tremor which varied in frequency or amplitude. In no instance was an intention tremor noted. Tremor production was dependent upon having the animal in a supine position (either in a wooden trough or supported on a table top) with a maximum degree of relaxation. In the 13 animals exhibiting a tremor, this disappeared when medication was stopped, and frequently reappeared upon readministration, on at least one occasion in all animals, and up to 6 times in certain ones during the period of observation. Tremor production bore only a partial relationship to dosage of the drug, in that doses below 500 mg human equivalents and above 2500 mg human equivalents were ineffective; amounts between these limits were effective at various times in different animals.

Other drug effects. Among the drug effects noted in this group were variable degrees of shivering, diarrhea, relaxation of the anal sphincter, lethargy, and frequent loss or diminution of the various postural and righting reflexes referred to earlier. However, there was no obvious change in the stütz responses, tendon reflexes, gait (except for infrequent impairment of motor function in the injected limb), or of pupil size and light response.

Absence of tremor. In the 15 cats which did not develop a tremor there was more pronounced lethargy and less response to stimulation than in the tremor animals. However, righting reflexes and postural responses remained relatively normal. Shivering, diarrhea, anal sphincter tone, gait, pupillary size and light response, and tendon reflexes were not noticeably different from those in the group with tremors.

Secondary observations. In 2 animals of the tremor group spontaneous movements which might be described as "swimming movements" were occasionally observed. In some instances these occurred in association with the tremor. These movements took the form of rhythmical alternating flexion-extension of the forelimbs accompanied by abduction of the toes and extension of the claws in the extended limb, and adduction of the toes and withdrawal of the claws in the flexed limb. When tremor accompanied this phenomenon, it was present in either position of the limbs, but exhibited greater amplitude and excursion in the flexing limb. In one animal it was present in the lateral, as well as the supine position. In four of the cats with tremor a stimulus such as a light tap on the footpad of a hind limb produced extension or flexion of a forelimb without regard to laterality. In all such instances the responses followed an ascending course, as forelimb stimulation did not elicit a hind limb response.

In another instance light constant tapping of a tendon at first produced no observable muscle contraction, but after several such stimuli a phasic tendon reflex appeared and increased progressively in amplitude of excursion for the next four to five taps, then showing a gradual decline until it could no longer be elicited. After a 60 second interval, the same phenomenon could be reproduced under similar conditions. A fourth effect occurring in varying degrees in four of the animals and independent of tremor production, was a lack of response to nociceptive stimuli, elicited by pinching or applying heavy pressure to the tail. The animals would either appear to be totally oblivious to the stimulus or would merely respond by a flick of the tail without turning the head in the direction of

the stimulus. This was in marked contrast to the behavioral responses of the controls.

Effects of prolonged administration. In 2 cats which received large continued doses of the drug (1500 and 2000 mg human equivalents) for 30 days, rigidity was observed in all 4 extremities and the trunk without any significant change in postural reflexes, righting reactions, and tendon reflexes. Upon cessation of medication the rigidity disappeared within ten days and the animals appeared to have returned to their normal state. In 2 other cats which were initially injected for 7 consecutive days with 300 and 400 mg human equivalents respectively, after which injections were discontinued for 14 days and then resumed in a like amount for 7 more days, no changes were observed as regards rigidity, tendon reflexes, righting reactions or postural reflexes.

Discussion. The tremor which we have observed with its variability in time, amplitude, regularity and anatomical location, but with a basic 6-8/sec. rhythm and appearing at rest, closely resembles that produced by the stimulation(11) and destruction(8,9) experiments of other workers, both in the same species and higher vertebrates, and that produced in man by the use of chlorpromazine(1-4). These earlier anatomical and physiological studies seemed to implicate a portion or portions of the reticular formation as a source for this tremor. More recent pharmacological studies (12,13), utilizing cholinergic, anticholinergic and ataraxic drugs which produce electrical changes in this region, appear to give additional support to the concept that activation of this anatomico-physiological system is in some way implicated in the appearance of a tremor at rest.

The dosage of chlorpromazine employed by us to produce tremor was in most instances comparable to that reported by Himwich and Rinaldi(12,13) as being within the range which produced a low voltage, fast record (activation) of the motor and limbic cortices, caudate nuclei, and the medial thalamic nuclei. We have noted that in the animals which did not develop tremor as compared to those which did there was reduced motor activity, drowsiness, and diminished interest in the immediate surroundings.

This behavioral observation links changes in electrical activity of the reticular formation induced by chlorpromazine, the state of alertness as dependent on the activity of this system(14) and the presence of a tremor at rest. We do not wish to imply that the reticular formation is necessarily the source of this tremor, but that it may merely reflect activity originating at more cephalic or caudal levels which traverses this region.

The appearance of parkinsonism with tremor in humans which may result from the use of chlorpromazine shows wide variation in time of appearance after institution of drug therapy, but in all cases has been reversible after discontinuance of the drug. In contrast to human observations the cats which react acquire a tremor after single injection; this difference may represent a species variation.

Since it is possible that sensitization to the drug may be a factor in the production of a tremor, the finding that initial doses sometimes produced tremor whereas interrupted doses did not, was felt to present some evidence that such a factor was not operative. Another observation of interest was the similarity of the "swimming movements" to those obtained by stimulation within the midbrain, which were termed tegmental responses by Ingram, *et al.*(15); however, it is possible that they might represent pleasure or nursing movements. The responses obtained by tactile stimulation and by repetitive tapping (of extensor tendons), were not unlike the irradiation and recruitment responses which have been described for spinal reflexes.

Summary. In a series of 28 cats injected with varying doses of chlorpromazine, 13 (46%) developed tremors. The basic tremor was present at rest and disappeared on movement. The rhythm was most often 6-8/sec., but a few animals displayed a faster or slower rate. The tremor was reversible and reproducible. The implications of these observations relative to human parkinsonism are discussed.

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1. Kinross-Wright, V., *Postgrad. Med.*, 1954, v16, 297.

2. Dundee, J. W., *Brit. J. Anaesth.*, 1954, v26, 358.

3. Bader, E., *Illinois M. J.*, 1956, v109, 28.

4. Ayd, F. J., Jr., *Dis. nerv. Sys.*, 1955, v16, 133.

5. Mettler, F. A., and Whittier, J. R., *Tr. Am. Neurol. A.*, 1947, 96.

6. Whittier, J. R., and Mettler, F. A., *J. Comp. Neurol.*, 1949, v90, 319.

7. Carrea, R. M. E., and Mettler, F. A., *ibid.*, 1947, v87, 169.

8. Ward, A. A., McCullouch, W. S., Magoun, H. W., *J. Neurophysiol.*, 1948, v11, 317.

9. Jenker, F. L., and Ward, A. A., Jr., *Arch. Neurol. and Psychiat.*, 1953, v70, 489.

10. Windle, W. F., Cammermeyer, J., Joralemon, J. T., Smart, J. O., Feringa, E., and McQuillen, M., *Fed. Proc.*, 1956, v15, 202.

11. Folkerts, J. F., and Spiegel, E. A., *Confinia Neurologica*, 1953, v13, 193.

12. Himwich, H. E., and Rinaldi, F., *Yale J. Biol. and Med.*, 1955/6, v28, 308.

13. Rinaldi, F., and Himwich, H. E., *Dis. nerv. Sys.*, 1955, v16, 133.

14. Magoun, H. W., *Physiol. Rev.*, 1950, v30, 459.

15. Ingram, W. R., Ranson, S. W., Hannett, F. I., Zeiss, F. R., and Terwilliger, E. H., *Arch. Neurol. and Psychiat.*, 1932, v28, 513.

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Ejaculatory Response Induced by Potassium Chloride in Small Mammals. (22492)

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Artificial ejaculation in laboratory animals has been produced by electrical stimulation and by drug action, the latter consisting of a hypnotic followed by a stimulant. No clear description of the properties of the secretions obtained has been reported, nor has either method been described as capable of producing a complete ejaculation containing sperm as well as secretions from the accessory organs of reproduction. A high incidence of ejaculation occurs in the final convulsions of a dying animal. Loewe(1) has termed this phenomenon terminal ejaculation in contrast to intravital ejaculation which neither coincides with death nor is followed by death after a short interval of time. Potassium chloride has been found to produce an intravital ejaculation in mice following intraperitoneal injection of sub-lethal doses. This investigation was undertaken to determine the mechanism by which the ejaculations were induced and the nature of the secretions.

Methods. The animal species employed were the albino mouse, albino rat (Wistar), hamster, guinea pig, pigeon, and ground squirrel. All animals used were adult males. The ground squirrels were trapped by snaring in the vicinity of Lafayette, Ind., and were kept in captivity about 6 months prior to their use. Other animals were obtained from breeders. Chemical agents were administered by intraperitoneal injection into the lower quadrant of the abdomen. Ejaculation time was measured from the time of injection until the first appearance of seminal fluid at the penis. Weights of ejaculates were made by collection on weighed glass microscope slides. Fructose analyses were made by the colorimetric method of Roe as modified by Mann(2). Seminal vesicles were removed through a midline incision in the abdomen cephalad the base of the penis. Isolated seminal vesicles were used to test the *in vitro* action of chemical agents by the method described by Stone and Loew(3). A seminal vesicle, placed in a bath of oxygenated Locke's solution to which

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