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SCREENING CENTER FOR PHARMACEUTICALS

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REPUBLIC OF KOREA

Technical Report: Development of Neuropsychopharmacology Screening Unit*

Prepared for the Government of the Republic of Korea
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Development Programme

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Vienna

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1. Introduction

The establishment of the screening center for pharmaceuticals in Korea is part of the ongoing programme of the development of new pharmaceuticals in Korea started in 1978.

In the past 20 years, Korea achieved a remarkable progress in Science and Technology with the rapid growth of national economy.

The organic chemical industry has shown a steady advancement of synthesizing agrochemicals, pharmaceuticals and other industrial chemicals domestically and is aiming for exporting the products in the future. This is to turn the tide of the most pharmaceuticals developed in the foreign countries used in Korea. The development of new pharmaceuticals in Korea should be a next step in order to compete with developed countries in the international market and to confront with the pressure of substantial patent system.

Organic synthesis, screening, and safety evaluation are the three steps essential for the new pharmaceutical product development. Rapid and accurate drug screening is closely related to the success of the synthesis in shortening the development period, lowering the costs, and providing guidance for the structure design and modification of the chemical molecule.

Recognizing the importance of the drug screening facility in the development of new pharmaceuticals, the Korean government has initiated this project with a skeleton staff of seven professionals and technical staff which needed to be expanded and strengthened.

2. Objectives and logic of project

The objectives of the project will be the establishment of a pharmacological screenig center, equipped with modern instruments, and a cadre of well trained technical staff.

The center will be capable of providing research services of screening newly developed neuropharmacological, cardiovascular, anticancer, immunological, and diuretic compounds. Research on the biotransformation and pharmacokinetics of these compounds will be followed.

3. Ongoing activities

The screening center is located on the compounds of Korea Research Institute of Chemical Technology (KRICT) at Daeduck Science Town, Choong Nam Province. KRICT has a land of 100,000 square meter. KRICT is established under the responsibility of the Ministry of Science and Technology, which provided a small seeding fund while the greater part came from various industrial companies. KRICT employs 600 persons at present, and 60 of which belong to Life Science Division. Life Science Division includes Toxicological Research Center (40 persons), Screening Center (7 persons), and Biotechnology Center (13 persons). The new building for the screening center under construction has three floors, a basement, and a roofing floor containing all the air conditioning units and otner engineering equipments. There will be a total of 6,000 square meter space. The neuropsychopharmacology unit will have 150 square meter of laboratory space. Animal housing, surgery, and other common equipment facilities will occupy additional spaces.

At present new antibiotics, antivirals, and some cardiovascular drugs have been tested through the screening center.

The ongoing screening tests in operation for neuropsycho-pharmacology are carragenin edema, acetic acid writhing, conditioned avoidance test, and exploratory and activity test.

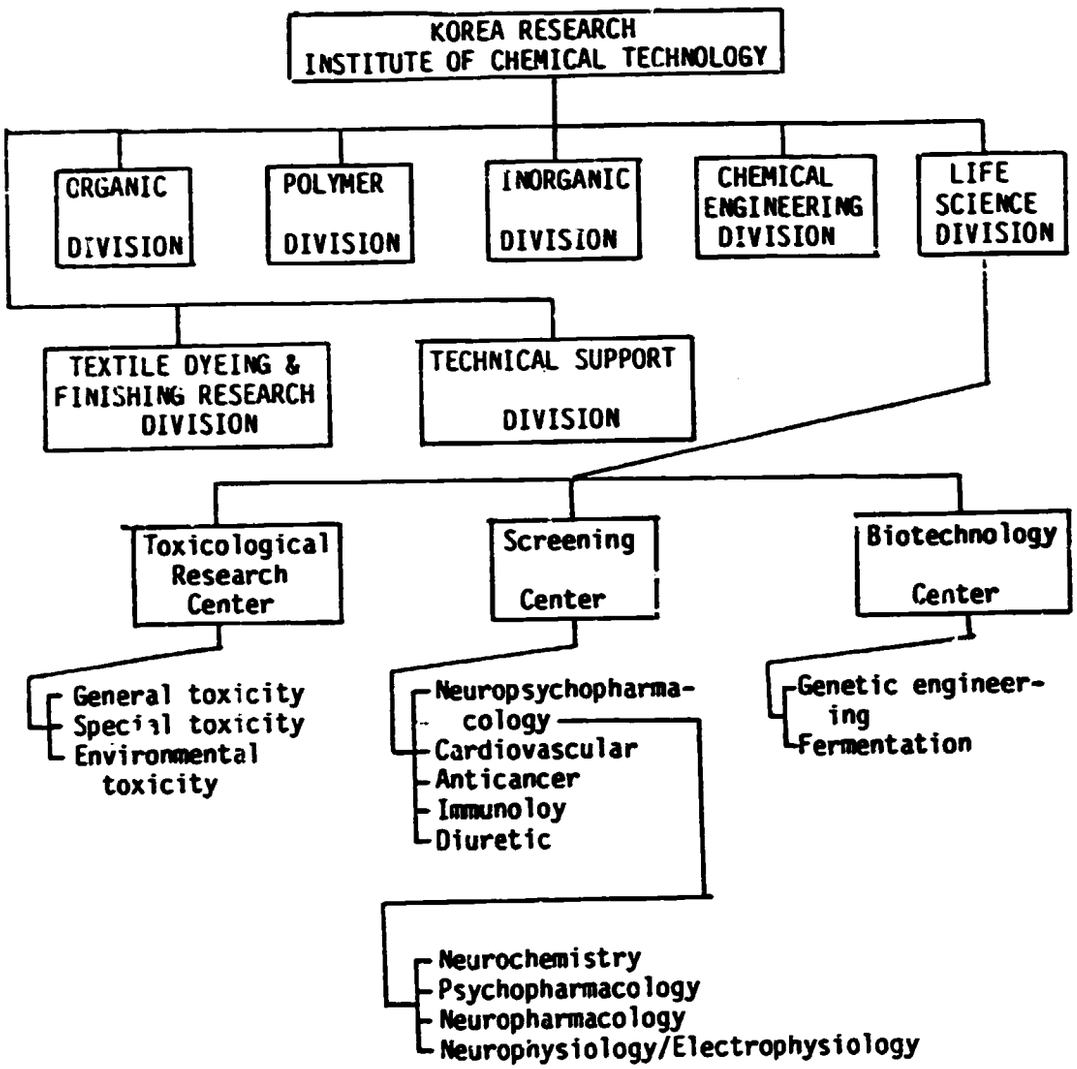

Figure 1: The Organization Chart of KRICT

4. RECOMMENDATIONS

A. Proposed organization of neuropsychopharmacology unit

It is proposed that the neuropsychopharmacology unit be subdivided into four sections as follows to take up different tasks.

1. Neurochemistry Section (2 prof, 4 tech)
   - Receptor binding by using isotopes
   - Monoamines, monoamine oxydase studies
   - Plasma corticoid level assay
   - Other neurohumonal assays

2. Psychopharmacology Section (3 prof, 6 tech)
   - Gross behavioral studies
   - Conditioned avoidance study
   - Locomoter activity study

3. Neuropharmacology Section (2 prof, 4 tech)
   - Analgesics
   - Anticonvulsant
   - Local anesthetic
   - Isolated tissue
   - Body temperature

4. Neurophysiology/Electrophysiology Section (3 prof, 6 tech)

   Electroenchephalography

   Sleep laboratory

Each section will be headed by a section leader. Under the section leader, two to three senior professionals with technical support will be needed as the screening tasks increase. The technical support will be added as the volume of screening increases. It is preferred that the senior members have an advanced degree, or are familiar with the specific testing method. A short term intensive training at an established laboratory or institution, domestic or abroad, may be the method of training.

B. Personnel

There is a senior scientist, Dr. Eun-joo KIM, a Ph. D. from des Fachbereichs Pharmazie, der Freien Universitaet Berlin, responsible for neuropsychopharmacology testing. She has a technical assistant. Dr. Kim is a neurochemist by training, and she has accepted present position right after her doctorate. Since then she has spent three months in Berlin for pharmacokinetic studies and another two weeks for medicinal chemistry conference. It is my opinion that one year training in the interdisciplinary fields of neuroanatomy, neurophysiology, psychopharmacology, electrophysiology, and neuropharmacology will bring her up to the state of the art in neuropsychopharmacology. I would like to recommend the Brain Research Institute and Neuropsychiatry Institute of the University of California at Los Angeles for her one year postdoctoral training.

C. List of chemicals

The following chemicals which are commonly used in neuro-psychopharmacological studies should be in stock at all time.

Amphetamine

Apomorphine

Chlorpromazine

Codeine

Diazepam

Haloperidol

Hexobarbital

6-Hydroxytryptamine

3-Mercaptopropionic acid

Morphine

Naloxone

Naltrexone

Norepinephrine

Parachlorophenylalanine

Pargyline

Pentobarbital

Pentylenetetrazol

Phenobarbital

Phenylparaquinone

Reserpine

Strychnine

D. Equipments

The following equipmencs are recommended for neuropsycho-pharmacology laboratory.

Stereotaxic instruments for rat, cat, monkeys

Stimulaters

Polygraph (multi channels, EEG, EKG, EMG)

Oscilloscope (2 traces)

Isolated tissue bath

Constant temperature bath

Animal recording box with one-way mirror, constant temperature, sound proove, and electric shielded.

Tail-flick equipment

Electroencephalography

Skinner box

Microcomputers for online data acquisition and processing

Surgical tables for big and small animals

E. Books

The following books are needed for neurophychopharmacology laboratory.

Rat brain atlas

Cat brain atlas

Rabbit brain atlas

Monkey brain atlas

Rhesus monkey

Squirrel monkey

Goodman and Gilman's The pharmacological basis of therapeutics 7th ed. 1985

The Merck Index 11th ed.

F. Methods and procedures in neuropsychopharmacology studies

1. Gross behavior assessment

Irwin's gross behavioral assessment procedure in mice is widely used for initial evaluation of psychoactive substances.

Method: Compounds were administered to groups of mice, six per dose level, and changes of function were rated on a 0 to 8 scale. Functional changes observed were impaired gait, impaired righting reflex, muscle tone reduction, sensoromotor responses, irritability avoidance, locomotor activity, finger approach and reduced behavioral arousal.

Minor tranquilizers caused impairment of gait and righting reflex. They also selectively reduced muscle tone.

2. Suppression of d-amphetamine lethality

The LD_{50} of amphetamine-treated mice can be reduced up to sixfold by confining the animals in a small space. This effect is counteracted by chlorpromazine and reserpine at low doses, by phenobarbital at high doses, and not at all by pentobarbital.

Method: Doses of test drug or vehicle are given orally or subcutaneously to groups of 18-hour ten fasted mice in geometrically spaced doses, 0.5 of 1.0 hour prior to interperitoneal dl-amphetamine sulfate. Each group of animals was placed in a separate wire mesh cage (15 X 25 X 10 cm) which was elevated by 5 cm legs to allow free air circulation. The animals were left undisturbed for 24 hours. Protective ED_{50} values were calculated using the method of Litchfield and Wilcoxon.

In the testing, the LD_{90} dose was administered to groups of mice.

This experiment gives constant results when performed in the room with temperatures of 20 to 21 ^{o}C with a relative humidity of 70 to 80%.

3. Inhibition of fighting behavior

Antiaggression and taming activity can be measured on aggressive animals. Aggressive behavior can be induced in mice by prolonged isolation or electroshock and in rats by electroshock or midbrain lesions.

Method: Mice was isolated for 21 days in 10 X 12.5 X 25 cm solid-walled cages such that the animals did not have visual contact with each other. After the 21-day period of isolation, the animals were individually exposed to several forms of aversive stimuli. They were gently prodded on the hindquarters with long forceps and their tails were pinched. After 5 minutes of aversive stimuli, a nonisolated "intruder" mouse was placed in the home cage of the isolated animal. The two mice were allowed to remain together for 5 minutes, or until the isolated animal attacked the intruder. Only animals which consistantly attacked were used in subsequent drug studies.

For studies, test compounds were administered in a geometrically spaced doses to groups of five isolated mice, and the incidence of attack directed toward an intruder was determined at preselected intervals (0.5, 1, 2, and 4 hours) thereafter.

Protective ED_{50} values were calculated and defined as the dose which blocked 50% of the isolated mice from attacking at the time of peak effect.

4. Blockade of apomorphine-induced emesis

All major tranquilizers are also potent antiemetic agents. Because of this, antiemetic activity was used in screening the unknown compounds for major tranquilizers. Most methods stimulate the chemoreceptor trigger zone by using apomorphine, morphine, digitalis, emetine, and copper sulfate in dogs.

Method: Adult mongrel dogs fasted for 12 to 18 hours were fed a standard diet 1 to 2 hour prior to apomorphine administration (0.31 mg/kg, s.c.). The percentage of animals totally protected from vomiting was used to calculate the protective dose 50% (PD_{50}) at preselected time intervals after administration of test compounds.

5. Inhibition of conditioned avoidance behavior

Chlorpromazine, a major tranquilizer, suppressed avoidance response in rats but did not impair escape behavior. In constrast, sedative and hypnotics are not selective both avoidance and escape behavior are blocked. This effect occurs only at large dose which produce marked impairment of coordination and spontaneuos motor activity.

Method: An electrically activated solenoid pushed a mouse off an elevated platform onto a grid floor. Five seconds later the grid became electrified (400 Hz, 1mA, 0.4 msec pulse width) and the animal escaped the shock by climbing back onto the platform.

Naive mice, placed in the apparatus for approximately 25 trials three or four times each day, quickly learned to escape the shock, and within 5 days were avoiding the shock 95 to 100% of the time.

6. Blockade of exploratory behavior

Major tranquilizers decrease exploratory behavior and locomotion in laboratory animals.

Method: Placing a naive male rat (200 - 250 gm) in the center of an "open field arena", the ambulation score during a 3 min observation period was determined at 30 min intervals. In addition the incidence of rearing during this time and the number of fecal boluses excreted were noted.

Using dose-response curves for drug-treated versus saline-treated, the dose which suppressed 50% of activity was determined.

The distinguishing characteristic of the major tranquilizers, compared to sedative hypnotics, in suppression of locomotor activity is the relative lack of other behavior suppression or neurotoxicity.

7. Ptosis

Major tranquilizer induces ptosis which is of central origin can be distinguished from peripheral mechanisms induced by sympathetic ganglionic blocker (hexamethonium), postganglionic alpha-adrenergic blocker (phentolamine), and interference or depletion of sympathetic nerve transmitters (guanethidine, bretylium).

Method: Rat was given the test compound and left undisturbed for up to 4 hours. At predetermined intervals the degree of ptosis was determined before and 90 sec after exteroceptive stimulation. By briefly handling the animal, spontaneous ptosis was eliminated. Stimulation reversed the ptosis induced by centrally acting agents and this reversal continued for approximately 1 minute. In contrast, peripheral agents which produced ptosis were generally non-reversible.

8. Body temperature

Major tranquilizers lowered the body temperature of mice kept in an environment of 25 ^{o}C.

Method: Mice and rats are commonly used. Each animal was individually housed and given free access to water. The testing compound was given in geometrically spaced doses to groups of eight animals each, with control animals receiving an equal volume of saline. Rectal temperatures were read at 0, 1, 2, 3, 4, and 5 hour. Up to 10 ^{o}C drop of body temperature had been reported.

9. Norepinephrine antagonism

Norepinephrine was believed to be responsible for psychotic states, because of the peripheral alpha-adrenergic blocking effects seen after various major tranquilizers in blocking norepinephrine induced lethality in mice.

Method: Rats were pretreated with test compound in geometrically spaced doses one hour prior to administration of lethal dose (1.25 mg/kg i.v.) of norepinephrine. The

protective ED_{50} value was calculated by probit analysis and compared with the protective ED_{50} value for the same test drug in blocking the compulsory gnawing or chewing movements induced by 10 mg/kg, i.v., of d-amphetamine. The ratio of ED_{50} of d-amphetamine over ED_{50} of norepinephrine was a measurement of the relative peripheral alpha-adrenergic blocking potency of the test compound. The haloperidol group has a very low peripheral adrenolytic effects.

10. Caudate mice

Unilateral lesioning of the corpus striatum (rich in dopamine) in rats, produces postural asymetries and pivoting toward the unoperated side (contralateral). When this animal is given apomorphine (dopamine receptor stimulant) will pivoting toward the operated side (ipsillateral).

Method: Rats lesioned on unilateral caudate nucleous were allowed to recover (5 to 14 days). Each rat was given 2 mg/kg i.p. of apomorphine and the presence or absence of body asymetries and pivoting was determined during the next 30 minutes. In animal with successful unilateral lesions, apomorphine caused the mice to pivot continuously toward the side of lesion. In the drug studies, only those animals which pivoted consistently on each of three drug trials one day

apart were used.

Major tranquilizers produced contralateral pivoting with unilateral caudate lesions. The endpoint for these studies may be either all-or-none response or a quantal response. Determining the number of animals with contralateral pivoting after major tranquilizer administration or the number of animals which did not show ipsilateral pivoting after administration of major tranquilizer plus apomorphine (2 mg/kg, i.p.). All major tranquilizers blocked the ipsilateral pivoting produced by apomorphine.

11. Self-stimulation

In general, parenteral administration of drugs which deplete brain monoamines (alpha-methyl-p-tyrosine) or which suppress adrenergic transmission (chlorpromazine, haloperidol) suppress self-stimulation whereas central administration of norepinephrine facilitates such behavior. Pentobarbital and meprobamate are without effect in these procedures.

Method: A pair of silver wires 0.2 mm in diameter and insulated except for the cross section of the tips was implanted in the lateral hypothalamus of male albino rats (300 - 400 gm). After recovery from surgery, animal were trained

to bar press for hypothalamic stimulation (60 Hz, sine wave at an intensity of 40 - 60 uA for 250 msec).

Chlorpromazine suppresses self-stimulation, whereas d-amphetamine, meprobamate, and LSD are without effect in altering the response.

There are reports indicating that self-stimulation was blocked by classes of drugs other than major tranquilizers. There was no correlation of inhibition of self-stimulation with tranquilizing activity, but rather with sleep-inducing activity.

12. Hypnotic activity

In rodents, the hypnotic activity can be measured by sleeping time, loss of righting reflex, potentiation of known CNS depressants such as hexobarbital.

The most appropriate for the evaluation is using neurophysiological techniques to measure drug effects on sleep stages of cats or monkeys.

13. Muscle relaxant activity

The methodologies used to evaluate muscle relaxant activity in animals involve either simple test systems such as antagonism of strychnine lethality, traction response, and inclined screen, or neurophysiological methods in decerebrate or high spinal cats.

14. Anticonvulsant activity

Convulsions induced by chemical agents such as pentylenetetrazol, nicotine, bemergride, 3-mercaptopropionic acid, and electroshock can be used to test anticonvulsant activity of a compound.

Method: Initial evaluation of anticonvulsant activity is usually made in groups of male mice. Convulsants such as pentylenetetrazol (85 mg/kg s.c.) and bemegride (40 mg/kg s.c.) are administered 30 minutes after test compounds and evaluations are made 15 minutes later.

15. Locomotor activity

Imipramine is differentiated from amphetamine by measuring locomotor activity. Both drugs inhibit exploratory behavior but amphetamine stimulates locomotor behavior whereas imipramine depresses it. Imipramine blocks defecation but amphetamine does not.

16. Catalepsy

This test is used to determine the incidence of extrapyramidal side effects of major tranquilizers. Haloperidol, a potent alpha-adrenergic blocking agent, causes profound depression of locomotor activity, loss of muscle tone, catalepsy, and marked hypothermia. Reversal of catalepsy makes a convenient endpoint to use as an indicator of antidepressant activity.

Method: One front foot of an adult rat is placed on a rubber stopper 3 cm high. Failure to correct the imposed posture

within 10 sec is considered a positive cataleptic response and a score of 0.5 is assigned. After using both forepaws, the stopper is removed and each forepaw placed, in turn, on a 9 cm high stopper. Failure to remove the paw from this stopper is assigned a score of 1.

Catalepsy scores are determined at 0.5, 1, 1.5, 2, 3, and 6 hour after drug administration. ED_{50} values are calculated and defined as the dose which produced one-half maximal response.

17. Inhibition of stereotypy

Amphetamine-induced stereotypy is believed to be due to central stimulation of dopamine receptors, and involves the nigrostriatal pathway. With exception of reserpine, all major tranquilizers block amphetamine-induced stereotypy in rats.

In this method, a score of "zero" was assigned to drug-treated mice whose behavior did not differ from that of saline-treated animals. Discontinuous sniffing with some locomotor activity in the animals resulted in a score of 1; behavior consisting of continuous sniffing, some head movement, and periodic locomotor activity was scored as 2. An animals with a stereotypic score of 3 appeared similar to an animal with a 2,

but discontinuous biting or chewing was also seen. Continuous gnawing, biting, or licking with no locomotor activity resulted in a score of 4.

Using this technique, drug effects in reducing the mean stereotypy score by 50% were determined when the maximal stereotypy dose of d-amphetamine was given.

18. Murcidal rat test

A small percentage of rats will spontaneously and instinctively kill a mouse within minutes of presentation. Antidepressants and certain antihistamines (e.g., triplennamine) and anxiolytics selectively block this behavior at doses which do not cause motor deficit.

Method: Male rats are isolated in individual cages, fasted 24 hours, and then each rat is challenged by placing a mouse in his cage. Those rats which kill the mouse within 30 minutes are selected for further intensification of their mouse-killing behavior. Eventually these preselected rats will kill mice within 30 sec of presentation.

In the testing, five rats per intraperitoneal dose of test drug are presented at 30 min intervals with mice. Mouse-killing behavior is considered blocked when the mouse is not attacked within 30 sec of presentation. Quantal ED_{50} values for prevention of muricidal behavior are calculated based on the 30 sec cutoff.

19. Hexobarbital sleep

Prolongation of sleep time from a fixed dose of a barbiturate can indicate the degree of potency of a drug as a CNS depressant or it can mean that the drug blocks metabolism of the barbiturate.

Method: Male rats in randomized groups of 10 are injected intravenously with 25 mg/kg hexobarbital sodium 30 minutes after oral or intraperitoneal test drug or venicle. Sleep duration is measured from time of loss to time of return of the righting reflex. An ED_{50}, the dose which increases sleep time to 150% of controls, may be calculated.

20. Phenylquinone writhing test

To detect analgesic activity the phenylquinone writhing test in mice is sensitive to all known analgesics including the narcotic antagonist analgesics. The simplicity of the procedure makes this test an excellent primary screening test.

Method: Male mice 14 to 25 gm are used. At various time intervals after administration of the test compound the animals receive intraperitoneally 0.1 ml/10 gm body weight of an 0.25 mg/ml solution of phenylparaquinone in 5% aqueous ethanol (2.5 mg/kg). Five minutes later they are placed in observation cages and the number of animals which do not perform a characteristic writhe during the next 10 minutes are recorded. Ten mice are used for each dose of each agent. Statistical analysis of the quantal data is performed by the logit method which generate an ED_{50}, slope, and 95% confidence limits.

21. Tail-flick procedure

Tnis test system is specific for entrally acting analgesics. It is operationally selective for the opiates and opiate-type analgesics. It does not detect those milder analgesics such as aspirin, indomethacin, chlorphentermine, and imipramine which are all active in the phenylquinone test. The tail-flick reflex requires CNS involvement, act predominently by a spinal mechanism.

22. Rotarod test

Performance of repetitive tasks and muscular coordination may be measured in mice on the rotarod. The rod consisting of a 1-inch wooden dowel was divided into 10 equal spaces of 3.5 inches by plastic disks of 8 inch diameter. The rod is operated by a kymograph motor. One end of the wooden dowel was fitted into a kymograph drive shaft and the other into a ball bearing supported by a vertical wooden support.

The animals were trained by placing them on the rotarod for 6 minutes. On test, the animals were injected with drugs and 30 minutes following injection, observing the performance of the animals on the rotarod for one hour, or until they fall off.

23. Antagonism of reserpine

Reserpine precipitates mental depression in man. It also produces the so-called "model of depression" in mice and rats. The degree of test drugs prevents or reverses the effects of reserpine has been used for screening.

G. Overall project

The screening center is in the process of expansion and the construction of the new building will be accomplished at the end of 1987. Dr. Ok-pyo JI has just been appointed as the new director of the center. In the next six months, the center will be moved to the new building. The recommended equipments, chemicals, books and staff will be purchased or filled. Most of the equipments and chemicals may have to be purchased abroad and recruitment of staff also takes time. It is suggested that the project needs to be extended to 1989 or even 1990 to accommodate the situation. A second visit of the expert shall be made after the center has the opportunity to implement the recommendations made in this report. This will make the visit effective, and the project successful.

5. Summary

The screening center for the pharmaceutical products of Korean Research Institute for Chemical Technology is presently testing new antibiotics, antiviral, and cardiovascular substances. The neuropsychopharmacology unit is now able to perform the following tests: carragenin edema, acetic acid writhing, conditioned avoidance, exploratory and activity tests. It is proposed that the neuropsychopharmacology unit should be subdivided into neurochemistry, psychopharmacology, neuropharmacology, and neurophysiology/electrophysiology sections. It is recommended that the senior scientists of neuropsychopharmacology be provided with a further training in the interdisciplinary field of neuroanatomy, neurophysiology, psychopharmacology, and electrophysiology. A list of chemicals, equipments, books, and test methods and procedures are given for further development of the neuropsychopharmacology unit.

The project should be extended to 1989 or 1990 to allow the center sufficient time for expansion and growth.

SEMINAR 안 내

임상화학실 에서는 neuropsycho pharmacology 의 전문가이신 Dr. Yng-shiuh Sheu (USA) 를 모시고 아래와 같이 group seminar 를 개최하오니 관심있는 여러분의 참석을 바랍니다.

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시 간 : 오 후 2 시 (약 1 시 간)

제 목 :

1st July : Study of Cholinergic drugs in central nervous system.

3rd July : Single nuronal activity studies in free behaving animals.

8th July : How abuse-liability of psychotropic drugs are assessed and controlled.

14th July : Preparation of experimental model by brain sugery (Surgical and chemical) for CNS drug screening.

* 6th July (월요일) 에는 세미나실에서 오후 2시부터 아래 제목으로 발표합니다.

6th July : Screening methods for the traqulizing substances.