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October 24, 1968

REF: Task Order No.
Contract No.

Dear Doctor

By this letter we should like to set down for the record the procedure that has evolved this year on the project for which you are the technical representative.

Arrangements were made with the to obtain small samples of newly synthesized chemicals of potential interest for your program. These samples were selected in conference with on one or more of the following bases:

(a) Preliminary indications of biological activity obtained from the screening program that would indicate a stimulation or depression of the central nervous system.

(b) Structural relationship to compounds of known pharmacological activity.

(c) The pharmacological class for which the compound had been synthesized; for example, analgesic, hypnotic, antidepressant, autonomic blocking agent, tranquilizer.

(d) Compounds not yet screened but having functional group or atomic spacings which should yield biological activity.

To date, forty-seven compounds have been supplied by

This laboratory over the past several years has developed a primary screen using mice that has been designed to detect pharmacodynamic properties of new chemicals. A background of experience in screening several hundred compounds, as well as a number of known sedatives, hypnotics, hallucinogens, central nervous system stimulants, etc., has been obtained. Consequently each candidate chemical received from , was initially subjected to this screen. In concept, the screen involves the intravenous injection of a series of graded doses from a dose producing no observable pharmacologic or toxicologic signs to a dose producing severe toxic signs or death. The data obtained make it possible to calculate the ratio of the LD_{50} dose to the dose producing minimal signs of biological activity (MED_{50}). Retrospective studies on therapeutic agents which have enjoyed extensive clinical use over the years, indicate ratios of the LD_{50} to the MED_{50} usually above 10. Other data obtained indicate the biological system of the body that is affected, the duration of effect, and a tentative classification of the pharmacological class of the compound.

A second primary screen quantitates in mice the effect of a compound upon spontaneous activity, thus further classifying compounds acting upon the central nervous system.

On the basis of the above two primary screens, compounds are selected for further study. Criteria of selection are LD_{50}/MED_{50} ratios of 10 or above, unusual pharmacological signs, and patterns of effect on spontaneous activity. Secondary screens involve compound induced behavioral changes in cats, effects on motivational responses in rats, and effects on trained behavior in rats. It is contemplated that compounds showing desirable characteristics in the secondary screens will be tested in non-human primates.

The accompanying information, prepared by discusses in detail the methodology used and results which have been obtained to date. As will be noted

from Dr. [redacted] report, several compounds show early indications of promising characteristics. I believe that such a high proportion of highly active compounds obtained by this acquisition and selection process proves the validity of this approach to your project.

Sincerely yours,

Enclosure

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TASK ORDER NO.

CONTRACT NO.

INTRODUCTION

The work of the year to date encompassed obtaining and screening 48 chemical compounds of potential interest, of which 47 were received from a commercial source under an agreement to protect the proprietary interest of the source.

Initially each compound was examined for preliminary (primary) screening by the Acute Toxicity Screen using unanesthetized and unrestrained mice as subjects. The rationale and methodology of this screen are described in the Method sections below. As part of the initial screening process, all compounds were evaluated by the spontaneous locomotor activity test in mice.

Compounds were also administered to unrestrained, unanesthetized cats and effects evaluated by physical and neurological examinations and gross behavior. Based on the results of these initial tests, compounds which appeared promising were evaluated by secondary screening procedures. Such compounds were administered to trained hooded rats, for a behavioral test which evaluates effects on three types of motivation: approach, avoidance, and escape.

Compounds which show exceptional activity are further evaluated by the sequential response method in hooded rats. Two units of equipment for conducting these tests have been recently completed in our Manufacturing facility. Very interesting materials will also be administered to monkeys under controlled conditions to further define the biological activity.

METHODS

ACUTE TOXICITY SCREEN - MICE

This procedure represents the first step in eventual acceptance or rejection of a compound. The screen is designed to eliminate from consideration compounds which do not possess sufficient biological activity, and to indicate types of promising activity. Criteria used in the selection of a compound include:

(a) Ratio of lethal dose to effective dose (safety factor)
(b) Speed of onset of pharmacologic signs
(c) Duration of action
(d) Type of action
(e) Completeness of recovery from the effects
(f) Degree of severity of signs observable

The effects sought are pharmacologic signs which are readily reversible in a progressive series of tests. Compounds are administered intravenously, and toxic signs are noted by gross observation or by manipulation and are recorded from among those listed in a List Of Reaction Signs and Standard Terms for Toxicity and Symptomology Reporting. If the LD_{50}/MED_{50} ratio is 10 or greater, the compound is tested by intravenous injection in cats and administered in behavior tests for secondary screening.

From previous experience, it has been found that with trained observers the mouse toxicity screen detects significant reaction signs (a prefered term to toxic sign) for compounds known to be mentally or physically incapacitating to man. The

performed in a "partially blind" manner; i.e., the observer is not informed of the structures of the compound to be screened, but is responsible for preparing solutions and dilutions, and injecting the mice. Technicians usually work in pairs, one person recording the data and whenever necessary helping to observe the mice, while the other technician serves as the regular observer. From time to time, known or standard compounds are introduced into the routine screening as unknowns to check the reliability and reproducibility of the screening technique.

PREPARATION OF COMPOUND FOR INJECTIONS

Solvents - In the absence of solubility information, very small, unweighed amounts of compound are tried in the following solvents and in the order listed until a suitable one is found. Heat may be used to aid solution.

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LOCOMOTOR ACTIVITY IN MICE

The spontaneous locomotor activity test measures the drive of an animal to move. This test is recognized as measuring a basic parameter for the screening of potential tranquilizers and sedatives (Jacobsen 1964) and excitants (Chen 1964). The apparatus used for this test,

For interpretation of the data, special attention is given the following parameters: -

(a) Dosage levels at which maximal, minimal, or no effect is seen.

(b) Time of onset of drug effect at each dosage level.

(c) Duration of a change in locomotor activity of treatment groups as compared to that of control groups.

REFERENCES:

Jacobsen, E., Tranquillizers and Sedatives, Chapter 10, Evaluation of Drug Activities, Vol. I, ed. by Laurence, D. R, and Bacharach, A. L., Vol. I, pp 215-237, Academic Press, N.Y., 1964

Chen, G., Antidepressives, Analeptics, and Appetite Suppressants, Chapter 11, Evaluation of Drug Activities, Vol. I, pp 239-260, Academic Press, N.Y., 1964

PHYSICAL AND NEUROLOGICAL EXAMINATION WITH BEHAVIORAL OBSERVATIONS OF CATS TREATED WITH CANDIDATE COMPOUNDS

In order to assess the overall physical, neurological, and behavioral effects of candidate compounds, a systematic examination of physical signs, sensory, motor and reflex reactions, and behavioral reactions to the observer is conducted in intact cats (McGrath, 1960, and Norton and deBeer, 1956). Each animal is examined before receiving drug, and periodically thereafter.

The examination consists of observation of heart rate, respiration rate, and body temperature, pupillary diameter (constricted or dilated), pupillary response to light, sensory response using sharp needle to scratch or poke the skin (superficial) or pinching the toe pads (deep), motor activity (walking), spinal reflexes (flexor reflex, knee jerk, extensor thrust, scratch reflex, crossed extensor reflex, spinal visceral reflex), and attitude and postural reactions (attitude reflexes: tonic neck reflex, tonic eye reflex; supporting reactions, righting reactions, placing reactions, and hopping reactions). The behavior of each animal and its reaction to the observer are noted during the examination period. Drugs are administered intravenously (on a weight basis) in a dosage of 0.1 of the LD_{50} level in mice. Cats are examined in most instances at approximate intervals of 0.5, 1.0, 2.0, 4.0, 6.0, and 21 hours after compound administration.

Unusual observations are presented in the tables which accompany the detailed reports. Although only positive significant effects are usually noted, the presence of a normal sign may be emphasized in order to indicate that an unusual effect was especially sought. As when dilated pupils are recorded, the pupillary light reflex is especially noted. Where both dilatation of pupil and paralysis

of the light reflex occur, a parasympatholytic effect may be assumed to be present. The hopping reaction seems to be an especially sensitive one for testing proprioception. Therefore, this test is individually recorded. In some cases, sedation or over-activity and hyperexcitability are noted. When the animal becomes hyperexcitable, it sometimes is impossible to do a complete examination. However, such observations which can be made are noted in these instances.

REFERENCES:

McGrath, J. T., Neurologic Examination of the Dog, 2nd Ed., Lea and Febiger, Phila., 1960

Norton, S. and deBeer, E. I., Effects of Drugs on the Behavioral Patterns of Cats. Annals of the New York Academy of Sciences, 64, pp 249-257, 1956

MOTIVATION TEST

This test is used for more detailed study of promising drugs, and is conducted according to the method of Barry and Miller (1965). Hooded rats are trained to run from a start box down a straight alleyway to a goal box. The response is measured under three motivational conditions: food approach, shock avoidance, and shock escape. Separate groups of hooded rats, approximately equally distributed by weight and age are initially randomly assigned to groups, and trained to approximately equal performance. Thus the motivational conditions are highly comparable (same response, approximately equal performance) yet independent of each other (separate groups).

Six rats are trained to each motivation. Each of the rats is run through six trials daily for five days per week. Start and

run times are recorded electronically to the nearest 0.01 second. The dosage schedule is as follows: On Monday, no injections are administered, but the rats run through the trials to "warm up" for the week. On Tuesday, saline injections are administered and "control readings" obtained. On Wednesday, and Thursday, drug injections are administered intravenously to two rats of each group, and saline to the remaining four, and trial runs are then conducted with all rats after an elapsed time period previously determined to be that of probable peak drug effect.

Compounds which show exceptional activity will be further evaluated by the Sequential Response Method in hooded rats (Polidora, 1963). Two units of the device for conducting these tests have been recently completed in our manufacturing facility.

REFERENCES:

Barry, H., and Miller, N. E., Comparison of Drug Effects on Approach, Avoidance, and Escape Motivation, Jour. Compar. Physiol. Psychol. 59, 18-24, 1965.

Polidora, V. J., A sequential Response Method of Studying Complex Behavior in Animals and Its Application to the Measurement of Drug Effects. Jour. Exper. Analysis Behav. 6, 271-277, 1963

RESULTS

Twenty-four of the following compounds were received on April 25, 1968, and examined by the toxicity screen and locomotor activity procedures. Detailed toxicity data were submitted in the first formal report for the contract year (June 24, 1968). In addition, [redacted] was purchased on the open market.