THE USE OF SEXUAL ATTRACTANT PHEROMONES TO INCREASE THE ACCEPTABILITY OF THE MALE CHEMOSTERILANT 3-Chloro-1,2-Propanediol BY WILD NORWAY RATS (Rattus norvegicus)

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THE USE OF SEXUAL ATTRACTANT PHEROMONES
TO INCREASE THE ACCEPTABILITY OF THE MALE CHEMOSTERILANT
3-Chloro-1, 2-Propanediol
BY WILD NORWAY RATS (Rattus norvegicus).

presented by

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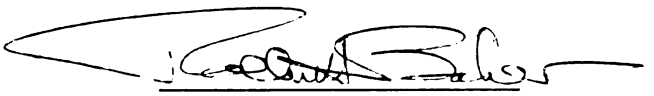

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ABSTRACT

THE USE OF SEXUAL ATTRACTANT PHEROMONES
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Numerous works have demonstrated the effectiveness of using sterilization techniques as methods of population control in pest species. Recently an alpha-chlorohydrin, 3-chloro-1,2-propanediol, which exhibits permanent chemosterilant properties in Norway rats, Rattus norvegicus, has been developed by R. J. Ericsson of the Upjohn Co. This material seems to be virtually one hundred per cent effective in male rats of this species, if ingested in doses of at least 35 mg/kg. Wild individuals which encounter the material, however, quickly develop a severe aversion which may inhibit sufficient food consumption of the material to induce sterility.

In an attempt to counteract this behavioral mechanism on the part of the rats, estrous urine collected from Long-Evans laboratory rats was mixed with the food containing the chemosterilant prior to introducing it to the wild rats, thus making it more acceptible to them and increasing the rate of consumption of the chemosterilant.

The following experiments were performed to demonstrate the attractiveness of this additive in a series of six tests. 1) Wild-caught animals were given a 1.0 per cent concentration of the chemosterilant in their food for a period of six days to determine the

severity of bait shyness. 2) These rats were given a similar 1.0 per cent diet for two days, then three ml. of estrous urine was added daily to the food containing the chemosterilant in order to determine its effect as an attractant. 3) Two groups of animals were given a 0.5 per cent concentration of the chemosterilant in the food simultaneously, but one group received three ml. of estrous urine daily while the other did not. 4) Several animals were given a 1.0 per cent mixture of the chemosterilant with the addition of three ml. of estrous urine daily and results were compared with those obtained from experiment 1. 5) Several females were separated into two groups. One group was given a 1.0 per cent mixture of chemosterilant with the addition of three ml. of estrous urine daily, while the other was given only the 1.0 per cent mixture without the addition of the urine. 6) A test was made to determine the existence of synergistic effects between the toxicity of the chemosterilant and a reduction of food consumption by rats.

There are two appendices to this paper. Appendix A involves a discussion of current rat control techniques practiced in Michigan, and contains suggestions for possible improvement of these policies. Appendix B deals with a graphic model of the ecology of rat populations within an urban ecosystem.

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By

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INTRODUCTION & LITERATURE REVIEW

The Norway rat (Rattus norvegicus) has long been a problem to mankind. The species, which is endemic to the Old World, became identified as a significant health hazard during the Middle Ages when it acted as a vector for the dread bubonic plague which revaged Europe during that period (Zinsser, 1935). Since that time it has become a recognized vector for some fifty or more diseases.

After being transported to the New World aboard ships of explorers and merchants (Darlington, 1957: 388), this rodent spread across the continents following the westward movements of human populations, and became firmly entrenched as a species and a semiparasite of man.

Presently, this highly adaptable murid rat has been successful in continuing to be a public health menace especially in cities but also in agricultural areas throughout the world, in spite of elaborate control operations. In the United States alone, the estimated total cost of damages caused by the gnawing teeth and filthy excrements of these animals lies somewhere between $900,000,000 and $1 billion annually (Bjornson et al., 1956).

In addition to the simple destruction of property, these animals are still significant factors in the transmission of diseases and also the cause of infant mortality and tissue damage to children through the lacerations and puncture wounds inflicted by their bites (Scott, 1965; Ruskin, 1968).

Currently, most urban areas in the United States are attempting to curtail their rodent problems through rat control programs of varying efficiency (See Appendix A). Due to the adaptability of these rodents, and to the relatively inefficient methods of instituted control measures, the problem persists, and in many instances, is believed to be increasing.

HISTORY OF RAT CONTROL

Historically, man initiated his attempts to control rat populations through the use of the simple rat traps. These were soon found to be relatively inefficient. This was mainly due to the rats' high learning capabilities which lead to the phenomenon of "trap shyness", or the avoidance mechanism utilized by rats when encountering traps (Brown, 1960; Calhoun, 1962a: 88).

The next attempt to control or reduce rat infestations involved the use of such poisons of the single dose variety as red Squill (Urginea maritima), strychnine (Strychnos sp.), arsenic, phosphorus, and more recently, alpha-naphthylthiourea (ANTU), sodium fluoracetate (1080), and flouracetamide (1081). These poisons work to some extent, but again lead to "bait shyness" on the part of the rats (Bjornson et al., 1965; Brown, 1960). In addition, many of these materials provided dangerous hazards to nontarget species, including humans, which chanced to come in contact with them (Ruskin, 1968).

The poisoning procedure was coupled as early as 1913 with more functional measures such as rat-proofing buildings, garbage removal, and other environmental manipulations (Simpson, 1913). These practices were found to be a very efficient combination, but unfortunately, urban ecosystems were not and are still not compatible with such a program to a

great extent (Calhoun, pers. comm.). This is due to several factors, but can be attributed primarily to building deterioration and housing policy (Beyer, 1965).

In 1944, Dr. Karl P. Link (Overmann et al., 1944) completed extensive research on the bovine hemmorrhagic sweet clover disease, which lead directly to the discovery of the multiple dose anticoagulent poisons. These poisons function very well as rodent control measures, and are still used today by most authorities. These have several drawbacks, however, such as the necessity of daily surveillance of the bait material by personnel. In addition, there is the simple biological fact that merely killing off adults in an increasing rat population may not be an effective control measure. This is partially due to the territorial behavior of the animals, and their propensity for immigration into an area recently vacated due to mortality (Calhoun, 1961; Davis, Emlem, and Stokes, 1948; Knipling, 1955). Howard (1967) has theorized that, in fact, the converse may occur, in that the simple introduction of mortality may actually cause an increase in density of rodent infestations.

In recent years the advent of antifertility agents (Knipling, 1955, 1960) or chemosterilants offered a new approach to possible control of rat populations (Bock and Johnson, 1956; Davis, 1961). Theoretically, this method of control should be superior to simply killing off adult or even juvenile animals, since lack of mortality in the sterilization process should result in no immigrational replacement activity. Conversely, the permanently sterilized dominant animals simply remain, thus effectively preventing any reproductive recruitment into the population, and simultaneously inhibiting the immigration of fertile

individuals due to the inherent territorial dominance of those present (Knipling, 1955).

Conceptually, the idea was highly desirable. Unfortunately, however, chemosterilants tested were generally found inefficient as sterilants (Kennelly et al., 1970), increased bait shyness (Marsh and Howard, 1969) or caused high mortality rates (Ericsson, 1970). An additional problem lies in the possibility of sterilization techniques creating an adverse effect by reducing the dominance and libido of the animals which ingest it (Howard, 1967; Calhoun, pers. comm.).

One of the latest advances in the field of chemosterilants has been made by Dr. Ronald J. Ericsson of the Upjohn Company. He has developed an alpha-chlorohydrin compound (3-chloro-1,2-propanediol) which causes temporary sterility, permanent irreversible sterility in male Norway rats, or mortality in both male and female rats, depending upon the amount ingested. The drug, labeled U-5897, has been found to be effective in this manner when administered abdominally, by gavage, mixed in food, or mixed in water (Ericsson, 1970; Ericsson and Connor, 1969). There is a marked advantage in the drug over many other experimental chemosterilants due to the fact that only a single dose of 35 mg/kg body weight is required for permanent sterility. This is a result of the formation of gross lesions and spermatic granulomata in the caput epididymus caused by the action of the drug, thus blocking sperm passage. At a dose level of 152 mg/kg, which has been determined to be the LD-50 dose rate in Norway rats (Ericsson, 1970), mortality occurs due to the development of lesions in the myelin sheath of the brain, and also to intestinal lesions and resultant hemorrhagic gastroenteritis (Ericsson, pers. comm.).

An important quality of the drug is the wide separation (2.5x10) between the minimal effective dose (MED) which causes temporary sterility (6 to 7 mg/kg) and the LD-50 dose. The importance of this fact lies in the greater likelihood of the target species being able to eat enough of the material to become infertile, but not ingesting enough to cause mortality, thereby removing it from the population of territorially functional male animals.

Ericsson (1970) also notes that the chlorohydrin chemosterilant has no observable effects in reducing either dominance or libido as have been demonstrated with other antifertility measures such as castration.

One of the problems which still exists with this drug is its effect in causing bait refusal by the animals (Ericsson, pers. comm.). This would tend to reduce its effectiveness in a wild population since it will not be consumed in sufficient quantities or by sufficient numbers of individuals to be effective in reducing the density of the population.

Related entomological problems in attracting insects to traps in field situations have been partially solved through the use of sexual attractant pheromones (Roelofs and Coneau, 1970; Brady et al., 1971). The effects of pheromones as sexual attractants in mammals have been noted in canines (Beach and Gilmore, 1949), but no detailed research has been conducted in that area. Other studies have demonstrated the use of mammalian pheromones as scent markers (Ralls, 1971), and as influencing mechanisms in reproduction (Parks and Bruce, 1961; Whitten, 1966; and Bronson, 1968). The research described in this paper is an attempt to utilize a sexual attractant pheromone to reduce bait shyness in wild Norway rats in conjunction with the alpha-chlorohydrin chemosterilants described above.

OBJECTIVES

In view of the previously stated inadequacies of current rodent control techniques, the research upon which this paper is based is an attempt to evaluate a new method. That is to say, it is an attempt to make a relatively new experimental technique more functional by making it more attractive to the rodents themselves.

The method described herein of utilizing olfactory sexual attractants or pheromones to make baits more inticing has been used previously in the control of insects (Roelofs and Coneau, 1970; Brady, et al., 1971). Little however, has been written which concerns the use of this technique with mammals.

As a supplemental appendix to my basic research problem, I would like to discuss the current rat control techniques as they are practiced in various cities in southern Michigan and attempt to suggest possible alterations or additions which might improve these existing techniques. These proposed suggestions will include additional methods of enumerating rat populations and a discussion of rodent breeding seasons which could lead to better evaluation of control efforts and to perhaps a more efficient reduction in populations through better synchronization of these control efforts with reproductive periods.

A second appended supplement to this paper will involve a graphic model which describes the ecological interactions of a rat population within the biological framework of an urban ecosystem. This graphic representation will be discussed at length for additional clarification of its structure.

METHODS AND MATERIALS

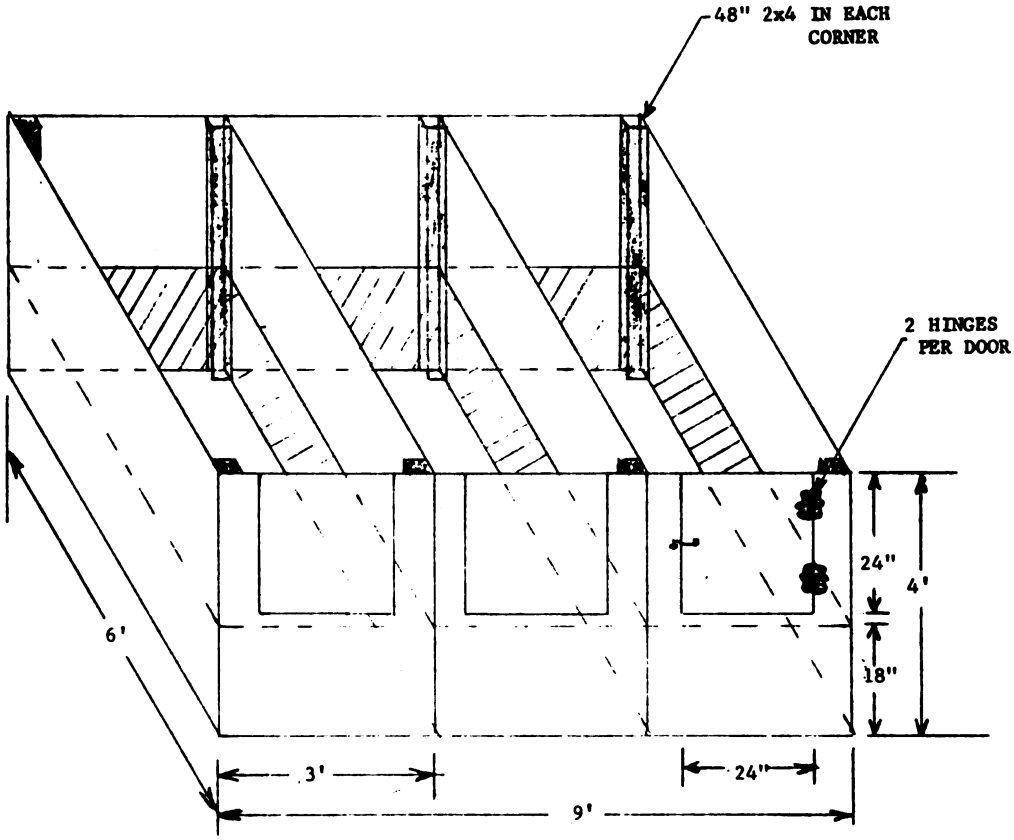
Forty-five wild Norway rats were caught in Tomahawk and National Wire live traps from various locations in the greater Lansing, Michigan, area, to be used as the experimental subjects for the testing of pheromones as attractants with chemosterilant baits. After capture, these rats were housed in quarantine cages prior to being placed in 3' x 6' experimental pens for the actual testing (Figure 1).

The experimental pens were constructed from 3/4" plywood sheets which were 4' in height. The lower 16" of all four sides of these pens were lined with 22 gauge galvanized sheet metal as a preventative measure against gnawing by the rats. All of the pens were kept on a light cycle of alternating twelve hours of light and twelve hours of darkness. The ambient temperature was regulated at 70°F.

Each pen was supplied with an unlimited quantity of water. Food was provided in the form of chicken feeder mash which was placed in 4" diameter finger bowls. The mash was used in preference to prepared lab rat chow in the form of nuggets, in order to prevent the animals from removing it from the feeding dish and storing it for later consumption (Marx, 1951). The enclosures also contained one galvanized tin nest can for each rat present in the pen, and an additional wooden nest box. The floors of the pens were covered with wood chips for sanitation purposes, but this material was only changed at the end of the testing period for each rat or pair of rats. The subsequent cage litter soiled by only that animal or pair of animals was allowed to accumulate in an attempt to increase or maximize the familiar home range or territorial behavior among the animals (Ralls, 1971).

FIGURE 1:

1 BLOCK OF 3 RAT PENS



Several types of experiments were conducted upon these wild rats in order to determine the effects of estrous urine from female laboratory rats in attracting wild rats to a chemosterilant bait.

The estrous urine used in the experiments was collected from female Long-Evans laboratory rats, in accordance with suggestions offered by John B. Calhoun (pers. comm.). Metabolic cages which separated and caught both the urine and the feces of the animals within them were used for the collection of this material.

The stage of the estrous cycle in the lab rats was determined daily by taking vaginal smears following the procedure outlined by Zarrow (1964). Only females actually in estrous, i.e. having cornified epithelial cells present in vaginal smears, were used for the daily collection of urine. Urine from all such females was mixed together and was used only on the day it was collected. Any unused portion was discarded rather than stored for use at a later date, thus preventing any possibility of decomposition of the hemical structure of the pheromone material due to aging.

Experiment I:

Fourteen adult rats consisting of six pairs and two single males were given a diet of plain or untreated normal mash for five days to determine their average daily food consumption. At the end of this period, the animals were weighed. The normal food was then replaced with a homogeneous mixture prepared by the Upjohn Company and obtained through the cooperation of Dr. R. J. Ericsson. This mixture consisted of the same chicken feeder mash but had the addition of 1 per cent by weight, U-5897. The animals were kept on this diet for five days in an attempt

to determine their acceptance of this material as food, and to determine their average daily consumption of the mixture. At the end of the experimental period all dead animals were weighed and examined to determine cause of death and the effects of the chemosterilant. This became standard procedure for each of the experiments, as did the initial several day diet of plain mash, and the recording of weights at the end of this period.

Experiment II:

Ten adult rats consisting of one single male and four pairs from Experiment I and one new single male were tested with a 1.0 per cent mixture of U-5897 after a 10 day average was taken for normal food consumption. The animals were placed on a diet of the 1.0 per cent mixture for two days, and the average consumption was calculated for this diet. At the end of this period, three ml. of estrous urine were added to the 1.0 per cent mixture of chemosterilant daily for two additional days, and the average consumption was again determined for the food with the pheromone added. This experiment was a preliminary attempt to evaluate the relative efficiency of estrous urine as an attractant when mixed with bait material which had been previously demonstrated to be undesirable to rats (Ericsson, pers. comm.).

Experiment III:

Twelve adult rats (four pairs and four single males) were once again given normal food for five days. On the sixth day, the normal food was replaced with the mixed chemosterilant bait, but the concentration of U-5897 was reduced from the previous 1.0 per cent to 0.5 per cent by weight. These rats were separated into two equal groups of two pairs and

two single males each. One group was then given only the 0.5 per cent chemosterilant mixture, while the other was given the same mixture, but with the daily addition of three ml. of estrous urine. The results of this experiment were graphed to determine any difference in the rates of food consumption between the two groups.

Experiment IV:

Three pairs of adult rats were tested with a 1.0 per cent mixture of the chemosterilant and the daily addition of two ml. of estrous urine. The average daily rate of consumption of this mixture was then compared with the results of the paired animals from Experiment I in order to determine the effect of the pheromone as an attractant at a 1.0 per cent concentration of chemosterilant.

Experiment V:

Five single females were tested at the 1 per cent concentration of the chemosterilant mixture after their normal food-consumption average was determined. Three of these animals were then placed on the plain 1.0 per cent mixture, while the two were placed on a diet of the same mixture, with the addition of three ml. of estrous urine daily. Although 3-chloro-1, 2-propanediol has no effect as a sterilant on female rats, it has been shown to be lethal to them when ingested in quantities equal to or in excess of the LD-50 dose rate of 152 mg/kg body weight (Ericsson, 1970). For this reason, it becomes important to determine whether female rats are also attracted by the pheromone in estrous urine if it is mixed into a chemosterilant bait.

Experiment VI:

After tabulating the results of the original five experiments, it became apparent that differential mortality occured between groups of animals that ate relatively large quantities of the chemosterilant and then ate little or no food afterward, and other groups which ate as much or more of the chemosterilant but ate a great deal of supplemental food. I proposed the hypothesis that food acts as somewhat of a buffer, thus delaying the lethal effects of the drug when large amounts of food are eaten with the chemosterilant. In order to test this hypothesis, the following experiment was devised and instituted.

Eight adult male Long-Evans rats were separated into 2 groups (Group A and Group B) which contained 4 rats each. Each group was given an unlimited supply of drinking water for the duration of the experiment, but all animals in Group A were denied all food from the date of initial injection until they died.

Group A was then split into 2 sub-groups, A-1 and A-2, each of which contained 2 animals. The animals in A-1 then received gavage doses of the U-5897 chemosterilant at the rate of 200 mg/kg on the first day, and 100 mg/kg on the following day. After this injection of 300 mg/kg, the animals were simply allowed to remain in their cages until they died. The length of this period was then recorded for comparison with Group B.

The animals in A-2 also received 300 mg/kg of the U-5897 in the same manner, however, they were injected at a rate of 75 mg/kg/day for the first four days of the experiment. Their survival time was also recorded.

Group B was treated identically with Group A except that an unlimited supply of food was made available to the animals, and their individual daily food consumption was recorded. At the conclusion of the experiment, results from all four sub-groups were compared.

RESULTS AND DISCUSSION

Experiment I: Results

The daily average food consumption of normal chicken mash was calculated to be 25.2 ± 1.6 grams per day for each individual rat tested in this group. For convenience and accuracy, the animals which were paired in pens were considered as a unit, and the unit or pair consumption averaged 50.2 ± 3.3 grams per day for the trial. This overall average food consumption was then broken down into the individual components which were found to be 24.1 ± 3.6 gms/day for males and 19.2 ± 5.1 for females (see Table 1). This computation was performed by calculating the ratio of the individual weights of each animal to the total weight of both animals in the pen. The total consumption of food per day was then multiplied by this fraction to determine the number of grams of food eaten by each animal. This method assumes that the rate of food injection per gram of rat is a constant.

All figures which refer to the rate of consumption of food for the animals being tested with the U-5897 will be stated in terms of a percentage of the normal daily rate of food consumption. This method eliminated any discrepancies in the absolute amounts eaten in grams, which are obviously functions of the sizes of the individual animals. All data include 95 per cent confidence intervals in the standard errors of the means.

On the first day of the introduction of the chemosterilant in the food during this experiment, food consumption dropped drastically for both the pairs and the single males (see Table 1 and Figure 2). On the second day of the test, the consumption continued to drop to less than

TABLE 1:
DAILY RATE OF CONSUMPTION OF 1.0 PER CENT MIXTURE OF U-5897
MEASURED IN PER CENT OF DAILY RATE OF CONSUMPTION FOR NORMAL CHICKEN MASH

EXPERIMENTAL GROUPS	BEGINNING SAMPLE SIZE	NORMAL RATE IN GRAMS (100%)	PERCENTAGE OF NORMAL FOOD CONSUMPTION PER DAY					
			1	2	3	4	5	6
PAIRS	N = 12	G = 50.2 ± 3.3	19.2 ± 5.1	0.55 ± 0.0	0.3 ± 0.1	0.0	0.2 ± 0.0	0.0
SINGLE MALES	N = 4	G = 24.1 ± 3.6	16.8 ± 5.1	0.0	0.0	0.4 ± 0.0	0.0	0.0

TABLE 2:
EFFECT ON DAILY RATE OF FOOD CONSUMPTION OF
1.0% U-5897 CAUSED BY ADDING 3 ml OF ESTROUS URINE DAILY

EXPERIMENTAL GROUPS	BEGINNING SAMPLE SIZE	NORMAL RATE IN GRAMS (100%)	PERCENTAGE OF NORMAL FOOD CONSUMPTION PER DAY			
			WITHOUT URINE ADDED		3 ml URINE ADDED DAILY	
			1	2	3	4
PAIRS	N = 8	G = 51.4 ± 4.4	0.9	2.8	8.4 ± 2.8	14.4 ± 5.6
SINGLE MALES	N = 2	G = 30.1 ± 1.8	6.0	0.0	5.3 ± 0.6	7.5 ± 0.0

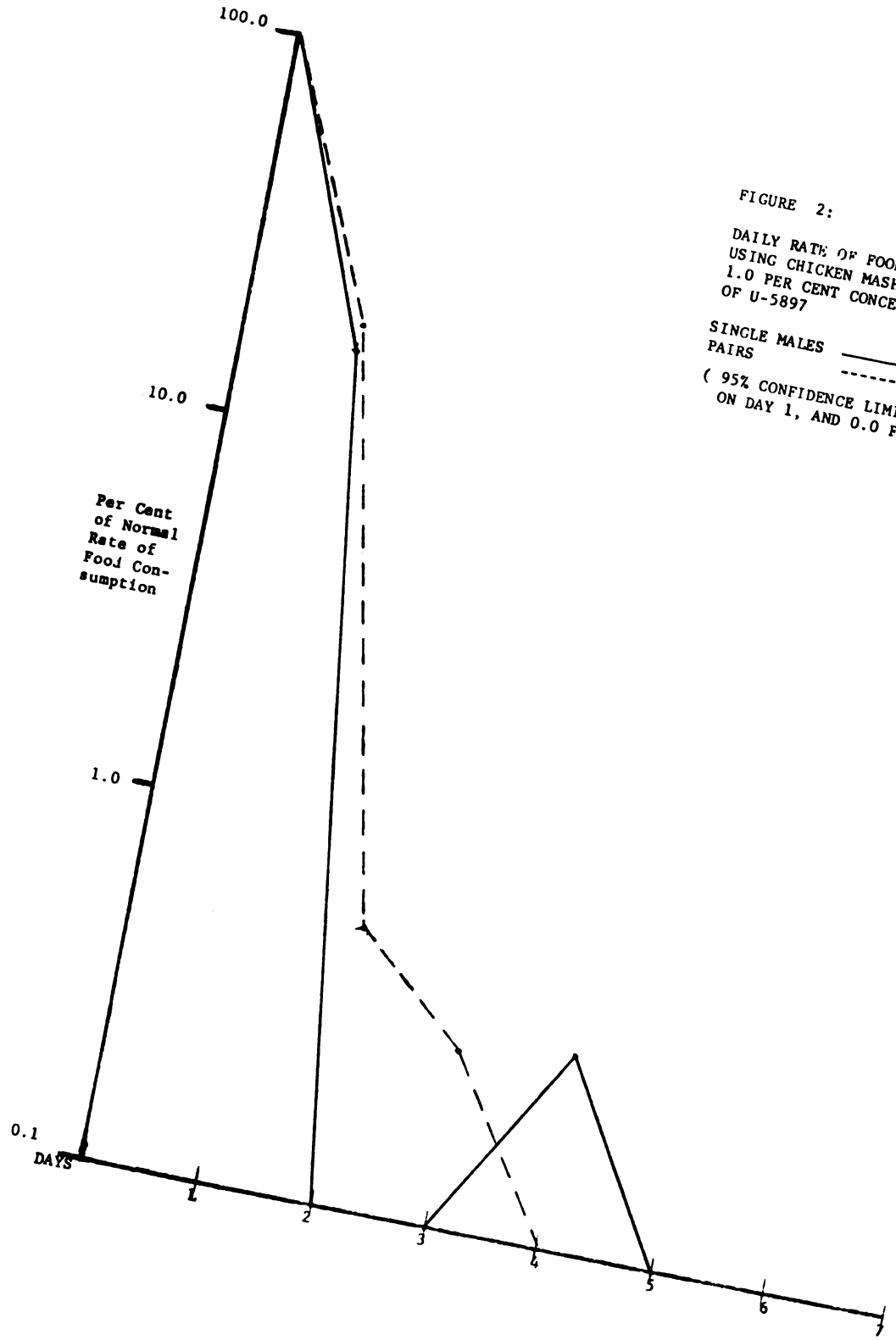


FIGURE 2:

DAILY RATE OF FOOD CONSUMPTION USING CHICKEN MASH CONTAINING 1.0 PER CENT CONCENTRATION OF U-5897

SINGLE MALES ——
PAIRS - - - - - -

(95% CONFIDENCE LIMITS ARE ± 5.1 ON DAY 1, AND 0.0 FOR ALL OTHERS)

1 per cent of normal, and remained there for the remainder of the testing period. There was possibly a somewhat greater effect upon single males than on paired animals; however, this could not be verified statistically due to the small sample size.

The lethal doses in Experiment I averaged 44.3 ± 9.5 gms/animal for each of the three males, and 45.0 ± 10.0 for the two females which died during the test period. This was approximately 122 mg/kg for these animals. The survival times for the animals which ingested lethal amounts of the chemosterilant were calculated to be 4.7 ± 0.3 days for the males, and 4.5 ± 0.5 for the females.

Post mortem examination with the assistance of Dr. Beverly Cockrell showed that each had died due to severe intestinal hemorrhaging or gastroenteritis and acute dehydration along with depletion of all body fat. All males tested had developed bilateral lesions in the epididymus, and exhibited bilateral atrophy of the testes. Lesions in the cranial myelin sheath similar to those found by Ericsson (pers. comm.) were sought but were not found in any of the rats examined.

At the end of the sixth day of the treatment with the chemosterilant, the remaining nine rats of the original group of 14, were returned to a plain mash diet. Since five cases of mortality had already occurred, thus making a prediction of the effect possible for the remaining rats, it was deemed more feasible to utilize these surviving animals as test subjects in Experiment II than to kill them at this time. This provided a supply of rats which were preconditioned or presensitized to the chemosterilant and therefore exhibited severe bait shyness.

On the first day of replacement of the 1.0 per cent mixture of U-5897 with normal or untreated mash, the food consumption immediately

increased to an average of 26.76 grams per individual. This represented an increase of nearly 2 grams over the original rate of consumption before the chemosterilant was introduced. These findings using wild rats are in contrast to those of Ericsson (pers. comm.) who found that when laboratory rats were treated similarly, their rate of consumption at the time of reintroduction to untreated food remained at a low level, and did not return to the original level for some time.

Experiment I: Discussion

The data gathered on these animals apparently demonstrate that after an initial ingestion of about 40 mg of the U-5897 (see Figure 2) per capita, when present in food at a 1.0 per cent by weight concentration, the rats developed extreme anorexia and refused to eat any more of the food containing this material. This is the phenomenon described previously by Calhoun (1962a) as bait shyness. In the case of five of the rats, this behavioral phenomenon was continued to the point of death. If the rest of the experimental animals had been allowed to remain on the same diet over a longer period of time, it can be predicted from the results obtained for those original five animals that the rest would also have died from similar causes.

Experiment II: Results

At the end of a ten day recuperation period from Experiment I, the nine remaining animals along with one new male were placed on a diet containing 1.0 per cent U-5897. The average consumption of normal food had been previously calculated to be 51.5 ± 4.4 for pairs and 30.1 ± 1.8 for single males.

The rate of food consumption dropped drastically at the first contact with the chemosterilant. The new male, however, which had not been presensitized to the material, only dropped to 12 per cent of normal on the first day, as compared with a far greater reduction in the case of all other animals (see Table 2, Figure 3). After a second day of very low consumption by all animals, 3 ml of estrous urine from the laboratory rats of the Long-Evans strain was added to the food containing the 1.0 per cent concentration of U-5897. This resulted in a sharp increase in food consumption in both pairs and single males (see Table 2 and Figure 3). The pairs increased their consumption to 8.4 ± 2.8 per cent of normal while the single males were somewhat lower at only 5.3 ± 0.6 per cent of normal. On the following day, each group continued to increase. The pairs climbed to 14.4 ± 5.6 per cent and the single males to 7.5 ± 0.0 per cent.

At this point, all animals were terminated for necropsy, and per cent weight loss was calculated for both male and female animals (see Table 6). Males were calculated to average a 13.5 ± 3.0 per cent weight loss, while females were recorded as averaging 12.2 ± 1.5 per cent.

The results of the necropsy were the same as those from Experiment I. All males had developed bilateral testicular atrophy. All animals exhibited some intestinal enteritis, although it had progressed to mortality in the case of only one male which died on the third day of the experiment.

Experiment II: Discussion

This experiment, like Experiment I, was a pilot experiment. It was designed to determine the effect of the estrous urine collected from Long-Evans laboratory rats when added to an undesirable food material for the

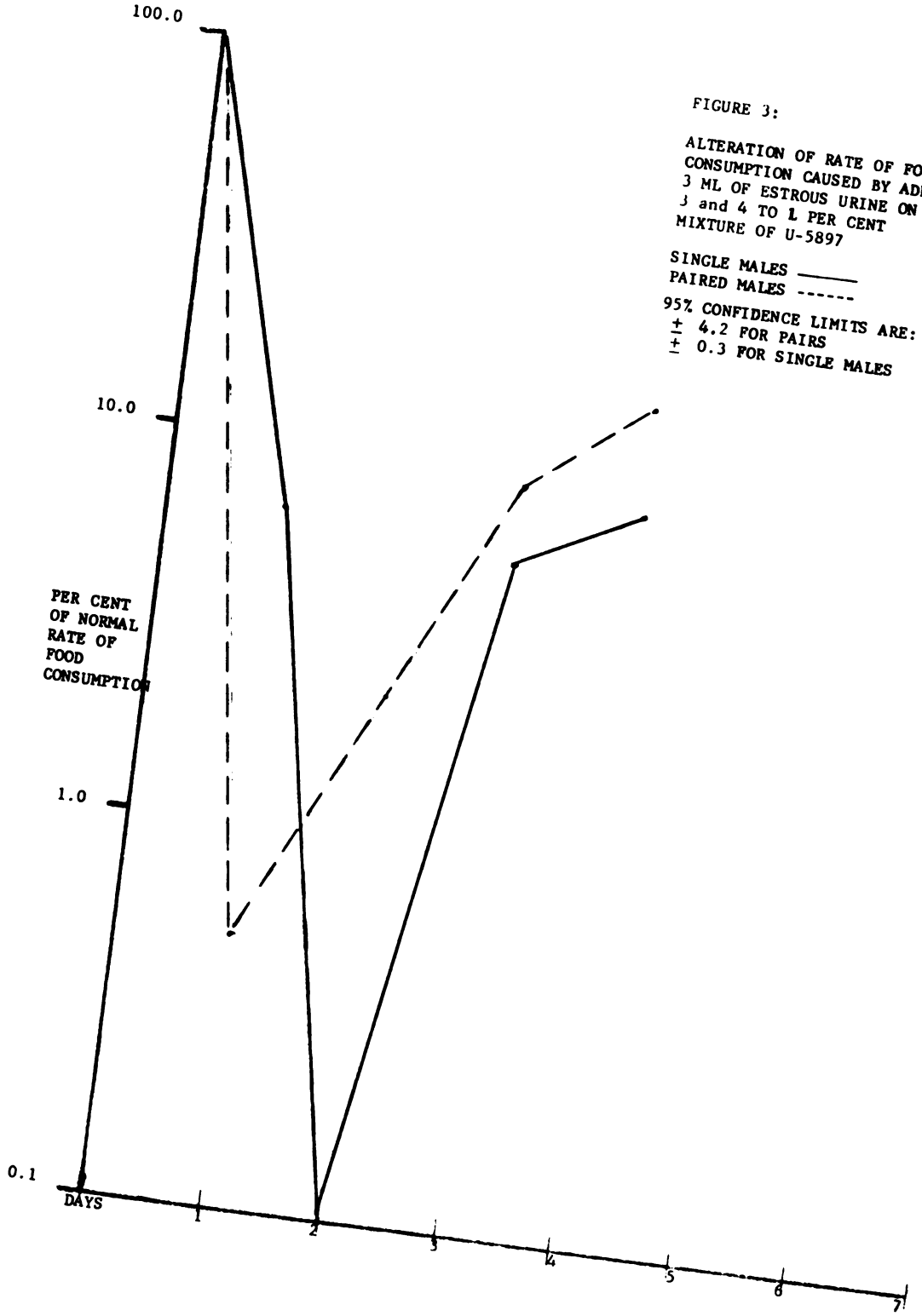


FIGURE 3:

ALTERATION OF RATE OF FOOD CONSUMPTION CAUSED BY ADDING 3 ML OF ESTROUS URINE ON DAY 3 and 4 TO 1 PER CENT MIXTURE OF U-5897

SINGLE MALES ———
PAIRED MALES ------

95% CONFIDENCE LIMITS ARE:
± 4.2 FOR PAIRS
± 0.3 FOR SINGLE MALES

TABLE 6:
MEAN WEIGHT LOSS PER INDIVIDUAL AS A PERCENTAGE OF ORIGINAL WEIGHT

INDIVIDUAL	SAMPLE SIZE	WEIGHT LOSS USING 0.5% U-5897 CONCENTRATION	INDIVIDUAL	SAMPLE SIZE	WEIGHT LOSS USING 1.0% U-5897 CONCENTRATION
MALES W/URINE	N = 4	27.7 ± 5.4	MALES W/URINE	N = 3	22.6 ± 0.0
FEMALES W/URINE	N = 2	17.3 ± 9.8	FEMALES W/URINE	N = 5	28.2 ± 1.7
MALES NO URINE	N = 4	23.2 ± 3.1	MALES NO URINE	N = 2	22.6 ± 2.3
FEMALES NO URINE	N = 2	19.5 ± 10.2	FEMALES NO URINE	N = 3	24.4 ± 2.5

TABLE 7:
MEAN LETHAL DOSE PER INDIVIDUAL

INDIVIDUAL	SAMPLE SIZE	MG/KG DOSE U-5897 1.0% CONCENTRATION	INDIVIDUAL	SAMPLE SIZE	MG/KG DOSE U-5897 0.5% CONCENTRATION
MALE W/URINE	N = 3	353.5 ± 9.9	MALE W/URINE	N = 4	425.5 ± 90.8
FEMALE W/URINE	N = 5	632.6 ± 274.4	FEMALE W/URINE	N = 2	424.3 ± 160.7
MALE NO URINE	N = 1	122.0 ± 0.0	MALE NO URINE	N = 4	418.1 ± 96.9
FEMALE NO URINE	N = 3	450.0 ± 251.0	FEMALE NO URINE	N = 2	396.1 ± 216.3

purpose of making it more attractive to wild Norway rats. The results accrued from this experimental manipulation of the food material apparently demonstrate that the estrous urine is indeed an effective attractant. This seems to be verified by the more than 500 per cent increase in mean food consumption rate which is induced by the addition of the urine.

This experimental addition of urine apparently caused no detrimental effects in the chemosterilant properties of the U-5897, since the male rat which was introduced to the chemosterilant for the first time during this test, developed bilateral lesions and testicular atrophy to a similar degree as those animals which had been preconditioned with up to 65 mg of the material in Experiment I.

Experiment VI: Results

In order to facilitate clarity in the discussion of Experiment III through Experiment V, the results of Experiment VI are interjected at this point, rather than in the normal sequential position.

After initial injections of the 1.0 per cent water solution of the U-5897, it was found that the animals getting the 300 ml in two days died on the third and fourth days after this initial injection. They had averaged a weight loss of 7.3 ± 0.8 per cent of their original weight. The animals, which were given similar doses in the same two day period but had supplemental food which they ate at a rate of 1.6 grams per day each, lived for three and six days after initial injection.

The weight loss for this second group of animals was also found to be somewhat lower than in the first group, being 6.15 ± 1.15 as compared to the previous 7.3 ± 0.8 per cent.

When comparing the animals which received the lethal dose at a rate of 75 mg/kg/day for four days, similar results occurred. Those which were permitted to eat nothing after injection, died on the sixth day after initial injection, and had lost an average of 33.45 ± 1.35 per cent of their normal weight. Those which were supplied with all the food they wanted to eat (an average of 10.9 grams per day per individual throughout the experimental period) were simply terminated at the end of nine days since they showed no sign of expiring. They had only lost an average of 11.25 ± 0.35 per cent of their original weight, or an average of 65.4 per cent less weight than the rats which did not receive food and therefore died quite rapidly.

Experiment VI: Discussion

This experiment was designed to evaluate the possibility of the existence of synergistic effects brought about by a lack of food coupled with ingestion of relatively large amounts of the toxic chemosterilant material. This theory would explain the difference in LD-50s recorded in the original set of experiments I through V. The results from Experiment VI indicate that synergistic effects are incurred if both conditions are met simultaneously.

Experiment III: Results

Group A: Pairs of animals without pheromone

Reactions exhibited by the two pairs of animals in this group when the chemosterilant was first introduced into their food at a 0.5 per cent concentration, were similar to those in the previous tests. On the first day of contact with the food mixture, the rate of food consumption in these rats dropped to 25.1 ± 1.3 per cent of the normal rate of 40.7 ± 3.5

grams per day per pair. On the second day the rate dropped to less than 2 per cent of their normal rate (see Table 3 and Figure 4), and remained at that level for the following day. On the fourth day of the testing period, food consumption increased sharply, rising to a level of 21.6 ± 21.4 per cent of normal. This rate then continued until the eighth day of treatment, when all animals in the group were dead. None of the animals in this group died before the end of the seventh day of treatment.

The average lethal dose ingested by these animals was calculated to be 418.1 ± 96.9 for the males, and 396.1 ± 216.3 for the females in the group. This total ingested dose of the chemosterilant was observed to be eaten in two major peaks. These occurred on the first day of the treatment, and then on the fourth through the final days of the experiment. Arithmetic computations demonstrated that an average of 222.0 ± 22.7 mg/kg were eaten by each pair before the sudden rise in consumption on the fourth day of treatment, of which an average of 89.4 per cent of this amount was eaten on the first day of the trial.

The apparent bait shyness, which immediately followed initial ingestion of the material on the first day of the trial, was probably due to a mild intestinal enteritis and irritation of the mucosa due to drug action. On the fourth day, however, the animals probably simply became hungary enough to overcome the bait shyness, and began to eat enough of food to sustain life, at the same time ingesting enough to poison themselves. This hypothesis can be partially supported by the amount of food, in grams, which was eaten by the individuals. After the first day, the rats ate only an average of 1.5 per cent of the normal consumption rate, or less than 0.3 grams of food per day per individual. This rate was maintained for both the second and third days of the test period.

TABLE 3:
DAILY RATE OF CONSUMPTION FOR 0.5 PER CENT MIXTURE OF U-5897
MEASURED IN PERCENTAGE OF DAILY RATE OF CONSUMPTION FOR NORMAL CHICKEN MASH

EXPERIMENTAL GROUPS	BEGINNING SAMPLE SIZE	NORMAL RATE IN GRAMS (100%)	PERCENTAGE OF NORMAL FOOD CONSUMPTION PER DAY							
			1	2	3	4	5	6	7	8
PAIRS W/ URINE	N= 4	G= 36.4 ± 2.5	21.1 ± 0.3	6.5 ± 0.9	21.1 ± 4.5	20.5 ± 16.0	31.5 ± 0.0	28.2 ± 0.0	0.0	
PAIRS NO URINE	N= 4	G= 40.7 ± 3.5	25.1 ± 1.3	1.8 ± 0.6	1.2 ± 1.0	21.6 ± 21.4	8.8 ± 7.8	19.5 ± 15.2	28.2 ± 19.5	0.0
SINGLE MALES W/U	N= 2	G= 32.2 ± 1.2	22.0 ± 1.4	25.8 ± 16.9	20.4 ± 12.2	7.9 ± 0.3	12.7 ± 6.1	6.9 ± 0.0	0.0	
SINGLE MALES N/U	N= 2	G= 30.5 ± 1.9	17.7 ± 3.8	9.6 ± 8.8	9.2 ± 7.7	23.7 ± 1.4	21.3 ± 2.5	18.7 ± 6.9	3.2 ± 0.9	0.0

TABLE 4:
COMPARISON OF DAILY CONSUMPTION RATES FOR 1.0 PER CENT MIXTURE WITH AND WITHOUT DAILY ADDITION OF 3 ml OF ESTROUS URINE

EXPERIMENTAL GROUPS	BEGINNING SAMPLE SIZE	NORMAL RATE IN GRAMS (100%)	PERCENTAGE OF NORMAL FOOD CONSUMPTION PER DAY							
			1	2	3	4	5	6	7	8
PAIRS W/ URINE	N= 6	G= 48.4 ± 4.4	14.2 ± 5.4	5.2 ± 1.6	7.5 ± 2.9	5.0 ± 1.5	5.1 ± 0.0	5.6 ± 0.0	4.9 ± 0.0	0.0
PAIRS NO URINE	N= 12	G= 50.2 ± 3.3	19.2 ± 5.1	0.6 ± 0.0	0.3 ± 0.1	0.0 ± 0.0	0.4 ± 0.0	0.0	0.0	0.0

TABLE 5:
EFFECT ON DAILY CONSUMPTION RATE BY FEMALES FOR 1.0 PER CENT MIXTURE OF U-5897
CAUSED BY THE DAILY ADDITION OF 3 ml. OF ESTROUS URINE

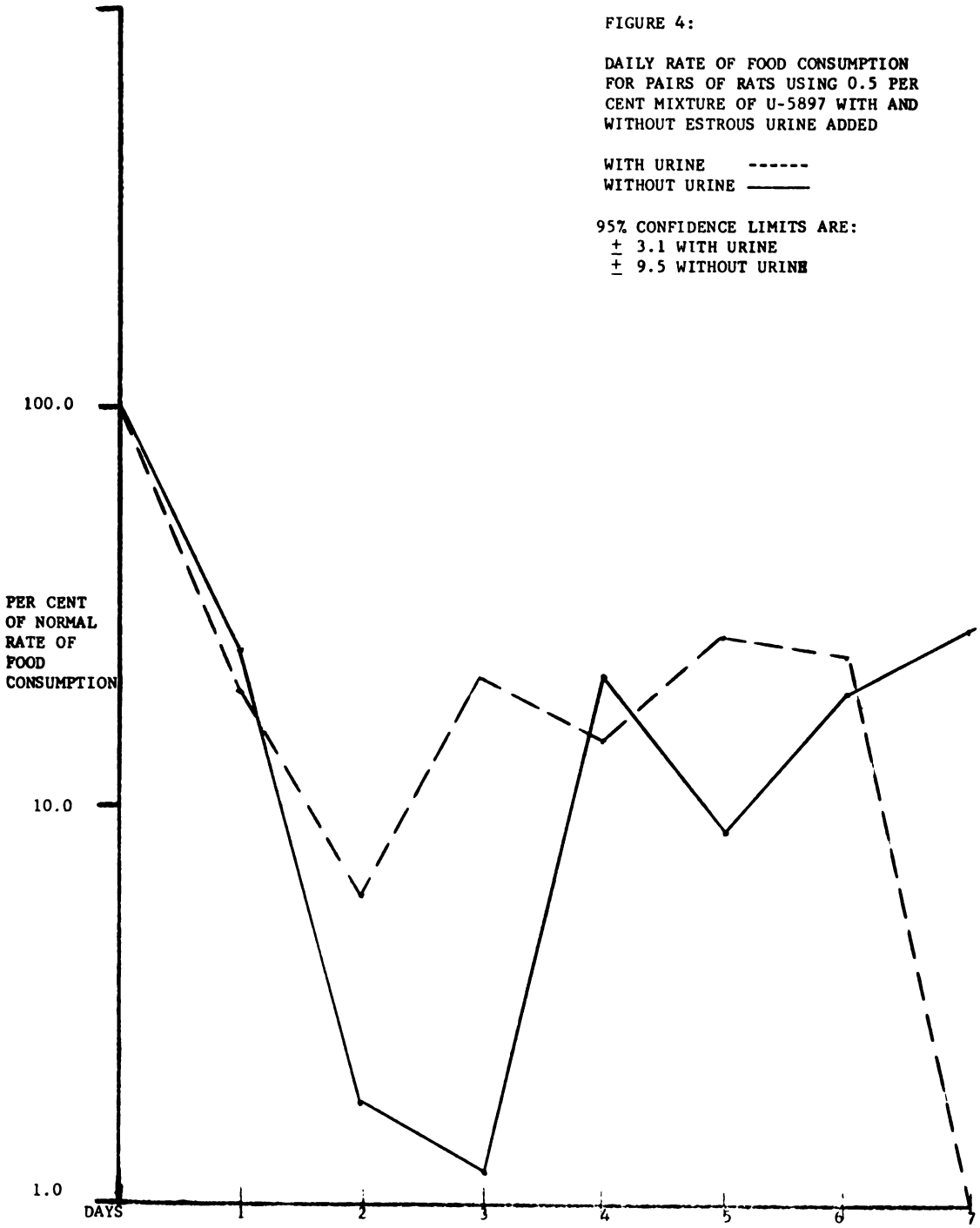
EXPERIMENTAL GROUPS	BEGINNING SAMPLE SIZE	NORMAL RATE IN GRAMS (100%)	PERCENTAGE OF NORMAL FOOD CONSUMPTION PER DAY								
			1	2	3	4	5	6	7	8	9
SINGLE FEMALES WITH URINE	N= 2	G= 19.2 ± 0.8	14.2 ± 5.7	16.4 ± 10.3	29.4 ± 19.9	45.2 ± 37.0	106.0 ± 0.0	36.9 ± 0.0	17.0 ± 0.0	0.0	0.0
SINGLE FEMALES NO URINE	N= 3	G= 19.2 ± 0.8	6.5 ± 1.2	1.5 ± 0.7	3.4 ± 1.8	5.0 ± 2.1	3.1 ± 0.4	8.4 ± 0.0	6.1 ± 0.0	3.7 ± 0.0	0.0

FIGURE 4:

DAILY RATE OF FOOD CONSUMPTION FOR PAIRS OF RATS USING 0.5 PER CENT MIXTURE OF U-5897 WITH AND WITHOUT ESTROUS URINE ADDED

WITH URINE ------
WITHOUT URINE ———

95% CONFIDENCE LIMITS ARE:
± 3.1 WITH URINE
± 9.5 WITHOUT URINE



Results of necropsy showed the same characteristics in these animals as in those from Experiments I and II. All animals had died as a result of extensive gastroenteritis and severe dehydration. All males in the group showed bilateral epididymal lesions and testicular atrophy. The weight loss for these animals was calculated to average 23.2 ± 3.1 per cent for the males and 19.5 ± 10.2 per cent for the females.

Group B: Pairs of animals with pheromone

This group of animals reacted to the material in a highly contrasting manner to those in Group A, in that they did not exhibit as great a reduction in food consumption. The initial drop on food consumption to 21.1 ± 0.3 per cent was similar to the drop in the animals which did not have the additive pheromone attractant, but, there was no subsequent drastic reduction on the second day of the treatment (see Table 3 and Figure 4). Instead, an average of 18.8 per cent of normal was maintained throughout the entire experiment until all animals had died on the sixth day of the trial.

This was again in contrast to Group A since all of the animals in Group B died on or before the sixth day of the trial, which was one day earlier than those without pheromone. This could quite probably be accounted for by the much higher total ingestion of the chemosterilant at an earlier date by those with pheromone attractants.

The animals in Group B all died from an average lethal dose of 424.9 ± 125.7 mg/kg, which had been ingested by the sixth day, as compared to a lethal dose for Group A of 412.1 ± 156.6 which was not attained until the seventh day of treatment in that group.

The results of the post mortem examination on these animals were similar to all previous results. Bilateral epididymal lesions and

testicular atrophy along with acute gastroenteritis were present in all animals in the group. Weight loss was calculated to be 27.7 ± 5.4 per cent for the males, and 17.3 ± 9.8 for the females.

Group C: Single males without pheromone

The animals in this group were found to react in a similar manner to those animals in Group A. Food consumption plunged to an average of 9.6 per cent of normal in the second day of treatment, after an initial drop to 17.7 per cent of normal on the first day (see Table 3 and Figure 5). It remained at approximately 9 per cent for the third day, and then increased to 23.7 per cent on the fourth day as did the consumption rate in Group A. This rate was then maintained until death on the seventh day, just as was the case with the animals in Group A.

The lethal dose was found to average 440.0 ± 120.5 for these animals, and their weight loss was calculated to be 25.2 ± 1.7 per cent of the original weight.

Group D: Single males with pheromone

These animals were found to be quite similar to those in Group B and greatly in contrast to those in Group C. Once again, the average consumption dropped drastically to 22.0 ± 1.4 per cent of normal on the first day of treatment, but rather than continue to drop on the second day, it rose from that level to 25.8 ± 16.9 per cent. The rate then began to tail off gradually as the animals apparently became quite severely affected by the U-5897. All animals were dead by the sixth day of treatment.

The lethal dose ingested by these animals was calculated to be 429.8 ± 150.3 per individual, and their weight loss was found to average 19.9 ± 0.2 per cent of the original weight.

The results of necropsy were again similar. All animals were found to be severely dehydrated, exhibited acute gastroenteritis, and the males were permanently sterilized in the same manner as all previously examined animals.

Experiment III: Discussion

This experiment was designed to answer four separate questions. 1. To determine whether there was a difference in acceptance of baits by rats when there was no pheromone added to the chemosterilant bait mixture, but the concentration of U-5897 was reduced from 1.0 per cent to 0.5 per cent. 2. To compare the effects of using the pheromone as an attractant at the 0.5 per cent concentration, with the effects of using the same addition of pheromone at a 1.0 per cent level as previously recorded in Experiment I. 3. To determine if the pheromone actually created better bait acceptance in animals whose food was treated with the substance, when compared with animals which were simultaneously given identical food material, but without the pheromone additive. 4. To demonstrate any differences in the attractant ability of the pheromone in regard to single males when compared to males which were mated to females.

1. The question concerning the difference in bait acceptance between the 1.0 per cent and the 0.5 per cent concentrations of the chemosterilant without the additional presence of any attractant is quite clearly answered by this experiment. In the case of the 1.0 per cent concentration, the daily rate of consumption dropped below 0.07 per cent of normal (see Figure 2), and stayed at that level until the termination of the experiment. In the case of the 0.5 per cent concentration, the food consumption never dropped as low as 1.2 per cent of normal, or more than 17 times higher than that of the 1.0 per cent concentration. At the

end of only two days of less than 2 per cent of normal consumption rate, that rate rebounded to more than 21 per cent and then averaged 19.2 per cent of normal for the remainder of the trial period (see Figure 4). It therefore appears evident that a mixture of 0.5 per cent concentration is highly more acceptible to wild rats than is a mixture of 1.0 per cent concentration of the U-5897.

2. The determination of the differences in the attractive ability of the pheromone when added to a 0.5 per cent mixture of U-5897 as compared with the same pheromone when added to a 1.0 per cent mixture can be obtained when the results of Experiment IV are studied. These results will be discussed at length later in this paper.

3. The comparison between the groups of animals (A & C) which received food containing the 0.5 per cent concentration of the U-5897 and the groups (B & D) which received the same food with the addition of 3 ml. of estrous urine daily, points to a probable positive attractive influence exhibited by the estrous urine. The rather drastic increase in food consumption on the fourth day by all animals on a diet which did not include the pheromone attractant can probably be attributed to a combination of three factors. First, these animals had not eaten enough of the chemosterilant on the first day to cause them to be irreversibly poisoned. Secondly, they had eaten enough food on the second and third days of the experiment to keep from losing strength and thus creating the synergistic effect of the U-5897 as previously discussed in Experiment VI. Thirdly, the animals while ingesting enough food to inhibit synergism for 2 days had eaten far below their normal rate of consumption, and were reaching a level of extreme hunger at this point, thus highly influencing their return to the food. It must be noted that in Experiment I, none of

these phenomena occurred i.e. the animals had already eaten enough of the chemosterilant to have lethal effects, or at least, they had eaten enough to make them so sick that the bait shyness was permanently established. In addition, these animals ate little or no food during the final five days of the experiment, and thus apparently fell victim to the combined effects of the drug and the lack of food.

4. The success of the pheromone as an attractant when used with single males as compared to mated males is demonstrated by Figures 4 & 5 and Table 3. The difference can be seen by comparing the rates of daily food consumption for each of the test groups. Mated males average somewhat higher overall with the average consumption of pheromone treated chemosterilant material being 21.5 ± 3.7 for mated males and 16.0 ± 6.2 per cent of normal for single males. Due to the rather large standard errors and the small sample size, the rates could not be statistically verified to be significantly different, although it is possible that paired males may have been more attracted to the pheromone than single males were.

Experiment IV: Results

The three pairs of animals which were given the 1.0 per cent concentration of the chemosterilant in this test were compared with the animals in Experiment I to determine the effect of the pheromone as an attractant at the 1.0 per cent level.

It was found that these animals with the pheromone attractant dropped in rate of consumption to 14.2 per cent of normal for the first day of the trial, and then averaged 5.6 per cent for the remaining six days of the experiment until all animals were dead by the seventh day.

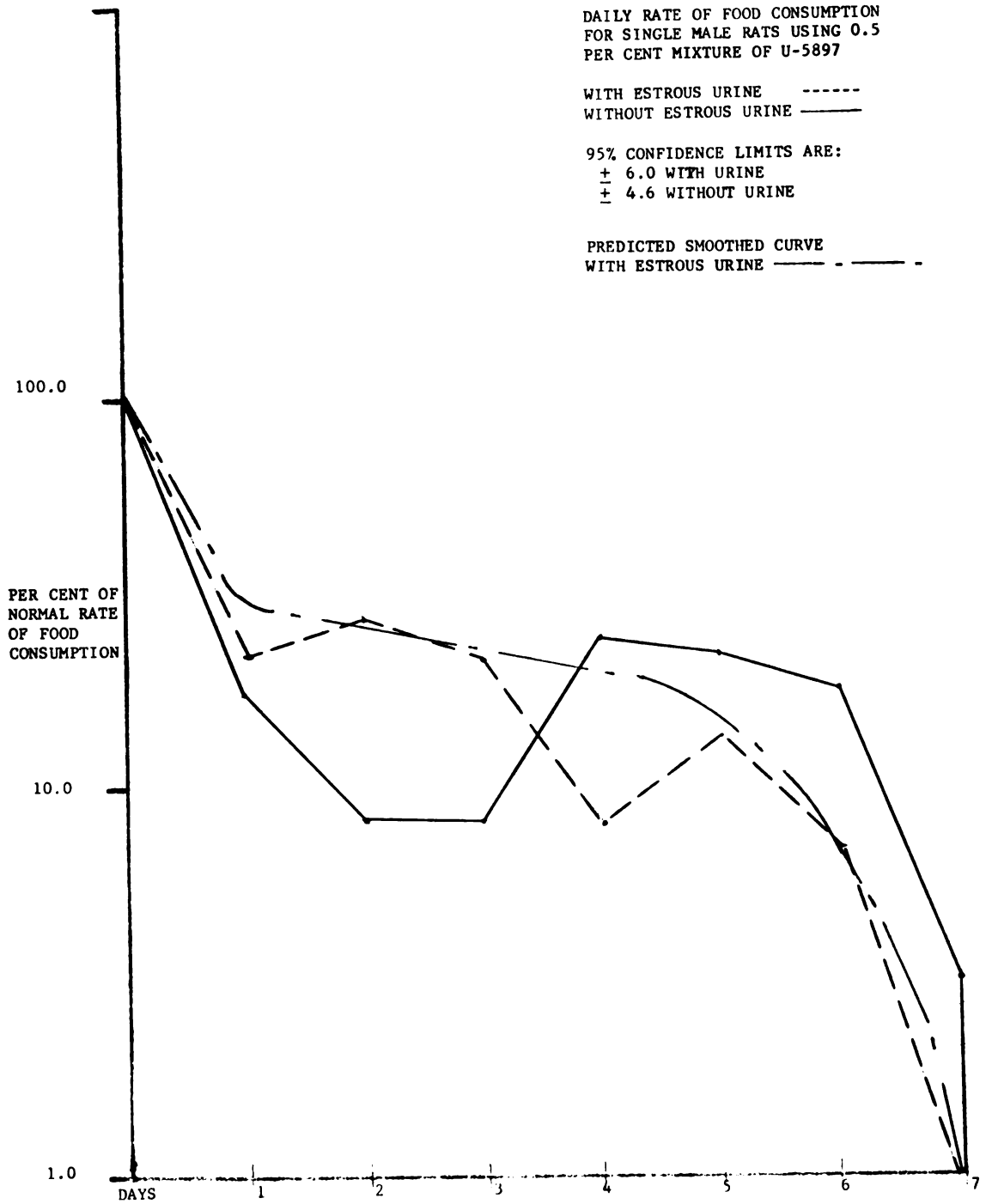
FIGURE 5:

DAILY RATE OF FOOD CONSUMPTION
FOR SINGLE MALE RATS USING 0.5
PER CENT MIXTURE OF U-5897

WITH ESTROUS URINE ------
WITHOUT ESTROUS URINE ———

95% CONFIDENCE LIMITS ARE:
± 6.0 WITH URINE
± 4.6 WITHOUT URINE

PREDICTED SMOOTHED CURVE
WITH ESTROUS URINE —— - —— -



Necropsy results were again similar to all other experiments, in that all animals exhibited severe hemorrhaging of the intestine and stomach, and all males had developed epididymal lesions.

The average per cent weight loss was calculated to be 22.6 ± 0.0 for the males and 22.6 ± 2.3 for the females.

Experiment IV: Discussion

When compared with animals which had received the same concentration of chemosterilant in their food for the same length of time, it appears that these animals which had the pheromone attractant added to the food found it far more acceptible than those in Experiment I which had no such additive. The original drop was slightly greater for the animals which had the pheromone present (14.2 per cent of normal as compared to 19.2 per cent for the other group). After the first day of treatment however, there was a very significant difference in the amounts of food eaten by each of the two groups. Those animals without pheromone averaged only 0.22 per cent of normal per day, and during three of the final six days of the experiment, they ate no food at all. This is contrasted with the animals in the group with pheromone additive. These animals never dropped below 4.9 per cent of normal, and averaged 5.6 per cent for the final six days of the experiment (see Figure 7).

In addition to the simple rate of food consumption, the lethal dose was considerably higher for these animals than for those which did not eat and subsequently developed synergistic effects between the action of the U-5897 and the lack of food (see Table 7). The animals without the attractant died after eating approximately 122 mg/kg of the material while these animals ate an average of 355.7 mg/kg before dying.

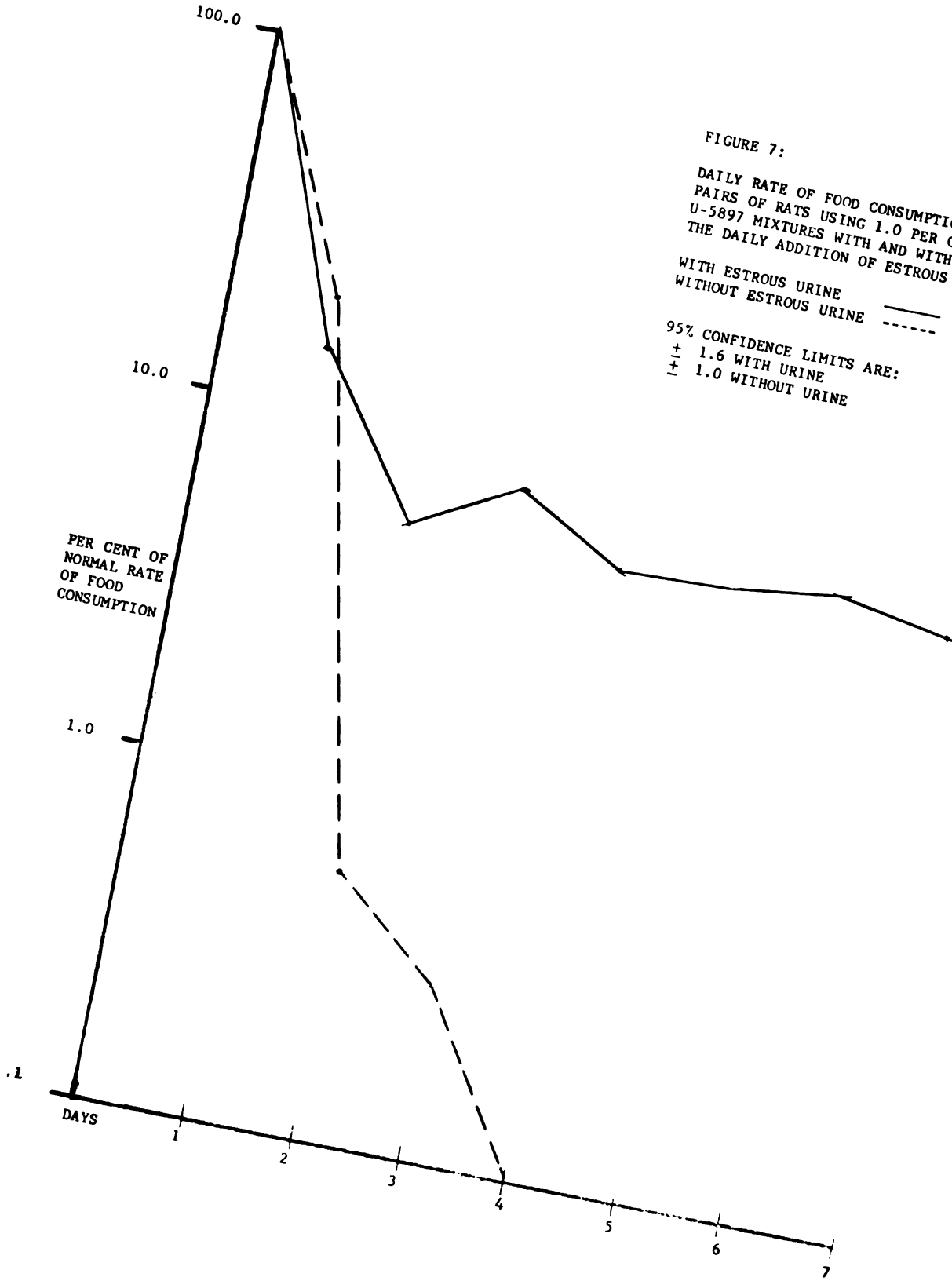


FIGURE 7:

DAILY RATE OF FOOD CONSUMPTION FOR PAIRS OF RATS USING 1.0 PER CENT U-5897 MIXTURES WITH AND WITHOUT THE DAILY ADDITION OF ESTROUS URINE

WITH ESTROUS URINE ——
WITHOUT ESTROUS URINE - - - - -

95% CONFIDENCE LIMITS ARE:
± 1.6 WITH URINE
± 1.0 WITHOUT URINE

A third comparison can now be made by evaluating the effectiveness of the pheromone in attracting male rats to a 0.5 per cent concentration of U-5897 with its effectiveness in attracting male rats to a 1.0 per cent concentration of U-5897 in food material. It can be demonstrated (see Figure 6) that the food consumption rate for animals which were given the 1.0 per cent concentration of the U-5897 which was treated with the pheromone attractant averaged only 6.8 per cent of their normal daily rate of food consumption over a 7 day period while those animals which were given the pheromone as an attractant to a 0.5 per cent concentration of the chemosterilant, ate 21.5 per cent of their normal rate on a six day average. This would seem to point toward a stronger attraction of the pheromone if used in a 0.5 per cent mixture of the chemosterilant.

Experiment V: Results

The two groups of female rats which were tested at the 1.0 per cent concentration level were found to react very favorably to the pheromone attractant if it was added to the food mixture. The rate of food consumption for the animals without the pheromone dropped to 6.5 per cent of normal on the first day of treatment, and then stayed at a similar low level (see Table 5 and Figure 8). In contrast, the females which had the pheromone additive dropped only to 14.2 per cent of their normal rate of food consumption on the first day, and then began to climb until they reached 106.0 per cent of their normal consumption on the fifth day of the test. At this point, they rapidly tailed off and all animals died by the seventh day of the test. The animals without the pheromone never reached as high as 10 per cent of their normal consumption rate, but due to their continual ingestion of small amounts of food daily, they continued to survive until the eighth day of the test.

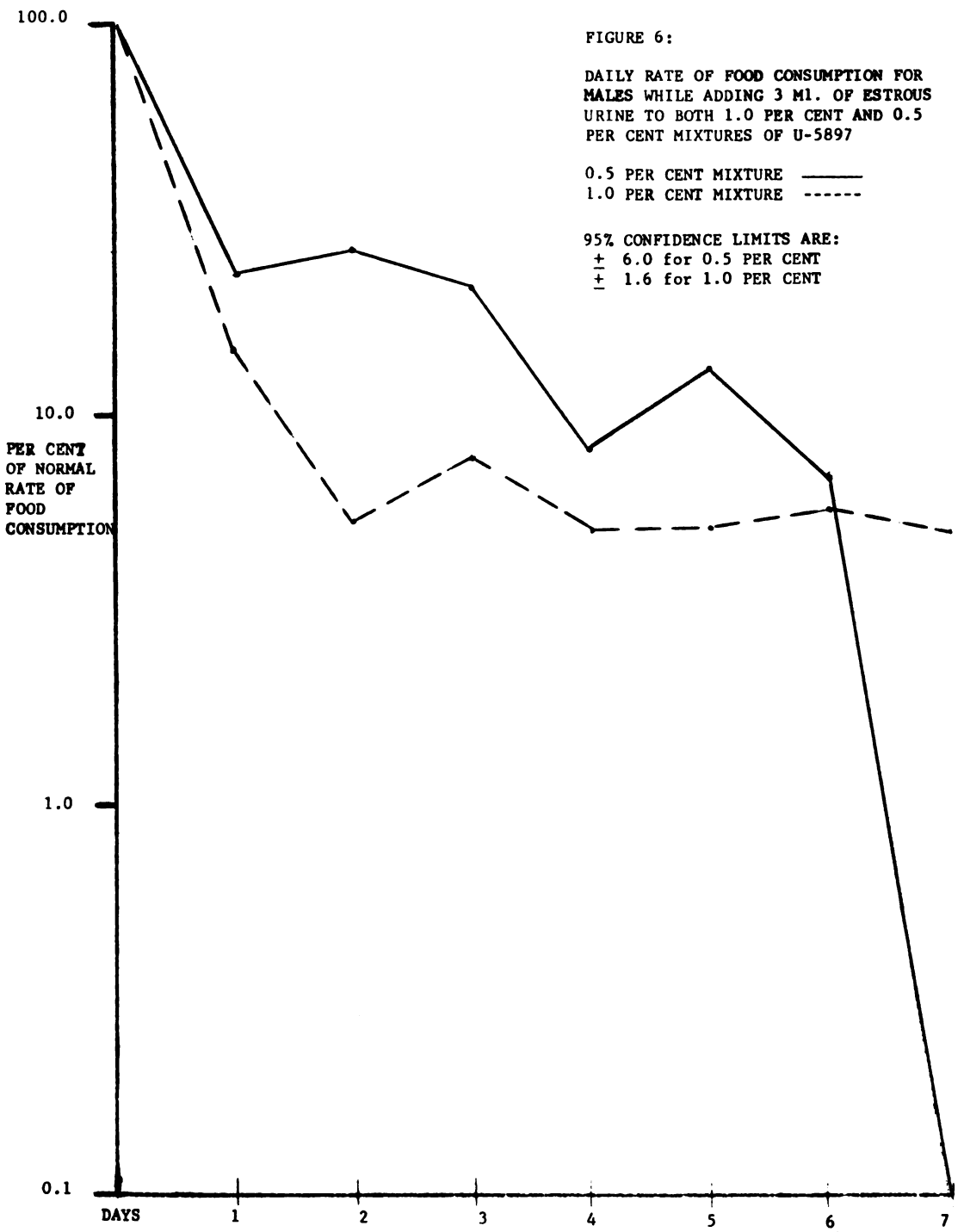


FIGURE 6:

DAILY RATE OF FOOD CONSUMPTION FOR MALES WHILE ADDING 3 Ml. OF ESTROUS URINE TO BOTH 1.0 PER CENT AND 0.5 PER CENT MIXTURES OF U-5897

0.5 PER CENT MIXTURE ———
1.0 PER CENT MIXTURE ------

95% CONFIDENCE LIMITS ARE:
± 6.0 for 0.5 PER CENT
± 1.6 for 1.0 PER CENT

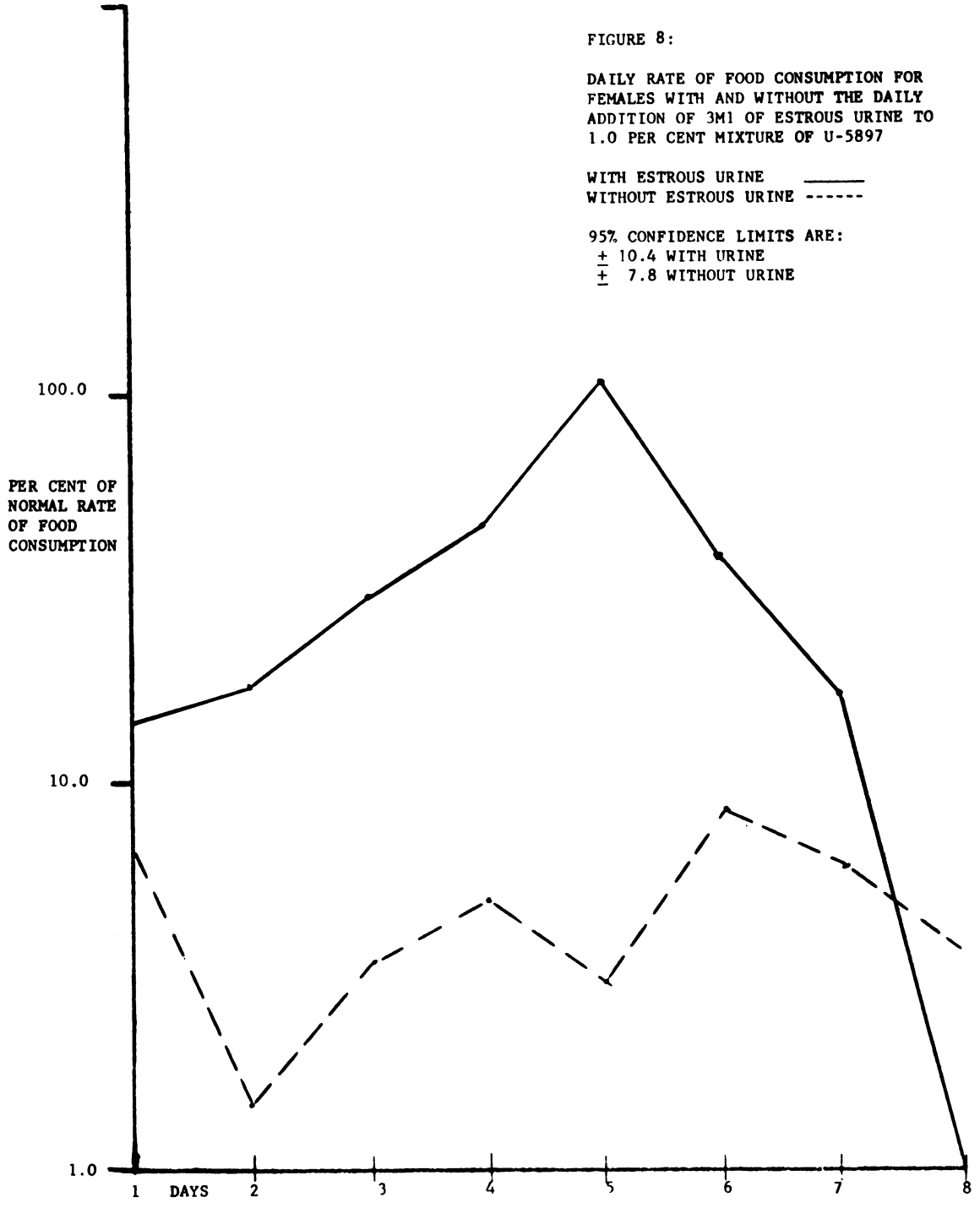


FIGURE 8:

DAILY RATE OF FOOD CONSUMPTION FOR FEMALES WITH AND WITHOUT THE DAILY ADDITION OF 3M1 OF ESTROUS URINE TO 1.0 PER CENT MIXTURE OF U-5897

WITH ESTROUS URINE ———
WITHOUT ESTROUS URINE ------

95% CONFIDENCE LIMITS ARE:
± 10.4 WITH URINE
± 7.8 WITHOUT URINE

The lethal doses were calculated to be 1044.7 ± 685.4 for the females with the pheromone, and 450.0 ± 251.0 for the females without any attractant. These figures are far greater than those for male rats on similar concentrations, and also nearly 700 per cent of those stated by Ericsson (1970) as being the LD-50 dose rate for lab rats.

Once again, necropsy showed that the animals had died from a combination of dehydration, loss of body fat, and extreme gastroenteritis.

Weight loss was calculated to be 25.6 per cent of the original weight for the animals with pheromone, and 24.4 per cent for those which were without any attractant.

Experiment V: Discussion

This experiment demonstrated that females are attracted even more than males to the estrous urine of laboratory rats, and thus, that the pheromone is a highly successful attractant for females when used in conjunction with the alpha-chlorohydrin chemosterilant. Although the compound is not effective in sterilizing female rats, the information may be valuable for future use because females may be expected to possibly eat enough of the material to be lethal to them. In certain wild situations they may also deprive males of enough of the material to be effective against them in the chemosterilant fashion in which it was intended. This possibility however, must be more thoroughly tested.

CONCLUSIONS

As previously stated, the basic purpose of this paper is to determine the relative efficiency of using the estrous urine from laboratory rats as an attractant to induce wild male Norway rats to ingest an undesirable chemosterilant poison.

In addition to supplying data concerning this question, this series of experiments has also suggested several properties of the alpha-chlorohydrin material which were not previously described.

SYNERGISM

From the results of Experiment VI, it appears that there are synergistic effects causing death in wild rats which ingest the 3-chloro-1, 2-propanediol mixture, thus causing wide differences in the LD-50 doses. Animals which eat relatively small doses of the material such as those in Experiment I, but eat very little food to act as a buffer substance in the intestinal tract, die with much greater rapidity and from smaller total ingested doses, than do those which eat large quantities of food along with the chemosterilant. These data are supported by the results of Experiment VI, in which animals which were given no food died much more rapidly than those which were given identical doses of the chemosterilant but supplied with unlimited food as a buffer.

Support of the hypothesis is also supplied when a comparison is made between the average lethal dose of rats which were given the chemosterilant at a 0.5 per cent concentration in their food (416.9 mg/kg), and those which were given food containing 1.0 per cent U-5897 by weight (321.7 mg/kg).

ACCEPTIBILITY OF U-5897 TO RATS AS A FUNCTION OF DOSE CONCENTRATION

A comparison of results of Experiment III in which the rats were fed a 0.5 per cent concentration of the chemosterilant, with the results of Experiments I, IV, and V, in which the concentration was at a 1.0 per cent level, seem to demonstrate that the alpha-chlorohydrin compound is more acceptable to the animals if it is present in the lower concentration. Apparently there is a noxious taste, odor, or some other property which makes the chemical unpalatible to rats, but this property is not as evident at lower concentrations in their food material. This is again supported by the results of the previously mentioned experiments, since it was found that the average food consumption for rodents at the 1.0 per cent level in which no pheromone attractant was present, averaged 4.2 per cent of the normal rate for all animals in the group, while in the group of animals which were given the 0.5 per cent concentration without the pheromone added the average rate of food consumption for all animals averaged 15.7 per cent of normal.

EFFECT OF USING THE PHEROMONE IN ESTROUS URINE AS AN ATTRACTANT

The primary question investigated appears to have been at least partially answered by this research. The pheromone material which was added to the chemosterilant was apparently effective as an attractant to the bait. In tests involving single males with the chemosterilant additive at both the 0.5 per cent and the 1.0 per cent levels, the animals which were fed the chemosterilant with the pheromone present were found to eat from 168.6 to 220.7 per cent more (see section on results and discussion, Experiment IV), for both concentrations. The actual numerical figures were 14.8 per cent of normal for the 0.5 per cent

concentration without the pheromone, as compared to 24.95 per cent of normal for the 0.5 per cent concentration when the pheromone was present. For the 1.0 per cent concentration, the figures were 2.9 per cent of normal for no pheromone, and 6.4 per cent of normal with the pheromone present.

Similar results were recorded when the mated males were compared in the same manner. In this case, the average consumption for males at 1.0 per cent with pheromone present was calculated to be 7.8 per cent of normal, or 229.4 per cent of the rate of consumption exhibited by the males on the same food but without the pheromone, who ate only at the rate of 3.4 per cent of normal.

This was also the case with the paired animals which were fed a 0.5 per cent concentration of the chemosterilant. Those males which were attracted by the pheromone ate an average of 21.5 per cent of their normal consumption, or 142.4 per cent as much as those males which had no attractant. These animals ate only at a rate of 15.1 per cent of their normal rate when given the chemosterilant material.

Female rats were also tested to determine the effect of the pheromone as an attractant to the chemosterilant material. This group showed the most drastic difference in consumption when the pheromone was added, as opposed to the same dose without the pheromone additive. Here, the test results show that females receiving no pheromone averaged 4.7 per cent of normal, while those receiving the pheromone attractant averaged 37.9 per cent of normal, or 806.4 per cent of the rate in the group without the attractant.

ATTRACTING ABILITY OF THE PHEROMONE AT 0.5% AS COMPARED TO 1.0% CONCENTRATION

A final comparison can be made to demonstrate that the ability of the pheromone to attract rats is nearly equal at each level of the chemosterilant additive. This ability was demonstrated in Experiments III and IV. The addition of a pheromone to the 1.0 per cent mixture of chemosterilant reduces the average drop in food consumption to about 7.4 per cent of normal as compared to less than 0.1 per cent of normal if urine is not added to the chemosterilant mixture at that concentration. The reduction in food consumption at the 0.5 per cent concentration was held at an average of 23.4 per cent of normal by the addition of the urine in comparison to a drop to less than 2.0 per cent of normal for the first two days when the urine was not added at that concentration of chemosterilant.

If compared on the basis of how much more readily the animals are attracted to the pheromone than to the plain food at each level, however, those animals which are given estrous urine at the 1.0 per cent level average 225.0 per cent greater food consumption than do rats given the same food material but without the addition of the urine. Animals given the urine attractant in a 0.5 per cent mixture of the U-5897 demonstrated only 155.5 per cent greater ingestion of the material than their counterparts without the pheremone additive. The effectiveness of the chemosterilant was apparently similar in both the 0.5 and 1.0 per cent concentrations. This is demonstrated by the fact that 100 per cent of all male rats tested at both levels developed bilateral lesions and testicular atrophy.

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APPENDIX

APPENDIX A

A METHOD FOR CONTROLLING COMMENSAL NORWAY RATS IN URBAN HABITATS

Contemporary efforts toward rodent control are often undertaken by individuals or even groups, who are not thoroughly familiar with the biological nature of the animals which they are attempting to regulate. It should be axiomatic, however, that a thorough knowledge of the target species be within the grasp of its human predator before he attempts to undertake any type of control effort. Logically, then, the opening sections of this appendix on rodent control should contain a discussion of the basic biology of the Norway rat.

A significant amount of research was done during the 1940's and 1950's concerning the population structure of wild populations of brown or Norway rats. Although most of this work was done on the East Coast in the Baltimore area, the data can probably be superimposed on the Michigan area without a great deal of error being introduced.

BREEDING AGE AND POTENTIAL OF NORWAY RATS:

According to Calhoun (1962a), male brown rats are ready to breed at the age of about 70-80 days, while females mature slightly more slowly (80+ days). After breeding, these rats have a gestation period of about 21-25 days (Silver, 1941). Female rats have an estrous cycle of about 4-5 days, so at the end of this period after parturition, they are ready for another mating and subsequent pregnancy.

Davis and Hall (1949) have shown that male brown rats are able to breed all months of the year, but that females, for the most part, have certain peak months for reproduction (Davis and Hall, 1951). Large adult

females seem to breed during all months of the year with equal probability, but smaller females have a bimodal breeding season with peaks in spring and summer (Davis and Hall, 1951).

MORTALITY

The average life span for wild Norway rats is about 7.1 months according to Davis (1948). He has stated, however, that there is a great deal of variability in survival of young dependent upon the time of parturition. There also seems to be differential survival rates between male and female rats, with the females seeming to live somewhat longer on the average. Mortality may be attributed to several different sources. Intraspecific competition is usually a significant factor because of territoriality and stress (Calhoun, 1962a). Other factors are predation by dogs, cats, owls, and man (Davis, 1948). Nutritional food requirements are frequently not met according to Davis and Winsor (1948), thus adding another increment to mortality.

CENSUSING

Emlen, Stokes, and Davis (1949) determined that by using bait stations, numerical censuses of urban rat infestations could be taken with 80 per cent accuracy. This figure was found to be highly preferable in terms of accuracy over that used by Davis (1948) in farm populations. His method was simply a modification of the Lincoln Index method, and subsequent studies (Calhoun, 1962a; Emlen, Stokes, and Davis, 1949) demonstrated that this was not accurate due to trap shyness and trap proneness on the part of once-trapped individuals.

A method of direct enumeration of the animals has been used by Calhoun (1962a). This method, which involves the capture of each

individual in the population, is obviously highly impractical for field work with wild populations of rats.

The only other type of census method available at the current time, is that of a relative index which is determined by the presence of visible rat signs such as rub marks, feces, gnawings, etc. (Littig et al., 1969). This method is used almost exclusively in nearly all rat control programs where censusing is attempted at all.

CURRENT MICHIGAN RODENT CONTROL PROGRAMS:

Rodent control operations of cities in southern Michigan vary a great deal in their modus operandi. The following is a description of the techniques used in the Michigan cities of Muskegon, East Lansing, Highland Park, and Lansing. These methods supply a cross section of the rat control programs used in Michigan. Unfortunately, only two or three cities in the state besides Lansing and Highland Park have programs which are on a similar scale of efficiency as these. The remaining metropolitan areas follow procedures similar to those of Muskegon and East Lansing.

Muskegon:

According to Jack Mason, the Chief of Environmental Health in Muskegon Co., the city of Muskegon has a relatively inefficient control program. The procedure is simply to mix up warfarin bait blocks (specified concentrations of warfarin mixed with corn and other materials, and encased within a parafin block for protection from the elements) and distribute them according to complaints about rat problems from the public. These baits are not checked on a daily basis due to lack of funding for hiring personnel. Instead, they are inspected only at infrequent intervals, and may or may not be replaced when they have been

completely consumed. No regular census is taken, and no attempt has been made at censusing the populations either before or after control efforts have been instituted.

East Lansing:

In East Lansing the control program, according to Donald Jenks who is head of the East Lansing Department of Sanitation, is approached in a manner which is somewhat more thorough than that in Muskegon. In addition to the complaint-reaction baiting program, there is a procedure instituted in this area which consists of assigning one man to inspect man-holes on a weekly basis, and dropping a warfarin mixture sealed in plastic bags into any holes which show evidence of rat infestations. This evidence is determined by the presence of feces, tracks, rub marks, etc. Frequently, if a man-hole does not contain running water, one or two bags of the warfarin mixture are dropped into it "just to be safe". Again no census attempts are made either before, during, or after control efforts.

Highland Park

Highland Park has one of the best approaches in the state to the problem of rat control. Here the procedure follows several steps which are under the direction of Deputy Health Officer Anthony P. Miano.

1) A team of sanitarians surveys a neighborhood to determine the rat infestation using the standard census procedure described by Littig et al. (1969). At the same time, they evaluate the amount of harborage and food material available to rats in the area.

2) At the completion of this step, the sanitarians then instruct the property owners and tenants in the neighborhood to clean up the property in terms of food and harborage which may support rats. The

penalty for non-compliance is the issuing of a citation and finally the levying of a fine.

3) After the area has been cleaned up, poisoning teams enter and distribute poison baits in a three-phase cyclic rotation. A sequence of red squill, diphacinon, and fumarin baits are used for this purpose. The teams simultaneously apply cyanogas powder to burrows and zinc phosphide in sewers where severe infestations are evident. The major problem lies in the fact that this program only takes place during the summer months when populations are at their peak density.

4) Additional inspection is made periodically by the sanitarians to see that there is no recurrence of harborage and food material in the area after the cessation of baiting.

Lansing:

The rodent control program in Lansing directed by John Ruskin and R. H. Goodsell is at least as good as, and possibly better than, any in the state, at least in the case of major neighborhood infestations. General complaints are simply handled on an individual basis in which a sanitarian or a rat control technician treats the area of the complaint with diphacinon-block poisons which are commercially prepared. The poison applied in this manner may or may not be rechecked on a regular basis.

Major infestations of entire one block or larger areas are treated on a large scale basis. Here each area is censused in the normal manner (Littig et al., 1969) and then the major locations of rat problems are mapped. Some "emergency baiting" is performed to reduce extremely severe infestations of rats at this time, thus reducing migration to human dwellings during the following step of the program.

Next, a full scale cleanup and ratproofing program under the supervision of the county health department and the city vector control department is initiated by the area residents.

The third step in the program is the placement of poison baits such as commercially prepared diphacinone. This bait is then checked on a regular basis by either the residents or the health department employees, and is replaced as necessary.

After a set period of 2-3 weeks, the poison material is removed to minimize the possibility of selection for genetic immunity. At this time, another census is made to determine the overall effect of the treatment program. If success was generally achieved, any remaining isolated infestations are treated on an individual basis with cyanogas and diphacinone. If the entire program is found to have had suboptimal results, full scale rebaiting is repeated throughout the entire target area.

The single most outstanding feature of the procedure lies in its year-round application. This factor makes it much more effective than a simple summer program, since it is possible to attack the rat population during the winter months, when they are at their lowest numerical densities.

A major fault, however, is that the program is not a regular or continuous event. It is, at best, a sporadic effort, relying on public pressure and allocations of funds for its institution.

SUGGESTIONS FOR AN ALTERNATIVE APPROACH TO A CONTROL PROGRAM

The literature clearly shows that any functional control program must be a multifaceted approach in which poisoning is only a supplement to environmental clean-up. The application of a chemosterilant material

rather than a purely toxic material may be a method of the future which will create a more desirable final end point; however, materials of this type are not yet available for widespread use, and therefore cannot be considered at the present time. The same status applies to the utilization of pheromone attractant materials such as those discussed previously in this paper.

The following is an outline of what I feel should be a theoretically sound and practical approach to urban rat control using only the currently available materials.

A. Pre-Treatment Survey

The proposed control area must be surveyed to determine the approximate extent of the rodent infestation. This should be done only by personnel trained in the techniques of making the survey and what to look for during the actual operation. Such a training program should last from two to three days, and should involve instructional pamphlets and films as well as classroom lecture.

The survey itself should be carried out by groups of five persons, one of which is a foreman who is responsible for transportation and for coordination of activities. The other four should work in pairs and survey one-block areas from both sidewalks and alleys. They should note signs of rat infestations, as well as units of harborage and food. Such information should then be recorded on standard check-off forms such as those used by the U.S. Public Health Service.

Sewer infestations which will be discussed later, must be determined in a similar but separate operation in which the survey team looks in manholes for rub marks and droppings, and along streetside curbs for burrows.

B. Analysis of Survey Results

The findings of the survey teams should be analyzed subsequent to the completion of the operation, and the location of infestations should be marked on a transparent overlay map of the urban area. The greatest concentrations of rodents, are then assigned the first priority, with the other areas being treated with as much emphasis as the monetary budget will allow.

C. Application of Poison

Surface Areas

After the target treatment areas have been assigned, a poisoning program can be initiated. This must be accomplished before cleanup operations in order to prevent dispersion of the rodents. Since anti-coagulent poisons of both coumarin and indandione derivatives have been demonstrated to be the safest and most effective when properly placed (Hayes and Gaines, 1959; Bjornson, Pratt, and Littig, 1968), these toxicants should be used exclusively in surface locations. Single dose poisons such as 1080 and 1081 are probably more effective baits for use in sewers for the most part (Bjornson, Pratt, and Littig, 1968).

A useful precaution in applying poison baits to an area is to use a system of rotation of toxic substances. This would effectively reduce any possibility of the rodents developing a tolerance to a specific poison as has been demonstrated in Great Britian (Lund, 1964).

A system should be used in which diphacinone or pival, which are indandione derivatives, are applied for a three week period, all remaining poison should be removed. This will maximize the deleterious effect on the rodent population, since nearly all rodents would have an opportunity to ingest the bait within this period. It will also minimize

the hazard to non-target animals, including children, since there will be no unconsumed bait remaining and available to them. As a further precaution, the occupants of all residences treated with poison should be informed of the project and poison by a form letter delivered at the time of bait placement.

At the close of this initial poisoning program, an environmental clean-up should be initiated as described below in part D. This should be immediately followed by the re-introduction of poison for another three-week period. This second poisoning program should employ warfarin or a similar hydroxycoumarin base anticoagulent, thus rotating toxic materials as previously suggested. Following this application, a two to three month waiting period should be observed, after which another poison application can be made if deemed necessary.

Sewers

Sewers can be treated simply by placing large quantities of single dose poisons in manholes where rats are in evidence. Since these areas are effectively removed from both pets and children, highly toxic compounds such as sodium flouracetate (1080) and flouroacetimide (1081) are recommended for use here. These poisons usually require fewer man-hours for supervision and a smaller quantity of bait since after the initial bait placement in all manholes which show signs of rodents, the bait need be checked only at monthly intervals. Because sewer populations of rodents are not frequently in direct contact with humans, and therefore not as serious a problem, some bait shyness can be tolerated if the financial cost is minimized.

D. Environmental Clean-up and Ratproofing

Upon completion of the initial poisoning program, an official should contact property owners and tenants in the target area and instruct them

to have all harborage removed or made rodent proof within a given period. They should also be required to use rodent proof garbage storage or disposal facilities and be informed of the immense value of these measures. It must be made clear to area residents that the poisoning program is not permanently effective without the subsequent destruction of all rodent harborage and food sources. It should also be clearly stated that future attempts at poisoning, or a continuation of the program, will not be made until the cleanup operation is completed. In extreme cases of non-compliance, legal action may be necessary, but should be used only as a last resort.

E. Expected Results of the Program

After the survey has been completed, an efficient overlay map of rodent concentrations in the area will be available for reference. This will provide the information necessary to maximize all control efforts in areas where they will be most effective and beneficial.

The application of poisons as outlined above, should reduce the rodent population by up to 100 per cent, depending on the efficiency of bait placement. An average result with proper placement and renewal of bait should approach 90 to 95 per cent mortality in any given area. According to Emlen et al. (1948), if a loss of these dimensions is incurred in a wild population of Norway rats, the rate of recovery is reduced to about 1 to 3 per cent per month. It should be noted that if the poisoning campaign occurs just before the winter, the rate of recovery will probably be even slower, since Davis (1950) found evidence of depressed reproduction rates from November to April.

The most lasting effect will be caused by the removal of harborage and garbage from the environment. Orgain and Schein (1953) demonstrated

that removal of either will cause drastic reduction of up to 50 per cent of the rodents without any additional control method being used. Elimination of both harborage and food sources such as garbage will effectively extirpate the population within a six-month period.

When this method of habitat manipulation is combined with an extensive poisoning program, the result should reduce populations to zero and it should remain at that level as long as sanitary measures prevail.

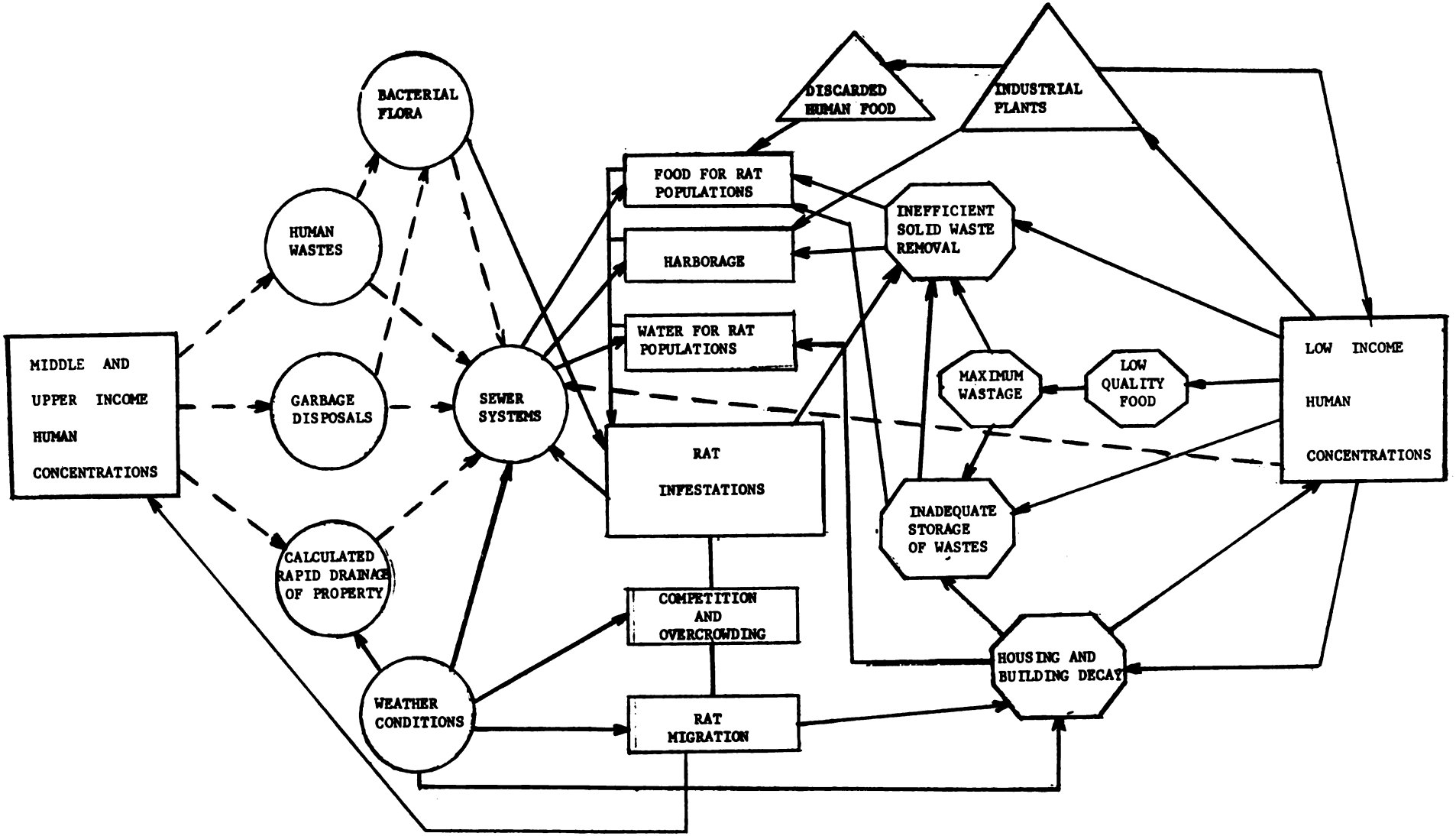
APPENDIX B

It was stated earlier in this paper that Norway rats can be regarded as a semi-parasite of human populations. The graphic model below (see Figure 9) is an attempt to describe the basic function or niche filled by rats in an urban environment or ecosystem. It must be realized that the human influences in this model can be expanded ad infinitum and probably are. This model, therefore, does not attempt to include all of these factors, e.g. industrial labor unions, political systems, social pressures, profiteering, etc., both for the sake of clarity and in the interests of time and space.

In defense of my statement describing these introduced rats as being semi-parasitic, it must be admitted that such entities as "feral" populations of rats exist in local areas far removed from human housing, but even these often depend upon the technology of man to provide them with their daily food through the medium of cultivated crops. It is within an urban ecosystem that rats truly become inextricably intertwined with human populations. They may function in a somewhat beneficial manner for human populations through their garbage removal actions. This example might cause them to be considered as somewhat commensal rather than truly parasitic. They might take on the title of competitor in another instance, when they consume goods which might otherwise be consumed by humans. The fact remains that they still depend upon man for a majority of their life supporting requirements. For this reason, I choose to label them as semi-parasites.

Rodent populations, similar to most other living organisms, must be supplied with three resources, each of which is a density dependent

URBAN RAT POPULATIONS



limiting factor. These factors are the most evident components of K, or the carrying capacity of the environment referred to by Smith (1952). The components under consideration are simply food, water, and harborage (Bjornson et al., 1956). Actually in a natural situation, water may be the least critical of the three limiting factors, and is only very infrequently of significant importance to the survival of the rodent populations. The model below graphically demonstrates the normal pathways through which these resources are acquired by the rodents. Its legend can be stated quite simply. The boxes are the significant factors which figure in the survival of rat populations in an urban ecosystem. The broken lines represent a man-directed, desirable relationship between the factors represented by the boxes, whereas the solid lines are representative of undesirable relationships from the viewpoint of human populations.

Part I: THE SUBURBS

By definition, a model of an ecosystem is complicated by interactions between each of the components. Perhaps the most appropriate place to begin a description of this model is in the left hand margin at the box labeled Middle and Upper Income Human Concentrations. This component of an urban ecosystem (probably this should be classified as the residential section at the outer fringe if an urban area, or the suburbs) exerts its effects upon rat populations in each of three major catagories, all of which are tied together in a common gathering facility--the sewer system. In order to understand these effects, certain inherent characteristics of an urban or suburban environment must be recognized.

First, the adversity caused by standing water from rain storms in any urban ecosystem must be considered. Large puddles in streets

virtually bring traffic to a halt. Similar puddles on private peoperty destroy expensive lawns or block sidewalks, residual stagnant water forms a breeding place for mosquitos, etc. All this creates a valid need for rapid drainage of property. This, of course, is accomplished through the medium of underground sewer systems in the majority of urban areas.

Secondly, the eating habits of most families at the middle to high income level described here must be considered. Normally, families within this economic bracket tend to select food which affords a minimal amount of waste, e.g. canned or boneless ham, relatively fat free meats (primarily beef), a large quantity of boxed or dehydrated foods, especially potatoes which do not require peeling, etc. (U.S.D.A. Report, 1956: 106-108, 190).

The relative abundance of garbage disposals in this economic class then becomes a factor. What waste remains from human food is rapidly ground into rather fine particulate matter and flushed into the city's sewer systems, often the same system as the excess run-off from the rain storms, since in many cases storm sewers and domestic sewers are not effectively segregated.

The third major consideration in the discussion of these middle and upper income families (which is also an obvious component of any economic class) is that of the biological human wastes or excrements which are also flushed into the sewer systems. In this case there is the additional factor of an enormous quantity of bacterial flora which is attached to and included within these excrements. Society has correctly placed premium importance upon flushing these bacteria away in an attempt to reduce the possibility of recycling within large human populations, thus creating the hazardous possibility of epidemiological disease.

In looking at these sewer systems, however, it becomes evident that for all of their beneficial function, they have a tremendous potential for causing highly detrimental effects to human populations. Due to their basic nature of construction, they form ideal harborage for rats. They are dark and offer a minimal amount of physical disturbance. They are of nearly constant warm temperature and are at nearly constant humidity. In addition to this harborage, the sewers, through design of purpose, afford an ample quantity of water for rodent populations, thus supplying two of the three basic requirements or limiting factors for rats. Now, if the contributions made by garbage disposals are added, a total set of necessary requirements are present. All of the ingredients have coalesced to support a subterranean rat population in the sewer systems of a city. But not only do these rodents have the benefits of food, water, and harborage from these sewers, they are also exposed to and live in the bacterial flora which human populations once attempted to dispose of by flushing down sewers.

Now comes the simple biology of the animal. Similar to mites on oranges (Huffaker, 1958), house mice (Southwich, 1955; Southern and Laurie, 1946) and muskrats (Errington, 1939), the rats expand in numbers until the food supply is depleted and intraspecific competition for this resource becomes a significant factor. Perhaps in a rainy season when sewers become flooded, competition for space becomes the governing agent. In either case, the animals begin to move out of the sewers and appear at ground level where, carrying large bacterial infestations, they must immediately begin searching to supply their three basic limiting factors or components of K.

Part II: THE INNER CITY

In order to facilitate the most meaningful description of this model, it becomes expedient to interrupt the apparent continuity of flow at this point, and address the reader to the extreme right hand margin, or the box labeled Low Income Human Concentrations. This area can probably be classified as the inner city, or the underprivileged urban residential area.

It must immediately be stated that this segment of the population has sewage facilities and systems probably much older but almost similar to those of the previously described economic segment. With the major exception of more garbage disposals in the latter segment, the systems are equal. The fact that these low income dwellings and residences are interconnected by the sewer systems with those of the higher income levels is significant however, as will become more apparent later in this discussion.

Normally, an urban human population which exists at an income level such as that described here, is made up of blue collar workers. This is demonstrated in the model by the broken arrow which represents a beneficial relationship between the urban population and the industrial plants, i.e. the availability of employment. The solid arrow from the industrial plants to the urban populations symbolically demonstrates that these workers are paid lower wages in comparison to those paid to residents of the suburbs, thus leading to the defined low income level. (It must be recognized that other occupations with low wages also exist in an urban ecosystem, but the factories are the best example for demonstrational purposes in this case.)

Inhabitants of these low income urban areas are the providers for and the victims of at least three more or less separate sources of rat infestations. Perhaps the most simple of the three are the rat infestations existing within the industrial plants which are quite frequently located very near, or adjacent to, low income housing. These plants are by nature large and impersonal structures which afford a maximum number of hiding places or harborage for rodent populations. They also supply a ready source of water from condensation of pipes, dripping faucets, wash rooms, etc. When any of the employees discard part of their lunch (multiplied by several hundred workers in the plant) there is a ready supply of food for the endemic rodent population. If this food is supplemented by any of innumerable sources in the general area of the plant, e.g. garbage cans, dog food, production of edible materials in the plant itself, etc., the rodent population is able to maintain itself, often in spite of poisoning operations or other control efforts within the factories.

The second source of rat infestations in low income areas is often brought about as a direct result of the low income of the inhabitants. Since the economic level is low by definition, frequently there is not enough money to purchase food of the quality consumed in higher income homes (U.S.D.A. Report No. 1, 1956). These lower quality and frequently deficient diets often include relatively high waste foods such as fatty roasts and cuts, or slabs or ribs. Other common high waste items are starchy foods such as fresh potatoes and sweet potatoes, or rather bony items such as chicken. All of these foods have a higher incidence of occurrence in homes with an income level of $5,999 and below, than in homes with a higher annual income level (U.S.D.A. Report No. 1, 1956:

66-76, 106-108, 190). Since a large portion of this food is not usable or used, e.g. fat and suet on meat, chicken bones, potato peelings, etc., it is normally discarded as garbage. This garbage may often be stored in paper bags or cardboard boxes since families may not be financially able to purchase proper garbage containers. These bags or boxes might then be stored in basements or back yards until such time as is convenient for removal to a suitable dump or other garbage disposal facility (Orgain and Schein, 1953). The time period involved here could conceivably be rather lengthy simply due to the financial burden of paying to dump the waste products, or to possibly paying to have either a professional or a friend with an automobile remove it. Such a removal system is obviously highly inefficient, and contributes tremendously to the availability of food for rat populations.

In assition, there is the third problem of the cheap, rented housing in which low income families frequently live. These dwellings are often well past their prime and are rapidly decaying or becoming dilapidated due to overuse, abuse, and lack of normal maintenance (Schorr, undated). These neglected structures not only permit nearly unobstructed entry for rats, thus giving them access to garbage and trash stored in cellars, but such dwellings also afford a nearly unlimited quantity of harborage for the high density of rodents the supply of food present.

The harborage provided by the deteriorated buildings in these areas is often supplemented by accumulations of abandoned appliances, automobiles, rubble, lumber, etc., between buildings. Consequently, rodents have very little difficulty in finding all of the requirements for survival.

Now it becomes feasible to return to those rats which have moved out of the sewer systems due to intraspecific competition. As they emerge from the sewers and begin to disperse, they are faced with the problem of finding harborage, food, and water. If they move into the suburbs, they are often attracted and supported by dog pens in which food pans are set out in the evening, and scraps of dog food remain for the rats to devour before the dish is taken away just prior to feeding time on the following day. In some cases, these moving rats may encounter uncovered garbage cans, and occasionally, they may find fruit from back yard apple trees. In no case is food impossible to locate if sufficient effort is exerted. Harborage, too, is quite accessible. Since Norway rats are normally burrowing animals, they are quite accustomed to digging holes under the foundations of buildings or along alleys. Water, as stated previously, is almost never of consequence.

A comparison of the availability of food, water, and harborage in suburban middle income residential areas with their availability in the inner city "ghettos" generally shows a greater quantity of these factors present in the latter areas. These environmental conditions probably provide a much higher carrying capacity for rodents in the inner city residential areas than environmental conditions prevalent in middle income suburban residential areas. Studies by Davis on city vs farm rats (1949) and by the Douglass Commission and published in transcripts of the Commission hearings (1967) on housing problems and the Commission report (1968) also seem to indicate that this may be the case.