THE EFFECTS OF LIFE CYCLE THERAPEUTIC DOSAGE ADMINISTRATION OF DRUGS TO ALBINO RATS

by

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INTRODUCTION

Purpose and Scope of the Experiment.

The drug feedings carried out during this two and one-half year experiment were organized for the purpose of emphasizing the factor of time rather than the amount of drug, in determining the possible effects of some commonly used drugs on the normal animal. The usual procedure is to give large or even heroic doses, singly, or over a short period of time, in an attempt to determine the effect or effects on the animal of drugs being investigated. Instances will be cited from the literature where doses many times a comparable human dose have been administered to test animals, conclusions as to the pharmacological action of the drug being drawn from the results obtained from such medication.

In the investigations to be presented herein, dosages comparable to human usage have been administered orally to normal animals which have been kept, as far as possible, in a constant normal environment with respect to food, housing, temperature, ventilation and general care. The test and control animals have been under almost constant observation throughout the life cycle. An exhaustive study of the literature reveals no precedent for this type of investigation. Sollman et. al.[1] have studied the effects of various alcohols for an average of twenty weeks, and growth curves for white rats are presented by the authors for sixty weeks. Nothing has been found, however, which presents life cycle changes

even for normal untreated animals.

Choice of Animals.

Only the Wistar strain of albino rat was used for these drug feeding experiments. Six pairs of breeders were obtained from the Wistar Institute and were mated in our own laboratory, twenty-one litters being obtained from the original breeding stock. The largest litter was seventeen young, the smallest, six. There were seven litters of fourteen and six litters of twelve young. One female raised thirty young from two successive litters. The average litter yield was eleven and five-tenths young as compared to the Wistar Institute yield of seven young or less.[2]

Rats were chosen as test animals for a number of important reasons. The animals are readily tamed and easily handled, an important point when the animals must be handled frequently as they were in these experiments. A rat colony may be maintained with reasonable economy as compared to other laboratory animals, and large numbers of rats may be housed in spaces which are relatively small. Cleanliness is an important factor when the colony must be kept in a building which is largely given over to general university pursuits, and our rat colony provoked no discomforts in this respect. Perhaps most important of all is the fact that the diet of rats resembles very closely that of man, more so than does that of any other experimental animal. Rats thrive on a diet that is normal for human consumption.

An additional factor favoring the rat as the animal of

choice for this type of investigation is well explained in the following paragraphs taken from Greenman and Duhring.[2]

"A rat of three years is equivalent in age to a man of ninety years. Both are at the close of the life span. The rate of growth in the rat is thus thirty times as rapid as in man. Development in the two forms is in the same stage when equal fractions of their life spans are compared.

Thus it is possible to verify or apply directly to man experimental data obtained on the albino rat. No other form is at present sufficiently well known to be utilized in this manner."[1 3]

Similar statements are found elsewhere in the literature.

Housing and Feeding of Animals.

The animals were housed in colony cages with twelve compartments in each, four on a level and three levels high. The compartments measure thirty inches deep, sixteen inches wide and fourteen inches high. They are covered, except at the back, and including the bottom, with one-half inch mesh hardware cloth. The back of each compartment is fitted with a nesting box built of wood, ten inches deep by sixteen inches wide and ten inches high. The bottom is also hardware cloth. The nesting boxes are readily detached from the cage thus making it possible to remove an entire group of rats from a given compartment without the necessity of catching the animals individually. A hinged door closes the back of the nesting box. This arrangement, together with a hinged door in

front, makes the entire compartment easily accessible. The animals spent much of the day in the nesting boxes which are thought to have added considerably to their contentment.

Removable galvanized iron pans beneath the wire floor of each compartment serve to catch animal and food refuse and make frequent cleaning a simple matter. Each compartment is equipped with an exercising turntable, ten inches in diameter, built on a bicycle hub. These exercisers were in constant use during the night and served to keep the animals in a healthy physical condition.

The laboratory housing the colony has a high ceiling and is well lighted through east and north windows, though the animals were never exposed to direct sunlight. The room temperature may be held within two degrees of 23° centigrade by a no-draft ventilating system through the use of an automatic shutter installed in a window. This system allows for maximum ventilation considering the amount of heat furnished to the laboratory. No attempt was made to control the room temperature when the outside temperature was above 23° centigrade. However, the laboratory was well ventilated during such periods by an eighteen inch exhaust fan.

All animals in the colony were fed a cooked ration which was prepared and seasoned as recommended by Greenman and Duhring.[2] The food was cooked in a large enameled dishpan on a steam kettle. Food was cooked every other day during cold weather and daily during warm or hot weather. The animals were not fed the same mixture of cooked food for more than two days in succession since experience has

shown[2] that a variety of food elements and combinations is necessary if a colony is to thrive as it should.

The following vegetables and grains made up the menu, at least two of each being used in each cooked mixture, -- canned peas, tomatoes, beans; fresh string beans, carrots, cabbage, cauliflower, onions, beets, spinach; cracked corn, whole wheat, wheat meal, whole oats, rolled oats. Canned salmon, hamburger and meat stock were alternated in the cooked mixtures.

The cooked food was fed to groups in one-half pound ointment jars with a two-inch round hole cut in the cover. The jars were clamped into six inch pie tins to avoid upsetting. The pans also caught much of the food dragged out of the jars during feeding, thus eliminating some of the loss.

The cooked ration was supplemented with Purina Fox Chow which was kept in the cage at all times, and which served as extra food supply if the quantity of cooked food proved insufficient. The outer leaves of head lettuce obtained from the local cafeteria were fed to the animals daily. Milk containing one minim of cod-liver oil per rat was fed twice daily. Butter and raw egg were fed with the milk twice weekly, a quarter of a pound of butter and four eggs being stirred into the warmed milk with an electric mixer. Once each week the animals were fed what is commonly called dog bone, waste obtained from local meat markets, cooked on the steam kettle. Cuttlebone was also supplied for the purpose of maintaining a healthy tooth condition. Greenman and Duhring[2] describe a faulty dentition occasionally

occurring in rats, resulting in an excessive and rapid growth of incisor teeth and requiring removal of a part of the tooth with bone forceps. No abnormalities of this sort have been noted in our colony of 200 full-grown animals.

Each compartment was fitted with the usual type of inverted water bottle. Two-hundred-forty c.c. oil specimen bottles were found to serve this purpose satisfactorily.

Formation of Test and Control Groups.

Only the largest of twenty-one litters were used for the drug feedings, the choice being based on the age and the number of males or females per litter. No litters were used which did not contain at least six males or six females. Several litters contained twelve males, while nine was the largest number of females in any litter. The males or females in a given litter were taken from the mother when twenty-five days of age and were divided, respectively, into three to five groups on a basis of weight. Enough litters were split in this manner to produce at least three groups containing an average of nine rats each, one group to be used as controls and the others for drug feedings. Each group contained rats from at least three litters, age variations between animals in test and control groups being not more than ten days. Animals were marked for litter and group record by notches in the ears. Table I lists the groups formed in this manner. This method of splitting litters reduced greatly the number of control

groups required.

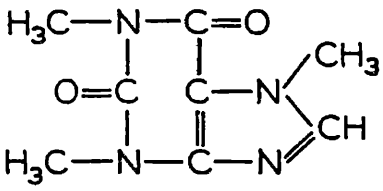
Properties and Source of Drugs Investigated.

Thirteen commonly used drugs were administered to albino rats during this two and one-half year feeding period. Sodium phenobarbital, aspirin and caffeine were fed to both males and females. Sodium barbital, sodium amytal, phenacetin, cincophen, acetanilid and phenophthalein were fed to males only, and sodium alurate, allonal, aminopyrine and antipyrine to females only.

One group of males called the "All" Group was given sodium barbital as a representative of the barbiturate drugs, together with each of the non-barbiturates used and listed above. The "All" Group therefore received sodium barbital, aspirin, caffeine, phenacetin, cincophen, acetanilid, phenolphthalein, aminopyrine and antipyrine, a total of nine drugs, in exactly the same dosage given the groups receiving just one of the drugs. The drugs and doses administered to the "All" Group are underscored in Table I.

A brief discussion of the drugs investigated follows, much of the material being taken from Pharmocotherapeutics[4] and the Epitome.[5] The structural formula of each drug, with the exception of allonal, is presented on pages 8 and 9.

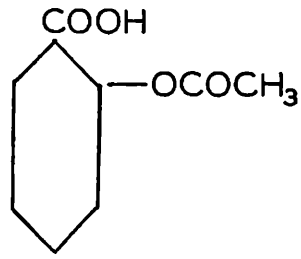
Caffeine, trimethylxanthine, is marketed as white silky needles. It is an alkaloid obtained from coffee and is used as a diuretic and as a cardiac, respiratory and psychic stimulant. It is used as a stimulant in poisoning by narcotic drugs, and with other



CAFFEINE

TRIMETHYLXANTHINE

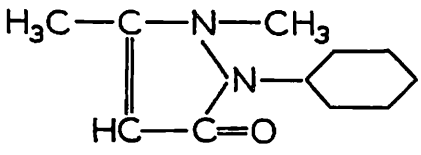
(MERCK)



ASPIRIN

ACETYLSALICYLIC ACID

(MERCK)

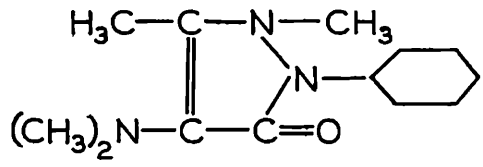


ANTIPYRINE

PHENAZONE

PHENYLDIMETHYLPYRAZOLON

(MALLINCKRODT)

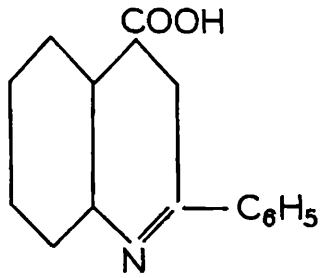


AMINOPYRINE

PYRAMIDON

DIMETHYLAMINOPHENYL-
DIMETHYLPYRAZOLON

(MERCK)

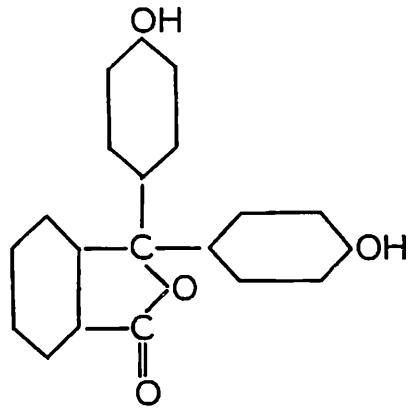


CINCOPHEN

ATOPHAN

PHENYLCINCHONINIC ACID

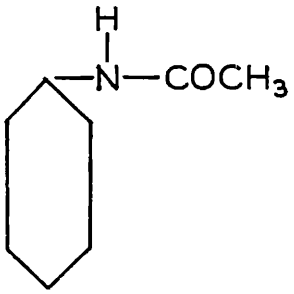
(MALLINCKRODT)



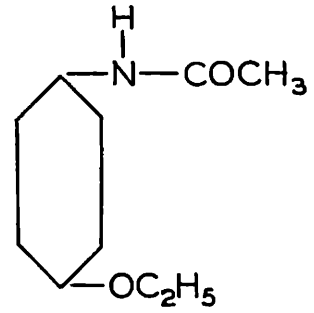
PHENOLPHTHALEIN

DIHYDROXY-TRIMETHYLMETHANE-
CARBOXYLIC ACID

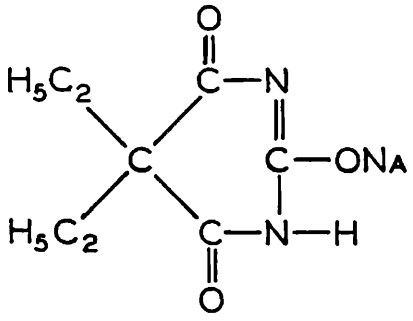
(MALLINCKRODT)



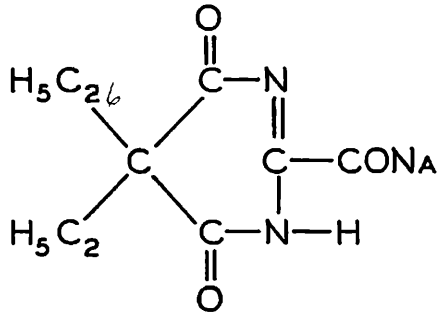
ACETANILID
ANTIFEBRIN
MONOACETYLANILINE
(MALLINCKRODT)



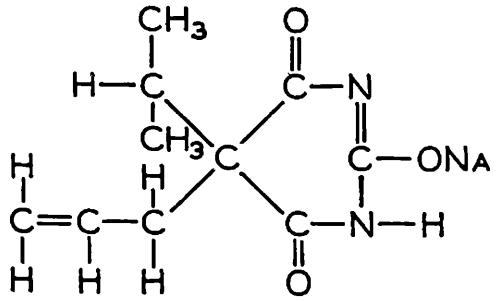
PHENACETIN
ACETOPHENETIDIN
PARAACETAMINOPHENETOL
(MERCK)



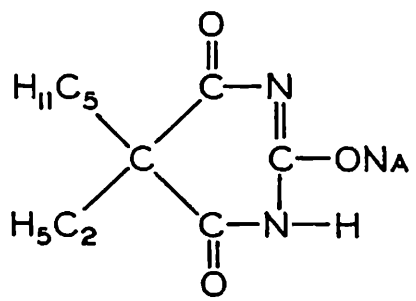
SOLUBLE BARBITAL
VERONAL SODIUM
SODIUM DIETHYLBARBITURATE



SOLUBLE PHENOBARBITAL
LUMINAL SODIUM
SODIUM PHENYLETHYLBARBITURATE
(MERCK)



SODIUM ALURATE
ALLYLISOPROPYLBARBITURATE-
SODIUM SALT
(ROCHE)



SODIUM AMYTAL
ISOAMYLETHYLBARBITURATE-
SODIUM SALT
(LILLY)

analgesics for the relief of headache.[5] Fatal cases of acute poisoning in man are apparently unknown but the fatal dose is probably above ten grams.[4] The average official dose is three grains. A cup of reasonably strong coffee contains about two grains of caffeine and therefore many individuals take several times the official dose of the drug daily.

Aspirin, officially known as acetylsalicylic acid, is a white, odorless chemical occurring in crystalline or powdered form. Its action resembles that of the salicylates. It is used as an antipyretic, analgesic, and antirheumatic, and especially for the relief of headache. It sometimes causes urticaria and dangerous acute edema of the respiratory passages (in humans).[5] The average dose for adults is five grains, usually taken in tablet form. However, fifteen grains is not an uncommon dose and the writer has known of individuals who have taken as high as forty-five grains in one day.[4] Hanzlik reports 120 grains per day as the toxic dose for humans. The early signs of overdosage from aspirin or from related salicylic acid derivatives are nausea, vomiting and sometimes diarrhea, headache, ringing in the ears or deafness, or mental excitement.[4] Very large doses produce weakness of the heart, depression of the respiratory and vasoconstrictor centers, and collapse. Albumin and casts are frequently found in the urine following excessive medication.[4]

Acetanilid, monoacetylaniline, sold under the trade name of Antifebrin, is a white, odorless, crystalline or powdered chemical. It is analgesic, antipyretic, and in excessive doses, a cardiac

depressant. It is used particularly to relieve headache and neuralgic pains. Its indiscriminate use in headache powders is dangerous.[4]

The acutely fatal dose in man cannot be stated with certainty, but disagreeable symptoms are likely to result from doses larger than four grains in afebrile persons and from even half this amount when there is fever. Chronic poisoning is much more common than acute poisoning and results either from repeated use of the drug internally or from continued use as a dusting powder, as in the treatment of chronic leg ulcer. Symptoms of overdosage are weakness, shortness of breath, hot flashes over the body, sweating and mental depression.[5] Digestion is not usually impaired. The average dose for an adult is three grains.

Phenacetin, officially known as acetophenetidin, is a white, odorless, slightly bitter chemical, occurring as crystals or powder. It is analgesic, antipyretic, and in excessive doses, a cardiac depressant. It has replaced acetanilid for the relief of headache and neuralgic pains and in the treatment of mild fevers. Its relation to acetanilid suggests similar caution in its use.[5]

Phenacetin is almost identical in its action with acetanilid except that there is less tendency to cyanosis and faintness. Habit is as readily induced with the drug as with acetanilid. The maximum single dose for humans is about eight grains, and the maximum daily dose is about sixty grains.[4] The average official dose is five grains.

Cincophen, phenylcinchonimic acid, sold under the trade

name of Atophan, is a white, odorless powder with a bitter taste. It is analgesic and antipyretic and is used especially in arthritis. Long continued use or overdosage may cause grave symptoms of intoxication or even fatal hepatitis.[5] In medicinal doses, up to five or even ten grams daily, cincophen usually causes no symptoms whatever.[4] The average official dose is eight grains.

Antipyrine, phenyldimethylpyrazolon, commonly known as Phenazone, is a white, odorless powder. It is antipyretic and analgesic in effect, similar to acetanilid. It should be given with even greater caution than acetanilid.[5] The drug is not, however, as toxic as acetanilid and reports of damage due to overdosage are rare. A normal adult can usually take thirty grains with no further disturbances than slight sleepiness with a tendency to chilliness and sweating.[4] The official average dose of antipyrine is five grains.

Aminopyrine, dimethylaminophenyldimethylpyrazolon, commonly known as Pyramidon, is a colorless or white crystalline substance closely related to antipyrine. It is an antipyretic and anodyne, acting somewhat more slowly than antipyrine, but with a more lasting effect. Granulocytopenia has followed the use of aminopyrine, and the administration should be stopped if skin eruption, dizziness or chill occurs.[5] In man eight grains causes redness of the face and slight sweating but usually no other symptoms. The fatal dose for humans is about eight grams.[4] The official average dose is five grains.

Phenolphthalein is a white or nearly white, odorless, tasteless

powder. It is a cathartic of variable efficacy and may cause some irritation to the rectum and lower bowel. It occasionally causes eruptions on the skin, some of which are quite persistent.[5] The average official dose is one grain.

Barbital, diethylbarbituric acid, Barbitone, Veronal, is a white crystalline powder with a slightly bitter taste. The sodium salt used in these feeding experiments differs from barbital only in being very soluble and much more rapid in its action. Sodium barbital, or soluble barbital, as it is called officially, is used as a sedative and hypnotic. Small doses induce sleep with little or no other effect while toxic doses cause a fall in temperature. It may produce skin eruptions in certain individuals.[6] The fatal dose of the drug is from eight to ten grams. Toxic symptoms are produced with much smaller doses, one gram or less.[4] The official average dose of sodium barbital is five grains. The official dose was eight grains when these experiments were started, and the dosage for rats was figured on that basis.

Soluble phenobarbital, the sodium salt of phenobarbital, phenylethyl barbituric acid, Luminal, occurs as flaky crystals, white crystalline granules, or a white powder. It has a bitter taste. It is used as a hypnotic in nervous insomnia and conditions of excitement of the nervous system, and chiefly as a sedative and antispasmodic in epilepsy. Long continued use, as in epilepsy, may give rise to toxic symptoms of diverse character including skin eruptions.[5] The usual dose in epilepsy is one to two grains daily, but some neurologists

regard from three to four and five-tenths grains daily as being within the limits of safety.[4] The official average dose is five-tenths grain.

Sodium amytal is an unofficial barbituric acid derivative, being sodium isoamylethyl barbiturate. It occurs as fine white crystals or powder. It is Council accepted.[6] Amytal differs from barbital in that one of the ethyl groups of the latter is replaced by an isoamyl group in the former. The actions and uses resemble those of barbital. The suggested sedative dose is one-third to three-fourths grain, the hypnotic dose one and five-tenths to five grains.[6]

Sodium alurate, the sodium salt of allylisopropylbarbituric acid, is also an unofficial compound. It is, however, Council accepted.[6] It differs from barbital in that both of the ethyl groups in the latter are replaced by an allyl group and an isopropyl group respectively. The actions and uses of alurate are essentially those of barbital, but alurate is more active and is used in correspondingly smaller doses. The suggested dose for mild cases of insomnia is one grain, and for obstinate cases, two grains.[6]

Allonal, an unofficial compound, is a mixture of alurate and aminopyrine, free and combined, in the proportion of one and sixty-six-hundreds parts of the barbiturate with one part of aminopyrine. It is dispensed in tablets containing two and two-thirds grains each. It is a safe hypnotic, analgesic and sedative, and is particularly serviceable in patients having affections and disorders

of the nerve system, including psychoses and psychoneuroses. It is said to be five times as active as barbital and the dose is proportional, varying from one to six tablets in twenty-four hours, depending upon the severity of the condition.[4]

It is interesting to note that allonal is the only drug used in the feeding experiments which has not been accepted into New and Nonofficial Remedies[6] by the Council on Pharmacy and Chemistry of the American Medical Association. The product was rejected because of "unwarranted therapeutic claims, non-descriptive name, and lack of satisfactory evidence that the administration of allylisopropylbarbituric acid and amidopyrine in fixed proportions is rational."[7]

Drug Feedings -- Dosage and Method.

Drug dosage for rats was figured on a basis of the official or recommended average dose for an adult human. Drug feedings were started when the animals had reached an age of ten weeks, this period being marked by A on the growth curves. From this period to 200 days of age, (B on the growth curves), the test groups were fed doses equivalent to the average human dose per kilogram, figuring on a basis of group weight as compared to a seventy kilogram adult. At 200 days of age the dose was doubled, and at 300 days (C on the growth curves) the original dose was trebled. Cincophen and sodium barbital were exceptions to this regular procedure because of the large official

dose of these drugs, it being five-tenths gram for each in the United States Pharmacopoeia X.

The test animals received a daily dose equivalent to the average single human adult dose for 130 days. They received the equivalent of a twice daily dosage schedule for a human adult for the next 100 days, and this schedule was continued throughout the rest of the feeding period for cinchophen and sodium barbital. Drugs other than cinchophen and sodium barbital were fed on a thrice daily human adult dosage schedule during the period beyond 300 days, about 400 days for most drugs. The average human dose and rat dosage schedule per kilogram of rat for the thirteen drugs investigated are given in Table I. The number of rats in each group, the feeding period in weeks, the number of doses per rat, together with the equivalent number of human doses, are given in Table II for females, and in Table III for males. The average human dose in milligrams per kilogram of body weight and the average of minimal lethal doses found in the literature for rats, in milligrams per kilogram of body weight, are also included in the tables.

From age ten weeks to 200 days, the drugs were given as a single dose in the morning. The drugs were fed in divided doses night and morning during the rest of the feeding period.

Drugs were fed to test groups in milk. The dose for a given drug was calculated on a basis of weight, the required amount of drug being thoroughly mixed with milk sugar in a mechanical mixer and by sieving, and filled into number 0 gelatin capsules. Each

capsule was prepared to contain the daily dose of the group during the period of the once-daily feedings, and one-half the daily dose during the remainder of the feeding period. A sufficient number of such capsules were filled to last for a two-week period, at the end of which new calculations were made on the weight of the group at that time, a new two-week supply being filled on that basis.

Test groups were fed the drug regularly at eight o'clock each morning during the period of once-daily dosage and at eight o'clock A.M. and at five o'clock P.M. after the daily dose was divided. A capsule containing the group dose was emptied into a narrow cylinder containing two c.c. of milk per rat, the contents being stirred vigorously with a hand model malted milk mixer. The drug this prepared was fed to the group in petri dishes. Controls were fed in the same manner, the capsules containing, however, only milk sugar.

This method of drug feeding has been found to be entirely satisfactory in every instance. In no case have the animals refused the milk containing the drug. All of the animals in a group were at the dish when the milk was poured and remained there until the last drop was consumed. Training and care are undoubtedly important factors involved in this method of drug administration. The feedings were started at an age when the animals were always hungry, apparently regardless of the amount of food in the cage. They gradually became aware of feeding time because of the noise made by the mixer, and would crowd to the front of the cage and remain there until the milk was

placed in their dishes. The animals were completely tamed through daily handling and evidenced no signs of fear toward regular attendants. They would, however, retreat to the nesting boxes on hearing the voices of strangers.

EXPERIMENTAL EFFECTS OF LIFE CYCLE DRUG ADMINISTRATION.

On General Welfare and Behavior.

Caffeine.

Caffeine has been given orally to both male and female rats, from the tenth to the one hundred fifth week for males and to the one hundred twentieth week for females. The number of animals originally present in the control and test groups is given in Tables II and III. The dosage schedule followed is presented in Table I.

Sollmann and Pilcher,[8] in studying the action of caffeine on the mammalian circulation, have found that the fatal dose of caffeine, when injected into the femoral vein, varies markedly, between 57 and 800 mg. per Kg. of body weight. They have accepted 175 mg. per Kg. as the mean fatal dose for dogs, cats and rabbits. Nine milligrams per kilogram was the maximum dose used during this investigation.

The males on caffeine have shown no significant variations from their controls during the greater part of the feeding period, i.e., from the tenth to the one hundredth week. No differences in general behavior attributable to caffeine medication were noticed,

although the animals were under almost constant observation. They possessed good appetites, but there was no evidence of an increased desire for food. There was no recognizable increase in activity during the day, the normal rest period for albino rats, as compared to their controls.

However, at about the one hundredth week, decreases in both appetite and activity were observed, the animals falling off rapidly in appearance and weight. The three animals remaining in the group at the end of the one hundred fifth week were actually killed to keep them from dying, in order that the internal organs and blood could be examined.

During this same part of the feeding period, the male litter mate controls were in good health, gaining thirty grams per rat while the males on caffeine were losing the same amount.

The caffeine females, in contrast to the caffeine males, showed an increased desire for food early in the experiment. This was evidenced by the fact that they crowded around the feeding dishes when the noises normally made at feeding time were duplicated at off-feeding periods. They would accept milk at almost any time of the day, while it was unusual if their controls could be aroused from their nesting boxes by similar experiences. There was, however, no evidence of an increase in total food consumption. Attempts were made to determine possible variations in food consumption accurately but the wasteful tendency of the rat makes such determinations of little value, in the experience of the author, at least.

The females on caffeine were carried through to the one hundred twentieth week and there was no evidence of delayed toxicity during the last several weeks as there was in the males. The animals did, however, lose some weight during the last two weeks.

The females on caffeine were also observed to be about the cage much more during the day than were their controls or any other animals in the colony. They were also observed to use the turntable exerciser much more during the day than did other groups. This observation as to increased activity in the caffeine females was proved more conclusively by their actions on the maze during maze learning, total time and travel time being considerably less for caffeine females than for other female groups run on the maze.

That there is a sex variation to the effects of caffeine has been shown by Horst and Willson.[9] These investigators have measured the amount of tremor in the index finger of young men and women after drinking coffee. They have found that a strong cup of coffee containing one and one-half to two grains of caffeine produced a certain increase in the amplitude in the tremor in women, but that it took more than twice this amount to produce the same effect in men.

Pilcher[10] has administered caffeine by stomach tube to cats in 5, 15, 30, and 60 mg. per Kg. doses, and has found that doses between 5 and 30 milligrams produced wakefulness in the animals. They were also thought to be a little more irritable. All animals receiving more than 30 mg. per Kg. were quarrelsome and restless.

One died four hours after the administration of a 30 mg. per Kg. dose and another died thirty-five minutes after a 120 mg. per Kg. dose. A dose of 200 mg. per Kg. produced death in from fifteen to twenty minutes in all of three animals treated. No increase in irritability has been evident in either the males or females on caffeine during the feeding period.

Eichler and Mugge[11] have subjected white rats to chronic poisoning with caffeine by giving daily doses of 100 mg. per Kg. of body weight. The animals have been observed through four generations of interbreeding and the authors state that no ill effects, other than a transient fall in weight occurring just after medication was begun, have been noted.

Schulte and Tainter[12] have found that 10 mg. per Kg. of caffeine sodium benzoate, when administered subcutaneously to rats, produced the threshold increase in activity. This compares favorably with the 9 mg. per Kg. dose administered orally during this investigation. These authors also found that 20 mg. per Kg. of caffeine sodium benzoate caused larger increases in activity and that 40 mg. per Kg. produced very marked stimulation. The latter dose killed one of the ten animals.

The literature cited above would seem to emphasize the fact that the dosage used for caffeine during this investigation was within the effective range for the drug, and that the sex variations observed during the experiment were entirely logical.

The author is fully aware that it would be unsafe to draw

any conclusions concerning the effect of caffeine on the length of the life span from the meager data presented herein. It is safe to say, however, that the litter mates controlling the males on caffeine were in a much better state of health than were those that had received caffeine during the ninety-five week period. The author is of the opinion that the controls would have outlived the caffeine males had the experiment been continued. The controls were kept for three weeks after the test animals were killed and showed no signs of senility at the end of the period.

Aspirin.

Many cases of mild and severe poisoning by aspirin have been reported in the literature though the dose in most cases has been extremely large. Krasso[13] states that the toxicity of the drug depends largely upon the individual tolerance, that some individuals show toxic symptoms even after 5 grains while others do not react after doses as high as 26 grains. Urticaria following the use of aspirin has been reported by a number of investigators[14] [15] after doses of from 5 to 15 grains. Urticaria and angioneurotic edema have been reported by Maloney[16] who attributes the idiosyncrasy to the phenyl group. The symptoms appeared also after the use of aminopyrine, antipyrine, phenacetin, quinine, luminal, allonal and phanodorn. Krasso[17] reports that the ingestion of 30 grains of aspirin during menstruation produced a very severe intoxication manifested by thirst, nausea, vomiting, difficult respiration, tachycardia, fall of blood

pressure and albuminuria.

Greensted[18] has reported a case, a girl in her early twenties, who took twenty-eight tablets containing aspirin, five powders containing aspirin, and two tablets of allonal over a period of seven hours in an effort to relieve a toothache. The patient finally resorted to dry heat because the medication had done no good.

Dyke[19] has listed nausea, tinnitus, deafness and mental wandering as symptoms occurring after a patient had taken 435 grains of aspirin. Recovery was complete, however. Lipetz[20] has reported a similar recovery after the ingestion of 600 grains of aspirin by a forty-eight year old man who experienced a rushing feeling in the head for a short time, but was able to work as usual in two days.

Neale[21] has listed four deaths resulting from aspirin overdosage. It was not known how much one case had taken, but the other three had taken 200, 750 and 1000 grains respectively.

Prickman and Buchstein[22] have reported sixty-two cases of hypersensitivity to aspirin and state that it is the most common form of drug allergy.

Results obtained from animal experimentation were almost as contradictory as reports following human medication. Robinson et al.[23] have administered doses ranging from 22 mg. to 623 mg. per Kg. of body weight to white rats varying in age from nine to twenty-nine weeks, the medication extending over a period of twenty-nine weeks. They report no effects on general physical condition, growth curves, appetite, activity, coat condition and appearance of the animals.

Perfectly normal litters were obtained by mating rats which had been receiving large doses of the drug for twenty-two weeks previously and throughout pregnancy. Four animals received doses in the excess of the beginning lethal range for humans, 280 mg. per Kg. for seven weeks without observed effect. Activity, growth, coat condition, tail and feces were all normal.

Schnedorf et al.[24] have found, however, that prolonged daily administration of aspirin, one hundred and fifty milligrams per kilogram twice daily, resulted in digestive disturbances in dogs. Alteration in nitrogen metabolism and a tendency toward acidosis also occurred.

Lehman[25] has studied the respiratory, inhibitory and paralytic effects of aspirin in dogs, cats and rabbits. He states that the drug produced only slight or no direct depressant or paralytic effects on the central or peripheral respiratory mechanisms when administered in reasonable doses. Large doses produced circulatory collapse as a result of cardiac arrest, and respiratory failure due to asphyxia. The respiratory paralytic dose for aspirin was stated to be between one and one and five-tenths gram per kilogram of body weight, the paralysis resulting in from eight to sixteen minutes.

Albricht[26] has compared the analgesic effects of aspirin, aminopyrine, antipyrine and phenacetin. He states that aminopyrine is undoubtedly the best analgesic, with aspirin next in effectiveness, and antipyrine third. Phenacetin, according to this author is the least effective.

Brownlee[27] has drawn attention to the necessity of comparing the antipyretic activity of the coal-tar antipyretics -- acetanilid, aminopyrine, phenacetin, antipyrine and aspirin. He has carried out a number of series of comparisons of different doses for each drug by oral administration to cats and rats. He has stated the observed antipyretic potency of these drugs in terms of phenacetin. Table IV is taken from his work. Brownlee states that if phenacetin is taken as 100, aspirin has a potency of 74, antipyrine 100, aminopyrine 134 and acetanilid 170. The toxic dose for rats takes the following order -- acetanilid, aminopyrine, phenacetin, aspirin and antipyrine. These results compare favorably with those reported by Albricht.[26]

Shimamura and Akira[28] have found that aspirin, when given by stomach tube to rats, caused stomach ulcers and bleeding. Bleeding alone resulted from similar medication in mice. Subcutaneous injection of aspirin produced no changes.

Thompson and Dragstedt[29] and Barbour and Porter[30] have reported gastritis and ulceration in dogs following large doses of aspirin. Barbour and Dickerson[31] have produced gastric ulcers in rats by the administration of 300 mg. oral doses of aspirin.

Drouthwaite and Lintott[32] have found that aspirin is a gastric irritant (in humans) and may cause acute indigestion and hemorrhage, or, if taken repeatedly, chronic gastritis. Wyllie[33] has reported similarly.

Both male and female rats have been given aspirin for 109 and 112 weeks respectively during this investigation, in doses

approximating those used by the human adult (Table I). There has been no evidence of blood in the feces of either group and the animals have shown no symptoms that would suggest abdominal disturbances as a result of this medication. The stomachs from seventeen animals from both groups have been examined very carefully after death and no indications of stomach ulcer have been found. Other internal organs were also found to compare favorably with those of the litter mate controls.

Neither males nor females on aspirin have evidenced any variations in appetites or activity as compared to their controls.

A rather marked laxative effect was noticed in both males and females on aspirin between the tenth and thirty-fifth weeks of feeding. The fecal material was more moist and much softer than that of the controls. It was also noticeably lighter in color. After the thirty-fifth week the laxative effect was no longer evident, but the color of the feces remained lighter throughout the feeding period. A positive salicylate test was obtained from the feces of both male and females on aspirin at the tenth week of feeding and at irregular intervals thereafter. The fecal material was extracted with water and to the filtered liquid was added a small amount of ferric chloride test solution. A violet color was considered positive for salicylate. Feces from the controls gave no such color.

During about this same period, from the tenth to the thirty-fifth weeks of feeding, noisy breathing was noticeable in both males and females on aspirin, but it was much more marked in the males.

Their breathing could be heard without difficulty from any point in the laboratory. There was no evidence of labored breathing and it seemed to cause little, if any, inconvenience.

Growths identified as fibroid tumors occurred frequently on the aspirin females, three of the eight animals being operated upon for the removal of such growths. The tumors first appeared under the skin over the abdominal area, being soft and without apparent form in the early stages. After about six weeks the tumor became firm and almost spherical and was easily peeled out through a short incision through the skin. A similar growth occurred on a litter mate control for the aspirin group, and on one other female in an entirely different group. The growths are thought to have no significance as regards drug effect although three out of five developed in the aspirin group. Two of those in the aspirin group appeared on litter mates. The control developing a growth was not a litter mate of those upon which growths appeared in the test group.

The aspirin males were without the drug for three weeks following the eighty-first week of administration. Withdrawal effects were not observed during this period. For this same three week period, amytal was added to the diet of the aspirin females. There were only three animals left in the group and amytal was added for the purpose of determining possible effects of the combination of drugs on the blood picture. No untoward symptoms were seen in the animals as a result of the added amytal.

Acetanilid.

Acetanilid has undoubtedly been the subject of more adverse criticism in the clinical literature of the past three decades than any other drug. As early as 1905 the Council on Pharmacy and Chemistry of the American Medical Association included in its reports[34] a warning of the possible dangers accompanying the use of mixtures containing acetanilid. The Federal Food and Drugs Act of 1906, and amended,[35] stated "that an article shall be deemed to be misbranded if the package fails to bear a statement on the label of the quantity or proportion of any alcohol, morphine, opium, cocaine, heroine, alpha or beta eucaine, chloroform, cannabis indica, chloral hydrate, or acetanilid, or any derivative or preparation of any such substances contained therein." The act of 1939, which displaces the act of 1906, prohibits the inclusion of acetanilid in proprietary mixtures.

The most frequent source of poisoning has been from its indiscriminate use for headache. Of 614 cases of poisoning reported by physicians to the United States Department of Agriculture, according to a detailed report on the summary of data collected from 1886 to 1910,[36] 325 cases or fifty-three per cent, occurred when the drug was taken without a physician's prescription.

The usually occurring symptoms of acetanilid are marked and prolonged cyanosis, great weakness, and loss of weight, prostration, coldness of extremities, profuse sweating and feeble, shallow respiration. Marked restlessness, convulsions and delirium may occur.

Vomiting frequently results and the pupils may be either dilated or constricted.[4] [5] [37] Lundstein et al.,[38] Wright and Montag[39] and Payne[40] consider acetanilid to be habit forming.

Helms[41] has stated that his experiments were performed to correct the erroneous impressions concerning the pharmacological and physiological action of acetanilid. This author has experimented with guinea pigs, rabbits, and rats, having determined the minimal lethal dose for each. The M.L.D. for guinea pigs and rabbits is given as 1500 mg. per Kg. of body weight, and for rats, 2400 mg. per Kg. All doses were given by stomach tube. Mice, according to this author, will survive up to 1350 mg. per Kg. of body weight when given hypodermically, and will take half that amount daily in their drinking water with no effect other than a delay in growth which is regained when the drug is withdrawn. Mice given 500 mg. per Kg. daily for one month tripled their weight. Helms fed rats from 5 to 10 mg. per Kg. per day in milk through four generations and found that the animals reproduced normally, developed normally, cared for the young in a normal manner, and showed no deviation in morbidity or mortality from the average of an unmedicated rat population.

To check the effects of acetanilid clinically, Helms gave ten adult humans four grains of acetanilid three times daily for a period of sixteen weeks. Weekly examinations revealed no physical changes and no changes in the nervous system, heart muscle, or basal metabolism. There was no detectable effect on kidney function, and blood chemistry results were normal. There was no blood cell

destruction and no methemoglobin formation. There was only a transitory effect upon hemopoiesis.

Helms sent a questionnaire to all hospitals and institutions in the United States and received replies representing a total of 25,000,000 admissions over a period of ten years. Total poisoning from acetanilid were five and six-tenths per million, resulting in sixteen hundredths per million deaths. The figures for the barbiturates were, respectively, ten and nine times as large, and Helms concludes that acetanilid is used in self medication while the barbiturates are prescribed by the physician.

Brownlee[27] (Table IV) has given 24 mg. per Kg. of body weight as the equivalent therapeutic dose of acetanilid when administered orally to rats. The toxic dose is given as 0.82 Gm. per Kg. The relative antipyretic potency for rats is also given in the table.

Higgins and McGuigan[42] have found that white mice could consume 325 mg. of acetanilid per day per Kg. of body weight with no significant effect on growth or health. A daily dose of 650 mg. per Kg. produced no effect other than a delay in growth. The latter dosage was continued for six weeks.

Fantus et al.[43] have fed acetanilid to white mice as two per cent of the food. The actual daily dose was 1443 mg. per Kg. of body weight. None of the animals survived after eleven days. These authors found that life could be prolonged about three times and that total consumption of drug could be increased about two and one-half times by decreasing the daily consumption of acetanilid fifty per cent.

Smith and Hambourger[44] have reported that acetanilid produces little tolerance and no addiction in monkeys, even after sixty-five days of daily administration of 100 to 500 mg. per Kg. of body weight.

Stanton and Agricola[46] have found that after daily doses by stomach tube of 700 mg. per Kg. to rats, no acetanilid was found in the feces. These authors have administered acetanilid to rats in gradually increasing doses from 20 mg. per Kg. up to 136 mg. per Kg., after which doses were rapidly increased to test tolerance. A gradual decrease in irritability was noted in all groups, but there was no increase in irritability on permanent withdrawal of the drug. These authors state that there is little or no cumulative effect from daily administration, and no addiction by dosages one-sixteenth to one and one-fourth times the minimal effective dose given daily for periods up to nine or twelve weeks.

Acetanilid has been given orally to male rats for eighty-six weeks during this investigation. There were six animals in the group originally and five survived the feeding period. Doses for the entire period are given in Table I. It is to be noted that the maximum daily dose of 9 mg. per Kg. given during the last 300 days of feeding was well below the minimal effective dose of 12.5 mg. per Kg. of Smith and Hambourger.[44] Additional data are given in Table III.

The group of rats on acetanilid was unquestionably the healthiest group of rats in the colony during the major part of the feeding period. While their litter mate controls and other groups in

the colony frequently experienced "sniffles," a condition which seems to be normal in white rats, the acetanilid animals never exhibited as much pathology as a sniffle. The fur of the animals was normal throughout the period and appetite and activity were comparable to that of the controls during the greater part of the eighty-six weeks. The feces and urine were normal in color and the feces in consistency during the entire feeding period. However, at about the eighty-second week of age, after seventy-two weeks of feeding, a decrease in appetite became apparent, and the animals fell off rapidly. They became hunched and dejected in appearance and paid little attention to care or feeding noises.

At the beginning of the seventy-sixth week of drug feeding, the drug was removed from the diet and discontinued for five weeks. There were no apparent withdrawal effects. The animals did not improve in general appearance and failed to regain lost appetites. Removal of the drug had no effect on the weight loss being experienced by the group at the time of withdrawal, and for four weeks preceding withdrawal. The drug was returned to the diet at the end of the eightieth week and its administration continued until the experiment was terminated, at the end of the eighty-sixth week. No change in symptoms was noted during this period.

One of the six acetanilid males died during the thirty-first week of drug feeding. A careful macroscopical examination of internal organs revealed no abnormalities. The five animals living after eighty-six weeks of acetanilid administration were also

carefully examined, the internal organs being compared with those of litter mate controls. No significant variations could be detected by macroscopical examination. Special attention was given to the liver, kidneys, and spleen of each animal and these organs appeared to be normal. The color and consistency of the blood was normal as compared to the blood of the controls.

Phenacetin.

This coal tar antipyretic is almost identical in its action with acetanilid. The dose, however, is almost twice ad great, and there is less tendency to produce cyanosis and faintness. Habit, according to Solis - Cohen and Githens,[4] is as readily produced by the drug, and is as disastrous. These authors consider that the fact that phenacetin is less broken down by the body than is acetanilid accounts for its reduced toxicity. Phenacetin is excreted chiefly as phenetidin and less as paramidophenol. The latter substance is given credit for much of the toxicity of acetanilid.

Mahnert[47] has found that, in rabbits and dogs, as much as 1.0 Gm. per Kg. of body weight may be given by mouth with no disturbances other than sleepiness, nausea, and shivering. Death, according to this author, results when a 3.0 Gm. per Kg. dose is given to rabbits by mouth. Wood and Wood[48] have reported that a dose of 0.5 Gm. per Kg. by vein will produce death in dogs.

Holst[49] has reported an interesting case of chronic phenacetin intoxication in which a patient had used phenacetin regularly for

thirty years in daily doses averaging 1.5 Gm. Anemia, cachexia, epistaxis and hemorrhages in the skin were noted as symptoms. This author states that, although chronic phenacetin intoxications are rare, this instance, together with cited cases of poisoning from other headache powders, warns that the use of these medicaments should be controlled.

Brownlee[27] has taken phenacetin as the standard for comparison of coal tar antipyretics and Table IV lists the principal results of his investigations. It is interesting to note that, with phenacetin taken as 100, aspirin, antipyrine, aminopyrine and acetanilid are given relative antipyretic potencies of 74, 100, 134 and 170 respectively. The oral toxic dose of phenacetin for rats is given as 125 mg. per Kg. of body weight, and the equivalent therapeutic dose 40 mg. per Kg., nearly double that for acetanilid. The ratio of toxic to therapeutic dose for phenacetin is thirty as compared to thirty-five for acetanilid. It is therefore evident from Brownlee's work that phenacetin should be nearly as toxic as acetanilid.

Phenacetin has been given orally to male rats for eighty-six weeks (see Tables I and II). The phenacetin rats were litter mates of those in the acetanilid group, both groups being controlled by the same group of litter mates. The period of drug feeding was identical for both drugs.

Although the animals on phenacetin never reached the weight peak gained by the acetanilid animals, other results of drug feeding were similar. There were six animals in the group originally and

four survived the feeding period as compared to five out of six in the acetanilid group. There were no symptoms attributable to drug medication during the first seventy-two weeks of drug feeding. However, at about the beginning of the seventy-second week, at the age of eighty-two weeks, and exactly simultaneously with the acetanilid group, the phenacetin males began to show evidences of toxic effects. Appetites decreased rapidly and the animals began to lose weight. The progress of symptoms was identical to that in the acetanilid animals, the phenacetin animals showing the same listless, hunched tendencies.

Drug feeding was also stopped at the beginning of the seventy-sixth week, and, as in the acetanilid animals, no correction of symptoms was noted. Medication was continued after a five-week abstinence interval, with no observed effect. The animals were still losing ground rapidly when the experiment was terminated at the end of the ninety-sixth week of age.

Three of the six phenacetin males died during the feeding period, two of pneumonia during the twenty-fifth week and one of unknown cause during the thirty-eighth week. The latter animal was examined carefully after death, as were the four rats surviving at the end of the feeding period. No lesions or abnormalities of any sort were detected in the internal organs by macroscopic examination.

It is interesting to note that acetanilid and phenacetin, being very closely related chemically, should have had such identical effects on the living animal during eighty-six weeks of administration. Both drugs were apparently, of benefit to the animal during seventy-two

weeks of drug feeding, toxic symptoms appearing in both groups only during the last fourteen weeks of medication.

Antipyrine.

Antipyrine is one of the most commonly used coal-tar antipyretics, chiefly because of its greater solubility. Its actions are similar to other members of the group, but it has more tendency to cause sweating and skin eruptions than has acetanilid and less tendency to cause cyanosis.[4] Although a normal adult can usually take about two grams without disturbances other than slight sleepiness, chilliness, and sweating, many individuals exhibit toxic phenomena after taking doses which are well within the therapeutic range. The most frequent of such toxic symptoms are eruptions of various sorts, sometimes urticarial with severe itching. Examination shows a weak, rapid heart; rapid, hyperpneic respiration; cyanosis; cold, wet skin, and low temperature. Convulsions frequently result after toxic doses in animals,[4] but are unusual in man.

In rabbits there is no effect from doses below 66 mg. per Kg. of body weight by vein, but with larger doses there is rapid heart, dilatation of the vessels of the skin, and some fall of temperature.[4]

Cats given one gram per kilogram by mouth show salivation and lacrymation, followed in a quarter of an hour by ataxia with increased reflexes, succeeded shortly by decreased reflexes and general weakness so great that the animals fall and are unable to

rise. The pupils are widely dilated, the respiration very rapid and deep, but finally growing weaker and weaker. Death either occurs in a convulsion or from paralysis of respiration.[50]

Ozaki and Mawatari[51] have found that intravenous injection of one-tenth and two-tenths grams of antipyrine produced, after a week of preliminary dilatation, a fairly consistent contraction of kidney vessels. The effect was qualitatively unchanged with the splanchnics cut or not.

Bernheim,[52] in studying the action of aminopyrine and antipyrine upon the oxidation of phospholipid by various tissues, has found that the oxygen uptake of rat tissues is inhibited twenty to thirty per cent by M/4500 solutions of aminopyrine, but that six to ten times this amount of antipyrine is without effect.

Gunn[53] has given 0.9 Gm. per Kg. of body weight as the M.L.D. for mice when injected intraperitoneally. The subcutaneous injection of 1.0 Gm. per Kg. in guinea pigs produced death in from one to one and one-half hours. The symptoms produced by minimal lethal doses described by this author are excitability, quickening of the respiration and tremors. In anesthetized cats the drug caused twitching of groups of muscles. Circulatory effects produced by the drug were similar in cats and frogs. In cats, 10 mg. per Gm. of body weight lowered the blood pressure and sometimes caused a definite, brief depression of heart action.

Brownlee[27] (Table II) has given 1.53 Gm. per Kg. as the oral toxic dose of antipyrine for rats and 1.66 Gm. per Kg. for mice.

It is to be noted from this table that antipyrine has a relatively low antipyretic potency for rats as compared to other members of the coal tar group, but that the ratio of toxic to therapeutic dose is relatively high.

Antipyrine has been given orally to female rats for ninety weeks during the study. The daily dose (Table I) has been well below the toxic dose determined for rats by Brownlee,[27] i.e., 1.53 Gm. per Kg. of body weight. The maximum daily dose administered during this 630 day feeding period was 12.9 mg. per Kg. of body weight, and was calculated on a basis of a three times daily dose schedule for a 70 Kg. adult human. Brownlee has found by actual experimentation that 40 mg. per Kg. is the equivalent therapeutic dose for male rats.

Toxic symptoms have not been observed in the group of female rats receiving antipyrine. There were no observable variations in appearance, appetite, or activity in the cage. During maze learning, however, the animals were appreciably less active than their litter mate controls. This observation will be discussed in a subsequent section.

Six of nine animals in the original group died during the feeding period, as compared to two of nine in the control group. All animals dying during the experiment, as well as the three animals living at the end of the period, were studied macroscopically for possible variations in the condition of internal organs. Litter mate controls were killed and examined and compared with the three surviving test animals when the drug feedings were stopped at the end of the

one-hundredth week of age. Variations attributable to antipyrine medication were not found in any of the test animals.

Abstinence symptoms were not observed in any of the antipyrine females during four and one-half weeks of withdrawal, from the sixty-ninth to about the seventy-fourth week of drug administration. There were six animals in the group during this period.

Aminopyrine.

Aminopyrine has received much attention in the literature during the last ten years and most reports have been concerned with the production by the drug of agranulocytosis and other blood cell disturbances. These reports will be presented with the discussion of the effects of life cycle drug feeding on the blood picture. It might be well to state here, however, that Kracke,[54] in discussing the relation of drug therapy to neutropenic states, has said that approximately eighty per cent of drug-produced agranulocytosis is caused by the administration of aminopyrine or one of its compounds.

Brownlee[27] has compared the antipyretic activity of the coal tar antipyretics, including aminopyrine, and Table IV is taken from his work. From this table it is seen that the equivalent therapeutic dose of aminopyrine for rats is 31 mg. per Kg. of body weight. The single official average dose for an adult human is 4.3 mg. per Kg. Table IV lists the toxic dose for rats as 1.15 Gm. per Kg. of body weight. Aminopyrine, according to Brownlee, ranks second in antipyretic potency when the latter is determined on rats.

Rose[55] has found 150 mg. per Kg. to be the minimal lethal dose for aminopyrine in mice, and 135 mg. per Kg. in rats. The drug was injected into the tail vein. This author defines the M.L.D. as the dose which produces death in at least three out of five animals treated.

Aminopyrine has been given orally to female rats for ninety weeks during this study, the feeding being started at the tenth week of age and stopped at the one-hundredth week. The dose administered per kilogram body weight of rat is given in Table I. The animals received the equivalent of three times the average human adult dose daily during the last 400 days of the feeding period. They were given the equivalent of the average human dose during the first 130 days of feeding, and twice the human dose from age 200 days to 300 days. The number of animals in the group, as well as other dosage data, is given in Table II.

The females receiving aminopyrine exhibited no unusual symptoms that might be attributed to aminopyrine medication, or that would serve to differentiate them from their litter mate controls. The animals had good appetites during the entire feeding period, as did their controls, and there was no observable difference in the activity of the group in the cage. During maze learning, however, a depressed activity was observed, the animals being less active and more sluggish than their controls.

As has been stated, aminopyrine and antipyrine are closely related chemically and should be expected to exert similar toxicities,

if any, under identical experimental conditions. Both drugs have been given orally to female rats (litter mates) in exactly the same dosage and for the same period of time. It is interesting to note that neither drug has produced toxic symptoms in the living animal. Death rates have, however, been markedly different in the two groups, as will be shown in the section on mortality.

The aminopyrine females were without the drug for the same period described for the antipyrine group. Abstinence symptoms were not observed during this four and one-half week withdrawal period.

Cincophen.

Many cases of cincophen poisoning have been reported in the literature during the last fifteen years. Straub[56] reports a fatal case after the daily ingestion of five-tenths gram tablets over a period of three months.[57] A total of 500 tablets were taken. Habs,[58] Peluse,[59] Anon,[60] Fraser,[61] Deutsch,[62] Perman and Goehring,[63] Johnson,[64] Perkel, and many others report deaths after the use of cincophen. Parsons et al.[65] reported in 1932 that thirty-two fatalities due to cincophen had appeared in the literature up to that date, twenty-six of which showed pathologic changes in the liver. One case was added to the list by these authors. Weir and Comfort,[66] in 1933, reviewed the case histories for 117 cases and stated that sixty-one patients had died and that many others were seriously ill.

Palmer and Woodall[67] reviewed the literature on cincophen

toxicity in 1936. They stated that 191 cases of jaundice following the administration of cincophen had been recorded in the preceding decade, eighty-eight or forty-six and three-tenths per cent of which ended fatally. These authors state that there is no safe method for the administration of cincophen.

Beaver[68] has stated that cincophen intoxication affects only the liver, resulting in acute or subacute atrophy, or in toxic cirrhosis if early death does not occur. Comfort[69] has reported four non-fatal cases in which the symptoms were painless jaundice, nausea, loss of weight and strength, clay colored stools and dark urine. All cases showed liver damage, one being very severe. Recovery was effected in from two to eight months by a diet high in carbohydrates and low in proteins.

Short and Bauer[70] have reported four cases of allergic reactions to cincophen in which allergy was evidenced by a generalized urticaria and bronchitis. Fifteen grams of the drug had been administered over a period of nine days.

Evans and Spence[71] stated in 1929 that cincophen had proved of great value in the treatment of gout and that proper dosage would eliminate toxic results. Davis,[72] in 1932, reported on 200 cases that had taken cincophen and neocincophen with no fatalities resulting. Thirty of the two hundred had slight digestive or circulatory upsets.

There are many reports in the literature concerning the experimental production of peptic ulcers in laboratory animals by the administration of cincophen. Stalker et al.[73] have produced ulcers

in ninety-five and eight-tenths per cent of their test animals by oral administration of large doses of the drug. Cincophen, administered by rectum, parentally, through intestinal fistulas, or orally, in dogs with fundic pouches, produced peptic ulcers in many instances, and these results were taksn as proof that ulceration occurred after the absorption of the drug. Except for the mild pathologic changes which accompany the toxemia produced in the first few days, no changes, either gross or microscopic, which could be attributed to cincophen, were seen by these authors in the liver, heart, spleen, lungs, pancreas, kidneys, gall-bladder or adrenal glands.

Churchill and Manshardt[74] have injected cincophen dissolved in olive oil directly into the jejunum of dogs. One dog received twenty-two 220 mg. per Kg. doses during as many days, resulting in a consistently bloody stool, and a large chronic ulcer in the pyloric region of the stomach. There was no ulceration at the point of injection. These authors also conclude that ulcer production is not due to a local toxic action on the gastric mucosa. A second dog died after the administration of ten doses, a large prepyloric ulcer being found two and one-half feet from the point of injection.

Schwartz and Simonds[75] have given cincophen orally in cotton-seed oil to cats, rabbits and guinea pigs, in doses calculated on a basis of 22 mg. per Kg. of body weight, which corresponds to a human dose of seven and five-tenths grains three times daily for a 150 pound adult human. Cats survived daily doses ten times the normal human dose for from two to five days. One cat survived the equivalent of

the normal human dose for sixty-two days. Four of six cats developed gastric ulcers, one of which perforated. None of the rabbits died from the effects of the drug and one rabbit survived sixty-six doses, each twenty-five times the normal human dose, without apparent injury. No ulcers developed in the rabbits. Guinea pigs survived daily doses ten times the normal human dose for two weeks or more, and one animal received ninety doses, each dose two and one-half times the average human dose. None of the guinea pigs developed gastric ulcers.

The maximum dosage used in the investigation being presented in this thesis was 14.2 mg. per Kg., or the equivalent of twice the average human dose. It will be observed in Table III that the feeding was continued for eighty-six weeks.

Churchill and Van Wagoner[76] have also calculated the average daily human dose on a basis of 32 mg. per Kg. of body weight, but have given dogs twenty-seven times this amount, or 595 mg. per Kg., mixed with food. The dogs refused to eat after eight or ten doses. Gastric ulcers were found in every case as well as yellowish areas over the liver.

Radroin and Lederer[77] have given 0.5 Gm. to 1.0 Gm. per Kg. of body weight as the lethal dose for cincophen for white rats. They found occasional abscesses in the lungs after cincophen medication, but no gross lesions in any of the organs after even 180 days of therapeutic dose administration. Most of the animals appeared ill, many lost weight and none gained as rapidly as the controls.

Reichle[78] has found that the subcutaneous administration of

1.0 Gm. per Kg. doses of cinchophen killed rats in twenty-four hours. Continued parenteral administration of smaller doses, 0.2 Gm. per Kg., did not cause death, but there was a period of excitement followed by collapse immediately following the injection. Respirations became deep and panting. Toxic cirrhosis was not discovered, but the author states that there was possibly some damage.

Myers and Goodman[79] have taken 30 mg. per Kg. of body weight as the average daily dose for man, and have given one dog the equivalent of that amount, two dogs twice that equivalent amount and a fourth dog five times that amount. Four rabbits were given ten times the equivalent of the average human dose. The drug was mixed with the food and administration was continued for seventeen days to dogs and for forty-five days to rabbits. Liver damage was produced in many instances, but the author made no statements concerning the general welfare of the animals.

Quick[80] has stated that toxicity determinations on lower animals are not an index of the toxicity for man as the conjugating mechanisms in the human organism respond quite differently in many instances.

Lehman and Hanzlik[81] have included cinchophen in the diet of white rats to the extent of five-tenths, one, and two and one-half per cent. The animals receiving the two and five-tenths per cent mixture refused to eat the food and died of starvation. The diet of the others was continued for twenty weeks. No injury resulted from the treatment or from similar medication with neocinchophen. Rabbits were given

doses of 0.2 to 0.6 Gm. per Kg. per week for thirteen weeks with no untoward results other than a decrease in the functional activity of the liver in two out of twelve cases. The authors concluded that there was no adequate experimental basis for establishing a direct cause and effect relationship in clinical cincophen toxicosis.

After considering the many reports of cincophen poisoning appearing in the literature during the past decade, there seems no room for doubt concerning the toxicity of the drug. Mention should be made of the fact, however, that in many of the experiments presented above, the dosage has been extremely high. The desire on the part of investigators seems to have been to give doses large enough to insure the production of pathologic disturbances or lesions, rather than to determine the effect of doses approaching what might be considered within the therapeutic range.

Cincophen has been given orally to male rats from the tenth to the ninety-eighth week of age during this investigation. The dose for the first 200 days of feeding was 7.1 mg. per Kg. of body weight (Table I and Table III). During the remaining 420 days of feeding the dose was held at 14.2 mg. per Kg. of body weight. The single human dose of 0.5 Gm. is equivalent to 7.1 mg. per Kg. for a 150 pound adult.

There was no evidences of cincophen toxicity during the first sixty-five weeks of feeding. Appetite, coat condition, activity and general behavior compared favorably with that of litter mate controls. Blood was absent from the stool during the entire feeding

period and the fecal material resembled that of the controls in color and consistency.

At about the sixty-fifth week of feeding, at seventy-five weeks of age, the animals began to show evidences of cincophen medication. The fur became coarse and rough and the animals gradually became less active, using the turntable exercisers very little during the last ten or fifteen weeks of the experiment. The desire for food, as compared to controls, lessened progressively after the seventieth week of feeding and the animals practically refused to eat during the last ten weeks of the 620-day feeding period. Three of the six animals survived the experiment. All of the animals were examined carefully after death, the internal organs being compared with those of litter mate controls. No significant variations were observed. There were no signs of lesions, healed or otherwise, in the stomach or intestines of any of the cincophen animals. What might be described as yellow spots were observed on the livers of two of the cincophen rats, but similar spots were found on the livers of litter mate controls and are therefore thought to have no significance. Similar spots were also observed on the livers of rats in other groups in the colony.

The possibility of abstinence symptoms was determined by withdrawal of the drug from the cincophen group for five weeks following the seventy-sixth week of feeding, at the beginning of the seventy-seventh week of age. Toxic symptoms had been noted for ten weeks before this, and the animals had been losing weight rather rapidly. No withdrawal effects were observed during the five week period without drug.

The toxic symptoms were not lessened and the animals continued to lose weight. Neither was a change in symptoms observed when the drug was returned to the diet after the withdrawal period. This lack of improvement during the five-week withdrawal period is taken as evidence that whatever damage had been done by the drug was irreparable.

Phenolphthalein.

Toxic reactions resulting from the use of phenolphthalein have been reported frequently in the literature of recent years. Ayers,[82] in 1921, reported on seven cases in which an eruption of the skin was associated with the oral administration of phenolphthalein as a laxative. Four of the seven cases were of the same type and corresponded clinically with erythema perstans.

Ely,[83] in 1932, described symptoms of swollen eyelids and lips and a red eruption occurring after a girl, aged two years and eight months, had received five tablets containing 120 mg. of phenolphthalein in each. Newman[84] has reported on nineteen cases of cutaneous eruptions due to phenolphthalein in which intense itching and burning were prevailing symptoms. Phillips[85] and Weiss and Kile[86] report similar cases.

Abramowitz[87] has stated, after a survey of the literature on reactions to phenolphthalein, that the number of reactions observed were small considering the extensive use of the drug. This author concludes that the nine reported cases of systemic disturbances resulting from overdosage were somewhat offset by eleven reported instances

of no apparent ill effects from overdosage. He assumes that there is a form of specific hypersensitiveness, allergic in nature, in patients who have eruptions after the use of phenolphthalein.

Sachs[88] has reported that the ingestion of a total of ninety-six grains of phenolphthalein by a three and one-half year old boy produced no ill effects aside from diarrhea and slightly increased temperature. Sachs concludes that this case lends support to the theory that phenolphthalein does not produce harmful disturbances in man.

Fantus and Dyniewicz[89] have examined one thousand samples of urine after the use of phenolphthalein by patients and report that medicinal doses of the drug do not produce albuminuria. They state further that phenolphthalein is not generally present in the urine after medicinal doses, but that a conjugated phenolphthalein is always present. The amount of free phenolphthalein found in the urine increases with the dose.

This author has been unable to find any statements of minimal lethal dose for phenolphthalein, for any animal in the literature. Wood[90] has found that doses equivalent to sixty to one hundred grains for human beings were quite harmless to dogs. Abel and Rowntree[91] have administered large doses of phenolphthalein (0.415 Gm. to 1.0 Gm.) orally, subcutaneously, and intravenously, to a dog over a three-month period without harmful effect.

Phenolphthalein seems to have had a toxic effect on male rats given the drug orally during this drug-feeding experiment. There

were four rats in the group originally and only one of the animals survived the eighty-six week feeding period. The dosage schedule for the drug is given in Table I and other pertinent dosage data in Table III. The maximum daily dose is seen to have been 3 mg. per Kg., three times the average single human dose. One rat died during the fourteenth week of feeding while on a 1.0 mg. per Kg. schedule. The second death occurred after 322 days of feeding, the animal having been on the 2 mg. per Kg. schedule for only twenty-two days. The third death occurred after 497 days of feeding and after 197 days on the 3 mg. per Kg. schedule. Therefore, for the greater part of the feeding period, three of the group of four received the equivalent of 2 mg. of the drug per Kg. of body weight. Only one animal received 3 mg. per Kg. for the major part of the experiment.

Sluggishness and a decreased desire for food were observed in the phenolphthalein group as early as the tenth week of drug feeding, the animals failing to gain weight as rapidly as did their controls. Phenolphthalein, or some substance giving a red color in alkaline solution, was found in the feces of the animals when the test was made after five weeks of feeding, while the animals were receiving only 1.0 mg. per Kg. of drug daily. Positive tests were obtained at irregular intervals thereafter. Similar tests performed on the feces of controls were always negative.

There was never any evidence of a laxative effect as a result of phenolphthalein administration. The fecal material was normal in color and consistency throughout the period of feeding.

A peculiar hypersensitivity to touch stimuli was observed in the phenolphthalein animals as early as the twentieth week of drug feeding. It was first evidenced when the animals became difficult to catch. The normal, tamed rat can be picked up without difficulty if the animal is aware that the hand is approaching. It merely assumes a slightly crouched position and offers little or no resistance. The phenolphthalein animals, however, seemed to object to being touched, and while they would assume the crouched position normally, they would frequently jump at the first touch of the hand. This apparent hypersensitivity became more marked as the experiment progressed, it being very difficult to catch the one animal that survived the entire feeding period.

This peculiarity was observed as a result of other stimuli such as being touched by other animals in the group or by lettuce or other food thrown into the cage. This excessive response to touch stimuli was not observed in any other group in the colony.

That this hypersensitivity was a result of phenolphthalein medication would seem to have been proved by the fact that symptoms were alleviated markedly by a five-week abstinence period which followed seventy weeks of medication. There was, however, only one animal left during this period. The drug was returned to the diet at the end of the seventy-fifth week of the feeding period, the symptoms gradually returning, but never becoming as marked as they were before the withdrawal period. The experiment was stopped at the end of the ninety-sixth week of age.

The internal organs of the three rats that died during the feeding period, as well as those of the one animal surviving the experiment, revealed no abnormalities under careful macroscopic examination.

A description of the toxic effects produced by the administration of phenolphthalein to white rats during this investigation has not been found in the literature.

The "All" Group.

As has been explained, this group of eight male rats received nine drugs, each in exactly the same dosage given to the groups receiving only one of the drugs. These nine drugs are underlined in Table I as are also the doses used for each during the three periods. Attention is called to the fact that these animals received as the maximum dose, for the last 402 days of the experiment, 128.9 mg. of drug per Kg. of body weight daily. If we are to consider the statements of Greenman and Duhring and others,[1 2 3] each rat received the equivalent of 315,600 adult human drug doses over a period equal to 2,580 weeks for human beings (Table III).

The lack of effect of this extended period of medication with a combination of diuretic, laxative, respiratory and cardiac-stimulating, antipyretic, analgesic and hypnotic drugs seems nothing short of amazing. While the group revealed some evidence of depressed activity on the maze, this effect of drug medication was not observed to any extent in the cage. Appetites were normal, as compared to the

controls, throughout the entire feeding period. The weight-depressing effect of caffeine seems to have been overcome by the stimulating effect of acetanilid, for the animals of the control group weighed less than those of the "All" Group during most of the experiment. There was no laxative effect as observed in the male aspirin group nor was there any of the noisy breathing described for the male and female aspirin groups.

Although the "All" Group received the same daily dose of phenolphthalein given to the phenolphthalein group, and for the same period, none of the peculiar hypersensitivity to touch stimuli observed in and described for the latter group were evidenced in the "All" Group. All of the animals in the last six groups listed in Table III, including the controls, were litter mates. The phenolphthalein, phenacetin, cincophen, acetanilid and "All" Group served as controls for each other as well as being controlled by litter mates. From Table I it is seen that the "All" Group received all four of the drugs mentioned above as well as caffeine, aspirin, aminopyrine, antipyrine and barbital.

A number of explanations could be given for the lack of toxic effects of this "shotgun" medic ation in the "All" Group. Downs and Eddy[92] have stated that barbital appears to protect the white rat to some extent against the lethal effect of cocaine, but that this protection is distinctly less than in other animals. Gilman and Barbour[93] have shown that, when phenobarbital is given orally to white rats, excitement, followed by hypnosis, is observed. The effects are

very marked with 50 mg. per Kg. doses. However, when 50 mg. per Kg. of aspirin are given with the barbiturate, neither excitement nor depression are produced, indicating that aspirin is antagonistic to phenobarbital. These authors have also shown that antipyrine antagonizes the toxic action of phenobarbital without diminishing its hypnotic effect. Rose[55] has shown, however, that the toxicity of aminopyrine was reduced approximately two-thirds when an effective dose of sodium amytal was given at the same time. It is conceivable, therefore, that the toxic effects of the antipyretics might have been antagonized by the presence of barbital in the drug mixture, or, according to Rose, that the reverse be true.

Modifications of solubility and absorbability of the constituent drugs in the mixture are undoubtedly factors involved in an explanation of the absence of toxic effects in the "All" Group. Many of the drugs in the mixture contain the benzene ring in the molecule and it is a well known fact that the body protects itself from such toxic substances by the formation of hippuric acid. In this connection, Astolfani,[94] has shown that caffeine increases the amount of benzoate that can be synthesized into hippuric acid. That there are optimum conditions for drug effect has been shown by Sleigmann et al.[95] who have found that bile is necessary for the activity of phenolphthalein in cats in which obstructive jaundice was produced experimentally.

Regardless of rhyme or reason, the administration of the nine drugs simultaneously to the "All" Group has not produced any of the toxic symptoms produced by the same drugs when given individually,

aside from a mild form of depressed activity made evident during maze learning.

Barbiturates (Literature References)

Reports of pathological disturbances and deaths resulting from the use of barbituric acid derivates are numerous in the literature of recent years. Lynch[96] reports that deaths in humans have been caused by a single 15 grain dose of barbital and of phenobarbital. He states that the average fatal doses are larger. Rylander[97] places the smallest lethal dose of barbital at 11 grains and the average fatal dose at 50 grains. Ravine[98] reports a case of barbital poisoning in which the patient had taken daily doses of 3 to 6 grains of the drug over a period of four to five years. The symptoms were tremor, weakness, restlessness and mental dullness, all of which disappeared after removal of the drug.

Birch[99] has reported toxic symptoms appearing in a case after one grain of phenobarbital had been given daily for 22 days. Holland et al.[100] report a dermatitis produced by phenobarbital after only three and one-half grain doses of the drug. Babington[101] states that the average daily dose for phenobarbital is 0.5 to 1.5 grains. He reports even the smallest doses as causing headache, nausea, and a rash resembling that of measles or scarlet fever.

Lagenbach[102] however, reports that two male and two female adults have taken an average of 3 grains of sodium amytal one to seven times weekly for from nine to twenty-four months with no untoward

reactions whatever. After intervals of six to thirteen months without the drug, all showed an acquired hypersensitivity to the compound, characterized by cutaneous eruptions on the face, neck, arms, hands, and mucous membranes of the lips and mouth. Free barbituric acid produced a similar result but phenobarbital failed to do so.

Larkum[103] has reported a case of probable allonal poisoning. A woman was found in a coma and died 6 days later from bronchopneumonia. Three empty bottles labeled allonal were found but no exact estimate of the dose, except a minimum of 60 grains, could be given. Loveman[104] reports a case of toxic reaction due to alurate, the barbituric acid derivative of allonal. The patient had been taking tablets of allonal for headache during the preceding 4 years. Experimental trial with other barbiturates, aminopyrine, phenolphthalein and other drugs failed to produce the characteristic reaction.

There are also many reports on the relative harmlessness of some of the barbiturates. Hoge[105] reports on a case, a woman, age 41 years, who had taken three or four 1.5 grain amytal tablets daily for over a period of 7 years with little or no harm. Lundy and Dixon[106] report a case as having taken a total of 600 Gms. of sodium amytal in 0.2 Gm. (3 grain) hourly doses over a period of four months with no harmful effects.

Bleckwenn[107] mentioned a case in which 250 daily doses of 1 to 1.4 Gm. of sodium amytal were injected intravenously with no pathological changes being noted and no habit formation suspected.

Emge and Hoffman[108] state that sodium amytal given during and after labor, 5 to 7.5 grains intravenously, produced no changes in sulfophenolphthalein excretion. They contend that sodium amytal does not constitute a menace to the patient whose kidney function is otherwise normal. Zerfas[109] states that there is considerable individual variation in patients with regard to susceptibility to sodium amytal. He considers, however, that 3 to 9 grains, not exceeding 15 grains, are comparatively safe limits for pre-anesthetic preparation.

Weiss,[110] in discussing the whole group of barbiturates, stated that if the scientific definition for the term were adhered to, no habit formation could be attributed to them.

Much work has been done in an attempt to establish some relationship between pharmacological action and chemical structure of the barbituric acid derivatives. Swanson[111] has studied over 50 barbiturates all of which were 5-5 substituted compounds similar in general formula to barbital. None of the barbituric acid derivatives used in these experiments were included in Swanson's group of 50. However, some very important facts concerning structure and pharmacological action were pointed out by the author. It was shown, for instance, that an increase in the number of carbon atoms in the alkyl group, either normal or secondary, resulted in a decrease in both the minimal anesthetic dose (M.A.D.) and in the minimal lethal dose (M.L.D.). Swanson[111] found, also, that when the alkyl radical is longer than 5 carbon atoms, the amount required to anesthetize or kill

rats again increases. The duration of action shows similar features according to this author; that is, it is shorter when the alkyl group becomes lengthened.

If the substituted group is phenyl, as in phenobarbital, the well known prolonged action results, but if the ethyl group is replaced by a methyl group the resulting substance, methyl ethyl barbituric acid, has a larger M.A.D. and M.L.D. and a shorter duration of action as compared with phenobarbital.

Fitch and Tatum[112] have obtained results similar to Swanson but these authors have reported a marked difference in the duration of hypnosis in relation to the mode of administration of the drug. For instance, the average duration of action of 60% of the M.L.D. (kills 50%) in rabbits orally was found to be 13 minutes and 36 seconds for alurate, 16 minutes and 30 seconds for barbital, 22 minutes and 40 seconds for amytal, and 36 minutes for phenobarbital. However, when the same size doses were administered intraperitoneally, the durations were, for amytal 3 minutes and 54 seconds, for alurate 6 minutes, for barbital 18 minutes and 10 seconds, and 22 minutes and 10 seconds for phenobarbital.

It is seen from the structural formulae on page 9 that the substituted alkyl group contains four carbon atoms in the alurate, two in barbital and five in amytal. The phenol group is substituted for ethyl in phenobarbital and it is the longest acting of all barbiturates fed during these experiments.

Fitch and Tatum[112] found the following toxicities in rabbits

orally -- phenobarbital 150 mg. per Kg. of body weight, alurate 160 mg. per Kg., barbital 275 mg. per Kg., and amytal 575 mg. per Kg.

In albino rats intraperitoneally they found the following toxicities -- alurate 100 mg. per Kg., amytal 115 mg. per Kg., phenobarbital 155 mg. per Kg. and barbital 300 mg. per Kg. of body weight.

All four of these barbiturates were used in these life cycle feedings but in doses which were only a very small fraction of these reported toxic doses, namely, alurate 0.088, amytal 0.0746, phenobarbital 0.0166 and barbital 0.0617, respectively, times the reported toxic dose. It is to be noted further that the intraperitoneal toxic doses of Fitch and Tatum required eleven and six-tenths days of feeding in our experiments for alurate, thirteen and four-tenths days for amytal, sixty days for phenobarbital and sixteen days for barbital.

Nielson, Higgins and Spruth [113] found the minimum fatal dose for barbital when given orally to cats in milk to be 0.4 Gm. per Kg. The same authors determined the toxicity, efficiency, and safety margin subcutaneously in albino rats for barbital, amytal, phenobarbital and alurate. The following table is taken from their work.

	Min. F.D. Gm. per Kg.	Ratio of Tox. Barb.= 1	Min. Effect Dose Gm. per Kg.	Ratio of Efficiency Barb.= 1	Safety % Margin
Barbital	0.310	1	0.225	1	27
Amytal	0.140	2 1/5	0.0575	3 9/10	59
Phenobarb.	0.140	2 1/5	0.11	2	21
Alurate	0.125	2 1/2	0.0525	4 1/4	58

The authors define the safety margin as the difference between the minimal effective dose and the minimal fatal dose, expressed

in per cent of the minimal fatal dose. They compare these four barbiturates as follows --

In order of increasing toxicity -- barbital, amytal, phenobarb., alurate
" " " " efficiency -- barbital, phenobarb., amytal, alurate
" " " " safety margin - phenobarb., barbital, alurate, amytal.

Gruhizt et al.[114] have administered sodium barbital to white rats and report their results as follows, in mg. per Kg. body weight --

	M.L.D.	M.T.D.	M.A.D.	M.S.D.
Intraven.	300	280	125	170
Intraperiton.	350	325	180	190
Orally	400	325	180	190

The terms used in the table are defined by the authors as follows -- M.L.D. produces death in 60% of the animals; M.Tolerated D. allows for survival in 60%; M.Anesthetic D. causes brief prostration; M.S.D. is the minimal surgical anesthetic dose. It is seen from this table that dosages do not vary markedly with different modes of administration.

Gower and Tatum[115] have reported that the majority of dogs treated recovered from an intravenous injection of 200 to 250 mg. per Kg. body weight of sodium barbital while a dose of 400 mg. per Kg. was fatal in one to five days in about 70% of the cases. The authors stated that it was apparent that the tolerant animal was one with a high urinary excretion rate, that the intolerant animal had a low rate of elimination.

Swanson and Shonle[116] have found the average oral M.L.D. of

sodium amytal for dogs to be 125 mg. per Kg. body weight when administered in capsules. The average M.L.D. of a 10% solution rectally was 200 mg. per Kg., and 70 to 75 mg. per Kg. intravenously. These authors treated a series of 350 dogs with 1 c.c. of 10% solution per minute intravenously without a single death and consider this the safest mode of administration.

Garry[117] states that, in laboratory animals, oral administration of amytal is unsatisfactory and that intravenous injections must be carried out slowly for fear of circulatory disturbances. Subcutaneous or intraperitoneal routes of administration are the best according to this author. The basic dose for complete surgical anesthesia in the cat and dog is given as 50 mg. per Kg. while 20 to 25 mg. per Kg. is said to be adequate in man. Garry reports that amytal lowers the body temperature but does not greatly diminish the minute volume of respiration. The drug has a definite toxic action on the heart, being more marked the more rapid the administration. It causes a definite decrease in intestinal motility but appears to have no detrimental effect upon the kidney. It does, however, inhibit water diuresis.

Kugel[118] has found that barbital, in doses of 0.12 Gm. per Kg., produces a deep sleep to light narcosis, an increased water diuresis with diminished sodium chloride diuresis results from such dosage. Dogs were used in his experiments.

Marx[119] reports that amytal has a strong antidiuretic effect on dogs, the effect being directly proportional to the amount of amytal

administered. The diuretic effect of urea is prevented in amytal anesthesia. This, according to this author, is likely due to a combination of three factors, (1) an impairment of renal function since there is a drop in phenolsulfonthalein excretion. There were, however, no evidence of renal lesions following the administration of the drug and neither casts or albumin were found in the urine. (2) The drug may have an effect on the cerebrospinal centers regulating water metabolism. (3) Amytal may influence the permeability of the endothelium of the capillaries in the kidney. A generalized edema was noticed in one dog.

Taylor and Lackey[120] found, in a series of 232 rats by subcutaneous injection of 4% solutions of sodium and magnesium phenobarbital, that the M.L.D. for both salts was approximately 215 mg. per Kg.

Foster[121] reports 265 mg. per Kg. of sodium phenobarbital as the average or median lethal dose for male mice. His figure for sodium alurate is 237 mg. per Kg. body weight. He defines the standard safety margin as the zone between the surely effective dose (E.D.99), i.e., effective in 99% of cases, and the lowest lethal dose (L.D.1), i.e., killing 1% of animals. This figure for sodium phenobarbital is 34% and for sodium alurate 105%. Alurate is said by this author to possess about twice the safety of phenobarbital.

Louvier[122] has found the minimal lethal dose of sodium phenobarbital when given intramuscularly to rabbits, to be 150 mg. per Kg. body weight. He states further that the drug attacks the respiratory

centers and produces a progressive decrease in body temperature.

Gilman and Barbour[123] state that oral doses of phenobarbital of 25 mg. per Kg. body weight in cats produced excitement followed by slight hypnosis. Doses of 50 mg. per Kg. gave more marked effects, depression lasting for several hours. They found aspirin to be antagonistic to the hypnotic effect of phenobarbital while phenacetin was not. Phenacetin did, however, antagonize the toxic effects of phenobarbital.

That there is a rather marked difference in the amounts of different barbiturates excreted by the kidneys has been shown by a number of investigators. Shonle et al.[124] report that amytal and pentobarbital are excreted in the urine of men and dogs only in traces, if at all, following the administration of their sodium salts. Under the same conditions, barbital and sodium barbital are excreted as such in the urine. The author states that, since both animals and men recover more rapidly from the effects of amytal and pentobarbital than from barbital, the body must necessarily destroy them rather rapidly and completely.

Koppanyi and Drop[125] state that neonal and amytal are not completely destroyed in the body but are excreted in the urine to the extent of about 8% of the administered dose. Koppanyi et al.[126] state that normal dogs, cats and humans excrete from 42 to 89% of barbital during 7 days following administration, from 13 to 16% of phenobarbital and 40% of neonal.

Much work has been done in an attempt to determine the

possibility of the development of tolerance to barbiturates.

Eddy[127] has reported that no tolerance to barbital developed in cats after long continued daily administration by stomach tube of hypnotic doses. After 6 weeks the drug produced just as much depression as when administered the first time. However, with repeated administration of any dose of barbital, a cumulative effect developed, being more marked the larger the dose. This effect disappeared as the dose was continued and the author suggested this as being due to the excretion gaining on absorption as is known to occur with bromides.

Fitch,[128] however, has reported that rabbits developed a high degree of tolerance to amytal, neonal, and noctal. Amytal, in doses of 550 mg. per Kg., killed two of four new rabbits while the same dose killed none of three addicts.

Stanton[129] has found that rats show no increase in abstinence irritability to sodium phenobarbital after daily injections of 5 and 15 per cent of the minimal fatal dose over a seven week period. Rats, according to this author, do not become addicted to sodium phenobarbital but tend to show evidences of some cumulation of depressive effect.

Swanson et al.[130] have given approximately one-third of the minimal lethal dose of sodium amytal, 40 mg. per Kg., orally to dogs in capsules three times a week for from two to four months and have noticed no egidence of tolerance, no withdrawal symptoms or decrease in toxicity. Similar results were obtained after intravenous injections in monkeys of an anesthetic dose, 35 to 40 mg. per Kg., three times a

week for 6 months. Each dose was followed by the characteristic hypnotic effects and the authors concluded that these results could be interpreted as proof against the possibility of habit formation resulting from prolonged use of amytal.[131] However, Oettel et al. have given barbital and phenobarbital to dogs in daily doses of 100 mg. per Kg. and 75 mg. per Kg. respectively, for a month. Habituation accompanied by increased excretion of the drugs is reported but there were no abstinence symptoms on withdrawal.

Sex variations have been reported in the literature. Holck and Kanan,[132] judging from sleep and mortality rate, have found that female white rats are more sensitive than males to amytal, nembutal, evipan, pernocton and hebral. White rats showed no sex difference in sensitivity to barbital or phenobarbital.

Nicholas and Barron[133] state that the female dosage for amytal in white rats is just one-half the male dosage. Immature rats require the lower dosage. Further, more concentrated solutions produce more uniform results, with the actual use of less amytal. They state that in the rat, the only contraindication so far found is a chronic respiratory infection. Death in case of overdosage is usually due to respiratory failure.

Moir[134] has found that very young female rats are more resistant than corresponding males to pentobarbital, but that mature females were less resistant than mature males.

The possibility of temperature and seasonal variations in the effects of barbiturates has been considered by various workers.

de Beer et al.[135] report the absence of significant seasonal variations in the toxicity of the sodium salt of ethyl n-hexyl barbituric acid. They state further that no detectable error in the determination of hypnotic potency is introduced into the experiment by making dosage proportional to body weight. They did, however,[136] find significant differences in the minimal hypnotic and minimal lethal doses in mice on two widely different diets, there being a marked difference in the duration of anesthesia.

Raventos[137] studied the influence of room temperature changes upon the action of sodium phenobarbital. Using male mice, he found that the median hypnotic dose in mg. per Kg. body weight was 105 at 30°C. and 90 at 20°C. He gives 234 mg. per Kg. and 162 mg. per Kg. as the median lethal dose at these respective temperatures. This is a decrease of 30% in the median lethal dose between 30°C. and 20°C. It would therefore seem necessary that the room temperature be rather carefully controlled during such experiments. (Writer's comment)

That diet is also a factor in the action of sodium phenobarbital has been shown by Nedzel[138] who has found that many rabbits on an oat diet would undergo a prolonged general narcosis with a given dose of the drug while only a few were found to do so on a diet of carrots. Complete narcosis was produced more quickly on a mixed diet than on either carrots or oats alone, but the effect faded comparatively soon.

Hirschfelder and Rice[139] have found that fear and excitement definitely diminish the soporific action of sodium barbital in white rats.

Holck and Cannon[140] have administered nostal to rats in doses sufficient to produce acute depression within a few hours from which the animals apparently recovered. However, most of them died later, usually on the second or third day, from pulmonary edema and fatty degenerative changes in the kidneys, heart and lungs. Such delayed deaths occurred occasionally even from subhypnotic doses. No such delayed deaths were observed after similar administration of amytal, barbital, phenobarbital and six other barbiturates.

Dille et al.,[141] in studying factors governing the distribution of barbiturates in the various tissues, found that the rate at which organs take up barbital is relatively faster after small than large doses. Fabre[142] fed barbital to a dog in daily doses of a gram for 10 days, and found 9.69 mg., 11.25 mg., 2.4 mg., 4.8 mg., 4.4 mg. and 5.79 mg. of the drug respectively in 100 Gms. of suprarenals, thyroid, testicles, pancreas, liver and brain. The amount in the suprarenals and thyroid was proportionately much greater than that in the liver and brain.

Olmsted and Ogden[143] have found that amytal, in doses as small as 18 mg. per Kg. in a heart-lung preparation, increased the diastolic volume, the venous pressure remaining constant. With 27 mg. per Kg., a greater dilation occurred with a diminution of useful outflow.

Schulte[144] has shown that pentobarbital and amytal injections given twice weekly to dogs for 205 days failed to produce specific skin lesions or damage to the liver, kidney, spleen, or heart muscle.

Barbiturates (Experimental)

General Welfare and Behavior

Four barbituric acid derivatives and one mixture of barbiturate and aminopyrine were given orally to white rats during this investigation. The dosage schedule for these drugs is given in Table I. The number of rats in each group, the feeding period in weeks and other dosage data are presented in Tables II and III.

Barbital and amytal (sodium salts) were administered to litter mate male groups, both groups being controlled by male rats from the same litters. Allonal and sodium alurate were fed to female groups which were litter mates, also controlled by the same group of litter mates. All animals comprising the groups described in this paragraph, both males and females, were from the same litters.

Sodium phenobarbital was fed to both males and females from identical litters, the controls being from the same litters.

Dosage data for the test groups described above may be summarized as follows --

Barbital, daily dosage range from 7.1 to 14.2 mg. per Kg. body weight over a period of ninety weeks, the average literature M.L.D. being 355 mg. per Kg.

Amytal, daily dosage range from 2.0 to 6.0 mg. per Kg. over a period of ninety weeks, the average literature M.L.D. being 137 mg. per Kg.

Phenobarbital, daily dosage range from 0.9 to 2.7 mg. per Kg. over a period of 100 weeks, the average literature M.L.D. being 178 mg. per Kg.

Alurate, daily dosage range from 2.0 to 6.0 mg. per Kg. body weight over a period of ninety weeks, the average literature M.L.D. being 107 mg. per Kg.

Allonal, daily dosage range from 1.7 to 5.1 mg. per Kg. of alurate and from 3.0 to 9.0 mg. per Kg. of aminopyrine over a period of ninety weeks. M.L.D. not reported in the literature.

Of the six groups of rats that were given barbituric acid derivatives, only the females on sodium phenobarbital evidenced any marked variations in appetite and they were observed to have a depressed desire for food during most of the feeding period. This group never reached the weight peak gained by their controls but were consistently below them in weight during the entire experiment. The males on sodium phenobarbital had appetites comparable to those of the controls during the first forty-six weeks of drug feeding but a depressed desire for food was observed during the last fifty-four weeks.

Reduced activity was observed in the amytal and barbital groups as early as the twenty-fifth week of drug feeding. This decrease in activity was very marked in the barbital group, the animals using the turntable exercisers much less than did their controls. As will be shown later, most of the barbital animals refused to run on the maze. The animals in the phenobarbital, alurate and allonal groups did not show any signs of reduced activity in the cage although the phenobarbital groups, both male and female, were considerably more sluggish on the maze than were their litter mate controls.

Twenty-seven of sixty animals originally present in the

barbiturate groups survived the feeding experiments. These rats, together with twenty-six from the control groups, were killed when the drug feedings were stopped at the end of the feeding periods. The internal organs of the test animals were subjected to careful macroscopic examination and were compared with those of the controls. No abnormalities attributable to barbiturate medication were found. The lungs of all test animals were examined with particular care and seemed to compare favorably with those of the controls. These post-mortem examinations revealed no evidences of barbiturate toxicity.

Mortality and Causes of Death

A death rate comparison for all groups of rats included in this investigation is presented in Table V. The death rate in the entire colony seems to have been rather high during the experiment. All groups in the colony totalled 203 rats at the beginning of the feeding period and there were ninety-one deaths during the experimental period. In terms of per cent, 44.8 per cent of the control and test animals died during the investigation and 55.2 per cent survived. Deaths in terms of mortality rate have been 448 per 1000.

Drugs were administered to 138 rats in seventeen groups and seventy-one of these animals died during the feeding period. The test animals were controlled by sixty-five rats and twenty of the latter died during the same period. On a basis of per cent comparison, 51.4 per cent of the test animals died as compared to 30.8 per cent of the control animals. It seems evident, therefore, that the drugs

administered during the experiment have exercised, as a group, a considerable degree of toxicity, judging, at least, from a comparison of death rates for the control and test animals.

As is seen in Table V, the highest death rate for all groups in the colony was experienced by the barbital males, only three of thirteen surviving the medication. The phenolphthalein animals were next with three deaths in a group of four. The acetanilid group suffered the least deaths of all test groups in the colony with only one death in a group of five.

The high death rate of four of eight females controlling the aspirin and phenobarbital female groups is accounted for by the fact that one of the deaths occurred after the removal of a growth, and a second as a result of hemorrhage after the end of the tail had been clipped for the purpose of obtaining blood for counting. When these two deaths are excluded, the actual death rate in the aspirin-phenobarbital female control group is two of eight or 25 per cent.

Two of the aspirin females died after the removal of growths not attributable to drug medication, since, as stated above, a similar death occurred in the control group.

Mention should also be made of the fact that two of the three deaths occurring in the aminopyrine group were due to bleeding after clipping the tail.

When these considerations are taken into account, there seems to have been a significant increase in the per cent of deaths in the following test groups, as compared to their controls -- barbital,

phenolphthalein, phenobarbital males, antipyrine, aspirin males, amytal, cincophen, phenacetin and alurate.

Significant variations in the death rate did not occur in the following test groups -- caffeine females, phenobarbital females, aminopyrine, allonal, "All" Group and acetanilid. Although the aspirin female group and caffeine male group each experienced higher death rates than did their controls, the variations are not sufficiently large to be considered significant. However, the fact that both female and male groups on aspirin had higher death rates than did their respective controls does appear to be significant.

Only two diseases were observed in the colony during the investigation, i.e., rat pneumonia and middle-ear disease. Both diseases are described by Greenman and Duhring.[2] Two males and three females developed the middle-ear disease during the experiment. One male was in the cincophen group and the other was in the "All" Group control group. Of the three females developing the disease, two were in the aminopyrine group and one was in the caffeine female group. The first indication of the disease is evidenced by the fact that the animal holds its head to one side and tends to go in a circle. When held by the tail the animal will spin rapidly, and will even twist the tail off if allowed to continue. Greenman and Duhring[2] describe the disease as an infection of the middle ear which may frequently result in mastoid abscess. Although some of the animals with the disease were observed for several months, there was no external evidence of an abscessed condition. The middle-ear animals seemed to have normal appetites and

continued to gain weight with the normal rats. This author doubts the possibility of infection from one animal to another because of the fact that the middle-ear rats were allowed to remain with the group for months without other cases developing.

So-called "rat pneumonia" was responsible for 53.8 per cent of all deaths in the colony during the investigation. The progress of the disease was much more rapid in our experience than in that of the authors mentioned above.[2] They describe a period of unnatural, noisy, labored breathing at the onset of the disease. In forty-nine cases of pneumonia observed during this investigation, there has been no sign of noisy breathing, but rather an apparent complete absence of breathing, when the disturbance was first noticed. In a number of instances the nostrils have been completely obstructed by the finger and little or no passage of air could be detected. The normal animal will not permit such treatment even for a second, but the pneumonia rat seemed to suffer no added discomfort even after minutes. This is taken as proof that the pneumonia rat was unable to breathe through the nose. The animals, as a rule, died in a few hours, presumably from suffocation.

We have, in a number of instances, cleared the nostrils with an ephedrine inhalant and have been able to keep some of the animals alive for as long as a week by feeding warm milk chocolate from a medicine dropper.

Many of the animals were examined carefully after death and, in every instance, the lungs were found to be badly congested with a watery fluid. The air passages were swollen almost to obliteration.

All of the abdominal contents had a bad odor even though the disease had been of but a day or so in duration. The blood was thick and dark in color and clotted almost immediately. Bacteria were cultured from the lung and lung contents.

In view of the fact that so many deaths in the colony were caused by pneumonia, it seems desirable to present a discussion of deaths in the different groups on this basis. Tables VI to X inclusive have been prepared from the data on deaths for this purpose.

It was evident quite early in the experimental period that many of the barbiturate animals were dying with pneumonia. This was especially true in the barbital group. A comparison of pneumonia deaths in all barbiturate groups is presented in Table VII. There were ten deaths in the barbital group and all of them were due to pneumonia. No other group in the colony suffered as high a per cent of pneumonia deaths as did the barbital group, seventy-seven and seven-tenths per cent of the animals dying with the disease. As shown in the table, the pneumonia death rate was higher in all of the six groups on barbituric acid derivatives than in their respective controls. The difference is least marked in the phenobarbital female and allonal groups. Allonal, as has been stated, contains aminopyrine and alurate. It should be noted that the per cent of deaths due to pneumonia was nearly four times as high in the alurate group as in the controls, while in the allonal group, it was nearly two times as high. The animals in the two test groups and in the control group were litter mates.

This rather marked difference in the per cent of pneumonia deaths in the alurate and allonal groups is especially interesting in view of the fact that allonal, as has been stated previously, has been refused acceptance by the American Medical Association Council.[7] The group of females on allonal has experienced the lowest death rate of all barbiturates tested. Table I shows that the allonal group received almost as much of alurate as did the sodium alurate group, and that they also received aminopyrine in dosages equal to three-fourths the dose given to the aminopyrine group. This would seem to indicate that aminopyrine has reduced the toxicity of allonal, and it will be shown later that aminopyrine, next to acetanilid, is the least toxic of the antipyretic drugs.

A comparison of pneumonia deaths for the different classes of drugs is given in Table X, and this comparison proves quite conclusively that the pneumonia death rate was extremely high in the barbiturate groups. It was, in fact, twice as high as for any other class of drugs.

A similar comparison is presented in Table VIII for the antipyretic drug groups. The variations in pneumonia deaths in these groups, as compared to their controls, are not as marked as in the barbiturate groups although every group of animals receiving an antipyretic drug, with the exception of the aspirin males and aspirin females, has suffered more deaths in terms of per cent than have their respective controls. The antipyrine females show the highest, 44.4 per cent, while their litter mates, the aminopyrine females, show the

lowest, 11.1 per cent. Both of these groups were controlled by a group of litter mates in which there were no pneumonia deaths.

Table X shows that the groups receiving antipyretic drugs ranked next to the barbiturate group in pneumonia deaths, although the per cent of pneumonia deaths is slightly less than half of that in the barbiturate group. The per cent of pneumonia deaths in the antipyretic group is also seen to have been twice that in all controls.

A comparison of pneumonia deaths in all groups receiving non-antipyretic and non-barbiturate drugs is given in Table IX. Of these drugs, phenolphthalein is the only one that shows a marked increase in the per cent of pneumonia deaths, there having been 50 per cent in the group as compared to 12.5 per cent in the controls. Table X lists this group of drugs (Other Drugs) third in per cent of pneumonia deaths.

Table X shows that the pneumonia death rate in all test groups was three times as high as in all control groups. Comparisons are also given in the table for female test groups, male test groups, female controls and male controls. There seems to have been no significant variation in pneumonia deaths between males and females.

Two rather striking statements may be said concerning the data presented above, first, that the barbituric acid derivatives increase the susceptibility of an animal to pneumonia, and, second, that the antipyretic drugs furnish no protection against it.

One additional point should be mentioned concerning the barbiturate animals and pneumonia. It has already been stated that

some of the pneumonia animals could be kept alive for as much as a week by the application of ephedrine inhalant to the nostrils and forced feeding with warm chocolate milk. The barbiturate animals with pneumonia did not respond to this treatment, and the duration of the disease was much shorter in these rats than in other groups. As a rule, the barbiturate rats would appear normal at the evening feeding, would be unable to breathe the next morning and would be dead by noon. Control animals, or rats in test groups other than the barbiturate, might live for one or two days without treatment, and might be kept alive for as long as a week by the ephedrine inhalant treatment and forced feeding. There seems to have been plenty of evidence to warrant the conclusion that the disease was more severe, as well as more frequent, in animals on barbiturates.

Effect on Growth and Maintenance of Weight.

The twenty-four groups of rats in the colony during this two and one-half year experiment were weighed weekly by litter mate groups during the period of rapid growth, and once every two weeks thereafter. The animals were weighed in a tared pasteboard box on a 2000 Gm. dietetic scale. The scale was calibrated at the beginning of the experiment with metric weights and was checked for accuracy each time it was used during the investigation. Growth curves for all groups are presented in Figures 1, 2, 3, 4 and 5. They are point to point curves and give the average weight for the group at the different points.

A careful study of the literature has revealed very little information concerning the growth and weight effects of the thirteen drugs investigated. However, Chase[145] has reported that caffeine, in doses of 0.119 mg. per Kg., produced a marked retarding of growth in chickens. The dose used by Chase is thirteen times the maximum dose given to rats during this investigation. Eichler and Mugge[11] have subjected white rats to chronic poisoning by the administration of large doses of caffeine, 100 mg. per Kg. per day, through four generations of interbreeding, and found that the drug produced no ill effects other than a transient fall in weight just after the medication was begun.

Higgins and McGuigan[42] found that white mice could consume 325 mg. per Kg. of acetanilid daily with no significant effect upon growth or health. A dose of 650 mg. per Kg. daily was continued for six weeks with no effect other than a delay in growth. The writer suggests that, since the drug has a slightly burning taste, the palatability of the food mixture would be changed sufficiently to reduce food consumption with a delay in growth as the result. Nine mg. per Kg. was the maximum dosage used in this experiment, one seventy-third of that given to rats by Higgins and McGuigan.

Helms[41] has found that white mice will take 675 mg. per Kg. of acetanilid, which is one-half the M.L.D., daily in their drinking water with no effect other than a delay in growth, which is regained when the drug is withdrawn. Mice, according to Helms, will take 500 mg. per Kg. daily for one month and will triple their weight

during that period.

Smith and Hambourger[146] have given 38 mg. per Kg. of acetanilid orally to rats six times a week for thirteen weeks with no significant variations in growth resulting. These authors found also, that 200 mg. per Kg. daily caused no significant differences, but when the daily dose was increased to 400 mg. per Kg. the animals grew more slowly than untreated animals.

Radwin and Lederer[77] have given white rats daily intramuscular injections of acetanilid equivalent to the human dose from periods varying from 140 to 180 days. Most of the animals lost weight and none of the animals gained as rapidly as did the controls.

Lehman and Hanzlik[81] have fed cincophen to white rats on a basis of 0.5 and one per cent of the food, the medication being continued for twenty weeks. Since the average food consumption for an adult rat is about 20 Gm. per day, the rats received from 100 mg. to 200 mg. per Kg., of cincophen daily (writers comment). The authors make no specific reference to growth effects but state that no injuries resulted from the medication.

Miller[147] gave sixteen dogs 300 mg. per Kg. of aminopyrine daily for four weeks. This author mentions no growth effects produced by the medication.

Swanson et al.[148] have given monkeys the minimal anesthetic dose of 35 to 40 mg. per Kg. of sodium amytal three times weekly for six months. They state that the animals continued to increase in weight although no mention is made of controls.

Robinson et al.[23] have administered aspirin in doses ranging from 22 mg. to 623 mg. per Kg. body weight to white rats varying in age from nine to twenty-nine weeks. They report that twenty-nine weeks of the medication produced no effects on the growth curves.

An inspection of the reports presented above would seem to indicate that caffeine may retard growth, and that acetanilid does likewise if the daily dose is extremely large. Growth effects of cincophen, aminopyrine and amytal are doubtful. Aspirin, as reported above, has no effect on growth. Nothing has been found in the literature which would indicate that the other drugs used in this investigation have any effect on growth or maintenance of weight.

Figure 1 presents the growth curve for the caffeine groups, both male and female, and their litter mate controls. These curves show conclusively that caffeine, in daily doses comparable to the adult human dose, has retarded weight gains in both males and females. Particularly noticeable is the fact that neither the males nor females ever reached the weight peaks gained by their respective controls. It is, however, interesting to note that three of the groups gained their respective weight peaks at exactly the same point in the feeding period, i.e., at the sixty-sixth week of age. At this point, the caffeine males were 38 Gms. per animal, or 6.6 per cent, below their controls in weight. The difference is more marked in the caffeine females, the latter being 21 per cent below their controls at this point. Attention is called to the fact that the caffeine females reached their weight peak at the one hundred eighteenth week,

after having gained rather steadily for about ten weeks preceding. However, the fact that the caffeine groups were consistently below their controls during most of the feeding period is considered more significant than are single period comparisons.

A very marked weight loss is observed for the caffeine males during the twenty-sixth to the thirty-third week. Both control and test animals were learning the maze during this period, and, while the amount of food per day was reduced during maze learning, it was reduced in exactly the same proportion for both the control and test groups. Cooked food was taken out of the diet during this period and the animals were given the usual milk twice daily, supplemented with Purina Fox Chow and libitum. The caffeine males lost 80 Gms. per animal as compared to less than 10 Gms. in the control group. The females were learning the maze during the same period but no difference is to be seen in the weight loss for the test and control groups.

It is interesting to note that both males and females on caffeine gained the lost weight rapidly after the cooked food was returned to the diet, and that each of the test groups passed their controls for a brief period at the thirty-fourth week for females and at the thirty-eighth week for males.

Because of the fact that there were only three rats left in the female control group after the eighty-seventh week, an auxiliary curve for all other female controls in the colony has been included from the seventy-sixth to the one hundred nineteenth week. Although the curve covers the same period with reference to seasons, the animals

averaged from sixteen to thirty-two weeks younger than the caffeine group. In spite of this difference in age, the four females in the caffeine group were considerably below the colony controls in weight during this period.

In discussing the effects of caffeine medication on general welfare and behavior, the statement was made that toxic effects were observed in the caffeine males during the last five weeks of the feeding period. The growth curve for this period offers striking evidence of this condition in the caffeine males. While both control and test animals lost weight between the ninety-second and one hundred-second weeks, the loss was considerably greater in the caffeine males than in the controls. The caffeine males continued to lose weight rapidly while the controls were gaining weight just as rapidly when the feeding was ended.

The growth curves presented in Figure 1 seem to show conclusively that caffeine has a weight depressant effect in both males and females and that this effect is greater in females than in males.

Growth curves for the aspirin-phenobarbital control groups, both male and female, are presented in Figure 2. Aspirin seems to have stimulated weight production slightly in the male group during the first half of the feeding period and to have depressed it during the latter third. The opposite effects seem to have been produced in the females on aspirin. The weight increase in the female aspirin group is quite marked from the sixty-seventh to the one hundred tenth week. The aspirin animals gained an average of 91 Gms. during this period

while their controls were gaining an average of 62 Gms. The three aspirin females averaged 48 Gm. more in weight than did the four controls when the experiment was terminated at the one hundred tenth week. It seems quite evident, therefore, that aspirin has stimulated weight gain in the females on aspirin after a questionable depression. Opposite, less marked effects were produced in the aspirin males.

As has been stated, amytal was added to the aspirin for the aspirin females from the ninety-fourth to the ninety-seventh weeks. This change in medication is not reflected in the growth curve although the animals grew rapidly heavier, as compared to the controls, during the following weeks. The aspirin males were without the drug from the ninety-first to the ninety-fourth week. No significant change in the growth curve is seen during this period.

The curves for the phenobarbital group, male and female, seem to show that the drug has depressed weight gains in both sexes. The effect of the drug is much more noticeable in the female group than in the male group since the female test animals were consistently below their controls after the first ten weeks of the feeding period. The phenobarbital females lacked an average of 50 Gms. per animal of gaining the weight peak reached by their controls at the ninetieth week. The test and control animals approached each other rather rapidly during the last ten weeks because of a more rapid loss of weight in the control group. There were three phenobarbital females and four litter mate controls in the respective groups when the experiment was ended at the end of one hundred ten weeks. The animals in the test group averaged

16 Gm. under the controls at the end of the feeding.

Weight depression was not observed in the phenobarbital males until the fifty-sixth week but was sufficiently marked from then on to be considered significant. The phenobarbital males also failed to reach the weight peak gained by the controls at the eighty-sixth week, averaging 75 Gm. below at this point. Attention should be called to the fact that the aspirin females exceeded the phenobarbital males in weight at the one hundredth week of age. This is the only instance in the author's experience where females have out-weighed litter mate males at any given age.

The phenobarbital males were without the drug from the ninety-first to the ninety-fourth week, the growth showing no significant variation from the controls during this period. The phenobarbital females were off the drug from the ninety-fourth to the ninety-seventh week. The growth curve shows a more rapid gain as compared to the controls during this period.

Growth curves for four barbiturate groups and their controls are shown in Figure 3. All of the animals in the six groups were from the same litters. Weight effects from barbiturate medication are not marked in any of the curves. It is interesting to note, however, that if the drug feedings had been stopped at the fortieth week, it would have seemed evident that sodium barbital had stimulated weight gain while sodium amytal had depressed it. During the last ten weeks of feeding, the barbital animals were considerably above the controls in weight. There were three rats in the barbital group,

five in the amytal group and eight in the control group when the experiment was stopped at the one hundredth week. A comparison of the curves for the test and control animals would seem to warrant the statement that barbital and amytal do not depress weight when given to rats in relative therapeutic doses. Withdrawal of the drug for both male groups (barbital and amytal) from the eighty-first to the eighty-fifth weeks is not reflected in the growth curves.

There were six allonal females, five alurate females and seven control females left in these groups when the experiment was stopped at the one hundredth week. During most of the feeding period, the test groups ranged above the controls in weight, the allonal animals gaining considerably above the controls during the last sixteen weeks. At the one hundredth week the allonal females averaged sixty grams heavier than the controls.

If allonal or alurate have had any effect on growth it has been a stimulating rather than a depressing effect, for both test groups were above the controls during most of the feeding period. Both female test groups were without the drug from the eighty-sixth to the ninetieth week. No abstinence effects were observable in the curves during the withdrawal period but there is some evidence of stimulation following this period.

Growth curves for the aminopyrine and antipyrine female groups and their controls have been drawn in Figure 4. The only consistent variation from the controls is exhibited by the aminopyrine group, this group being consistently below the controls during most of

the feeding period. The animals lacked 50 Gms. of reaching the weight peak gained by the controls at the eightieth week and were above the controls only twice during the feeding period, i.e., at the thirty-ninth and ninety-second weeks.

There were six females left in the aminopyrine group at the end of the one hundredth week as compared to three in the antipyrine group and seven in the control group. At this time the aminopyrine animals averaged 15 Gms. per animal below the control group while the antipyrine group averaged 30 Gms. above the control group. The antipyrine animals gained very rapidly on the controls during the eightieth to eighty-fourth weeks and held the gain until the feeding was stopped at the end of the one hundredth week. Both test groups were off the drug from the seventy-ninth to the eighty-third week. Weight gain was stimulated markedly in the antipyrine group during this abstinence period. The average weight of the group reached the extremely high level for females of 380 Gm. per rat. Both test groups and control groups experienced weight gains during this period but the gain was most marked in the antipyrine group.

While the variations are not marked for either aminopyrine or antipyrine animals, it seems safe to conclude that aminopyrine depressed weight gains very slightly while antipyrine had the opposite effect.

Also included in Figure 4 are growth curves for the acetanilid group, the "All" Group and their controls, all of which are male groups. Growth curves for phenacetin, cincophen and

phenolphthalein groups are presented in Figure 5. All of the rats represented by the curves in Figures 4 and 5 were from the same litters. The male rats in the phenacetin, cinchophen, acetanilid, phenolphthalein and "All" Group groups were controlled by the same group of litter mates. The male control curves in Figures 4 and 5 are therefore identical.

In the discussion which was given at the beginning of this section on weight effects, four references were made to literature reports all of which stated that acetanilid, in extremely large doses, caused a delay in growth. [41 42 77 146] Two of the references stated that smaller doses had had no effect on the animals treated. [41 146]

A study of the curves for the acetanilid and control groups in Figure 4 seem to prove conclusively that acetanilid, when given orally to white rats in doses equivalent to human usage, stimulates weight production. The acetanilid animals were consistently above their controls during the major part of the feeding period. At the weight peak, gained by the acetanilid animals at the seventy-fourth week and by the controls at the eighty-second week, the average weight of the acetanilid animals was 567 Gm. as compared to 480 Gm. for the controls. The acetanilid animals averaged 18 per cent heavier than did the controls at the respective weight peaks.

Reference has been made to the appearance of toxic effects in the acetanilid group after the eighty-second week of age. The growth curve for the group from this point to the end of the feeding period reveals ample evidence of such effects for during this fourteen week

period the animals lost an average of 120 Gms. They actually lost as much during this fourteen week period as they had gained during the preceding fifty-eight weeks. During this same fourteen week period the controls gained 15 Gm. per animal.

That portion of the curves for male groups between the thirty-sixth and fiftieth weeks represents the weights of the different groups during the hot months of July, August and September when the laboratory temperature frequently rose to 100°F. The animals in the colony ate very little besides their milk during this period, but attention is called to the fact that the acetanilid animals lost appreciably less weight than did their controls or the "All" Group. This would suggest, at least, that acetanilid had protected the animals from the heat effects experienced by other groups.

The growth curve for the "All" Group, Figure 4, shows without question that the 129.8 mg. per Kg. daily dose of a mixture of nine drugs did not depress growth. The animals were above the controls during most of the feeding period, being significantly above during the fifty-fifth to seventy-fourth weeks. The animals lost weight rapidly after the seventy-fourth week and averaged 35 Gm. below the controls at the end of the ninety-sixth week. The animals in the "All" Group received caffeine and phenolphthalein, both of which depressed weight gains rather markedly when given singly, and they also received acetanilid and phenacetin, both drugs having stimulated weight gains just as markedly. Curves for phenolphthalein and phenacetin are presented in Figure 5. Other drugs included in the "All" Group

medication, i.e., cinchophen, aspirin, barbital, aminopyrine and antipyrine have had little or no effect. It will be pointed out later, however, that cinchophen and phenacetin caused rather marked weight losses during the last few weeks of medication. The "All" Group lost only a little more weight during this period than did the controls and were gaining weight, as were the controls, when the experiment was stopped at the end of the ninety-sixth week. It seems reasonable to say that the combination of nine drugs given orally to the "All" Group have caused a slight but appreciable weight stimulation, and further, that the delayed weight loss effects produced by several of the drugs when given singly have been observed only slightly in the "All" Group.

The weight curve for the phenacetin males (Fig. 5) is very similar to the weight curve for the acetanilid males (Fig. 4), the important difference being that the acetanilid animals were considerably heavier than the phenacetin animals during the entire feeding period. As compared to the controls, the curve for the phenacetin group shows a rather marked stimulating effect during most of the feeding period. However, in contrast to the acetanilid animals, the phenacetin group reached the weight peak for the controls at almost exactly the same point and weight.

Like the acetanilid group, the phenacetin animals were much less affected by the hot months of July, August and September. Also, as in the acetanilid group, the phenacetin group suffered a marked loss in weight after the eighty-second week of age, this period

coinciding exactly with a similar loss period in the acetanilid group. The phenacetin animals lost an average of 75 Gm. during this fourteen week period.

Attention has already been called to the close similarity of these two drugs, acetanilid and phenacetin, chemically. When this close similarity of chemical composition is considered, it is perhaps not surprising that the two drugs should have almost identical effects upon the normal animal. Both drugs have been found to stimulate weight grain, both drugs have protected the animal from excessive loss during extreme hot weather, and both drugs have caused a marked weight loss during the last weeks of medication. Acetanilid is most effective in stimulating weight production, and while the latent effect of weight loss was more marked in the acetanilid animals than in the phenacetin animals, much of the difference was probably due to the fact that the acetanilid animals were considerably heavier when the toxic effects became evident.

Variations in the weight curve for the phenolphthalein males (Fig. 5) can have but little significance because of the small number of animals in the group. There were four animals at the beginning of the feeding period and only one at the end of the experiment. The curve shows, however, a rather marked depression during the fifteenth to the eighty-second weeks. There were four animals in the group up to the fiftieth week and they are seen to have experienced very little loss during the hot weather between the thirty-sixth and fiftieth weeks, probably because of the fact that weight had been depressed so

markedly preceding this period. There was only one animal left in the group at the end of the feeding and it weighed slightly less than the average of the controls.

Cincophen seems to have had little effect on the growth of male rats during the first fifty-five weeks of feeding. The growth follows that for the controls rather closely up to sixty-five weeks of age. From this point on, however, the delayed toxic effects mentioned under general welfare and behavior are quite evident for the cincophen animals fall rather markedly below the controls, averaging 55 Gms. per animal below when the experiment was ended at the ninety-eighth week.

In summarizing the weight effects presented above, it seems reasonable to conclude that caffeine depresses weight gain in both male and female rats. There is a limited amount of evidence to show that phenolphthalein depresses weight gain also.

Of the five barbituric acid derivatives, phenobarbital depresses weight gain in both sexes, the effect being more pronounced in female rats. Allonal and alurate have a tendency to stimulate growth in females, as evidenced by weight increase, while barbital and amytal have little if any effect on growth in male rats.

Of the antipyretics, acetanilid and phenacetin stimulated growth markedly during the major portion of the feeding period, the former more than the latter, the stimulating effects being followed by marked depression during the last weeks of the feeding period. Aspirin has little if any effect on the growth of male rats but

stimulates growth rather markedly in females after a mildly depressant effect during the first half of the experiment.

Aminopyrine and antipyrine, although closely related chemically, have had opposite effects on the growth of female litter mates. While the effects are not marked, aminopyrine has depressed weight throughout most of the feeding period. Antipyrine had practically no effect during the major part of the experiment but seems to have stimulated growth rather markedly during the last sixteen weeks. Cincophen caused marked weight reduction during the last thirty-three weeks of the experiment.

As has been stated previously, there are few instances of life cycle observations of albino rats in the literature. Slonaker,[148] in 1912, studied the life cycle activity of normal rats and has given the maximum weight of one control male rat as 331 Gms., reached at the age of fifty-six weeks. The average maximum weight for females is given as 200 Gms., reached at an age of forty-eight weeks.

Greenman and Duhring[2] have given weight records, for both male and female rats, through 150 days only. The average maximum weight listed for males at this age is 294 Gms. and for females, 209 Gms.

Sollman et al.,[1] in 1920, surveyed much of the literature pertaining to normal weight of albino rats, and 320 Gm. is given as the maximum weight for males at sixty weeks of age, and 250 Gms. for females at fifty weeks of age.

Weight summaries for both male and female groups are presented in Tables XI and XII. In every group, the average weight at 150 days

is much greater than that given by Greenman and Duhring.[2] The peak weights for all controls are considerably greater than any found in the literature. The plus and minus signs are for the purpose of comparison with the controls.

Blood Picture Effects of Life Cycle Drug Feeding

On The White Cell Count

As was mentioned earlier in this thesis, the author is not aware of any precedent for this type of investigation. There has been, however, an enormous amount of work published on the blood picture of the normal albino rat. Adams and Shevket,[150] in 1929, tabulated much of the material published for normal animals up to that time and have called attention to the wide variation in values reported by different investigators. They have also reported rather wide ranges in values for rats of different strains. They give 13,379 total white cells as the average for Wistar males and 9,997 as the average for Mount Holyoke males. Females are given similar values of 12,250 and 10,750 cells respectively. These values are reported from their own work and are considerably higher than values given by other authors quoted in the table, the average for all sexes being near 10,000. However, Robinson et al.[151] have given 14,493 as the average total white cell count for eight normal animals.

Scarborough[152] has tabulated the literature of the blood

picture of normal laboratory animals and gives 11,590 as the average of 134 counts reported in the literature up to 1931.

Abbott and Ahmann[153] have studied the blood picture of albino rats through ages varying from very young to mature animals and have reported 6,700 white cells per cu. mm. as the average at ten weeks of age, and 8,000 at forty-six weeks of age. These few of many reports, found in the literature show a wide range of from 8,000 white cells per cu. mm. to 14,493 cells for the normal white rat.

Of the thirteen drugs included in this investigation, only a few have been found reported on in the literature from the point of view of possible blood picture effects. Smith and Hambourger[146] have given 19 to 38 mg. of acetanilid per Kg. body weight to white rats six times a week for thirteen weeks with no significant changes in the blood picture resulting. Doses as high as 200 mg. per Kg. were also without effect.

Smith[154] has given 500 mg. per Kg. of acetanilid daily to monkeys by stomach tube over a period of 107 days without significant effect on the white cell count.

Wilson[155] has studied the effects of large doses of aminopyrine and of phenobarbital separately and of the two drugs combined on the leucocyte count of adult rats. Daily doses of aminopyrine which were ten to fifteen times the average oral dose for man were administered subcutaneously to white rats for thirty-eight days. One rat showed a gradual increase from 8,100 to 14,000 white cells per cu. mm. in two weeks. Three other rats showed increases of from 2,000 to 3,000 cells

during the thirty-eight day period. Wilson found that daily doses of phenobarbital equivalent to one hundred times the average daily dose for man produced no changes in the white cell count of rats. Marked increases were produced, however, when both drugs were given simultaneously, one animal showing an increase of from 6.800 to 18,200 cells. Three other animals experienced increases varying from 4,000 to 6,000 cells per cu. mm. of blood.

Smith and Mack[156] have found that the total white count of albino rats weakened by a deficient diet could be reduced 50% by the oral administration of a mixture of amytal and aminopyrine.

Bolton[156] has studied the effect of aminopyrine upon the blood cell count in dogs and reports that a mild leucopenia was observed in one of the animals. Stier and Levy[157] have studied the influence of narcotics, including sodium luminal, on the white cell count of rabbits. These authors found a moderate leucopenia occurring sometimes during and after anesthesia. In some cases there was no change and more rarely a slight leucocytosis was observed.

Robinson et al.[151] have administered aspirin to white rats in doses varying from 22 to 625 mg. per Kg. body weight for eighteen weeks without producing a significant change in the white cell count. On the second day following the removal of the drug there was, however, a total rise of about 60% in the white cell count, the count returning to normal after the sixth day.

Smith and Hambourger[146] have given white rats daily doses of 100 mg. of caffeine per Kg. body weight for thirteen weeks and

have observed no significant changes in the white cell count.

From the references given above it seems reasonable to accept 10,000 white blood cells per cu. mm. as the average for adult rats.

The above references would also seem to indicate that acetanilid, aspirin, phenobarbital and caffeine have no effect on the white cell count of albino rats, but that aminopyrine increases the count rather markedly.

Total white and red cell counts were made on the blood of all test and control animals, with the exception of the phenolphthalein groups, at varying intervals during this investigation. Bureau of Standards pipettes were used for making dilutions according to accepted methods for blood counting.

Blood for the counts was obtained by removing about one-fourth inch of the tail with a razor blade. Scarborough [152] has stated that this method of obtaining blood gives valid results only when the tail clipping is done at sufficiently long intervals to eliminate the possibility of trauma, with a high-grade inflammatory reaction resulting in hyperleukocytosis. This possibility has been eliminated in the present instance by the fact that the shortest interval between clippings was two weeks which allows ample time for the tail to heal. Most intervals were of ten weeks or more. The clipped tail heals very quickly in the rat, there being no evidence of the operation after a week.

After sufficient blood was obtained, the tail was dipped

in 10% ferric chloride solution to prevent excessive bleeding. Only three of 800 such operations resulted in hemorrhage after the animal was returned to the cage.

Countings were started at about eight o'clock in the morning, the test and control animals being deprived of the morning feeding. Test animals were given the drug as usual the night before. Test and control animals were taken alternatingly during a given series of counts in order to eliminate the factor of time with reference to the last feeding period.

White cell counts for groups are presented in Tables XIII to XX. The average age of the group, together with the number of animals in the group at that time, and the lowest count, the highest count, and the mean count are included in each table. The mean count for each test group has been compared with that of the controls for the same period and is presented in the tables as the variation from controls. A.D. stands for average deviation from the mean within the individual group.

Caffeine Groups - Male and Female (Table XIII)

There seems to have been a gradual depression of the white cell count in both males and females on caffeine up to the ninety-fifth week. The variations were consistently negative, the maximum variation being reached in each group at the ninety-fifth week. The marked change in the count at the one hundred sixth week may possibly be

attributed to the production of tolerance to the drug, or it may be due to the fact that the white cell count in rats increases rather rapidly with age.[3 153] This increase is quite obvious in the controls as well as in the test animals. The author favors the latter hypothesis since he has already stated that the males on caffeine were failing rapidly at the time these counts were taken.

Barbituric Acid Derivatives

Phenobarbital - Males and Females (Table XIV)

White cell counts have been determined in the phenobarbital groups, both male and female, at six points during the feeding period. The results for all groups, including the litter mate controls, are presented in Table XIV.

It is difficult to say whether or not the drug has caused a significant variation in the male rats on phenobarbital, for the variations range from a +13.3 per cent after sixty-six weeks of drug feeding, to a -14.8 per cent after seventy-two weeks of feeding, as compared to litter mate controls. The positive variation at the seventy-sixth week of age may have been due in part, at least to a low count in the controls at this point, for it is observed that the controls experienced the lowest average count of the six at seventy-six weeks. The phenobarbital males were without the drug for three weeks preceding the counts taken at the ninety-fourth week of age. It is impossible to say whether or not this abstinence period has affected

the white cell count. The phenobarbital females were off the drug for three weeks preceding the count taken at the ninety-seventh week. Attention is called to the fact that the variation as compared to controls changed from negative to positive in each test group. The fact that the last three counts show a positive variation may be significant of a gradual increase in the white count.

The series of counts for the phenobarbital males seems to indicate a rather definite increase in the white cell count as compared to controls. It is, however, obvious that if the counts at the ninety-ninth and one hundred eleventh weeks had not been taken, a mild depression would have been suggested. The consecutive positive variations of 13.9 per cent and 23.9 per cent for the last two periods are, it seems, more significant especially in view of the fact that the males on phenobarbital show positive variations for these same periods. These comparisons at least warrant the conclusion that if phenobarbital has an effect on the white cell count, the tendency is toward an increase.

Amytal-Barbital Groups (Table XV)

There seems to be little question as to the effect of amytal and barbital upon the white cell count of male rats. The first counts were made after thirty-nine weeks of drug feeding and the last count after ninety weeks of feeding. All of the six counts taken during the experiment show an increase in the white cell count for both test groups as compared to litter mate controls. The maximum variation for

the barbital group of -36.1 per cent was reached at the seventy-ninth week of age, after sixty-nine weeks of feeding. The barbital animals were without the drug for four weeks preceding the eighty-fifth week and the effect of this abstinence period is reflected in the white cell count. The counts showed a positive variation of 36.1 per cent when taken at the seventy-ninth week, two weeks before the withdrawal period started. At the end of the withdrawal period the positive variation was only 3.5 per cent.

The maximum positive variation of 29.2 per cent was reached in the amytal group at the one hundredth week. These animals were also off the drug for four weeks preceding the counts taken at the eighty-fifth week. However, the count at this time seems to have been unaffected by the abstinence period, the positive variation being even greater that that obtained six weeks before. Five weeks after the end of the withdrawal period the positive variation was the smallest of the six determinations, and the one obtained ten weeks later was the largest.

It is possible that this apparent delayed effect of withdrawal of amytal after prolonged administration of the drug, as compared to the more immediate effect after withdrawal of barbital, may be accounted for by a difference of storage in or fixation by the tissues. Koppanyi et al.[126] have reported that normal dogs, cats and humans excreted from 42 to 89 per cent of barbital during seven days as compared to 13 to 16 per cent of phenobarbital.

Shonle et al.[124] have reported that amytal and pentobarbital

are excreted only in traces if at all in the urine of men and dogs following the administration of their sodium salts. Under the same conditions they have found that barbital and sodium barbital are excreted as such in the urine. They assume from this difference in the rate of elimination in the urine of these barbiturates, and from the fact that both animals and man recover more rapidly from the effects of amytal than from barbital, that the body must necessarily destroy amytal more rapidly.

The delayed withdrawal effect evidenced by the lower white cell count at the ninetieth week suggests the possibility that not only is amytal eliminated to a less extent by the urine than is barbital, but that it is fixed more firmly or more completely by the body tissues.

Allonal-Alurate Group (Table XVI)

It will be remembered that allonal is a mixture of alurate, or allylisopropylbarbituric acid, and aminopyrine. White cell counts for the allonal group are presented in Table XVI. Variations in the count as compared to controls are observed to have been consistently negative from the fiftieth week, when the first counts were taken, to the ninetieth week when the variation became slightly positive. The drug seems therefore, to have depressed the white cell count during this period. The animals had been off the drug for two and one-half weeks preceding the count taken at the eighty-eighth week. The count at this time showed a negative variation as compared to controls of

7.2 per cent. Four weeks previous to this, and one and one-half weeks before the withdrawal period started, the significant negative variation of 19.9 per cent was obtained. At the ninetieth week, after four and one-half weeks of withdrawal, the variation had changed to slightly positive and was found to be slightly positive thirteen weeks later, at the one hundred third week.

The series of six counts taken during the feeding period seems to prove without question that allonal reduced the white cell count in female albino rats. In the introduction to this section the statement was made that Wilson[155] had found that a mixture of amino-pyrine and phenobarbital increased the white cell count.

A consideration of white cell counts for the alurate animals (Table XVI) shows quite conclusively that the drug has increased the white cell count in female rats. The negative variation of 11.6 per cent obtained at the fiftieth week would probably be significant if considered alone. Any possible significance that it might have is, however, overshadowed by the fact that the five counts taken after the fiftieth week were consistently positive.

Withdrawal effects are very evident in the alurate table, for the positive variation of 22.8 per cent at the eighty-second week was reduced to 4.1 per cent at the eighty-eighth week, after two and one-half weeks without the drug. No further reduction was produced by an additional two weeks without the drug.

From the material presented in the preceding pages the author feels that the following conclusions are warranted -

Allonal, a mixture of a barbiturate and an antipyretic, lowers the white blood cell count in female albino rats.

Phenobarbital effects are less conclusive than are those of other barbiturates used in the investigation but the results seem to indicate that the drug causes an increase in the white cell count in both male and female rats.

Barbital, amytal and alurate produce marked increases in the white cell count of albino rats, there being little if any difference in the action of the three drugs in this respect.

Antipyretic Drugs

Aspirin Groups - Male and Female (Table XVII)

The effects produced by aspirin on the white cell count in the blood of both male and female rats is presented in Table XVII. Counts taken at six different periods during the investigation show quite conclusively that aspirin has stimulated white cell production in both sexes. In only one instance has the count shown a negative variation as compared to the controls and that occurred at the fifty-eighth week in the female group. This marked difference from the general trend toward an increase cannot be explained by the author. It will be recalled, however, that the drug had a similar effect on weight in females, first depressing weight slightly and then causing a rather marked increase in weight gain.

When the counts were taken at the ninety-seventh week, the

females had been given a mixture of amytal and aspirin for three weeks preceding. The marked increase in count to a positive variation of 59.4 per cent conforms with Wilson's report that a mixture of phenobarbital and aminopyrine (barbiturate and antipyretic) caused a marked increase in the white cell count.

The males on aspirin were without the drug for three weeks preceding the counts taken at the ninety-fourth week. This withdrawal period is reflected in the marked lowering of the positive variation.

Aminopyrine-Antipyrine Group (Table XVIII)

The results presented in Table XVIII conform with those obtained by Wilson[155] after continued daily administration of aminopyrine to white rats. He, however, gave much larger doses for a much shorter period (38 days).

The positive variation observed at the fifty-eighth week, 73.7 per cent, is the maximum variation observed for any group of animals during this investigation.

The aminopyrine animals were without the drug for four and one-half weeks preceding the counts taken at the eighty-third week. The counts taken at this time and at the eighty-first week show a significant decrease in the positive variation.

The close similarity in chemical structure of aminopyrine and antipyrine is shown on page 8. This similarity of chemical structure is not reflected in the effects of the two drugs on the white cell count, for the effects of antipyrine cannot be said to have been

consistent in any direction. Here is another instance that reveals the inadvisability of relying upon a single determination for drawing a conclusion, for if only the counts obtained at the fortieth week were considered it would seem quite conclusive evidence that the drug had caused a decrease in the white cell count. The author cannot account for the marked increase in the white cell count for the antipyrine group at the fifty-eighth week as compared to the count at the fortieth week. It might be possible that the animal body had improved in its ability to metabolize or eliminate the drug. There is unquestionably a rather marked increase in the count from the seventy-third to the ninety-fifth week, there being a change toward the positive side of 48.6 per cent.

The animals were without the drug for four and one-half weeks preceding the counts obtained at the eighty-third week. This abstinence period is not reflected in the white cell count.

Acetanilid-Phenacetin Group (Table XIX)

Both of these antipyretic drugs have caused a marked increase in the white cell count of male rats as evidenced by counts determined at six points during the feeding period. The maximum positive variation as compared to the controls was obtained in the phenacetin group at the eighty-fifth week and in the acetanilid group at the seventy-ninth week. The fact that all variations from the controls are positive is considered as being most significant. Both groups were off the drug for five weeks preceding the counts made at the end of the

ninety-first week and this abstinence period has resulted in a rather marked lowering of the white cell count in each group.

Phenacetin and acetanilid are very closely related chemically, the former being the ethoxy derivative of the latter. It is, therefore, not surprising that the similarity of effect observed on general welfare and weight gain should be also evidenced in their effect on the white cell count.

Cincophen-"All" Group (Table XX)

The effects of cincophen upon the white cell count of male rats is rather mixed, for the first count taken at the forty-second week showed a positive variation as compared to controls of 22.1 per cent. Twenty-seven weeks later, at the sixty-ninth week, the count showed a negative variation of 10.3 per cent. The variation was also negative at the seventy-first week. The possibility of the animals having developed a tolerance to the drug might be suggested as a cause for this marked change if it were not for the fact that the animals showed the maximum positive variation of 23.0 per cent at the eighty-fifth week.

The cincophen animals were off the drug for five weeks preceding the counts obtained at the ninety-first week. The positive variation is observed to have been reduced rather markedly during this abstinence period, from -23.0 per cent one week before the withdrawal period started to -8.29 per cent at its termination. If this change was due to withdrawal, and it is not of course possible to

say definitely that it was because of the fact that even more marked changes were observed between the forty-second and sixty-ninth weeks, the change would suggest that cincophen had caused an increase in the white cell count.

White cell count data for the "All" Group is also included in Table XX. The "All" Group received barbital as a representative of the barbiturate drugs and all of the antipyretics included in the preceding discussion. The drugs and dosage schedule for the "All" Group are underlined in Table I.

The data presented in the preceding pages would seem to have proven rather conclusively that barbital, aspirin, aminopyrine, acetanilid and phenacetin have caused marked increases in the white cell count of albino rats when given singly in doses which are relatively therapeutic, over a long period of time. The effects of antipyrine, cincophen and caffeine have been less conclusive but the tendency in these three drugs has also been toward stimulation of white cell production. Since the animals in the "All" Group received all of these drugs in exactly the same dosage given to individual drug groups, a marked increase in the white cell count should be expected.

The animals in the "All" Group did experience a consistent increase in the white cell count but the increase was not as marked as in several of the groups receiving the drugs singly, as a comparison of the tables will show. A drug withdrawal period of five weeks duration just before the counts obtained at the eighty-fifth week is evidenced by a marked lowering of the count at this period, the variation being

-5.5 per cent as compared to a positive variation of 21.9 per cent, obtained one week before the drug was withdrawn.

Blood Picture Effects of Life Cycle Drug Feeding on Red Cell Count and Hemoglobin

The per cent of hemoglobin (Gms. per 100 c.c.) in the blood of control and test rats was determined by the acid hematin method, the acid dilutions being compared with a Newcomer place. The same plate, the same strength of acid and the same colorimeter were used for all determinations. The method used for obtaining blood for cell counts and for hemoglobin determinations was given in the preceding section.

The author has been unable to find anything in the literature that would indicate that any of the drugs used during this investigation have an effect on the red blood cell count or the hemoglobin concentration of the blood of albino rats when given in relative therapeutic doses over a long period of time.

Wilson [155] has found that daily doses of 50 mg. per Kg. of aminopyrine produced no change in the red cell count of albino rats after thirty-eight days. This author found, however, that while similar treatment with phenobarbital (150 mg. per Kg.) produced no change in the red cell count, a mixture of the two drugs reduced the count about one million per cu. mm.

Robinson et al. [151] have observed a decrease in the red cell count on the second day following withdrawal of aspirin which had been

given to white rats for eighteen weeks. Blood dilution is suggested as a possible cause for this change. Kerti[160] has observed a decrease of about 10 per cent in the red cell count after two to four days of aspirin administration to human beings.

Smith and Hambourger[146] have found no change in the number of erythrocytes in the blood of rats after the administration of 300 mg. per Kg. doses of acetanilid six times weekly for ten weeks. Rats receiving 400 mg. per Kg. daily for an average of seventy days did, however, show a significant lowering of the red cell count and the hemoglobin concentration.

Young and Wilson[158] observed more than a 50 per cent drop in the red cell count within five days following intravenous injection of an acetanilid solution into a dog, the animal dying four days later in a respiratory death.

Payne[40] has produced a rather marked anemia in dogs by the daily administration of 1.2 Gm. of acetanilid. The red cell count decreased as much as 59 per cent during a two week period of feeding. This author found, however, that after prolonged administration of the drug, the rate of cell formation became greater than the destructive rate, and the number of cells and hemoglobin increased.

The following values for red blood cells and hemoglobin in the blood of normal, mature albino rats are quoted from the literature.

	Literature References	R.B.C.	Hemoglobin	
Scarborough	(152)	8,500,000	100	Sahli
Smith and Hambourger	(146)	7,730,000	14.4	Newcomer
Adams and Shevket	(150)	10,000,000	100	Tallquist
Donaldson	(3)	9,400,000	103	Sahli
Robinson et al.	(151)	7,916,000	Not given	
Smith and Mack	(156)	Not given	14.5	Newcomer
Drubken and Fitz-Hugh	(159)	8,500,000	18.4	Newcomer

Caffeine Groups - Male and Female (Table XXI)

Red cell counts were determined for the caffeine groups at three different periods during the investigation. The summary of results is presented in Table XXI. Although the per cent variations between the values given for the test and control groups are consistently positive, with the exception of the last counts taken for the female test group, the variations are not sufficiently great to be considered significant. The writer concludes therefore, that caffeine has had little or no effect on the red cell count in the blood of male and female white rats.

Hemoglobin determinations were not run on the blood of the caffeine groups.

Barbituric Acid Derivatives

Phenobarbital Males and Females (Table XXII)

Erythrocytes were counted in the blood of male rats on phenobarbital at three different points during the investigation, after forty-seven, sixty-six and seventy-two weeks of drug feeding. The results presented in Table XXII would seem to indicate that the drug had first stimulated red cell production, this effect being followed by one of slight depression. The positive variation of 11.5 per cent is possibly significant, while the negative variations of 2.5 per cent and 6.9 per cent are unquestionably not when considered singly. However, the two successive negative variations are probably more significant because of the sequence.

The per cent of hemoglobin is observed to have been lower in the phenobarbital males than in the controls at each of the five determinations. None of the negative variations could be considered significant when taken singly but the consistency of negative variations would seem to indicate that the per cent of hemoglobin was lowered by the continuous use of the drug. This fact might also be considered as strengthening the possibility of a lowered red cell count following an initial rise.

The picture is, however, entirely different for the female animals on phenobarbital for there is no indication of a significant variation in either the red cell count or the hemoglobin percentage. The results suggest a gradual reduction in the red cell count together

with an increase in the hemoglobin percentage.

The phenobarbital females were off the drug for three weeks preceding the determinations obtained at the ninety-seventh week. The red cell count is observed to have become lower as compared to the controls while the hemoglobin percentage has increased rather markedly. A similar abstinence period in the male group preceding the ninety-fourth week had no apparent effect on the hemoglobin percentage. Red cell counts were not obtained at this time.

Barbital - Amytal Groups - Male (Table XXIII)

In each of these groups on barbituric acid derivatives an apparent increase in the red cell count has occurred, this increase being followed in each case by a rather marked reduction of the count. These effects seem to have been more marked in the amytal group than in the barbital group. Both groups were without the drug for four weeks preceding the determinations taken at the eighty-fifth week, a decrease in the negative variation being observed in each instance.

The hemoglobin percentage was not affected by either of the drugs.

Allonal - Alurate Groups - Female (Table XXIV)

Neither of these drugs can be said to have caused a significant variation in the red cell count of female rats. Attention is called to the fact, however, that the first counts taken at the fiftieth week show

positive variations as compared to controls and that these positive variations are followed by negative variations. A similar sequence has occurred in each group of rats on barbituric acid derivatives with the exception of the phenobarbital females. This similarity in results in the different groups emphasizes the possibility already suggested that barbiturate drugs produce an increase in the red cell count, the increase being followed by a decrease in the count. Both test groups were off the drug for four and one-half weeks preceding the counts taken at the ninetieth week. The red cell count was considerably lower at this time as compared to the controls, the variation being minus 8.8 per cent. There is no indication that this abstinence period was reflected in the red cell count for the alurate group or in the hemoglobin percentage for either group.

Significant variations in the hemoglobin percentage have not been observed in the groups on allonal and alurate.

The data presented in Tables XXII to XXIV inclusive and in the preceding pages would seem to have proven rather conclusively that none of the barbituric acid derivatives examined during this drug feeding experiment have had what might be called a marked effect on the red cell count or hemoglobin percentage of the blood of albino rats. When the results obtained from all drugs are considered as a whole, these results suggest the possibility that barbituric acid derivatives cause first an increase in the red cell count which is followed by a decrease, this being especially true for barbital and amytal. In only one instance was there any suggestion of an effect on the hemoglobin percentage,

this being in the phenobarbital male group, the hemoglobin percentage being consistently lower through five group determinations obtained at different intervals.

Antipyretic Drugs

Aspirin Groups - Male and Female (Table XXV)

The results presented in Table XXV would seem to permit a conclusion that aspirin has increased the red cell count in the blood of both male and female rats. Significant positive variations of 29.3 per cent and 19.3 per cent for males and females respectively, as compared to controls, were obtained at the fifty-seventh week, after forty-seven weeks of drug feeding. This increase is seen to have become reduced markedly when the second series of counts was taken nineteen weeks later, the variation being negative at this period for the female group. Determinations obtained at later periods indicate that the increase was followed by a rather marked lowering of the count below normal in the female groups.

The hemoglobin percentage is observed to have been consistently below that of the controls in the aspirin males. A withdrawal period of three weeks preceding the determinations made at the ninety-fourth week is not reflected in the hemoglobin percentage obtained at that time.

The series of hemoglobin percentages obtained for the aspirin females reveals nothing of significance in the way of variation from control values. There is, however, the suggestion that the percentage was gradually decreased, the average of the last determinations being

considerably lower than any previous ones.

The aspirin females were given both amytal and aspirin for three weeks preceding the ninety-seventh week. No effects of this change in medication are observed in the determinations obtained at this time. As has been stated, Wilson [155] found that a mixture of phenobarbital and aminopyrine (barbiturate and antipyretic) reduced the red cell count about 10 per cent.

Aminopyrine - Antipyrine Groups - Female (Table XXVI)

Erythrocytes have been counted at six intervals during the aminopyrine feeding period. The mean counts for the group show a consistent positive variation, as compared to the controls, for the first four determinations. The animals were off the drug for four and one-half weeks preceding the counts taken at the eighty-third week. The counts taken at the end of the withdrawal period show a negative variation as compared to the controls, and the variation was also negative at the ninety-fifth week when the last counts were taken. The negative variations are not sufficiently large to be significant but they undoubtedly emphasize the fact that the drug had caused an increase in the red cell count during the period before withdrawal.

The hemoglobin percentage shows a significant positive variation of 13 per cent at the seventy-third week. The next count, at the eighty-first week, was taken after the animals had been off the drug for two and one-half weeks, the variation being -7 per cent at this time. This is a change of 21 per cent as compared to the controls. The

variation was also negative (-9 per cent) two weeks later after the animals had been off the drug for four and one-half weeks. The results would seem to indicate that aminopyrine had caused an increase in the hemoglobin percentage and that a rather marked reduction resulted after withdrawal.

The effects produced by antipyrine are similar to those described for aminopyrine. However, the withdrawal of the drug for four and one-half weeks preceding the counts taken at the eighty-third week did not produce a negative variation as compared to controls although the count was reduced appreciably after this abstinence period.

The hemoglobin picture for antipyrine is almost identical to that produced by aminopyrine although the withdrawal effects are not as marked. At the seventy-third week, before the withdrawal period, the variation from controls was plus 8 per cent. At the end of the four and one-half week withdrawal period the variation was minus one per cent.

The author realizes that many of the comparisons presented in Table XXVI would not be significant if considered alone. However, the series of determinations would seem to warrant the conclusion that both of these antipyretic drugs, aminopyrine and antipyrine, have caused an increase in the red cell count. Effects on hemoglobin are less significant but the results suggest a possible increase in this blood constituent also, the increase being followed by a decrease when the drugs were withdrawn.

Acetanilid - Phenacetin Groups - Male (Table XXVII)

The red cell count was determined in these groups at three intervals during the feeding period. Acetanilid has unquestionably caused a rather marked decrease in the count, the maximum negative variation being 18.4 per cent. The average counts were consistently lower than the controls each time the counts were taken. These results conform with those of Smith and Hambourger,[146] Young and Wilson,[158] and Payne,[40] although all of these investigators used massive doses to produce the decrease. The gradual decrease in the negative variation may also be explained by the work of Payne.[40] He found that the number of cells and hemoglobin percentage increased following prolonged administration of massive doses of the drug, which had produced as much as a 59 per cent reduction in the cell count during the first two weeks of administration. He attributes this marked change to the fact that the rate of cell formation became greater than the destructive rate.

The hemoglobin percentage has not been reduced by acetanilid. The results presented in the table show no significant trends or variations. In view of the marked reduction in the red cell count a corresponding reduction in hemoglobin concentration would seem inevitable. The author can suggest no explanation for this inconsistency.

Phenacetin, the ethoxy derivative of acetanilid, seems to have had an opposite effect on the red cell count for the count is observed to have been consistently above that in the controls for all determinations. The hemoglobin determinations are, on the other hand,

consistently below those for the controls with the exception of the first one. The author is familiar with no instance in the literature which would explain this apparent contradiction in results.

"All" Group - Cincophen Groups - Male (Table XXVIII)

Consistent variations in the red cell count or hemoglobin percentage were not observed in the "All" Group. It will be recalled that these animals received barbital, caffeine, phenolphthalein, aspirin, aminopyrine, antipyrine, acetanilid, phenacetin and cincophen, each drug being administered in exactly the same dosage as when given singly to individual test groups.

Cincophen, the results for which are also included in Table XXVIII, has possibly has a slight depressant effect on red cell production but has caused an increase in the hemoglobin as compared to controls. All of four determinations show rather significant positive variations as compared to values obtained in the control group at the same time.

By way of summary of antipyretic drug effects on the red cell count and hemoglobin percentage, the following statements seem to be permissible -

Aspirin causes a rather marked increase in the red cell count, this increase being followed by a gradual decrease. Effects of the drug on hemoglobin percentage are questionable but the results presented above suggest a decrease in the value.

Aminopyrine and antipyrine cause an increase in the red cell count, an increase in the hemoglobin percentage being indicated also.

Acetanilid causes a marked decrease in the red cell count with no significant effect on the hemoglobin percentage.

The results for phenacetin indicate an increase in the red cell count with a decrease in the hemoglobin percentage.

Cincophen causes a rather marked increase in the hemoglobin percentage without significant effect on the red cell count.

White Cell Differentiation

Much of the clinical literature of recent years pertaining to the effects of different drugs on the blood picture in human beings has been devoted to the production or non-production of granulocytopenia by these drugs. Aminopyrine, of all drugs studied during this investigation, has been most prominent in these reports.

Plum,[161] in 1935, stated that a review of the literature up to that time showed one hundred twenty-eight cases in which aminopyrine was the cause of agranulocytosis. Seventy deaths were enumerated by the author. Ruther,[162] reported three cases of agranulocytosis and cited others from the literature in 1936 to refute the statements of various authors that no aminopyrine produced agranulocytosis had been observed in Germany and Austria. Many similar reports are to be found in the literature.

Squier and Madison[163] stated in 1936 that a review of the literature indicated that aminopyrine granulocytopenia must be due to an allergic rather than to a toxic response since the incidence of the disease was low as compared with the use of the drug. The severity of the reaction was independent of the dosage and in certain patients the drug had no ill effects when taken during acute primary granulocytopenia.

Simon and Metz[164] have reported that no significant decrease in the number of leucocytes or granulocytes occurred in one hundred three patients after the use of aminopyrine in therapeutic doses over an extended period of time. Rawls[165] reported agranulocytosis in one per cent of a series of four hundred patients receiving aminopyrine medication. Marcus[166] has reported similarly on one case receiving the drug for many weeks.

The writer has not found any reports in the literature in which aminopyrine agranulocytosis has been produced in laboratory animals. Thorington[167] stated in 1934 that experiments on animals had not proved that aminopyrine, either alone or combined with barbiturates, had produced granulocytopenia. Stenn[168] in 1935, was unable to produce the abnormality in guinea pigs, rabbits and monkeys, and Kunde et al. reported similar results in rabbits.

Paranjpe and Kanitkar[170] have given aminopyrine daily to ten rats for more than three months and have observed no reduction in the granulocytes. They state that there is evidence that granulocytopenia is not the result of the ordinary toxic action of the drug but that it is due to an idosyncratic reaction.

The writer has found a few references in the literature concerning the effect of drugs other than aminopyrine on the differential count. Smith and Hambourger[146] have found that doses of acetanilid as high as 400 mg. per Kg. daily for a period of thirteen weeks produced no change in the ratio of white cells in the blood of albino rats.

Shapiro and Lahman[171] have reported a case in which agranulocytosis similar to that produced by aminopyrine appeared at the end of a three week period during which cincophen was administered three times daily in 7.5 grain doses. They state, however, that countless other patients receiving cincophen in varying dosages failed to show any change.

Fisher[172] has reported a case of agranulocytic angina following the use of allonal.

Bonsdorff,[173] in a discussion of the subject of granulocytopenia after aminopyrine medication in 1935, stated that several cases of granulocytopenia had been reported following the use of antipyrine. He also stated that phenobarbital had been reported as causing a decrease in the granulocytic value in animals. No references to published articles were given.

Robinson et al.[151] have shown that doses varying from 22 mg. to 623 mg. per Kg. daily for twenty-nine weeks produced no significant change in the differential count in the blood of white rats.

Experimental

The differential counts for all test and control groups, with the exception of the caffeine groups, are presented in Table XXIX. All test groups had been on their respective drug for seventy weeks or more when these counts were obtained.

It is quite obvious that there is no suggestion of an agranulocytosis in any case. Neither is there any significant variation in the white cell ratio between control and test groups. The allonal females show the highest value for total granulocytes with 55 per cent. The barbital males show the lowest with 25 per cent. There are, however, two control groups with similar low values.

The writer concludes that the white cell ratio has not been affected by any of the drugs included in the table.

Estrus Cycle Determinations

The estrus cycle of the female white rat may, according to Long and Evans,[174] be followed with a high degree of accuracy by the daily examination of the vaginal contents. A smear of the contents is easily prepared by introducing the tip of a small medicine dropper containing a drop of normal saline into the vagina and compressing and releasing the bulb. The dropper is then withdrawn and the contents transferred to a microscopic slide and examined under the low power of a microscope.

Long and Evans[174] state that the succession of cell types occurring in the vaginal smear at various stages during the estrus

cycle is invariable, and that the stages may be appropriately named from the cell content of the smear as follows -

1. The stage of the sudden appearance of a mass of uniform sized nucleated epthelial cells dehisced from the surface of the vagina.

2. The stage of few large cornified cells.

3. The stage of extremely abundant cornified cells.

4. The stage of many leucocytes admixed with cornified cells.

5. The stage of leucocytes with scanty epithelial cells.

Stages two and three together represent the period of definite heat.

The estrus cycle for all female test and control groups has been determined during five periods for the caffeine group and for the aspirin-phenobarbital groups during four periods for the aminopyrine-antipyrine and allonal-alurate groups. Summaries for each group are presented in Tables XXX to XXXIII inclusive. Each table includes the time of year of the different periods, the length of the period in days and the age and length of time on the drug for each group.

Each table also presents a comparison of the number of animals in the test and control groups experiencing no cycles during the different periods. The average number of cycles for all animals present during all periods is given also together with the number of four day cycles experienced by these animals.

Long and Evans[174] have found that the average length of the estrus cycle in the sexually mature albino rat is four days. Branch and Moss[175] have used this rhythmical sequence as a criterion for determining the effect of nicotine administration to female white rats. These authors report that the number of four day cycles occurring

during a given experimental period is reduced by nicotine medication.

The author has found no other report in the literature dealing with the effects of drugs on the estrus cycle.

The summary of 15,707 individual smear determinations presented in Tables XXX to XXXIII suggest only one possibility of a drug effect, that being in the table for the aspirin-phenobarbital group. All three of the animals remaining in the aspirin group during the last period of cycle determination showed no evidence of extrus during the forty day period. During the same time however, two of five controls also failed to show any evidence of heat.

The writer concludes from the data presented in the tables that caffeine, aspirin, aminopyrine, antipyrine, phenobarbital, allonal and allurate have had no effect on the estrus cycle of female albino rats.

Maze Learning - Re-learning - Maze Activity

The maze was used during this study primarily for the purpose of determining the effect of life cycle drug feeding on the activity of treated animals as compared to that of litter mate controls.

Shirley[176] has found the revolving cage to be an excellent tool for studying the activity of the normal rat but the writer has found it extremely inaccurate as a means of determining activity variations. He is of the opinion that the elevated T maze, or possibly some modification of it, expresses with a high degree of accuracy variations in activity drive, for it places the animal in a position which approaches

the normal, at least. He may run or not as he wishes.

A very simple pattern of elevated T maze was used because of space limitations. A diagram of this maze is shown in Figure 6. The maze was constructed of seventeen sections presenting sixteen culs-de-sac. The running strips were one inch wide and the total direct distance between the starting point and the goal was thirty-five feet.

Manually controlled drops were placed at seven points on the maze, the locations of which are shown in the diagram. These drops were used to prevent re-tracing. The author felt that this factor should be controlled if travel time and total time on the maze were to have any significance. The drops were constructed of light tin plates which were cushioned with rubber to insure a minimum of noise. The drops were operated individually by strings.

The same maze procedure was used for all test and control groups run on the maze during the study. Experiments for individual drug groups were started about eight o'clock in the evening, an animal from a control or test group being placed at the starting point at this time. Thereafter the animals in the control and test groups were alternated on the maze until all animals completed a run during the same evening or night. The animals were not touched after being placed on the maze. Food consisting of powdered cracker and milk was placed at the end of the maze but the animals were not aware of its presence there until they had completed the first run. Similar dishes of food were placed at the right and left of the center of the maze

in an attempt to rule out the sense of smell as a factor in maze learning.

The maze was cleaned thoroughly each night after a group had completed a run with a pineoleum antiseptic. Sections to which drops were not attached, exclusive of the last section, were reversed at this time.

Groups to be run on the maze were deprived of the regular evening drug feeding which was given to the animals after the runs were completed. Cooked food was taken from the diet during the maze learning period and Purina Fox Chow bricquets ad libitum were substituted for it.

The animals were placed on the maze on successive nights until each rat in the group had completed three consecutive trials without error. A record of trials, total errors, travel time, i.e., time in motion in any direction, and total time on the maze were kept for each animal.

A summary of the results obtained during these determinations is presented in Table XXXIV to XXXVIII inclusive. The values listed are average values in each instance and represent the average number of trials required before three successive perfect runs were completed, except as otherwise indicated in the tables and in the discussion. In learning the maze, for instance, the caffeine males required an average of fifteen trials and made an average of sixty errors before three consecutive perfect runs were completed. The values for travel time and total time were obtained by the same procedure. The age of

all groups and period of drug feeding are included in the tables. The dosage schedule for all test groups is given in Table I.

Caffeine Groups - Male and Female (Table XXXIV)

Reiman[177] has proved conclusively that the power of adult humans to form associative bonds is increased by coffee, and that there is also an increase in the value of the speed factor.

Lashley[178] has given caffeine to female albino rats in doses corresponding by weight to four and eight grain doses for man. The drug was given daily ten minutes before maze training was begun. Lashley states that caffeine retards learning in direct proportion to the size of the dose, and that large doses of the drug result in increased activity and reduced accuracy of performance.

Macht[179] has given caffeine to trained adult rats in doses varying from 10 to 50 mg. per Kg. He reports that excitement was produced in 71 per cent of the experiments while depression resulted in 24 per cent. No effect was observed in 5 per cent of the experiments.

From the results presented in Table XXXIV it would seem that caffeine, as administered during this experiment, has stimulated activity in female rats rather markedly, for the travel time and total time are respectively, 37 and 59 per cent below the values obtained for the controls. This marked increase in activity was readily observed when the animals were learning the maze. They actually appeared to be running at full speed even before the maze was learned and would frequently run into a cul-de-sac so rapidly that they would

be unable to stop. The rate of learning seems also to have been affected for the values for both trial and errors are significantly below the control values.

Values presented for the males are less significant, for the number of errors was increased considerably as compared to the controls, while the number of trials was less. An increase in activity is also observed for the caffeine males.

In re-learning the maze the caffeine females are still seen to have made significantly fewer errors than did the controls. The marked decrease in travel time and total time for caffeine females is still evident during the re-learning period.

The caffeine males were observed to be markedly inferior to their controls during the re-learning period. During the six trials of re-learning the caffeine males made respectively, five, seven, eight, two, four and three errors while the five controls made respectively, one, five, one, one and one errors. This seems conclusive proof that the drug reduced re-learning ability in the males. Variations in travel time and total time are probably not significant for the males although the total time was 38 per cent greater than for the controls.

Barbituric Acid Derivatives

Five barbital males were placed on the maze along with the amytal males and the "All" group. None of the five animals showed

more than a suggestion of activity during the first seven trials. One hour before the eighth trial was to begin the barbital group was given 5 mg. of caffeine per rat. When placed on the maze following this dose of caffeine, three of the animals climbed down immediately, one left the maze at the eighth intersection and the fifth animal completed the run after ten minutes and sixteen seconds, making sixteen errors. On the following evening, three of the animals left the maze at once while two completed the run. In ten trials for each of the five animals in the group only four completed runs were made, two by each of two animals, all after the administration of a dose of caffeine as stated above.

Omwake [180] has obtained entirely different results after the injection of 100 mg. per Kg. doses of barbital on alternate days for four and one-half months, for this author concludes that this medication produced only a slight decrease in the activity of male and female adult rats. The ability to locate a reward in a maze within thirty minutes showed now significant differences between treated and control animals but the percentage of barbital rats successful in five minutes was definitely lower, according to this author.

As regards the effect of the single dose of caffeine on the barbital animals, it is difficult to say whether or not a stimulation resulted. The effect was not as great, at least, as that experienced by normal animals after a similar dose of the drug. The barbital males were made extremely irritable by the caffeine. They resented any attempt to keep them on the maze or to force them along.

Phenobarbital Groups - Male and Female (Table XXXV)

The table for these barbiturate drug groups indicates that the drug had a strong depressant effect on all phases of maze learning. The effect on learning is observed to have been more marked in the female group than in the male group. The effects of the drug on re-learning are especially significant. Every comparison of test and control achievement for both sexes in learning and re-learning favors the control animals. These effects produced by phenobarbital confirm the results obtained by Williams.[181] He administered 87 mg. per Kg. doses of the drug by intraperitoneal injection to white rats and found a marked and consistent tendency for the drugged groups to be inferior to the control group in both learning and re-learning when measured by both time and errors.

"All" Group - Amytal Group - Males (Table XXXII)

These male groups, together with their controls were placed on the maze for twelve trials only. The animals in the "All" group show the effect of life cycle administration of nine drugs more markedly in their behavior on the maze than in any other test to which they were subjected. They were extremely sluggish on the maze although they did not refuse to run as did the barbital males. They had received the same amount of barbital.

The amytal males were also significatnly depressed in all the phases of maze learning although the increases for travel time and

total time were not as marked as in the "All" Group animals. The number of errors was also considerably lower in the amytal group than in the "All" group animals, but both were markedly higher than in the controls.

Antipyretic Drugs

Jones[182] has attempted to determine the effect of aspirin on learning in human beings. He states that the drug seems to have a neutral effect.

Macht and Bloom[183] have determined the effect of antipyretics on the behavior of rats in the circular maze. Drugs used in their experiments were quinine sulfate, salol, sodium salicylate, acetanilid, phenacetin, antipyrine and pyrimadon. These authors administered the drugs in relatively small doses, 10 mg. or less. They determined the performance of the animals before the drug was given, one-half hour after, and three hours after. They concluded that all of the antipyretics depressed the behavior and the memory habit of the rats. Antipyrine and aminopyrine were the most powerful in this respect, according to these authors.

The effect of three antipyretic drugs on maze activity and maze learning and re-learning has been studied during this investigation. Two of these, ahtipyrine and aminopyrine, were included in the reference presented above. The third antipyretic, aspirin, is very similar in its actions to the salicylates, two of which were included above.

Aspirin (Table XXXV) is seen to have had little or no effect

on learning but the effects on the time factors are rather marked, especially in the male group.

The aspirin females seem to have been depressed more markedly than were the males during re-learning. The travel time for each test group is observed to have been increased significantly while the total time on the maze was reduced during re-learning. Errors and average trials were increased in the aspirin females during re-learning.

Aminopyrine - Antipyrine Groups - Female (Table XXXIV)

Aminopyrine has had no significant effect on the learning ability of female rats. The total time on the maze was reduced significantly. Antipyrine has, however, caused a depression in all maze data, the time factors being affected most. During re-learning marked depression is observed in all determinations for both drug groups.

The results presented above conform with those determined by Macht and Bloom.[183]

Summary

Thirteen commonly used drugs have been administered orally to albino rats in doses equivalent to normal human usage. Drug feeding periods have averaged ninety weeks. Test animals were controlled by litter mates and all animals in the colony received the same care and attention during the study.

The effects of the following drugs were studied during the investigation -

Antipyretics -

Aminopyrine, antipyrine, phenacetin, acetanilid, aspirin, cinchophen.

Barbituric acid derivatives -

Barbital, phenobarbital, amytal, alurate (all as the sodium salt) and allonal.

Other drugs -

Caffeine, phenolphthalein.

A group of male rats called the "All" Group received all of the drugs underlined above in exactly the same dosage given to test groups receiving only one of the drugs.

Conclusions

Caffeine increases daytime activity in female rats but has no observable effect on males. Weight gain is depressed markedly in both sexes, the effect being more marked in females. Males show a toxic effect of the drug by a rapid loss of weight after eighty weeks of feeding. There is no significant difference in the death rate of caffeine fed rats although the caffeine males were unquestionably more senile than the controls when the experiment was ended. The drug produces no significant variations in the white cell count, red cell count, hemoglobin percentage, or in the estrus cycle. Caffeine stimulates learning and activity on the maze in females, but has a tendency to depress both in males.

Phenolphthalein depresses weight gain in males (not given to

females) and apparently increases the death rate rather markedly. The drug produces a peculiar type of hypersensitivity to touch stimuli in rats.

Barbiturate Drugs

None of these drugs, barbital, phenobarbital, amytal, allonal and alurate, have any effect on the general behavior of albino rats in the cage. Neither do they have any significant effect on weight gain. All of these drugs increase the incidence of pneumonia, barbital being most effective in this respect, all of ten deaths in a group of thirteen being due to this disease.

Barbital, amytal and alurate increase the white cell count rather markedly. The effect of phenobarbital on the white count is less significant but there is a tendency toward an increase.

Allonal, a mixture of barbiturate and antipyretic, decreases the white cell count rather markedly.

The barbiturate drugs tested have a tendency to increase the red cell count, this increase being followed by a decrease. Phenobarbital caused a consistent decrease in the hemoglobin percentage in male rats. None of the barbiturates tested affect the white cell ratio. They definitely do not cause an agranulocytosis.

The estrus cycle of female rats is not affected by phenobarbital, allonal or alurate.

All phases of maze learning are depressed by those barbiturates tested, i.e., phenobarbital and amytal. Barbital fed animals refuse to run on the maze.

Antipyretics

The antipyretics used in this study provide no protection against so-called "rat pneumonia." The per cent of pneumonia deaths were significantly higher in the antipyrine and phenacetin groups.

Aspirin produces a noisy breathing in both sexes during the first months of feeding, this abnormality disappearing during later months. No effects on general behavior are observed in either sex. The other antipyretics, i.e., acetanilid, phenacetin, aminopyrine, antipyrine and cincophen, have no effects on general behavior that distinguish the animals given these drugs from the controls.

Acetanilid and phenacetin stimulate weight gain during the first seventy-five to eighty weeks of administration, this effect being followed by marked weight loss. The latter effect is accepted as evidence of a delayed or latent toxic effect of the drugs.

Aspirin causes marked stimulation in weight gain in females during the last forty weeks of drug administration. Aminopyrine has a tendency to depress weight gain while antipyrine has no apparent effect. Cincophen is without effect during the first seventy weeks of administration, but a depression is observed during the last twenty weeks.

The white cell count is very markedly increased by aspirin, aminopyrine, acetanilid and phenacetin. Effects on the white cell count are less significant for antipyrine and cincophen.

The red cell count is rather definitely increased by aspirin, the increase being followed by a decrease. Aminopyrine, antipyrine and phenacetin also increase the red cell count. Aspirin decreases

the hemoglobin percentage in males while cincophen increases the percentage.

None of these antipyretics have any effect on the white cell differential. No agranulocytosis is produced.

Aspirin, aminopyrine and antipyrine have no effect on the estrus cycle when administered to females.

The three antipyretics tested on the maze, i.e., aspirin, aminopyrine and antipyrine, reduce all phases of maze efficiency, the effects being most marked during re-learning. Aminopyrine and antipyrine are more effective than aspirin and the depressant effect of these drugs is more noticeable during the re-learning period.

The "shot-gun" administration of nine drugs to a group of male rats produced no more effect or effects than when the drugs were given singly to individual groups. The number of deaths due to pneumonia was significantly lower even though the group was receiving barbital. The white cell count was increased rather markedly but the red cell count and hemoglobin percentage were not effected appreciably.

The animals were definitely more sluggish on the maze than were their controls.

Table I

Drugs and Drug Dosage per Kilogram of Rat

Drug	Average human dose	From 10 weeks to 200 days	From 200 to 300 days	From 300 days to end of period
Caffeine	0.20 Gm.	3.0 mg.	6.0 mg.	9.0 mg.
Aspirin	0.30 "	4.3 "	8.6 "	12.9 "
Acetanilid	0.20 "	3.0 "	6.0 "	9.0 "
Phenacetin	0.30 "	4.3 "	8.6 "	12.9 "
Cincophen	0.50 "	7.1 "	14.2 "	14.2 "
Antipyrine	0.30 "	4.3 "	8.6 "	12.9 "
Aminopyrine	0.30 "	4.3 "	8.6 "	12.9 "
Phenolphthalein	0.06 "	1.0 "	2.0 "	3.0 "
Sod. Barbital	0.50 "	7.1 "	14.2 "	14.2 "
Sod. Phenobarb.	0.03 "	0.9 "	1.8 "	2.7 "
Sod. Amytal	0.15 "	2.0 "	4.0 "	6.0 "
Sod. Alurate	0.15 "	2.0 "	4.0 "	6.0 "
Allonal (Barbiturate	0.12 "	1.7 "	3.4 "	5.1 "
Allonal Aminopyrine	0.20 "	3.0 "	6.0 "	9.0 "

All Group received all drugs and doses underlined.

Table II

Summary of Drugs, Dosage and Feeding Periods (Female Groups)

Drug	Rats in group	Feeding period in weeks	Equiv. period for human (30-1)	No. of doses per rat	Equiv. no. of human doses	Max. dose used in mg. per Kg.	Literature M.L.D. for rats mg. per Kg.
Caffeine (Fem.)	7	109	3270	1719	51570	9.0	200+
" Controls	5	109	3270				
Aminopyrine	9	90	2700	1320	39600	12.9	1150+
Antipyrine	9	90	2700	1320	39600	12.9	1500+
" " Controls	9	90	2700				
Phenobarbital(Na)	8	100	3000	1530	45900	2.7	178
Aspirin	8	100	3000	1530	45900	12.9	600+
" " Controls	8	100	3000				
Alurate (Na)	10	90	2700	1320	39600	6.0	107+
Allonal	9	90	2700	1320	39600	14.1	not known
" " Controls	9	90	2700				

Table III

Summary of Drugs, Dosage and Feeding Periods (Male Groups)

Drug	Rats in group	Feeding period in weeks	Equiv. period for human (30-1)	No. of doses per rat	Equiv. no. of human doses	Max. dose used in mg. per Kg.	Literature M.L.D. for rats mg. per Kg.
Caffeine (Males)	7	95	2850	1425	42750	9.0	200+
" Controls	7	95	2850				
Aspirin	9	100	3000	1530	45900	12.9	600+
Phenobarbital	9	100	3000	1530	45900	2.7	178
" " Controls	9	100	3000				
Barbital (Na)	13	90	2700	990	29700	14.2	355
Amytal (Na)	11	90	2700	1320	39600	6.0	137
" " Controls	10	90	2700				
Phenolphthalein	4	86	2580	1236	37080	3.0	1250+
Phenacetin	6	86	2580	1236	37080	12.9	2000+
Cincophen	6	86	2580	990	29700	14.2	750+
Acetanilid	5	86	2580	1236	37080	9.0	2400+
All Group	8	86	2580	10520	315600	129.8	
""""Controls	8	86	2580				

Table IV

Drug	Equivalent therapeutic dose		Toxic doses		Ratio of toxic to therapeutic dose Rats	Relative antipyretic potency Rats
	Cats mgm. per kgm.	Rats	Rats Gm. per kgm.	Mice		
Acetanilid	16.5	24	0.82	3.23	35	170
Amidopyrine	25	31	1.15	1.32	40	134
Phenacetin	33	40	1.25	1.38	30	100
Antipyrine	33	40	1.53	1.66	40	100
Aspirin	54	54	1.24	1.36	20	74

Table V

Death Rate Comparison for all Groups

Drugs	Deaths	% deaths in group	% deaths in controls
Barbital males	10 of 13	80.0	20.0
Phenolphthalein "	3 " 4	75.0	22.2
Phenobarbital "	6 " 9	66.7	22.2
Antipyrine Females	6 " 9	66.7	22.2
Aspirin "	5 " 8	62.5	50.0
Caffeine males	4 " 7	57.0	43.0
Aspirin "	5 " 9	55.6	22.2
Amytal "	6 " 11	54.5	20.0
Cincophen "	3 " 6	50.0	22.2
Phenacetin "	3 " 6	50.0	22.2
Alurate females	5 " 10	50.0	22.2
Asp. Phenobarb. fem. controls	4 " 8	50.0	
Caffeine females	3 " 7	43.0	40.0
Caffeine male controls	3 " 7	43.0	
Caffeine females	2 " 5	40.0	
Phenobarb. "	3 " 8	37.5	50.0
Aminopyrine "	3 " 9	33.3	22.2
Allonal "	3 " 9	33.3	22.2
Asp. Phenobarb. male cont.	3 " 9	33.3	
All Group males	2 " 8	25.0	22.2
All controls	2 " 8	22.2	
Aminop. Antip. fem. controls	2 " 9	22.2	
Allonal Alurate " "	2 " 9	22.2	
Barbital Amytal male controls	2 " 10	20.0	
Acetanilid "	1 " 5	20.0	22.2
All test animals	71 "138	51.4	30.8
All control "	20 " 65	30.8	

Table VI

Death Due to Pneumonia

Drugs	Group no.	No. of rats in group	Total deaths	Pneum. deaths	Pneum. % of total deaths	Pneum. deaths % of group
Barbital	1	13	10	10	100.0	77.7
Phenolphthalein	2	4	3	2	66.7	50.0
Phenobarb. males	3	9	6	4	66.7	44.4
Antipyrine fem.	5	9	6	4	66.7	44.4
Alurate "	4	10	5	4	80.0	40.0
Amytal	1	11	6	4	66.7	36.4
Phenacetin	2	6	3	2	66.7	33.3
Phenobarb. fem.	6	8	3	2	66.7	25.0
Aspirin males	3	9	5	2	40.0	22.2
Allonal fem.	4	9	3	2	66.7	22.2
Cincophen	2	6	3	1	33.3	16.7
Caffeine fem.	7	7	3	1	33.3	14.3
Caffeine males	8	7	4	1	25.0	14.3
All Group	2	8	2	1	50.0	12.5
Aspirin fem.	6	8	5	1	20.0	12.5
Aminopyrine fem.	5	9	3	1	33.3	11.1
Acetanilid	2	5	1	0	0.0	0.0
Controls for	3	9	3	2	66.7	22.2
" "	7	5	2	1	50.0	20.0
" "	2	8	2	1	50.0	12.5
" "	6	8	4	1	25.0	12.5
" "	4	9	2	1	50.0	11.1
" "	1	10	2	1	50.0	10.0
" "	8	7	3	0	0.0	0.0
" "	5	9	2	0	0.0	0.0

Table VII

Pneumonia Deaths in Barbiturate Groups

Drugs	Group no.	No. of rats in group	Total deaths	Pneum. deaths	Pneum. % of total deaths	Pneum. deaths % of group
Barbital	1	13	10	10	100.0	77.7
Phenobarb. males	3	9	6	4	66.7	44.4
Alurate	4	10	5	4	80.0	40.0
Amytal	1	11	6	4	66.7	36.4
Phenobarb. fem.	6	8	3	2	66.7	25.0
Allonal	4	9	3	2	66.7	22.2
Controls for	3	9	3	2	66.7	22.2
" "	6	8	4	1	25.0	12.5
" "	4	9	2	1	50.0	11.1
" "	1	10	2	1	50.0	10.0

Table VIII

Pneumonia Deaths in Antipyretic Groups

Antipyrine	5	9	6	4	66.7	44.4
Phenacetin	2	6	3	2	66.7	33.3
Aspirin male	3	9	5	2	40.0	22.2
Cincophen	2	6	3	1	33.3	16.7
Aspirin fem.	6	8	5	1	20.0	12.5
Aminopyrine	5	9	3	1	33.3	11.1
Acetanilid	2	5	1	0	0.0	0.0
Controls for	3	9	3	2	66.7	22.2
" "	6	8	4	1	25.0	12.5
" "	2	8	2	1	50.0	12.5
" "	5	9	2	0	0.0	0.0

Table IX

Pneumonia Deaths in Non-antipyretic and Non-barbiturate Groups

Drugs	Group no.	No. of rats in group	Total deaths	Pneum. deaths	Pneum. % of total deaths	Pneum. deaths % of group
Phenolphth.	2	4	3	2	66.7	50.0
Caffeine males	8	7	4	1	25.0	14.3
Caffeine fem.	7	7	3	1	33.3	14.3
All Group	2	8	2	1	50.0	12.5
Controls for	7	5	2	1	50.0	20.0
" "	2	8	2	1	50.0	12.5
" "	8	7	3	0	0.0	0.0

Table X

Comparison of Pneumonia Deaths in all Groups

Barbiturate	60	33	26	79.0	43.3
Antipyretic	52	26	11	42.3	21.2
Other drugs	26	12	5	41.7	19.2
All controls	65	20	7	35.0	10.8
All test groups	138	71	42	60.0	30.4
Female test "	60	28	15	53.9	25.0
Male " "	78	43	27	63.0	34.6
Male controls	34	10	4	40.0	11.7
Female "	31	10	3	30.0	9.7

Table XI

Weight Summary for Male Groups
(Av. wt. in Gms.)

Group	150 Days	Peak & wk.	Max. * var. wk.	Termination
Caffeine	+390	+536 - 66	-19% - 104	+517 - 104
Controls	385	570 - 66		536 - 107
Phenobarb.	+356	-455 - 85	-8.4% - 67	-400 - 112
Aspirin	+385	-465 - 98	+14% - 23	-430 - 112
Control	346	485 - 98		458 - 112
Barbital	+388	-503 - 65	+10% - 96	+483 - 100
Amytal	+378	+527 - 65	- 5% - 39	-452 - 100
Control	375	507 - 75		460 - 100
Acetanilid	+426	+567 - 74	+17% - 62	-469 - 96
"All" Group	+418	+548 - 74	+16% - 74	-492 - 96
Phenacetin	+428	-512 - 74	-17% - 98	-446 - 96
Phenolphth.	-366	-498 - 93	-16% - 38	-498 - 96
Cincophen	+400	-471 - 74	-14% - 98	-432 - 96
Controls	390	521 - 96		521 - 96

* Compared to controls

Table XII

Weight Summary for Female Groups
(Av. wt. in Gms.)

Group	150 Days	Peak & wk.	Max. * var. wk.	Termination
Caffeine	-220	+366 - 66	-27% - 66	-300 - 119
Controls	232	308 - 117		360 - 119
Phenobarb.	-255	-348 - 100	-15% - 90	-342 - 112
Aspirin	+265	+430 - 100	+20% - 104	+400 - 112
Controls	260	380 - 90		370 - 112
Aminop.	-248	-340 - 92	- 9% - 80	-330 - 100
Antip.	-260	+380 - 84	+13% - 90	+378 - 100
Controls	263	360 - 80		345 - 100
Allonal	+260	+386 - 100	+19 - 90	+386 - 100
Alurate	+265	+374 - 92	+ 9 - 92	-320 - 100
Controls	253	341 - 92		324 - 100

* Compared to controls

Table XIII

White Cell Count

Caffeine

		Low	High	Mean	A.D.	Variation from cont.
Males						
Caffeine						
52 wks	6*	7960	9910	9180	530	- 0.7%
76 "	6	8920	10080	9390	414	- 2.4%
95 "	5	10640	11880	11456	309	-12.0%
106 "	3	10880	16200	14053	2115	+ 3.2%
Controls						
52 wks	5	8680	9450	9250	214	
76 "	5	9320	9800	9616	211	
95 "	5	11400	14400	13004	1109	
106 "	4	12120	16960	13612	1673	
Females						
Caffeine						
52 wks	7	7340	8420	7830	370	- 8.6%
76 "	5	7280	10480	7968	1005	- 7.7%
95 "	5	7720	8640	8088	234	-18.4%
106 "	5	8320	12280	10624	1571	- 0.7%
Controls						
52 wks	5	7760	9080	8570	390	
76 "	4	8480	8800	8630	130	
95 "	4	8640	12440	9910	1240	
106 "	3	10200	11600	10700	560	

* = No. of animals

Table XIV

White Cell Count
Sodium Phenobarbital Groups -- Male & Female

		Low	High	Mean	A.D.	Variation from cont.
Males						
Phenobarb.(Na)						
57 wks	6*	8240	11520	9640	773	- 1.7%
76 "	5	7120	14640	11136	1629	+13.3%
82 "	5	8000	12480	9702	1237	-14.8%
94** "	5	10200	15200	12950	1375	+ 6.1%
98 "	4	10550	12500	11512	537	+ 6.5%
108 "	4	10375	13200	11594	831	+ 9.9%
Controls						
57 wks	6	9120	10760	9807	553	
76 "	6	9280	10440	9827	320	
82 "	6	10240	11760	11138	418	
94 "	6	11060	13600	12210	730	
98 "	5	9550	13350	10814	1069	
108 "	5	9950	12400	10548	740	
Females						
Phenobarb.(Na)						
58 wks	6	7800	12320	9510	993	+ 2.1%
76 "	6	8080	9920	8707	1067	- 4.1%
84 "	6	7360	11200	8977	1370	- 9.3%
97** "	4	10450	12650	11600	1050	- 6.3%
99 "	4	11400	15200	13163	1113	+13.9%
111 "	4	12100	17600	14038	1782	+23.9%
Controls						
58 wks	5	8000	10440	9312	838	
76 "	5	7320	10560	9072	1030	
84 "	4	8560	11200	9820	740	
97 "	4	11400	13100	12338	488	
99 "	4	9600	13450	11550	1125	
111 "	4	11325	13100	11325	1813	

* = No. of animals
** = 3 wks off drug

Table XV

White Cell Count

Barbital -- Amytal Groups -- Male

		Low	High	Mean	A.D.	Variation from cont.
Barbital						
49 wks	6*	9040	11600	10297	947	+11.0%
69 "	6	9320	13920	12187	1044	+16.9%
79 "	5	13960	18840	16368	1670	+36.1%
85** "	4	7200	14000	10900	1850	+ 3.5%
90 "	3	9900	13000	11717	1211	+ 5.3%
100 "	3	13200	14210	13645	377	+26.7%
Controls						
49 wks	7	8880	10200	9270	331	
69 "	7	9240	11760	10337	642	
79 "	7	9080	13680	12023	987	
85 "	7	9400	13400	10523	980	
90 "	7	9700	12800	11221	1459	
100 "	6	9750	12210	10764	767	
Amytal						
49 wks	7	9400	10320	9871	255	+ 6.4%
69 "	7	10280	14280	11920	926	+15.3%
79 "	7	13520	16360	15040	779	+25.1%
85** "	6	10600	14500	11857	1475	+ 5.8%
90 "	6	10100	19000	13433	2911	+27.6%
100 "	4	11225	16215	13913	1645	+29.2%

* = No. of animals
** = 4 wks off drug

Table XVI

White Cell Count

Allonal -- Alurate Groups -- Female

		Low	High	Mean	A.D.	Variation from cont.
Allonal						
50 wks	8*	8600	9960	9480	400	- 4.1%
67 "	6	6604	9760	7782	678	-19.5%
82 "	6	8000	9930	8885	618	-19.9%
88** "	5	9750	10850	10400	360	- 7.2%
90*** "	5	10050	11950	11020	744	+ 2.3%
103 "	4	9900	13300	11650	1650	+ 3.1%
Control						
50 wks	7	8600	12000	9886	1003	
67 "	7	9320	10200	9676	278	
82 "	7	9760	15140	11097	1156	
88 "	6	8750	13300	11207	1377	
90 "	6	9970	12160	10770	680	
103 "	6	10210	12550	11290	763	
Alurate						
50 "	7	7040	9440	8731	715	-11.6%
67 "	6	7960	13040	11133	1302	+15.0%
82 "	6	11430	14800	13638	882	+22.8%
88** "	6	9750	13650	11675	1912	+ 4.1%
90*** "	5	9650	13200	11390	888	+ 5.7%
103 "	5	10400	18500	14890	2272	+31.8%

* = No. of animals
** = 2½ wks off drug
*** = 4½ wks off drug

Table XVII

White Cell Count
Aspirin Groups -- Male & Female

		Low	High	Mean	A.D.	Variation from cont.
Males						
Aspirin						
57 wks	8*	10120	12320	11339	802	+15.6%
76 "	7	9440	16120	13726	1716	+39.7%
82 "	7	12880	17760	15383	1266	+38.1%
94**"	5	11600	15200	13460	1436	+10.2%
98 "	4	12250	16900	15287	1519	+41.3%
108 "	4	13200	17200	15367	1084	+45.7%
Controls						
57 wks	6	9120	10760	9807	553	
76 "	6	9280	10440	9827	320	
82 "	6	10240	11760	11138	418	
94 "	6	11060	13600	12210	730	
98 "	5	9550	13350	10814	1069	
108 "	5	9950	12400	10548	740	
Females						
Aspirin						
58 wks	6	6160	7920	7443	428	-25.1%
76 "	6	7960	11600	9593	900	+ 5.7%
84 "	3	10980	11920	11300	413	+15.0%
97***"	3	13000	23100	19667	4444	+59.4%
99 "	3	13500	18450	15250	2133	+32.0%
111 "	3	10650	15200	12583	1744	+11.1%
Controls						
58 wks	5	8000	10440	9312	838	
76 "	5	7320	10560	9072	1030	
84 "	4	8560	11200	9820	740	
97 "	4	11400	13100	12338	488	
99 "	4	9600	13450	11550	1125	
111 "	4	11325	13100	11325	1813	

* = No. of animals
** = 3 wks off drug
*** = 3 wks on Amytal & Aspirin

Table XVIII

White Cell Count

Aminopyrine -- Antipyrine Groups -- Female

		Low	High	Mean	A.D.	Variation from cont.
Aminopyrine						
40 wks	10*	8400	17680	12756	3284	+46.3%
58 "	8	12400	26320	15548	2707	+73.7%
73 "	8	10960	16520	14150	1510	+43.5%
81** "	7	10940	15050	13419	1349	+24.7%
83*** "	7	9850	17100	13014	2088	+15.4%
95 "	7	8600	17300	13801	2263	+23.9%
Controls						
40 wks	7	7680	10600	8714	931	
58 "	6	8040	9760	8953	513	
73 "	6	8320	11920	9860	807	
81 "	6	10050	12500	10758	611	
83 "	6	10100	13250	11275	671	
95 "	6	8400	14500	11135	1393	
Antipyrine						
40 wks	8	6000	8040	7370	448	-18.2%
58 "	6	8320	14080	10688	2208	+19.3%
73 "	6	7760	10000	8973	587	- 9.8%
81** "	4	9700	11750	10525	619	- 2.2%
83*** "	4	10600	13800	11825	988	+ 4.8%
95 "	3	13750	15350	14400	633	+29.3%

* = No. of animals
** = 2½ wks off drug
*** = 4½ wks off drug

Table XIX

White Cell Count

Acetanilid -- Phenacetin Groups -- Male

		Low	High	Mean	A.D.	Variation from cont.
Acetanilid						
42 wks	5*	10920	15940	12708	1474	+31.6%
69 "	5	11940	19200	15958	2950	+26.9%
79 "	5	12500	21800	17420	3816	+51.4%
85 "	5	12500	22050	17290	3288	+39.2%
91** "	5	11540	16920	13374	1418	+11.7%
Controls						
42 wks	8	9240	10760	9650	475	
69 "	6	12000	13840	12575	539	
79 "	5	9760	12200	11496	694	
85 "	5	10200	13700	12490	916	
91 "	5	10860	13200	11964	869	
Phenacetin						
42 wks	5	10720	11320	11152	246	+15.5%
69 "	3	11150	14320	12893	1162	+2.5%
79 "	3	12030	20300	15110	3127	+31.4%
85 "	3	16450	19650	18217	1178	+45.8%
91 "	3	11360	17400	14320	2053	+19.6%

* = No. of animals
** = 5 wks off drug

Table XX

White Cell Count

"All" Group -- Cincophen Groups -- Male

		Low	High	Mean	A.D.	Variation from cont.
"All" Group						
42 wks	8*	9520	12200	11095	795	+14.9%
69 "	7	12600	13600	13099	325	+ 4.0%
79 "	7	12760	15100	14023	729	+21.9%
85** "	7	10200	14150	11829	1161	- 5.5%
91 "	5	11340	13210	12222	546	+ 2.1%
Controls						
42 wks	8	9240	10760	9650	475	
69 "	6	12000	13840	12575	539	
79 "	5	9760	12200	11496	694	
85 "	5	10200	13700	12490	916	
91 "	5	10860	13200	11964	869	
Cincophen						
42 wks	6	10240	13440	11787	996	+32.1%
69 "	4	10460	12340	11395	543	-10.3%
79 "	4	10300	12600	11200	700	- 2.6%
85 "	3	10550	18350	15367	3211	+23.0%
91** "	3	11320	14250	12957	1091	+ 8.3%

* = No. of animals
** = 5 wks off drug

Table XXI

Red Cell Count
Caffeine

	No. of animals	Low	High	Mean	Variation from cont.
Males					
Caffeine					
52 wks	7	8.12	9.72	8.78	+ 0.2%
76 "	6	8.00	10.80	9.08	+ 4.7%
95 "	5	6.87	9.14	7.96	+ 2.8%
Controls					
52 wks	5	8.40	9.10	8.76	
76 "	5	8.34	8.98	8.67	
95 "	5	6.94	8.35	7.74	
Females					
Caffeine					
52 wks	7	8.23	9.78	8.79	+ 5.0%
76 "	5	8.56	9.10	8.81	+ 8.6%
95 "	5	7.14	8.41	7.94	-11.2%
Controls					
52 wks	5	7.88	8.90	8.36	
76 "	4	7.87	8.27	8.11	
95 "	4	8.15	9.64	8.94	

Table XXII

Red Cell Count (Millions) and Hemoglobin (Gms. per 100c.c.)
Sodium Phenobarbital Groups -- Male & Female

		Red Cell Count				Hemoglobin			
		Low	High	Mean	Variation from cont.	Low	High	Mean	Variation from cont.
Males									
57 wks	6*	5.93	8.94	6.85	+11.5%	--	--	--	--
76 "	5	7.53	8.45	8.08	- 2.5%	12.9	15.4	14.1	- 4.1%
82 "	5	7.30	8.65	7.87	- 6.9%	12.2	14.4	13.3	- 8.3%
94**"	4	--	--	--	--	13.7	15.4	14.4	- 6.5%
98 "	4	--	--	--	--	12.4	15.4	13.5	- 9.4%
108 "	4	--	--	--	--	12.2	15.1	13.7	- 5.5%
Controls									
58 wks	6	5.71	6.46	6.14		--	--	--	--
76 "	6	7.82	8.65	8.29		13.5	15.8	14.7	
82 "	5	7.90	9.00	8.46		13.5	15.9	14.5	
94 "	6	--	--	--		14.0	16.7	15.4	
98 "	5	--	--	--		13.7	15.7	14.9	
108 "	5	--	--	--		13.5	15.4	14.5	
Females									
58 wks	6	7.77	8.97	8.41	+ 3.4%	--	--	--	--
76 "	6	7.16	8.86	7.86	+ 2.3%	12.4	14.7	13.5	- 0.7%
84 "	6	8.17	8.90	8.40	+ 1.0%	12.4	13.5	12.9	0.0%
97**"	4	7.15	8.62	7.91	- 4.5%	12.9	15.4	14.3	+ 7.1%
99 "	4	--	--	--	--	15.4	16.2	15.8	+ 1.3%
Controls									
58 wks	5	7.34	8.97	8.13		--	--	--	--
76 "	5	6.70	8.15	7.67		13.2	15.0	13.6	
84 "	4	7.96	9.06	8.32		12.4	13.5	12.9	
97 "	4	7.94	8.64	8.29		13.7	16.2	15.4	
99 "	4	--	--	--		12.4	14.7	13.6	

* = No. of animals
** = 3 wks off drug

Table XXIII

Red Cell Count (Millions) and Hemoglobin (Gms. per 100c.c.)
Barbital -- Amytal Groups -- Male

		Red Cell Count				Hemoglobin			
		Low	High	Mean	Variation from cont.	Low	High	Mean	Variation from cont.
Barbital									
49 wks	6*	6.63	8.39	7.69	+ 7.1%	--	--	--	--
69 "	6	7.95	9.46	8.27	+12.1%	13.7	15.8	14.9	+ 1.4%
79 "	5	--	--	--	--	14.0	15.8	14.9	+ 5.0%
85**"	4	7.17	9.15	8.17	- 9.5%	13.7	16.7	15.4	+ 1.3%
90 "	3	--	--	--	--	13.7	15.4	14.5	- 2.7%
100 "	3	--	--	--	--	13.7	15.8	15.0	0.0%
Controls									
49 wks	7	6.56	8.54	7.18		--	--	--	
69 "	7	8.85	10.13	9.41		13.7	15.8	14.7	
79 "	7	--	--	--		13.5	15.1	14.2	
85 "	7	8.17	9.65	9.03		14.0	16.2	15.2	
90 "	7	--	--	--		13.7	15.7	14.9	
100 "	6	--	--	--		13.5	16.2	15.0	
Amytal									
49 wks	7	7.05	9.63	8.56	+19.2%	--	--	--	--
69 "	7	7.14	9.20	8.03	-14.6%	13.7	15.8	14.7	0.0%
79 "	7	--	--	--	--	14.0	15.8	14.6	+ 2.8%
85**"	6	8.15	9.60	8.72	- 3.4%	14.4	16.2	15.4	+ 1.3%
90 "	6	--	--	--	--	12.9	15.4	14.7	- 1.3%
100 "	4	--	--	--	--	14.0	15.8	14.9	- 0.7%

* = No. of animals
** = 4 wks off drug

Table XXIV

Red Cell Count (Millions) and Hemoglobin (Gms. per 100c.c.)
Allonal -- Alurate Groups -- Female

		Red Cell Count				Hemoglobin			
		Low	High	Mean	Variation from cont.	Low	High	Mean	Variation from cont.
Allonal									
50 wks	8*	7.48	9.68	8.14	+ 3.2%	--	--	--	--
67 "	6	7.29	10.61	8.46	- 0.2%	13.5	17.6	15.5	- 6.6%
82 "	4	--	--	--	--	13.7	19.8	16.4	+ 1.9%
88 "	4	--	--	--	--	13.5	16.2	15.2	- 7.3%
90**"	4	7.15	8.10	7.71	- 8.8%	13.2	16.7	14.9	- 6.3%
103 "	4	8.15	8.80	8.51	+ 2.6%	--	--	--	--
Controls									
50 wks	7	6.87	8.84	7.88		--	--	--	
67 "	6	7.57	9.02	8.48		15.8	17.6	16.6	
82 "	7	--	--	--		15.4	16.7	16.1	
88 "	6	--	--	--		13.7	19.8	16.4	
90 "	6	7.95	9.12	8.46		15.4	16.7	15.9	
103 "	6	7.96	8.64	8.29		--	--	--	
Alurate									
50 wks	7	7.42	8.65	7.91	+ 0.3%	--	--	--	--
67 "	6	7.10	9.25	8.33	+ 1.7%	14.4	19.8	17.2	+ 3.6%
82 "	5	--	--	--	--	14.4	16.7	16.0	- 0.6%
88 "	5	--	--	--	--	14.0	17.6	15.8	- 3.3%
90**"	5	7.64	9.11	8.42	- 0.5%	14.0	17.6	16.1	+ 1.3%
103 "	5	8.11	9.13	8.67	+ 4.5%	--	--	--	--

* = No. of animals
** = 4½ wks off drug

Table XXV

Red Cell Count (Millions) and Hemoglobin (Gms. per 100c.c.)
Aspirin Groups -- Male & Female

		Red Cell Count				Hemoglobin			
		Low	High	Mean	Variation from cont.	Low	High	Mean	Variation from cont.
Males									
57 wks	8*	7.65	8.17	7.94	+29.3%	--	--	--	--
76 "	7	8.61	9.74	8.85	+ 6.7%	13.5	15.8	14.5	- 1.4%
82 "	7	7.80	8.65	8.29	- 2.0%	13.5	15.5	13.8	- 4.8%
94**"	5	--	--	--	--	13.5	15.4	14.4	- 6.5%
98 "	4	--	--	--	--	13.7	14.7	14.2	- 4.7%
108 "	4	--	--	--	--	12.4	15.1	13.9	- 4.1%
Controls									
57 wks	6	5.71	6.46	6.14		--	--	--	
76 "	6	7.82	8.65	8.29		13.5	15.8	14.7	
82 "	5	7.90	9.00	8.46		13.5	15.9	14.5	
94 "	6	--	--	--		14.0	16.7	15.4	
98 "	5	--	--	--		13.7	15.7	14.9	
108 "	5	--	--	--		13.5	15.4	14.5	
Females									
58 wks	6	9.30	10.28	9.75	+19.9%	--	--	--	--
76 "	6	6.52	7.97	7.21	- 5.9%	12.4	15.4	13.9	+ 2.2%
84 "	3	7.47	8.37	7.53	- 9.4%	12.9	14.0	13.3	+ 3.1%
97***"	3	6.23	8.01	7.36	-11.1%	13.5	15.4	14.7	- 4.5%
99 "	3	--	--	--	--	15.4	16.2	15.8	+ 1.3%
111 "	3	--	--	--	--	10.0	14.0	12.3	-11.5%
Controls									
58 wks	5	7.34	8.97	8.13		--	--	--	
76 "	5	6.70	8.15	7.67		13.2	15.0	13.6	
84 "	4	7.96	9.06	8.32		12.4	13.5	12.9	
97 "	4	7.94	8.64	8.29		13.7	16.2	15.4	
99 "	4	--	--	--		15.1	16.2	15.6	
111 "	4	--	--	--		12.5	15.0	13.9	

* = No. of animals
** = 3 wks off drug
*** = 3 wks on Amytal & Aspirin

Table XXVI

Red Cell Count (Millions) and Hemoglobin (Gms. per 100c.c.)
Aminopyrine -- Antipyrine Groups -- Female

		Red Cell Count				Hemoglobin			
		Low	High	Mean	Variation from cont.	Low	High	Mean	Variation from cont.
Aminopyrine									
40 wks	10*	8.18	9.86	8.84	+ 8.2%	--	--	--	--
48 "	9	8.28	10.01	9.28	+19.4%	--	--	--	--
58 "	9	7.80	9.22	8.40	+ 8.5%	12.2	15.8	14.7	- 2.0%
73 "	9	6.83	10.54	8.32	+11.0%	13.7	19.8	15.7	+12.0%
81 "	7	--	--	--	--	11.3	15.4	13.4	- 7.0%
83** "	7	7.05	8.06	7.47	- 3.4%	11.9	15.4	13.5	- 9.0%
95 "	7	5.61	9.64	8.27	- 2.7%	13.5	16.0	14.6	0.0%
Controls									
40 wks	7	7.64	9.26	8.17		--	--	--	
48 "	7	6.65	8.81	7.77		--	--	--	
58 "	6	7.08	9.16	7.74		13.7	15.8	15.0	
73 "	6	6.56	9.14	7.50		11.1	15.1	14.0	
81 "	6	--	--	--		12.9	15.7	14.5	
83 "	6	7.17	8.43	7.74		14.0	15.7	14.9	
95 "	6	7.90	8.73	8.50		14.0	15.0	14.6	
Antipyrine									
40 wks	8	7.00	8.52	7.71	- 5.6%	--	--	--	--
58 "	6	7.25	8.67	7.97	+ 2.9%	12.4	15.4	13.8	- 8.0%
73 "	6	7.27	10.45	8.53	+13.7%	14.0	15.8	15.1	+ 8.0%
81 "	4	--	--	--	--	12.9	15.7	14.1	- 3.0%
83** "	4	7.38	8.95	8.17	+ 5.5%	13.7	16.2	14.7	- 1.0%
95 "	3	7.25	9.80	8.92	+ 4.9%	13.0	15.0	14.2	- 3.0%

* = No. of animals
** = 4½ wks off drug

Table XXVII

Red Cell Count (Millions) and Hemoglobin (Gms. per 100c.c.)
Acetanilid and Phenacetin Groups -- Male

		Red Cell Count				Hemoglobin			
		Low	High	Mean	Variation from cont.	Low	High	Mean	Variation from cont.
Acetanilid									
42 wks	5*	6.26	8.71	7.14	-18.4%	--	--	--	--
69 "	5	6.40	8.80	7.45	-12.3%	13.5	16.2	14.6	+ 4.3%
79 "	5	7.20	8.56	7.90	- 9.1%	13.5	15.4	14.7	- 0.7%
85**"	5	--	--	--	--	12.2	16.7	14.7	+ 4.5%
Controls									
42 wks	8	8.09	9.85	8.74		--	--	--	
69 "	6	8.00	9.63	8.61		11.5	18.0	14.0	
79 "	6	8.14	9.22	8.70		12.4	16.7	14.8	
85 "	5	--	--	--		12.9	15.7	14.1	
91 "	5	--	--	--		12.8	16.2	14.2	
Phenacetin									
42 wks	5	9.08	9.56	9.30	+ 6.6%	--	--	--	--
69 "	3	9.00	10.20	9.54	+ 9.8%	13.7	14.7	14.3	+ 2.1%
79 "	3	9.29	9.56	9.42	+ 8.3%	11.7	15.4	13.9	- 6.1%
85**"	3	--	--	--	--	12.4	15.1	13.4	- 4.7%
91 "	3	--	--	--	--	12.4	13.7	13.2	- 7.0%

* = No. of animals
** = 5 wks off drug

Table XXVIII

Red Cell Count (Millions) and Hemoglobin (Gms. per 100c.c.)
"All" Group and Cincophen Groups -- Male

		Red Cell Count				Hemoglobin			
		Low	High	Mean	Variation from cont.	Low	High	Mean	Variation from cont.
"All" Group									
42 wks	8*	7.67	9.57	8.47	- 3.0%	--	--	--	--
69 "	7	7.93	9.52	8.64	+ 0.3%	12.6	15.6	14.1	+ 0.7%
79 "	7	8.15	10.33	9.07	+ 4.2%	13.5	16.2	15.2	+ 2.7%
85** "	7	--	--	--	--	13.5	16.2	15.0	+ 6.4%
91 "	5	--	--	--	--	12.4	15.8	14.1	- 0.7%
Controls									
42 wks	8	8.09	9.85	8.74		--	--	--	
69 "	6	8.00	9.63	8.61		11.5	18.0	14.0	
79 "	6	8.14	9.22	8.70		12.4	16.7	14.8	
85 "	5	--	--	--		12.9	15.7	14.1	
91 "	5	--	--	--		12.8	16.2	14.2	
Cincophon									
42 wks	6	7.49	8.97	8.20	- 6.2%	--	--	--	--
69 "	4	7.90	8.65	8.25	- 4.2%	15.4	16.2	15.8	+12.8%
79 "	4	8.32	9.95	9.26	+ 6.4%	15.8	17.6	16.3	+10.1%
85** "	3	--	--	--	--	15.0	16.2	15.7	+11.3%
91 "	3	--	--	--	--	15.0	15.9	15.5	+ 9.1%

* = No. of animals
** = 5 wks off drug

Table XXIX

White Cell Differentiation

Group	No. of Animals	Neutrophils	Eosinophils	Basophils	Total Gran.	Lymphocytes	Monocytes
Males							
Acetanilid	5	38.2	2.6	0	40.8	59.2	0
Phenacetin	4	29.5	5.2	0	34.7	65.2	0
Cincophen	3	31.3	3.7	0	35.0	65.0	0
"All" Group	5	39.6	0.6	0.2	40.4	58.4	1.2
Controls	4	37.0	1.0	0	38.0	60.5	1.5
Aspirin	5	32.7	0	0	32.7	67.3	0
Phenobarbital	4	42.0	0.7	0	42.7	57.3	0
Controls	6	25.8	0	0	25.8	74.0	0.2
Amytal	7	28.2	0.9	0	29.2	70.3	0.5
Barbital	5	25.0	0	0	25.0	75.0	0
Controls	7	27.8	0	0	27.8	71.8	0.3
Females							
Aspirin	3	29.7	2.0	0.3	32.0	67.0	1.0
Phenobarbital	4	30.7	3.0	0	33.7	66.2	0
Controls	4	33.5	3.5	0	37.0	62.7	0.2
Allonal	4	48.0	2.5	0	55.0	49.5	1.0
Alurate	5	37.5	1.4	0	38.9	60.1	1.0
Controls	4	36.0	0.2	0	36.2	62.5	1.2
Aminopyrine	7	39.0	1.4	0	44.0	58.3	1.3
Antipyrine	3	33.0	0.7	0	33.7	65.3	1.0
Controls	4	32.0	3.5	0	35.5	63.5	1.0

Table XXX

Estrus Cycles -- Caffeine Group

		Oct. -- Dec. 61 days Age 34-43 wks Drug 9-15 ''	Jan. -- Feb. 42 days Age 46-52 wks Drug 36-42 ''	May -- June 30 days Age 62-66 wks Drug 52-56 ''	Sept. -- Oct. 40 days Age 81-87 wks Drug 71-77 ''	Mar. -- Apr. 20 days Age 108-112 wks Drug 98-102 ''
Caffeine	Max.	17	12	7	8	2
	Min.	9	5	0	0	0
	Mean	13.8	9.9	2.9	3.6	0.6
Controls	Max.	15	11	6	6	3
	Min.	11	8	0	0	0
	Mean	13.7	10.6	3.5	2.5	1.0

Animals Showing no Cycles during Periods

	1st period	2nd period	3rd period	4th period	5th period
Caffeine	0 of 7	0 of 7	2 of 7	1 of 6	3 of 6
Controls	0 of 5	0 of 5	1 of 4	1 of 4	2 of 3

Average cycles for animals present thru all 5 periods (192 days)
Caffeine 26 (6 rats) Controls 24.9 (3 rats)

Average 4 -- day cycles for animals present thru all 5 periods
Caffeine 15.6 Controls 15.0

Table XXXI

Estrus Cycles Aminopyrine - Antipyrine Group

		Age 10-14wks Jan. - Feb. 42 days Drug 0-4wks	Age 27-31wks May - June 30 days Drug 17-21wks	Age 61-65wks Jan. - Feb. 31 days Drug51-55wks	Age 72-76wks Mar. - Apr. 27 days Drug62-66wks
Aminop.	Max.	11	6	7	5
	Min.	10	2	0	0
	Mean	10.3	3.6	2.4	1.4
Antipyrine	Max.	11	7	7	5
	Min.	10	1	0	1
	Mean	10.3	3.5	3.9	1.9
Controls	Max.	11	8	6	4
	Min.	8	0	1	0
	Mean	10.0	4.4	3.5	1.7

Animals Showing no Cycles during Periods

	1st period	2nd period	3rd period	4th period
Aminopyrine	0 of 9	0 of 8	3 of 7	3 of 7
Antipyrine	0 of 9	0 of 9	0 of 6	2 of 6
Controls	0 of 9	0 of 9	0 of 7	0 of 6

Av. cycles for animals present thru all 4 periods (130 days)
Aminop. 17.6 (8 rats) Antip. 21 (6) Controls 19.8 (6)

Av. four-day cycles for animals present thru all 4 periods
Aminop. 10.25 Antip. 10.7 Controls 8.7

Table XXXII

Estrus Cycles -- Aspirin -- Phenobarbital Group

		Nov. -- Dec. 50 days Age 17-24 wks Drug 7-14 "	Jan. -- Feb. 42 days Age 26-33 wks Drug 16-23 "	May -- June 30 days Age 43-47 wks Drug 33-37 "	Sept. -- Oct. 40 days Age 62-68 wks Drug 52-58 "	Mar. -- Apr. 40 days Age 86-92 wks Drug 76-82 "
Aspirin	Max.	13	10	6	7	0
	Min.	6	1	0	1	0
	Mean	10.7	6.9	3.0	4.2	0
Phenobarb.	Max.	13	11	7	6	9
	Min.	9	6	0	1	0
	Mean	11.4	9.1	3.6	3.3	3.0
Controls	Max.	12	10	6	7	6
	Min.	9	1	0	3	2
	Mean	10.6	6.2	3.3	4.4	3.7

Animals Showing no Cycles during Periods

	1st period	2nd period	3rd period	4th period	5th period
Aspirin	0 of 8	0 of 8	2 of 8	0 of 6	3 of 3
Phenobarb.	0 of 7	0 of 7	1 of 7	0 of 6	0 of 4
Controls	0 of 7	0 of 7	3 of 7	0 of 5	2 of 5

Average cycles for animals present thru all 4 periods (162 days)
Aspirin 23.3 (3 rats) Phenobarb 23.1 (5) Controls 24.4 (5)

Average 4 -- day cycles for animals present thru all 4 periods
Aspirin 11.8 Phenobarb 15.3 Controls 10.6

Table XXXIII

Estrus Cycles -- Allonal -- Alurate Group

		Jan. -- Feb. 42 days Age 19 - 25 wks Drug 9 - 15 "	May -- June 30 days Age 35 - 39 wks Drug 25 - 29 "	Jan. -- Feb. 31 days Age 70 - 74 wks Drug 60 - 64 "	Mar. -- Apr. 27 days Age 80 - 84 wks Drug 70 - 74 "
Allonal	Max.	10	8	6	2
	Min.	4	2	1	0
	Mean	8.5	5.3	2.5	1.2
Alurate	Max.	11	8	8	4
	Min.	5	0	0	0
	Mean	8.0	4.7	1.8	2.0
Controls	Max.	10	7	4	4
	Min.	7	2	0	1
	Mean	8.6	4.8	1.8	1.6

Animals showing no Cycles During Periods

	1st period	2nd period	3rd period	4th period
Allonal	0 of 9	0 of 9	0 of 6	2 of 6
Alurate	0 of 9	0 of 9	3 of 6	1 of 6
Controls	0 of 9	0 of 9	1 of 7	0 of 7

Average cycles for all animals present thru all 4 periods (128 days)
Allonal 18 (6 rats) Alurate 17.5 (6) Controls 16.3 (7)

Average 4 -- day cycles for all animals present thru all periods
Allonal 8.5 Alurate 7.3 Controls 7.6

Table XXXIV

Maze Learning and Activity
Caffeine Groups -- Male and Female

Learning

	Males			Females		
	Age 28 wks -- 18 wks on drug			Age 29 wks -- 19 wks on drug		
	Test(6)*	Variation from cont.	Control(5)	Test(7)	Variation from cont.	Control(5)
Av. Trials	15	-12.8%	17	18.4	-24.0%	24.4
Av. Errors	60	+22.0%	49	55.0	-29.0%	78.0
Trav. T.	9.7'	-12.0%	11'	9.7'	-37.0%	15.4'
Tot. T.	48.0'	- 6.0%	51'	29.3'	-59.0%	71.0'

Re-learning

	Males			Females		
	6 weeks after learning			11.5 weeks after learning		
	Test(6)	Variation from cont.	Control(5)	Test(7)	Variation from cont.	Control(5)
	6 trials		6 trials	6 trials		6 trials
Av. Error	4.8	+16.7%	1.8	13.0	-22.0%	16.6
Trav. T.	146 "	- 6.0%	156 "	144 "	-30.0%	208 "
Tot. T.	10.2'	+38.0%	7.3'	9.9'	-40.0%	16.7'

* = No. of animals

Table XXXV

Maze Learning and Activity
Phenobarbital Groups -- Male and Female

Learning

	Males			Females		
	Age 70 wks -- 60 wks on drug			Age 72 wks -- 62 wks on drug		
	Test(6)*	Variation from cont.	Control(6)	Test(6)	Variation from cont.	Control(6)
Av. Trials	21	+ 8%	19.6	19.4	+29%	15
Av. Errors	126	+20%	104.7	93.5	+37%	70
Trav. T.	15.4'	+24%	12.7'	9.7	+20%	8.1'
Tot. T.	93.7'	+28%	72.6'	64.0	+19%	54.0'

Re-learning

	Males			Females		
	10½ wks after learning			12½ wks after learning		
	Test(6)	Variation from cont.	Control(6)	Test(6)	Variation from cont.	Control(6)
Av. Trials	16	+37%	11.7	13.0	+59%	8.7
Av. Errors	36	+53%	23.5	33.0	+106%	16.3
Trav. T.	6.7'	+67%	4.0'	6.0'	+37%	3.9'
Tot. T.	63.3'	+155%	24.4'	65.4'	+250%	18.5'

* = No. of animals

Table XXXVI

Maze Learning and Activity
"All" Group & Amytal Groups -- Males
79 weeks of age -- after 69 weeks of drug feeding

	"All" Group(5)*		Control(10)		Amytal(5)
	12 Trials	Variation from cont.	12 Trials	Variation from cont.	12 Trials
Av. Errors	91	+61%	56.5	+26%	71
Trav. T.	11.1'	+68%	6.6'	+41%	9.3'
Tot. T.	105'	+113%	49.3'	+44%	71.0'

* = No. of animals

Table XXXVII

Maze Learning and Activity
Aspirin Groups -- Male & Female

Learning

	Males			Females		
	Age 70 wks -- 60 wks on drug			Age 52 wks -- 62 wks on drug		
	Test(7)*	Variation from cont.	Control(6)	Test(6)	Variation from cont.	Control(6)
Av. Trials	21	+ 7%	19.7	17	+13%	15
Av. Errors	106	+0.9%	105	68	- 3%	70
Trav. T.	16'	+29%	12.4'	8.5'	+ 5%	8.1'
Tot. T.	118'	+62%	72.6'	68'	+26%	54'

Re-learning

	Males			Females		
	10½ wks after learning			12½ wks after learning		
	Test(7)	Variation from cont.	Control(6)	Test(6)	Variation from cont.	Control(6)
Av. Trials	11.8	+ 1%	11.7	10.0	+15%	8.7
Av. Errors	23.0	- 2%	23.5	18.7	+15%	16.3
Trav. T.	5.6'	+40%	4.0'	5.3'	+36%	3.9'
Tot. T.	20.7'	-16%	24.4'	17.6'	- 5%	18.5'

* = No. of animals

Table XXXVIII

Maze Learning and Activity
Aminopyrine -- Antipyrine Groups -- Female

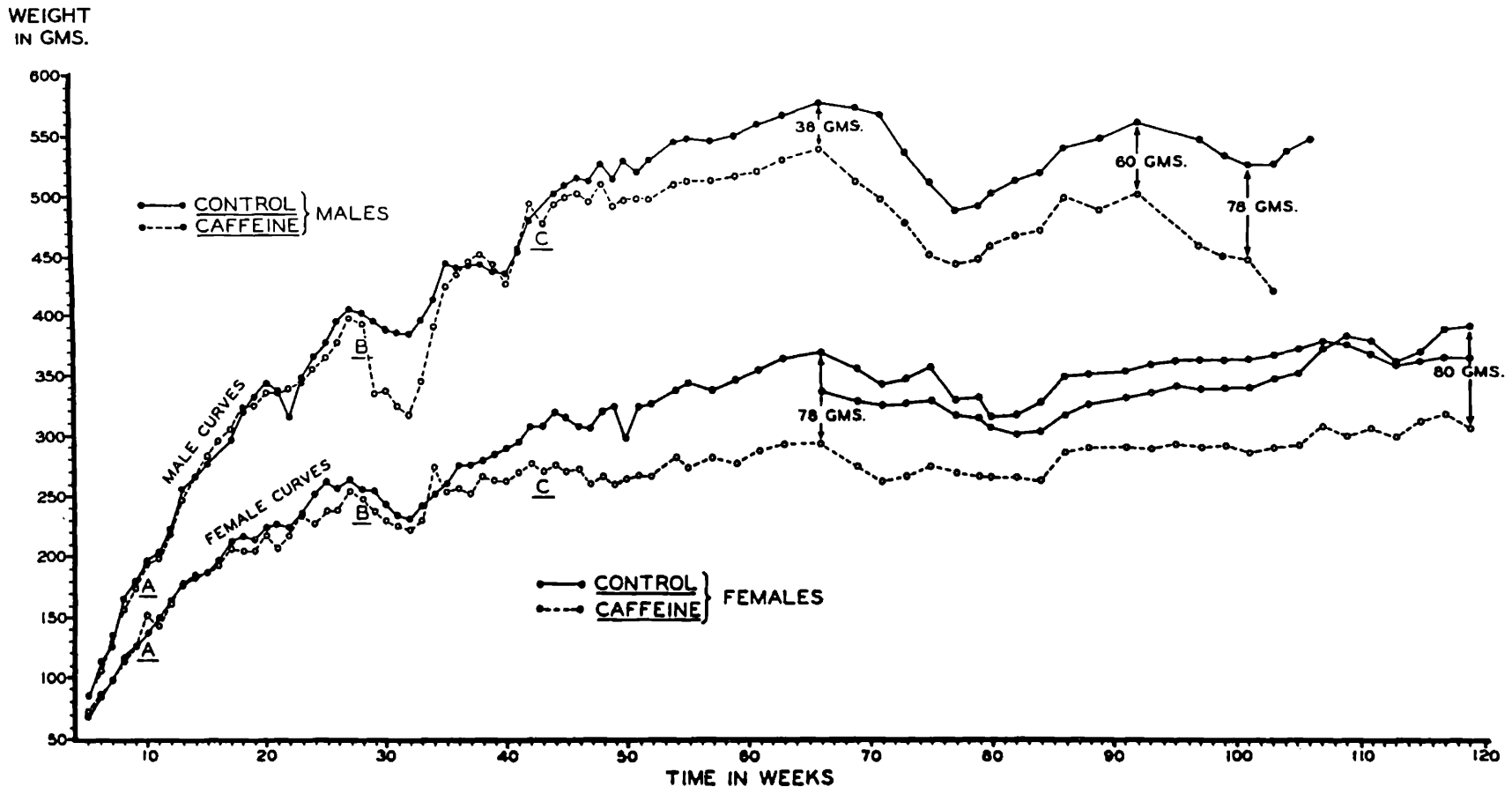
Learning

	Aminopyrine			Antipyrine	
	Age 59 wks -- 49 wks on drug Test(8)*	Variation from cont.	Controls(7)	Age 59 wks -- 49 wks on drug Test(7)	Variation from cont.
Av. Trials	17.3	+ 2%	16.9	18.0	+ 6%
Av. Errors	79.0	-0.5%	79.4	86.4	+ 9%
Trav. T.	7.5'	+ 3%	7.3'	8.7'	+19%
Tot. T.	53.4'	-17%	64.6'	74.3'	+15%

Re-learning

	Aminopyrine			Antipyrine	
	12 weeks after learning Test(8)	Variation from cont.	Controls(7)	12 weeks after learning Test(7)	Variation from cont.
Av. Trials	14.9	+26%	11.8	16	+36%
Av. Errors	43.1	+75%	24.6	55	+124%
Trav. T.	7.3'	+30%	5.6'	7.9'	+41%
Tot. T.	46.2'	+113%	21.7'	43'	+98%

* = No. of animals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IN GMS.
600
550
500
450
400
350
300
250
200
150
100
50
CONTROL
CAFFEINE
MALES
38 GMS.
60 GMS.
78 GMS.
MALE CURVES
FEMALE CURVES
A
B
C
78 GMS.
80 GMS.
CONTROL
CAFFEINE
FEMALES
10
20
30
40
50
60
70
80
90
100
110
120
TIME IN WEEKS

Fig. 1.

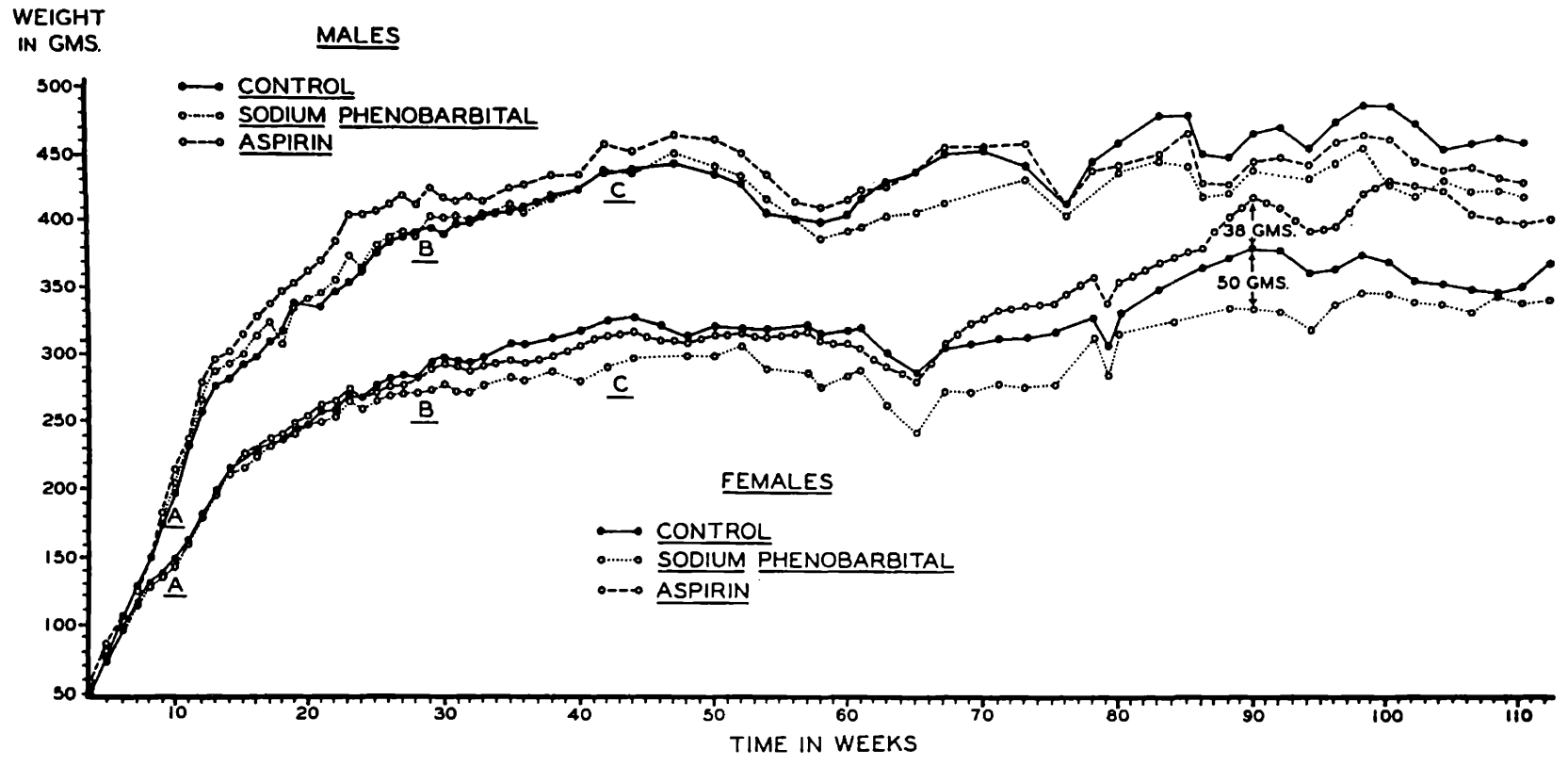

WEIGHT
IN GMS.
MALES
CONTROL
SODIUM PHENOBARBITAL
ASPIRIN
500
450
400
350
300
250
200
150
100
50
A
B
C
38 GMS.
50 GMS.
FEMALES
CONTROL
SODIUM PHENOBARBITAL
ASPIRIN
10
20
30
40
50
60
70
80
90
100
110
TIME IN WEEKS

Fig. 2.

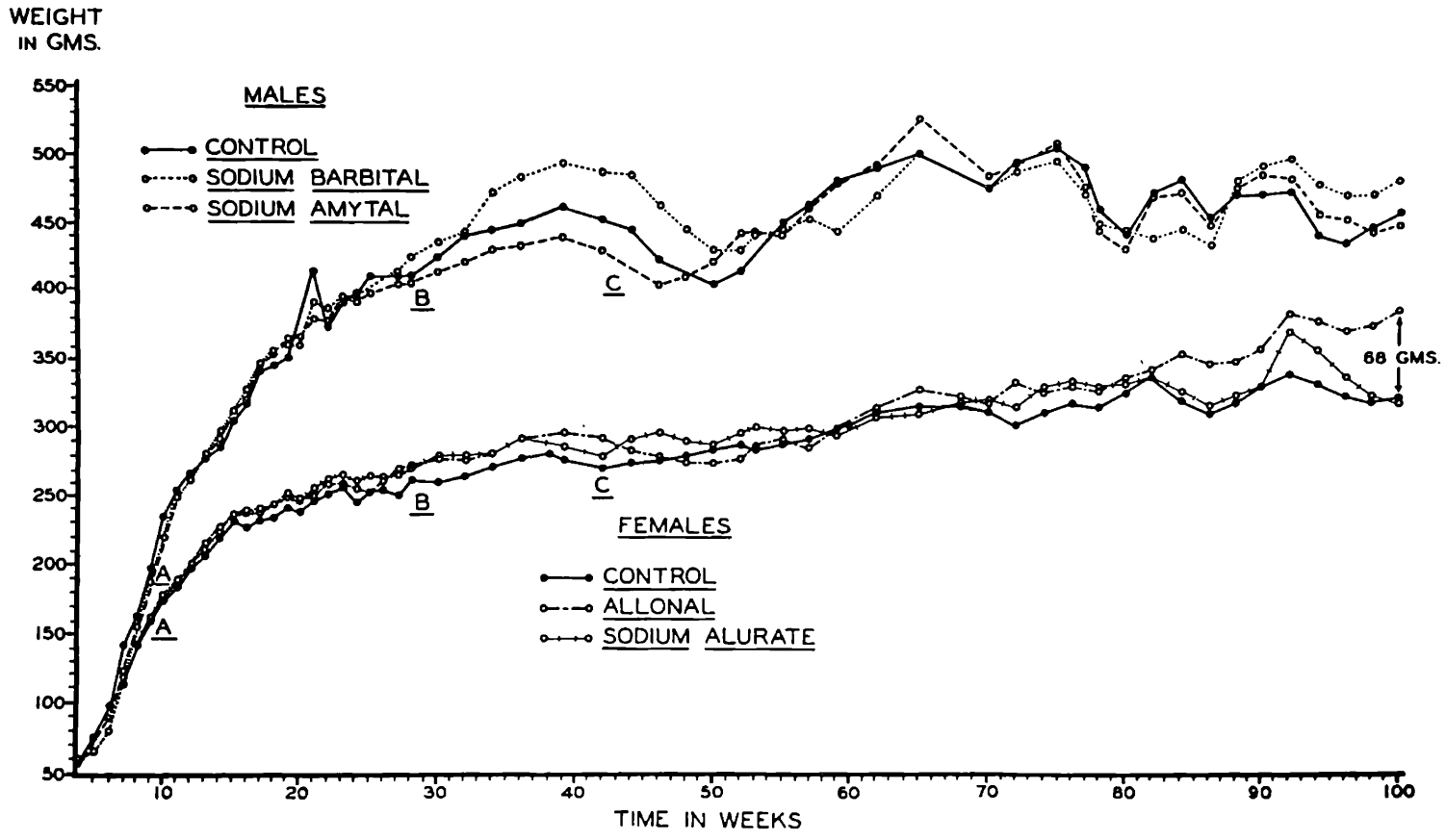

WEIGHT
IN GMS.
MALES
CONTROL
SODIUM BARBITAL
SODIUM AMYTAL
A
B
C
68 GMS.
FEMALES
CONTROL
ALLONAL
SODIUM ALURATE
550
500
450
400
350
300
250
200
150
100
50
10
20
30
40
50
60
70
80
90
100
TIME IN WEEKS

Fig. 3.

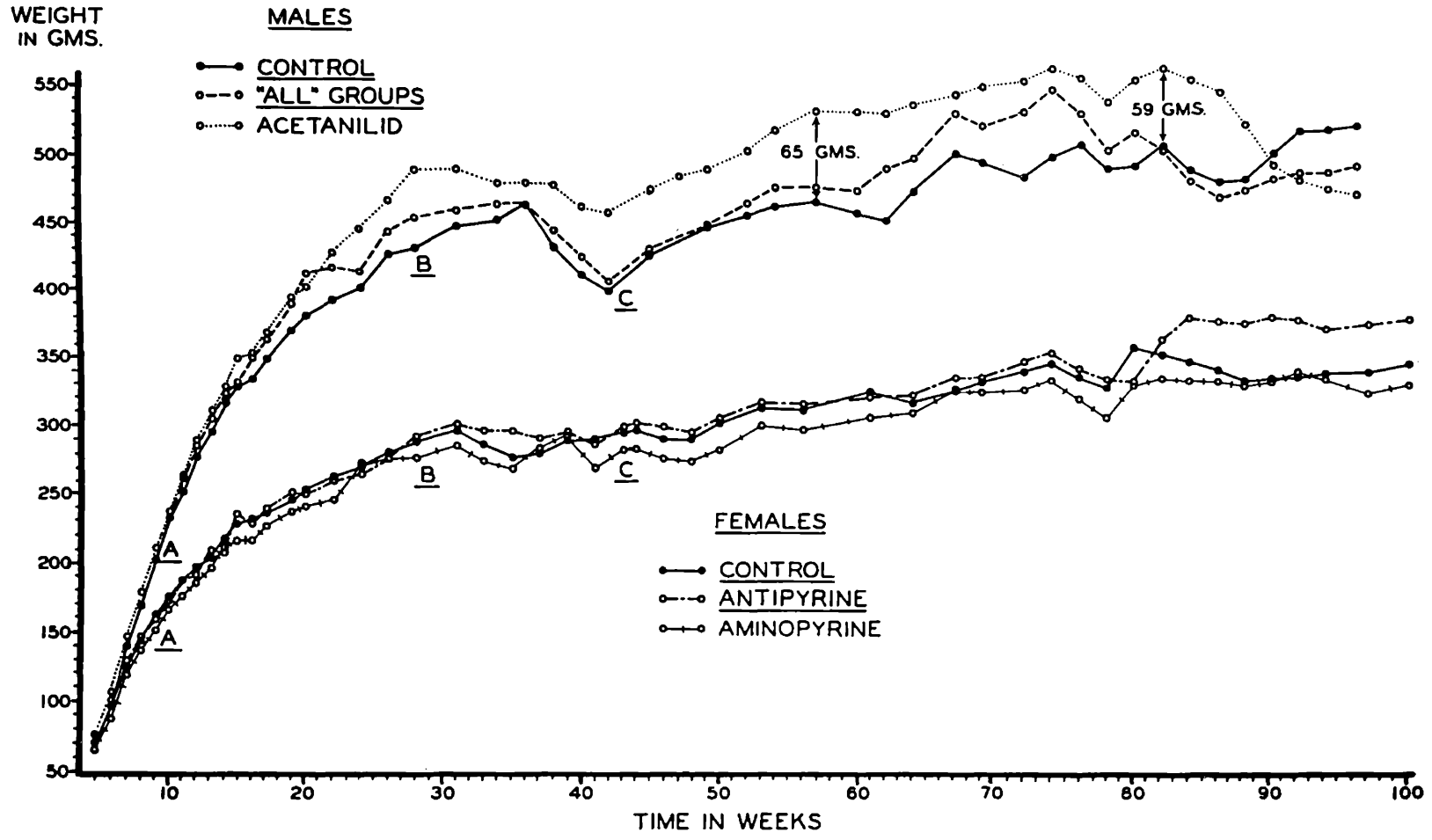

WEIGHT IN GMS.
MALES
CONTROL
"ALL" GROUPS
ACETANILID
65 GMS.
59 GMS.
A
B
C
FEMALES
CONTROL
ANTIPYRINE
AMINOPYRINE
550
500
450
400
350
300
250
200
150
100
50
10
20
30
40
50
60
70
80
90
100
TIME IN WEEKS

Fig. 4.

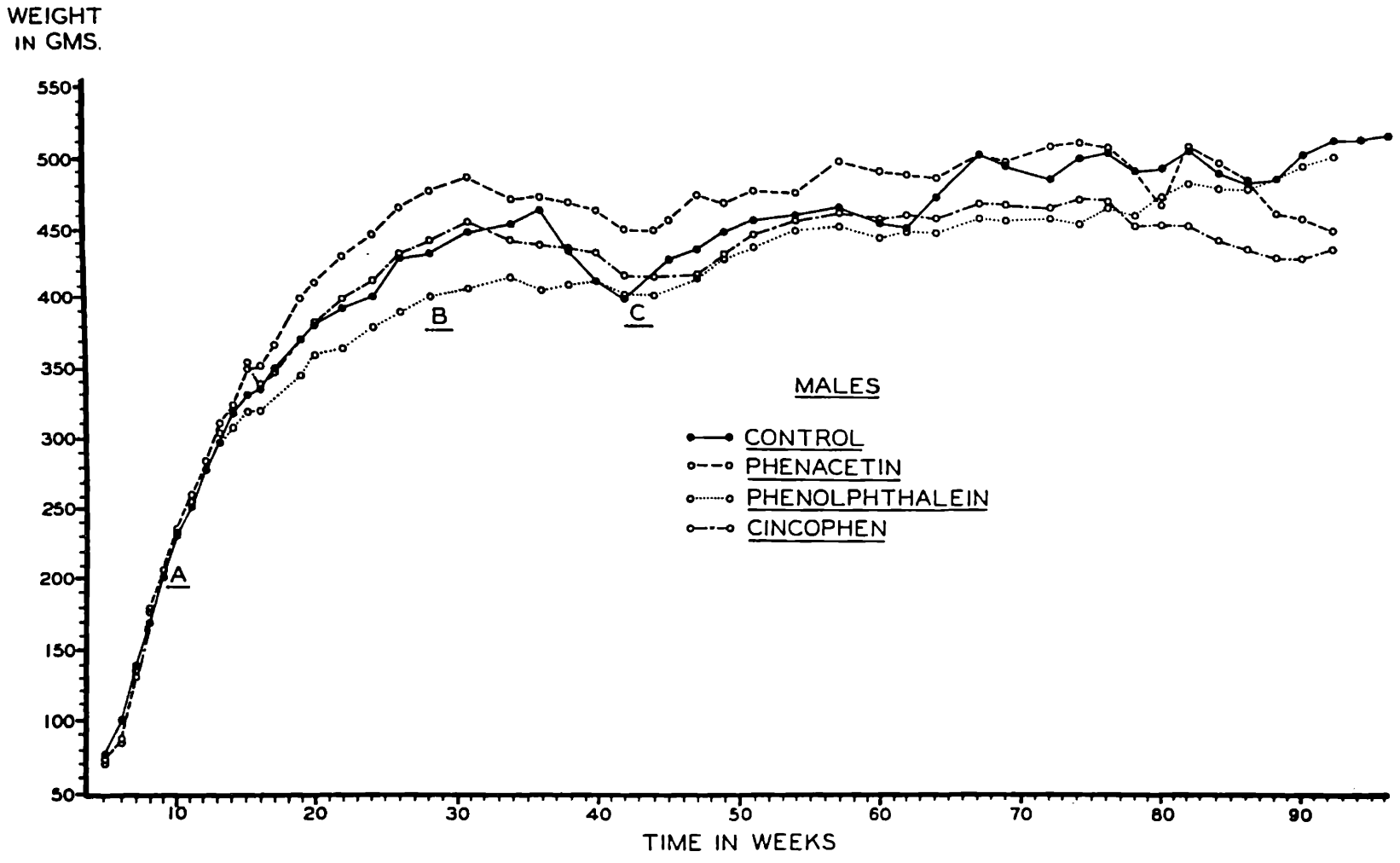
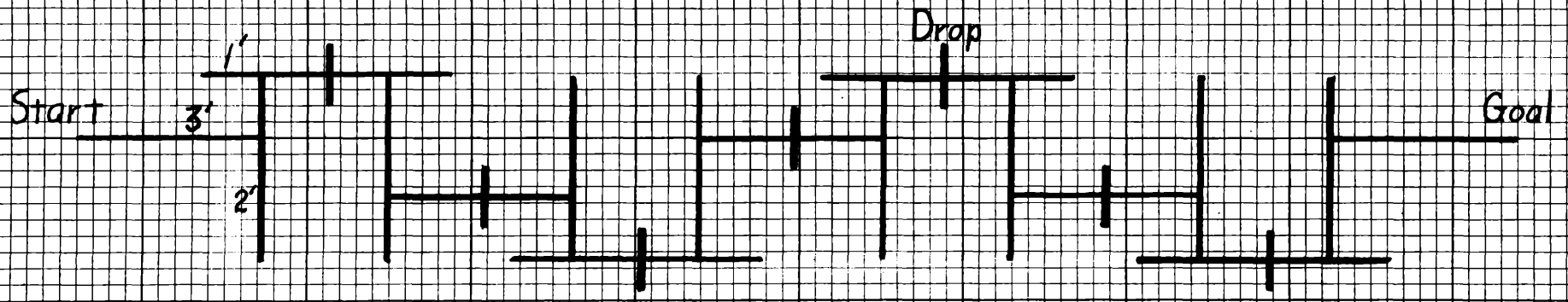

WEIGHT
IN GMS.
550
500
450
400
350
300
250
200
150
100
50
10
20
30
40
50
60
70
80
90
TIME IN WEEKS
A
B
C
MALES
CONTROL
PHENACETIN
PHENOLPHTHALEIN
CINCOPHEN

Fig. 5.



Maze Pattern

Fig. 6.

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