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C00021831

February 26, 1969

Dear Sirs:

Enclosed in quadruplicate is a proposal for the extension of work being conducted under Contract [Task Order No.] for the period February 28, 1969, through February 28, 1970.

This proposal provides for a substantial increases in the level of effort in order to accomplish more advanced pharmacological characterizations of compounds showing interesting properties by the primary screening procedures. Since the advanced techniques will emphasize the use of nonhuman primates, it is anticipated that the results obtained will have good predictive value.

Sincerely yours,

Enclosures

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TECHNICAL PROPOSAL

[redacted] submits this unsolicited proposal for continuation of the program on pharmacological screening of potentially useful new agents.

ABSTRACT

Each new compound will be examined by primary screening in mice using acute toxicity screen and locomotor activity tests, as well as by effect on the physical and neurological examination in the cat. Promising candidate materials will be evaluated by secondary screening, including a motivation test and a sequential response test using hooded rats. Further tests will be done with very promising materials to evaluate effect on social behavior of squirrel monkeys, mechanism of action, effects of antagonists, and detailed analysis of effects on behavior using suitable animal species with emphasis on primates.

TECHNICAL PROPOSAL

[redacted] submits this unsolicited proposal for continuation of the program on pharmacological screening of potentially useful new agents.

HISTORY:

Since renewal of contract No. [redacted] Task Order No. 01 [redacted] has through the contracting agency arranged with a pharmaceutical company for the acquisition of compounds for screening. At the time of preparation of this proposal, 48 compounds received from this source have been screened by primary screening methods including mouse toxicity screen, spontaneous locomotor activity screen, and evaluation of behavioral and neurological profile in cats. Secondary screening is now in progress. At least eight of the initial compounds show promising activity as listed in our second interim report. The initial secondary screening program consists of a motivational test and a sequential response test, both using hooded rats. For the latter test, two apparatuses were constructed, and are now in operation. Additionally, selected promising compounds are administered to selected monkeys for evaluation of the effect on physical, neurological, and behavioral profile as with the cats. Behavior is evaluated by the effect on such characteristics as curiosity, aggressiveness, and fear. Seven squirrel monkeys (Saimire sciuresus) have been obtained, and are trained in a test situation to evaluate changes in social behavior and performance in situations which involve both positive and negative reinforcement according to the method of Plotnik (Plotnik, R., Jour. comp. physiol. Psycol. 66 (2),

369-77, 1968). In each of two groups of three monkeys a hierarchal pattern of aggression or submissiveness has emerged. The monkeys are ranked according to this characteristic. Changes in aggressiveness, dominance, or submissiveness induced by the most promising candidate drugs are evaluated in submissive or aggressive monkeys, respectively.

PROPOSAL FOR THIRD YEAR:

Each compound will be examined by primary screening methods in mice and cats as described below.

I. PRIMARY SCREENING PHASE

1. Acute Toxicity Screen - Mice

This procedure respresents the first step in eventual acceptance or rejection of a compound. The screen is designed to eliminate from consideration compounds which do not posses 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 preferred term to toxic sign) for compounds known to be mentally or physically incapacitating to man. The test is 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:

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.

2.

2. 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, the

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., 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

3. 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 systemic 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, body temperature, pupillary diameter (constricted or dilated), pupillary response to light, sensory response using a 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 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.

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Thus 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

II. SECONDARY SCREENING PHASE

1. 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 intially 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 (Policora, 1963). Two units of the device for conducting these tests have been recently completed in our manufacturing facility.

Sequential Response Behavior Test

The sequential response behavioral test (Policora, V. J., Jour. Exper. Anal. Behav. 6, 271-277, 1963) is a method useful for the study of detrimental drug effects on complex behavior using hooded rats as experimental subjects. The test compartment is cylindrical in shape, and contains at the periphery, four response pedals separated by 90° angles. Above the response pedals are a signal light and a fountain to yield liquid rewards, after a correct sequence of pedals is pressed by the experimental subject. Subjects are maintained at a stable response level by 2 or 3 hour water deprivation, one-half hour

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ad lib water, and frequent practice sessions. Baseline data control are obtained by administration of saline solution, in place of drug, and obtaining characteristic response and reward rates at that time.

REFERENCES:

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

Polidora, V. G., 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

III. TERTIARY SCREENING PHASE

1. Social Behavior in the Squirrel Monkey

The effect of very promising drugs will be evaluated on social behavior of squirrel monkeys using the method of Plotnick (Jour. Comp. physiol. Psychol. 66 (2), 369-377, 1968). The apparatus consists of two boxes with plexiglass sides connected by a tunnel, which is separated from the boxes by guillotine doors. The floors of each box and the tunnel contain parallel rods, which may be electrified. Food pellets can be administered at the end of each box.

In this test groups of squirrel monkeys are trained to respond to a cue (light, for negative reinforcement, sound for positive reinforcement) by running through the tunnel to the opposite compartment. Observations are made of running order and aggressive interactions, to determined the hierarchy of dominance-submission. Food deprivation is maintained to furnish the drive for positive food reinforcement. The effects of candidate drugs on aggressive and submissive behavior, will

2. Drug Classification Studies

Suitable studies will be conducted to determine class of drug action, mechanism of action, and antidotes of promising test materials.

Such tests in rats and other species will include effect of drugs on threshold to produce convulsions with metrazol or electric shock, ability to produce catalepsy, potentiation or blockage of drug effect by neurohumoral agents and potentiation or blockage of known active materials by test compounds. Discussion of the applicability of these tests is given in several chapters of Animal Behavior and Drug Action, ed. by Steinberg, H., Little Brown & Co., 1964, especially the discussion by Janssen (p. 392).

3. Advanced Behavioral Analysis on Nonhuman Primates

Method development using primates as test subjects will continue for the purpose of application of suitable tests for a complete description of behavioral and central nervous system effects of promising drugs.

Submitted: February 26, 1969

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COST BREAKDOWN

THIRD YEAR

Month	Per Cent Expenditure of Total	Accomplishment
1	5	Primary Screening Phase
2	5	Primary Screening Phase
3	7	Primary and Secondary Phases
4	7	Primary and Secondary Phases
5	9	Primary, Secondary, Tertiary Phases
6	9	Primary, Secondary, Tertiary Phases
7	9	Primary, Secondary, Tertiary Phases
8	9	Primary, Secondary, Tertiary Phases
9	10	Primary, Secondary, Tertiary Phases
10	10	Primary, Secondary, Tertiary Phases
11	10	Primary, Secondary, Tertiary Phases
12	10	Primary, Secondary, Tertiary Phases

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COST ESTIMATE

TECHNICAL PROPOSAL - THIRD YEAR

PERSONNEL:

Classification	Hours	Rate Per Hour	Total Cost