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An investigation of the toxicology and residues of rabon in poultry.

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AN INVESTIGATION OF THE TOXICOLOGY AND RESIDUES OF RABON IN POULTRY

A Dissertation Presented

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TABLE OF CONTENTS

INTRODUCTION

Pest insects have always been a problem to man. They attack his crops, stored food products, are ectoparasites on him and his animals, transmit various disease producing micro-organisms, and are a constant source of annoyance.

In the continuous combat against insect pests, man has been using a wide variety of control measures but has been depending largely on chemicals. However, a chemical, though very effective, becomes useless if a population of resistant insects develops. Also certain very promising insecticides show high toxicity to animals being treated or leave undesirable residues in the crops or animal tissues following the treatment. These are some of the major problems which contribute to the search for newer insecticides that are effective against insects but are less hazardous to man and other animals.

Rabon[1] is such a new chemical, possessing very low toxicity to mammals but high toxicity to several species of phytophagous insects, ectoparasites of animals and dipterous insects breeding in animal manure.

Poultry is the second largest agricultural industry in Massachusetts. Rabon has been shown to be very effective against ectoparasites of poultry and also the flies breeding in manure. Federal law requires that any pesticide must conform with the residue tolerances set up by the United States

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[1]Rabon 2-Chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate is a product of Shell Chemical Company.

Food and Drug Administration before being recommended for use. Since no information was available on the potential residues of Rabon in edible tissues and egg yolks of poultry, the present investigation was undertaken to;

i) observe the effects of Rabon on general appearance and behavior of hens.

ii) develop a cleanup procedure for determining the presence of Rabon in tissues and egg yolks by gas chromatography.

iii) determine the amount of Rabon present in the edible tissues and egg yolks of hens fed known amounts of the insecticide, and

iv) determine the occurrence and rate of disappearance of Rabon in the egg yolks following the treatment.

REVIEW OF LITERATURE

Evaluation of the potentials of Rabon as an insecticide, with special reference to its efficacy against poultry pests is necessary prior to determining its residues in the tissues and egg yolks.

A. Use on Poultry

Insecticides have been used to control the ectoparasites of the hens or the dipterous insects which breed in chicken manure. To check the flies breeding in the manure, residual sprays are generally used but inclusion of certain insecticides in feeds or water has successfully prevented the development of dipterous larvae in poultry droppings; (Burns et al. 1965; Sherman and Komatsu 1963, 1965; Sherman and Ross 1959, 1960a, 1960b, 1961; Sherman et al. 1962; Simco and Lancaster 1966; Sherman et al. 1967, and Loomis et al. 1968). An ideal insecticide for use on poultry should provide good control of ectoparasites and flies, display good residual activity, should not impair egg production and must not leave any residues in the tissues or eggs, Kraemer (1959).

Brady (1966) reported that a suspension of Rabon wettable powder applied to 9 m^2 plots of manure at a rate approximating 1 g. of insecticide in 0.8 liters of water/m^2, gave 99-100 percent reduction of flies one day after the treatment and 47-100 percent reduction 3-4 days after treatment.

Hoffman et al. (1967) observed that 5 percent Rabon dust applied to litter at the rate of 50 g./m^2 killed 91 out of a total of 93 chicken lice of three species; body louse, Mecanthus stramineus (Nitzch), shaft louse, Menopan gallinae (L.) and wing louse, Lipeurus caponis (L.) and was better than 5 percent Ronnel granules, 2 percent Bromophos granules and 2 percent dimetilan dust.

Sherman et al. (1967) reported that Rabon added to feed at the rate of 400 ppm produced toxic droppings resulting in 99 percent mortality of larvae of housefly, Musca domestica (L.), 50 percent mortality of Fannia pusio (Weidmann), 49 percent mortality of Chrysomya megacephala (F.) and 59 percent mortality of Parasarcophaga argyrostoma (Robineau-Desvoidy), whereas only 10 ppm of Rabon mixed directly into manure gave 100, 86, 83 and 14 percent control of M. domestica, F. pusio, C. megacephala and P. argyrostoma respectively.

B. Use on Cattle

i) Against ectoparasites. Drummond et al. (1967) used several insecticides for the control of screwworm, Cochliomya hominivorax (Coquerel) on cattle and reported that Rabon 0.45 percent sprayed at the rate of 2.5 gal./head gave complete control of one and two-day old larvae.

Drummond (1967) working on the control of cattle grubs, Hypoderma spp. found that both a pour on treatment of 16 percent Rabon in oil (100 mg/kg) and three sprayings of Rabon 75 percent WP at a concentration of 0.5 percent (115 mg/kg)

gave better than 90 percent control of these larvae. However, Drummond (1968) in his later work observed that one or two sprayings of 0.25 percent (60 mg/kg) was not effective against the cattle grubs H. lineatum and H. bovis.

Drummond et al. (1967) reported that 0.1-0.5 percent Rabon sprayed on the cattle gave more than 90 percent reduction of lone star tick, Amblyomma americanum (L.) one day after treatment.

Rogoff et al. (1968) stated that Rabon showed moderate systemic activity against northern cattle grub, Hypoderma bovis (L.) and the common cattle grub, H. lineatum (de Villers) when applied to calves by the pour on procedure. They mentioned 89-100 percent control in their earlier test but in the later tests only 41-60 percent control was found on the animals treated at the rate of 30 g./head. The toxicant was dissolved in acetone and a maximum of 60 ml. was poured on each animal. No significant cholinesterase depression, indication of toxicity or difference in weight gain was observed.

Drummond et al. (1967) used 26 insecticides against cattle ear tick, Otobius megnini (Duges) and reported that most of the insecticides including Rabon E.C. (0.5 percent concentration) killed 95-100 percent of the ticks at one week post-treatment. But at the same concentration, Rabon killed only 66 percent of ticks after one month of treatment. Drummond et al. (1968) found that 0.1 percent Rabon as a spray gave 100 percent control of the cattle tick, Boophilus

annulatus (Say) and 99.9 percent control of B. microplus (Canestrini). In dipping experiments, they indicated that freshly charged vats containing 0.25 percent Rabon controlled ticks satisfactorily but the effect was less satisfactory after six weeks of aging and moderate use.

ii) Against flies. Treece (1964) discussed an experiment in which cattle were fed several dosages of insecticides mixed with grain. Dung collected from these cattle was infested in the laboratory with newly hatched larvae of face fly, Musca autumnalis (DeGeer) and survival to the pupal stage was observed. They reported that the lowest effective dose of Rabon was 0.5 mg/kg.

In similar work Drummond et al. (1967) added larvae of the housefly, Musca domestica or hornfly, Haematobia irritans (L.) or both to manure from cattle fed 11 insecticides for ten days and found that Rabon 10 mg/kg/day was effective against both housefly and hornfly larvae and that only Rabon and bromophos controlled larvae of both species.

Mathis and Schoof (1965) working on housefly control observed that a residual treatment with suspension of Rabon at the rate of 1 g./m^2 to all the interior potential housefly resting surfaces in a dairy with the exception of milk and feed rooms, gave effective control for only one week. Six weeks later the dairy was re-treated at the rate of 2 g./m^2. At one dairy six weeks of excellent control was followed by seven weeks of satisfactory control.

Brady et al. (1966) in a field test for housefly control

determined that a spray of Rabon emulsion 100 mg/ft^2 gave 74-97 percent control after one day, 6-60 percent after 3-4 days and 0-14 percent after 7-8 days.

LaBrecque et al. (1967) in a screening test of contact sprays for control of adult houseflies indicated that based on LC_{50} values Rabon was one of the 61 compounds that were more effective than standards (malathion or ronnel). The LC_{50} value against Orlando regular colony was as follows: Rabon 0.06 percent, Ronnel 0.08 percent and malathion 0.05 percent and LC_{50} against Cardon P (insecticide resistant) colony was 0.30, 1.27 and 7.24 percent, respectively.

Hansen et al. (1967) tested insecticides for three years in one dairy barn and two years in another barn, and reported that Rabon as 1.0 percent WP applied to all walls, ceilings and other exposed surfaces gave 6 to 10 weeks of fly control.

Mathias and Schoof (1968) applied a number of insecticides as residual spray, by treating all potential resting sites for Musca domestica in milking barns and cattle sheds and by selective treatment of night resting sites in the same structure. Moban and Rabon in full coverage treatment were the most effective materials tested, giving up to 14 weeks satisfactory control.

Hansen et al. (1968) reported that when sprayed at the rate of 15.4 ug/cm^2/min. Rabon gave 20 to 57 percent mortality of houseflies.

Morgan and Blume (1966) reported that Rabon after 24 hours of treatment at a concentration of 0.1 percent, gave 90 percent

mortality for newly emerged hornfly adults Haematobia irritans (L.) when the latter were exposed for one minute on treated paper and was very effective as compared to methoxychlor.

Morgan (1967) worked out an electrochemical device for control of hornfly, Haematobia irritans, which used ultraviolet radiation as an attractant and a cotton curtain impregnated with 5 percent aqueous solution of Rabon as toxicant. This attractant-toxicant device was found to be very satisfactory in reducing the population of adult hornflies.

Mount et al. (1967) in a work on control of adult and larval stable flies, Stomoxys calcitrans (L.) reported that Rabon was highly effective with LC_{50} value of 0.91 ppm and 0.021 percent against larvae and adults, respectively.

Seawright and Adkins (1968) installed self regulatory devices consisting of shed protected dust bags in 24 pastures to control face fly, Musca autumnalis (DeGeer) on beef cattle and reported 73.6 percent reduction of this fly with a 5 percent Rabon dust.

Rabon has been reported to have killed 100 percent of housefly larvae seeded into manure of dairy cattle, fed a ration mixed with 36 ppm Rabon (Anonymous 1968).

C. Use on Crops.

Rabon has proved very promising for controlling certain insect pests of crops and ornamentals. Among insects that have been controlled successfully are hornworm, Protoparce sexta (Johanson) (Rabb and Guthrie 1964); oyster shell scale, Lepidosaphes ulmi (L.) (Boulanger et al. 1965); fall army worm,

Spodoptera frugiperda (J.E. Smith) and corn ear-worm, Heliothes zea (Boddie) (Young et al. 1966); red banded leaf roller, Argyrotaenia velutinana (Walker) (Cox 1966); gypsy moth, Porthetria dispar (L.) (Doane 1966); case bearer, Chlamisus cribripennis (Le Conte) (Wood 1966). It has also shown effectiveness against some stored grain pests (Strong et al. 1965, 1968; Lemon 1967).

Rabon has proved less promising against some crop pests such as green peach aphid, Myzus persicae (Sulzer) (Thurston 1965); citrus rust mites, Phyllocoptruta oleivora (Ashmead) and Aculus pelekassi (Keifer) (Reed et al. 1967); potato tuber worm, Phthorimea operculella (Zeller) (Shorey et al. 1967) and pickle worm, Diaphania nitidalis (Stoll) (Waites and Habeck 1968).

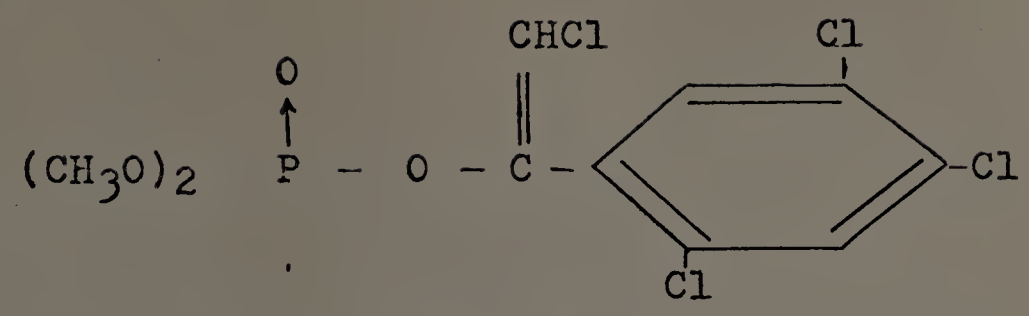
D. Properties of Rabon

The physical and chemical properties of any toxicant affect its use as an insecticide and govern its behavior in analytical systems. Therefore, it is necessary to examine the physical and chemical properties of Rabon prior to reviewing the analytical procedures for its residue determination.

Rabon, formerly known as Gardona or Shell SD-8447, chemically is 2-Chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate. It has shown high toxicity to several species of insects but is relatively safe to the laboratory mammals (Whetstone, et al. 1966). It has the following empirical formula and structure:

Empirical formula; $C_{10}\ H_9\ O_4\ Cl_4\ P$

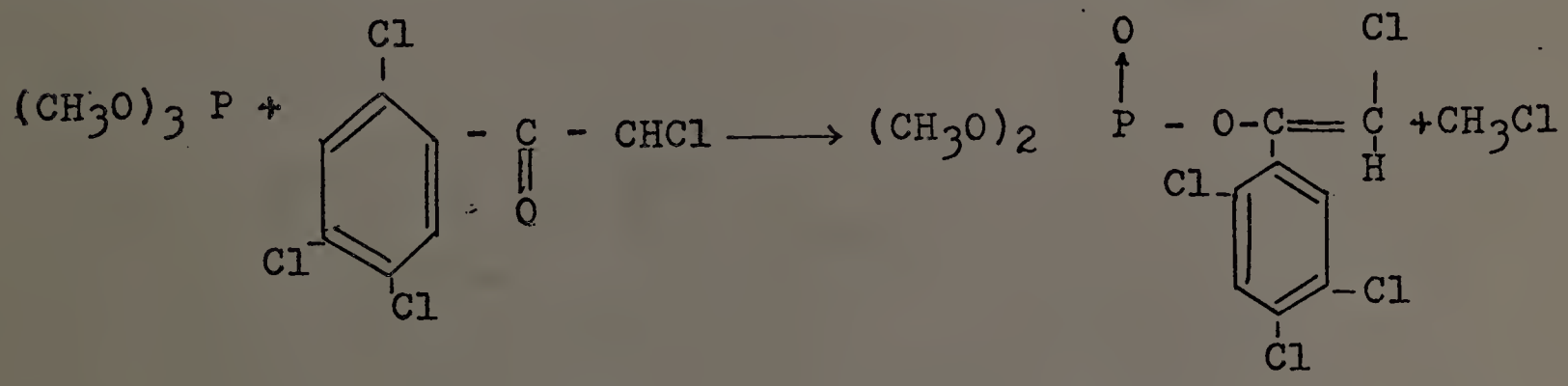
Chemical Structure:



Technical Rabon is a tan-brown solid with a molecular weight of 265.98 and melting point of 97.98° C. Its solubility in various solvents is reported as below:

Solvent	Solubility % wt at 21° C.
Chloroform	40
Methylene chloride	40
Acetone	20
Xylene	15
Hexane	5
Water	0.001 (11 ppm)

Whetstone et al. (1966) reported that it is decomposed slowly and has hydrolytic half life of 37 hours at pH 9.1 and 7200 hours at pH 1.1. They described its preparation by the following reaction. With chlorine in 2 and 4 positions of the benzene ring of the ketone intermediate the reaction is highly selective for β isomer, presumably for steric reasons.



Describing the synthesis they mentioned that the intermediate acetophenones were prepared using the Friedel Craft ketone synthesis. Dichloroacetyl chloride was added in 10

minutes to a stirred slurry of 88 gms (0.66 mole), of anhydrous purified powdered aluminium chloride in 109 grams (0.60 mole) of 1,2,4-trichlorobenzene. The mixture was heated slowly to 90° C., held at this temperature for four hours, cooled and poured on to mixture of ice and a few milliliters of hydrochloric acid. This product was extracted with ether, washed, dried and distilled to give 134 g. 77 percent yield of 2,2,2, 4,5-pentachloracetophenone, b.p. 103-105° C.

Rabon was prepared by addition of 23 g. (0.49 mole) of trimethyl phosphite to 50 g. (0.17 mole) of the pentachloroacetophenone at 30 to 50° C. in 30 minutes. The mixture was then heated at 110° C. for 30 minutes, cooled and poured into about 100 ml. ether. After being cooled in ice, the solid was filtered and washed with pentane to give 49 gms. (79 percent yield) of white solid 2-Chloro-1-(2,4,5-trichlorophenyl) vinyl dimethyl phosphate.

E. Analytical procedure

Adequate methodology is a very important aspect of measuring residues. A method to be used must have adequate sensitivity to determine a significant level of residue and should be applicable to the product being analyzed.

Methods for analytical measurement can conveniently be grouped into biological, chemical and physical means. Currently useful or promising methods of residue measurement according to Gunther (1962), include:

1. Biological measurements:

   a. Bioassay

      i) living organisms or plants
      ii) isolated organ systems or tissues

   b. Biochemical

      i) enzyme system
      ii) gross respiration
      iii) specific plant or animal elaborates

2. Chemical measurements:

   a. Color formation or blanching
   b. Gas generation or absorption
   c. pH changes
   d. Precipitation
   e. Radiotracer

3. Physical measurements:

   a. Chromatographic (retention time or volume, Rf value or related constants)

      i) columnar (inorganic and organic adsorbents including wax adsorbents)
      ii) gas (as volume, thermal conductivity, ionizability)
      iii) ion exchange
      iv) paper (as cylinder, disc, strip, centrifuged)
      v) strip of adsorbent on glass (so-called thin layer)

   b. Electrophoretic (as paper, zone)
   c. Nuclear magnetic resonance
   d. Polarographic
   e. Spectrometric

      i) fluorescent
      ii) gamma ray from neutron activation technique
      iii) infra red
      iv) ultraviolet (from 186 mμ to 350 mμ)
      v) x-ray
      vi) visible

   f. Titrimetric (as amperometric, potentiometric)
   g. Tracer with all isotopes

All the above methods and techniques can be useful in any residue problem. Selection of appropriate combinations of them for a particular problem requires confidence in the ability to exploit all reasonable techniques and appropriate knowledge of potentialities and limitations of each method for final detection of the chemical.

There is generally one instrumental technique best suited for each type of problem. Work done on plants and cattle indicates that Rabon can be detected by gas chromatographs equipped with electron capture device. But no information has been available on the extraction and cleanup of Rabon from tissues and egg yolks of hens for detection by gas chromatography.

Claborn and Ivey (1965) developed a gas-chromatographic method for determining the minute quantities of Shell compound 4072, 2-chloro-1-(2,4-dichlorophenyl) vinyl diethyl phosphate in tissues and milk of cattle. They hydrolized the compound with dilute sulphuric acid to 2,2,4-trichloroacetophenone and examined it by means of gas liquid chromatograph equipped with an electron capture detector.

Beroza and Bowman (1966) described a procedure by which Shell compound 4072 and Rabon may be determined by gas chromatography without a breakdown, on a 90 cm X 4 mm i.d. stainless steel column containing 5% w/w purified silicon grease on acid washed chromosorb W. at 190° C. They could determine the residues of compound 4072 and Rabon in corn extract up to 0.02 ppm by electron capture and 0.002 ppm by flame photometric detection. They hydrolized and identified the product

formed by acid hydrolytic cleavage as 2,2,4-trichloroaceto-phenone from compound 4072 and as 2,2,4',5'-tetrachloroaceto-phenone from Rabon.

Ivey et al. (1966) worked on the residue analysis of Rabon in cattle tissues and described the extraction and cleanup procedure for Rabon from fat (unpublished).

From the above information it is evident that Rabon is a promising insecticide for the control of a wide variety of insect pests and is very safe for use on warm blooded animals. Therefore, it would seem to be very suitable as an insecticide against ectoparasites of poultry as a direct application material. It appears to be suitable as a residual application for controlling adult houseflies in and around the poultry houses and also as a feed additive for reducing the popu-lation of fly larvae developing in manure. Rabon may prove to be an ideal insecticide provided it does not leave any residues in poultry products following its applications.

MATERIALS AND METHODS

A. Treatment of the Birds

In practical application the most probable sources through which insecticides may enter body tissues of poultry are: 1) direct application on the body, 2) feed in which insecticide has been intentionally or unintentionally mixed, and 3) drifting of sprays and dusts.

Hence, in experiments on the determination of residues, it would be desirable to apply insecticides in all three ways, but time did not permit. In order to avoid loss and breakdown of the toxicant in the bird's external environment, to obtain uniformity in treatment of each bird and to make accurate estimation of the amount of insecticide deposited in the tissues from the amount applied, it is preferable to administer the insecticide orally to the birds. In the present work the experiments on birds were limited to the breeds available.

i) Experiment I. A preliminary test was conducted to determine the amount of Rabon necessary to feed hens orally to result in tissue and egg yolk residues. On March 18, 1968 three White Leghorn hens were brought from the university poultry farm to the test room and placed randomly in a wire cage having separate bird units. The room was maintained on a 16 hour photoperiod. Birds were numbered and randomly selected for treatment. Bird No. 1 and No. 2 received Rabon 75 percent WP at the rate of 3 and 30 mg of active ingredient

per kilogram of body weight per day. Bird No. 3 was left untreated as control. Rabon was placed in gelatin capsules and force fed to birds daily for one week. The control bird received only empty capsules. The general appearance and behavior of the birds was noted. A daily record of the egg production was maintained. All three birds were slaughtered 24 hours after the final day of treatment. Eggs were collected daily, the yolks separated, placed in plastic bags, numbered, sealed and frozen. The number on the bag corresponded to the hen. Thus each yolk could be traced to its origin.

ii) Experiment II. Based on the data from the initial test a second experiment was conducted on April 15, 1968. Thirty Fayoumi hens which had just come into production were obtained from the university poultry farm and placed randomly in a wire cage having individual bird units. These birds were kept under similar conditions as birds utilized in Experiment I. The birds were banded and the number on the bird corresponded to the number on the cage unit. These birds were very excitable and stopped feeding and drinking for a few days before they learned to use the existing feeding and watering devices. As a result all the birds went out of production and it was not possible to run any test on residues in eggs. Therefore, it was decided to feed massive oral dosages of Rabon over an extended period of time to determine chronic lethal dosages.

Rabon 75 percent WP was fed to birds at the rate of 94, 188, 376, 752 and 1505 mg of active ingredient/kg body weight/ day. Five birds were randomly selected for each of the above mentioned treatments. A group of five randomly selected birds was untreated as a control. The insecticide was orally administered directly into the crop of the birds through a plastic tube having a funnel at the outer end. The measured amount of Rabon was transferred into a 50 ml. beaker containing 10 ml. of water, stirred with a glass rod and poured into the crop of the bird through the tube. An additional 10 ml. of water was used to rinse the beaker, funnel and tube. Control birds were given 20 ml. of plain water through another similar tube. Thus all the birds received an equal amount of water. Two persons were needed for this operation, one to hold the bird and the other to administer the insecticide.

A daily record of the weight of each bird was maintained during the treatment period and the amount of insecticide to be fed was based on the daily weight of each bird. The general appearance, behaviour and mortality of birds in each treatment was noted. The treatment started on May 7, 1968 and ended on May 19, 1968. Two birds from each treatment were randomly selected and slaughtered 24 hours after the last day of treatment. Approximately 20 g. samples of tissue (fat, liver, breast muscles and leg muscles) were removed, placed in polyethylene bags, labelled, quick frozen on dry ice and stored in a deep freezer for future analysis. Similarly, 2 birds from each treatment were selected randomly 8

days after the final day of the treatment and slaughtered. Remaining birds were slaughtered 15 days after the last day of treatment. The tissues were removed, frozen and stored as above.

Since the dosages administered to the birds were higher than would be expected in practical application, only the tissues collected from birds from the first slaughter were analyzed for residues. A third experiment using more appropriate dosages was performed.

iii) Experiment III. Due to lack of suitability of Fayoumi hens for the experiment originally planned, a third experiment was performed using Comet pullets. On June 6, 1968, 30 young hens were purchased from a private farm in Connecticut. These birds were randomly separated into single bird units of the wire cage, banded and placed in the same room and under the same light conditions as mentioned previously. When all the birds came into production, they were randomly divided into five equal groups. All the birds of each group received one of the following dosages: 00, 25, 50, 100 and 200 mg active Rabon/kg of body weight/day for a seven day period. The control birds (0 mg/kg) received 20 ml of water. The treatment began July 23, 1968 and continued until July 29, 1968. The Rabon 75 percent WP was administered orally through a plastic tube as described in Experiment II.

A daily pre-treatment, treatment and post-treatment record of weight, water consumption and egg production for each bird was maintained. The general appearance and behavior of birds under each treatment was observed. A daily

record of the droppings of the birds during and after the treatment was also maintained for an indirect approximation of food consumption. Birds were fed a complete commercial layer ration. Two birds from each treatment were randomly selected and sacrificed 1, 7 and 14 days after the final day of the treatment. Tissues were removed, quick frozen and stored as described in the second experiment.

The selection of dosages in the third experiment was based partly on results of the first experiment and partly on a trial made on the development of little housefly larvae in manure collected from the birds treated orally with Rabon during the second experiment. In the manure of the birds receiving 100 mg/kg of Rabon, 25 newly hatched larvae were released and mortality was noted. All 25 larvae in treated manure died as compared to only 2 in manure from control birds.

B. Analytical Method

Since no work has been done on the method of analysis of Rabon in poultry tissues and egg yolk it was necessary to develop a reliable and sensitive method for the present work.

Ivey et al. (personal communication), described a method for cleanup of Rabon in cattle tissues which involved the use of silicic acid column. Ivey et al. (1967) described a method of extracting Zytron from tissues and egg yolk of poultry in which the extract was also cleaned on a silicic acid column. Therefore, it was decided to combine the method of extraction

for Zytron with the cleanup method for Rabon. This combined procedure gave very satisfactory results and was used in the investigation. It is described below:

i) Reagents and apparatus

a) Acetonitrile nanograde, dichloroethane, n-hexane (redistilled)

b) Silicic acid 100 mesh powder specially prepared for chromatography (Mallinckrodt)

c) Celite as filter aid

d) Chromatographic column 20 mm i.d. by 400 mm overall length with fritted disk

e) Gas chromatograph, Aerograph HYFI model 600 C of Wilkins Instrument and Research Inc. equipped with electron capture detector and a g.l.c. pyrex column 5 ft X 1/8"

ii) Analytical procedure and gas chromatography. The method of analysis is based on hydrolysis of Rabon with 12 N sulfuric acid to form 2,2',4',5'-tetrachloroacetophenone and determination of the latter by gas chromatography. The analyses were made using a Wilkins Instrument and Research Inc. Aerograph HYFI model 600 C chromatograph. The column was packed with 5% S.F. (silicone fluid) 96 coated on chromosorb W, 60-80 mesh, conditioned isothermally at 240° C. Purified grade nitrogen at an inlet flow rate of 50-60 ml/minute was used as carrier gas. The temperatures of the column and injector were 200° C. and 220° C., respectively. At the above settings the retention time for Rabon was about 2 minutes. A series of standards utilizing hydrolized Rabon (2,2,4,5-tetrachloroacetophenone) in n-hexane were prepared ranging from 0.1 to 1.0 ppm. Two µl of these solutions were injected into the gas chromatograph. Standard solutions were

run with every analysis in such a way that the height of at least one standard closely approximated the height of each unknown.

The most commonly used methods of calculating the results from mechanically recorded curves resulting from known and unknown samples are: 1) comparison of the area under two curves drawn by recording instrument equipped with disc integrator; 2) triangulation, and 3) comparison of peak heights. When peaks are clean and sharp the third method is very convenient for all calculations.

iii) Extraction

a) Muscles, liver and eggs. A 20 g. sample of meat, liver or egg yolks was extracted by blending with 150 ml. acetonitrile, transferred to a 600 ml. beaker, stirred with 2 g. celite and filtered. Blendor and beaker were washed with additional 75 ml. of acetonitrile. The extract was concentrated to 50 ml. on a steam bath and transferred to a 500 ml. separatory funnel containing 150 ml. n-hexane. Additional 50 ml. hexane was used to complete the transfer. The funnel was shaken for one minute and the phases were allowed to separate. The acetonitrile phase was drained into a second 500 ml. separatory funnel containing 100 ml. hexane, shaken for a minute and left to let the phases separate. The acetonitrile phase was then drained into a 125 ml. flask. This extraction was repeated with additional 50 ml. of acetonitrile and both extracts were combined in the same flask and concentrated to 10 ml. on a steam bath. The

last traces of acetonitrile were removed by adding and evaporating four 25 ml. portions of hexane. At no time were residues allowed to go to dryness. The residue was dissolved in 5 ml. of hexane and reserved for cleaning and partitioning on chromatographic column.

b) Fat. A 20 g. sample of fat was blended with 20 g. anhydrous sodium sulfate and 150 ml. hexane, transferred to a 600 ml. beaker, stirred with 2 g. of celite, heated on steam bath to nearly boiling. The liquid was decanted onto a folded filter paper and collected in a 500 ml. Erlenmeyer flask. The blendor, beaker and filter paper were washed with 100 ml. hot hexane. This extract was concentrated to 150 ml. on steam bath, cooled to room temperature and transferred to a 500 ml. separatory funnel. Additional 50 ml. hexane was used to complete the transfer. One hundred ml. hexane was added to another 500 ml. separatory funnel and the dissolved fat was extracted four times with 50 ml. fractions of acetonitrile. Each time the acetonitrile extract was drained into the second separatory funnel and shaken with hexane. All four fractions of acetonitrile extract were combined in a 300 ml. beaker, concentrated to 100 ml., transferred to a 125 ml. flask and further concentrated to about 10 ml. on the steam bath. The last traces of acetonitrile were removed as described for eggs, etc. and the residue was stored in 5 ml. hexane for cleanup.

iv) Cleanup of extract. A chromatographic column was prepared by first adding 0.5 inch anhydrous sodium sulfate, then 12 g. silicic acid, followed by 1.0 inch of sodium sulfate. The silicic acid used was activated at 225° C. for 16 hours, cooled and deactivated by adding 14 percent water. The silicic acid was packed by tapping the column tube with a wooden block from below. A solvent mixture was prepared by mixing dichloromethane and n-hexane in ratio of 3:1. Of this mixed solvent 100 ml. was used to pre-wash the column. Then the sample which had been stored in 5 ml. hexane was transferred to the column using two 10 ml. fractions of the mixed solvent. At this point, the receiver was changed and Rabon was eluted with 230 ml. of the same solvent. The eluate was concentrated to 50 ml. on a steam bath, transferred to a 125 ml. flask with hexane and evaporated to nearly 1 ml. on a steam bath. The last traces of mixed solvent were removed by blowing air into the flask. The residue was dissolved in 0.5 ml. methanol and 10 ml. of 12 N sulfuric acid was added to it. The flask was connected to a reflux condenser for one hour. After completion of hydrolysis the heat was turned off and condenser was washed down with 5 ml. of distilled water. The flask was cooled to 15-20° C. with water and disconnected from the condenser. Five ml. hexane was added to the flask, shaken for a minute and transferred to 60 ml. separatory funnel. After separation of water and hexane phases the bottom layer of water was drained completely and hexane solution was transferred to a 50 ml. beaker containing a small amount of anhydrous

sodium sulfate. The solution was then transferred to a 10 ml. glass stoppered tube. An aliquot of this was injected into the gas chromatograph and the amount of Rabon was determined by injecting Rabon standard of approximately the same concentration based on peak heights.

The above procedure of cleanup was followed in Experiment III. But in Experiment I and II silicic acid was deactivated by adding 20 percent water instead of 14 percent and mixed solvent was prepared in the ratio of 4:1 instead of 3:1. Also, only 50 ml. of mixed solvent was used to wash the column after transferring the sample and Rabon was eluted with only 175 ml. of mixed solvent. This procedure was an attempt to save time and chemicals needed for the cleanup. The recoveries with this combination were as good as with the previous combination but the extract was not entirely clean and was gradually affecting the sensitivity of the detector. Also, more time was needed to bring the machine to the zero line after each run.

The recovery studies were made by fortifying the tissues with known amounts of Rabon and carrying them through the above mentioned procedures of extraction and cleanup. The results were very satisfactory and are reported in a later section.

RESULTS

A. Effect on General Appearance and Behavior of Birds

i) Experiment I

a) Effect on general appearance. All the birds remained active throughout the 7-day treatment period, with no marked visual difference between the control and the treated birds. However, a loss in weight of treated birds was noted as evident in Table 1.

Table 1.--Effect of oral dosages of Rabon on weight of White Leghorn hens.

Treatment* mg/kg	Weight in grams		
	Initial weight	Weight before slaughter	Loss in weight
Control	1600	1590	10
3	2350	2070	280
30	2060	1940	120

*One bird per treatment

There was a difference between the weights of treated and control birds but it did not appear to be directly related to the amount of insecticide administered, since the bird receiving only 3 mg/kg lost more weight than the one fed 30 mg/kg dosage.

b) Effect on egg laying. The treatment did not markedly affect the egg laying by the bird fed 30 mg/kg. There was a 50 percent reduction in egg laying by the bird fed 3 mg/kg, but since only one bird was used, it was impossible to determine the significance of this data. The egg production data are presented in Table 2.

Table 2.--Effect of continuous oral dosages of Rabon on egg laying of White Leghorn hens.

Treatment mg/kg	Number of eggs laid								
	Days after first day of treatment								
	0	1	2	3	4	5	6	7	8
Control	0	1	1	0	1	0	1	1	1
3	1	1	0	1	0	0	0	0	0
30	1	0	1	0	1	0	0	1	1

ii) Experiment II

a) Effect on general appearance and mortality. The treatment started on May 7, 1968 and the daily doses were continued until May 19, 1968. Control birds and those receiving 94 mg/kg appeared normal. However, those receiving 188, 376, 752 and 1504 mg/kg dosages became quite inactive and lethargic by the third day of treatment. Birds receiving 752 and 1504 mg/kg dosages reduced feeding and drinking. Their droppings were very loose, mixed with mucus and in some cases

Birds receiving 1504 mg/kg dosage showed symptoms of toxicity. Their combs appeared dry and whitish as compared to the reddish color of control birds. The affected birds could no longer stand but sat trembling with their heads down and eyes closed. Initial mortality occurred after administering the third dose and all birds in this group died before receiving the seventh dose.

Contrary to the above, the general appearance and activity of the remaining groups up to and including 376 mg/kg improved after the administration of the fifth dose. However, as the treatment advanced, the condition of birds receiving 752 mg/kg again gradually started deteriorating. These birds sat with their heads turned underneath the wing or between the legs. They were unable to respond to simple sound or vibrations unless tapped directly on their body. Their eyes were closed and mouths were full of saliva. The first bird in this group died after receiving the fourth dose and a total of three birds died by the fourteenth day after receiving 13 doses of Rabon. The chronic LD_{50} of Rabon for 6 days was 1064 mg/kg (Figure 1).

In the rest of the treatments (94, 188, 376 mg/kg), all the birds appeared to improve, and on the seventh day of treatment these birds were drinking and feeding the same as control birds.

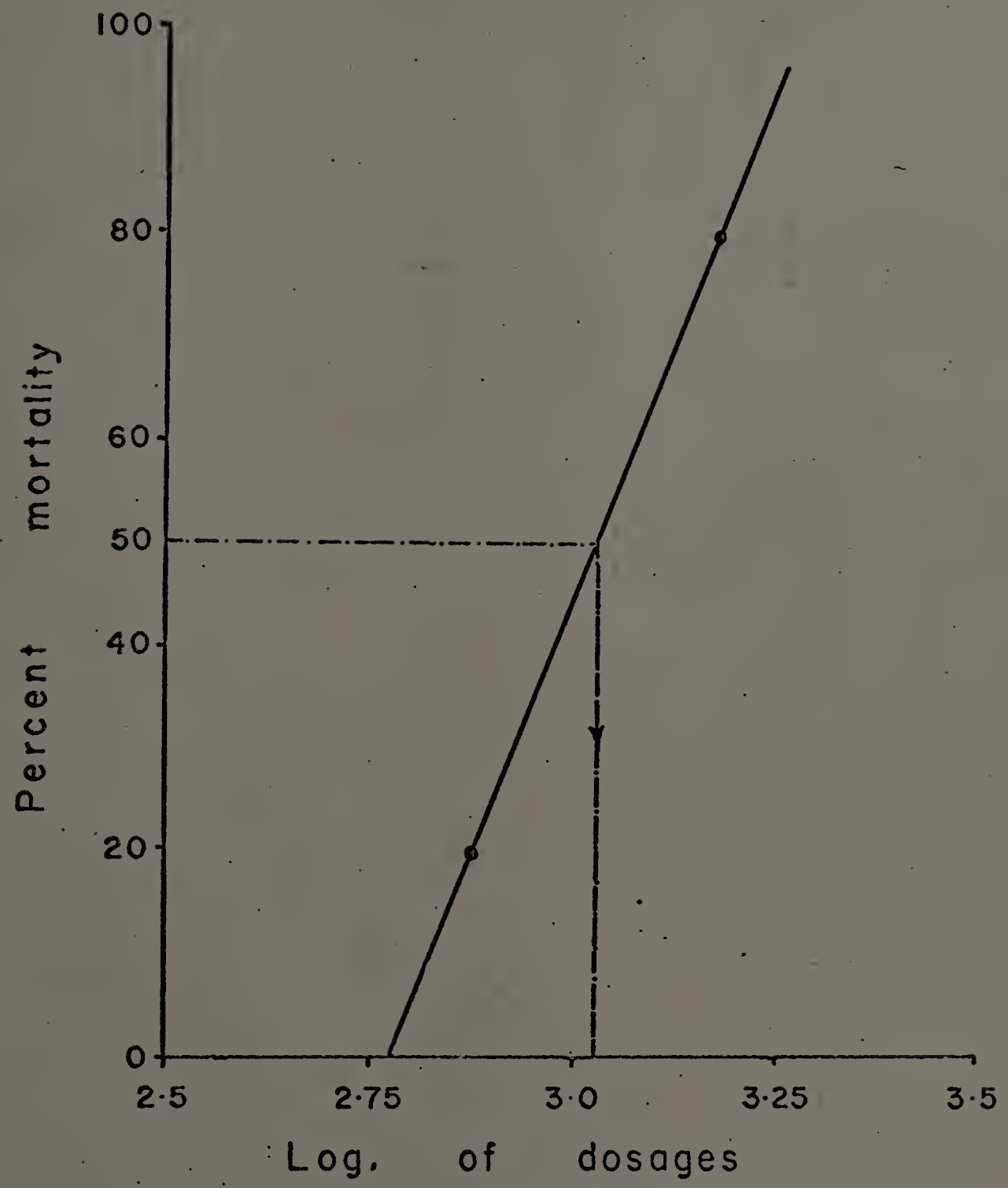
Mortalities of birds receiving different treatments are presented in Table 3.

Table 3.--Cumulative percent mortality of Fayoumi hens receiving daily doses of Rabon.

Treatment mg/kg	Percent Mortality on days of Treatments													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0	0	0	0	0	0	0
188	0	0	0	0	0	0	0	0	0	0	0	0	0	0
376	0	0	0	0	0	0	0	0	0	0	0	0	0	0
752	0	0	0	20	20	20	20	20	40	40	40	40	60	60
1504	0	0	40	40	60	80	100	-	-	-	-	-	-	-

Figure 1

Chronic LD_{50} of Rabon for Fayoumi hens for six days



b) Effect on weight. The control birds and those receiving 94 mg/kg gained weight by the end of the treatment period (Table 4). The maximum average gain was 2.3 and 4.2 percent respectively (Figure 2). The remaining birds gradually lost body weight. Considerable weight was lost by birds receiving 376, 752 and 1504 mg/kg. After the fifth day of treatment, birds receiving 188 mg/kg began to regain weight. Toward the end of the treatment period weight gain was also observed in birds receiving 376 mg/kg dosage. Those receiving 752 and 1504 mg/kg continued losing weight and by the seventh day of treatment 100 percent of the birds receiving 1504 mg/kg dosage died. Sixty percent of birds receiving 752 mg/kg died by the end of the treatment period.

Post-treatment records show that all surviving birds continued to gain weight for another week when most of them were slaughtered.

iii) Experiment III. In the third experiment 30 Comet pullets were purchased and divided in five groups of six each. However, two birds, one in control and one to be fed 200 mg/kg, became ill prior to treatment and were discarded, leaving only five birds in each of these treatments. The remainder of the treatments (25, 50 and 100 mg/kg) contained six birds each. The treatment started on July 23 and continued until July 29, 1968.

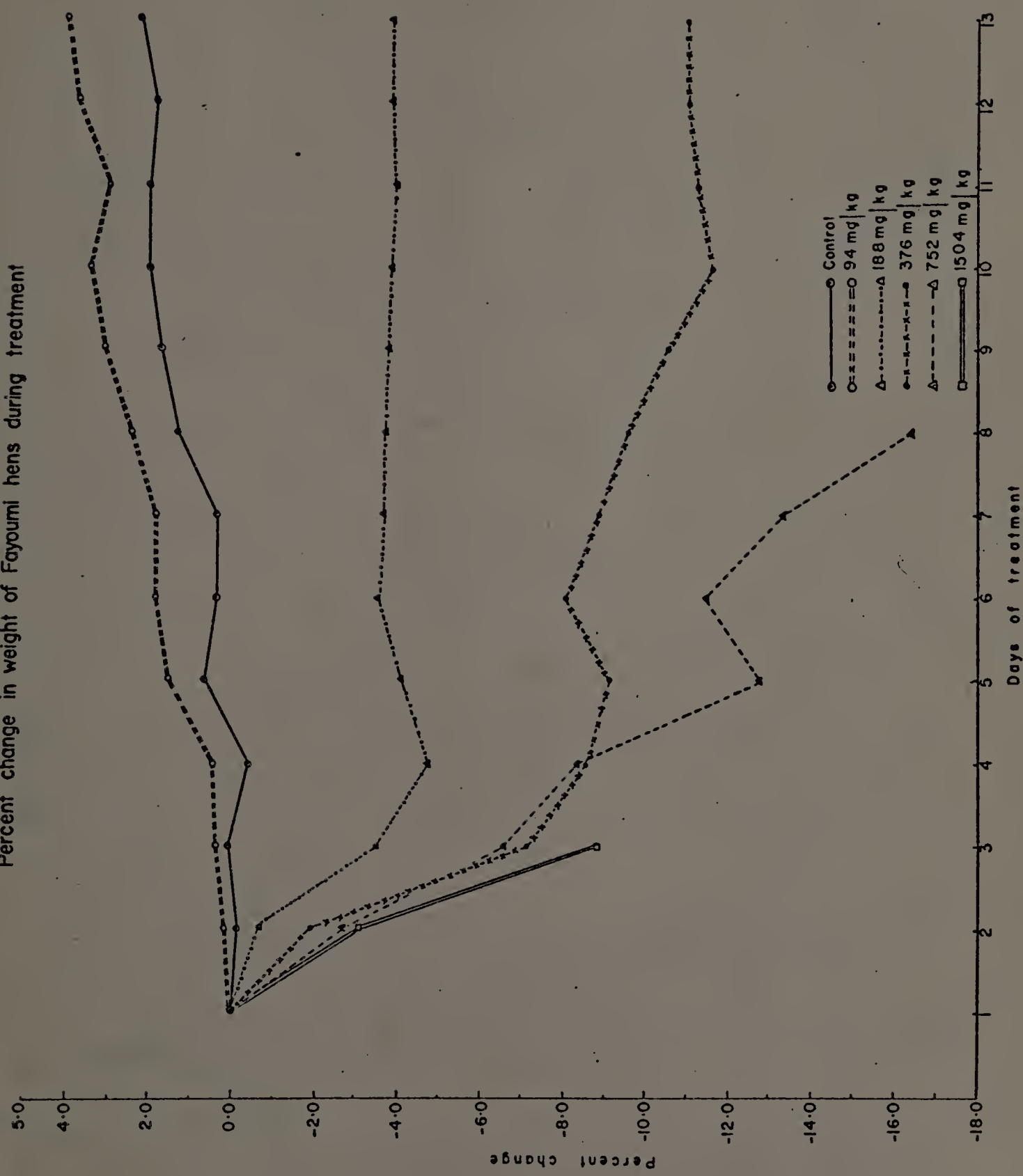
Table 4

Effect of oral doses of Rabon on average weight of Fayoumi hens

TREATMENT	No. of Birds	WEIGHT in grm.	TREATMENT PERIOD IN DAYS												POST TREATMENT PERIOD IN DAYS							
			1	2	3	4	5	6	7	8	9	10	11	12	13	1	2	3	4	5	6	7	8
CONTROL	5	Average weight	1658	1656	1660	1652	1670	1666	1666	1680	1688	1692	1692	1690	1696	1810	1813	1810	1800	1800	1787	1790	1793
		% Change	0·0	-0·12	+0·12	-0·36	+0·72	+0·48	+0·48	+1·33	+1·81	+2·05	+2·05	+1·93	+2·29	0·0	+0·17	0·0	-0·53	-0·55	-1·30	-1·10	-0·94
94 mg | kg	5	Average weight	1516	1518	1522	1524	1538	1544	1544	1554	1564	1570	1562	1576	1580	1696	1700	1697	1707	1707	1707	1703	1720
		% Change	0·0	+0·13	+0·36	+0·52	+1·45	+1·85	+1·85	+2·31	+3·17	+3·56	+3·03	+3·96	+4·22	0·0	+0·24	+0·06	+0·65	+0·65	+0·65	+0·53	+1·40
188 mg | kg	5	Average weight	1596	1684	1636	1616	1628	1636	1634	1634	1632	1632	1630	1634	1634	1566	1547	1547	1557	1557	1580	1563	1583
		% Change	0·0	-0·71	-3·54	-4·72	-4·01	-3·54	-3·66	-3·66	-3·77	-3·77	-3·89	-3·77	-3·77	0·0	-1·20	-1·20	-0·57	-0·57	+0·89	-0·19	+1·10
376 mg | kg	5	Average weight	1508	1478	1402	1380	1372	1386	1376	1364	1350	1332	1338	1342	1342	1420	1443	1443	1461	1477	1493	1503	1510
		% Change	0·0	-1·99	-7·03	-8·49	-9·02	-8·09	-8·75	-9·55	-10·48	-11·67	-11·27	-11·01	-11·01	0·0	+1·62	+1·62	+2·90	+4·00	+5·10	+5·80	+6·30
752 mg | kg	4	Average weight	1445	1405	1348	1325	1260	1280	1253	1208	1220 (3)	1180 (2)	1137 (2)	1097 (2)	1063 (2)								
		% Change	0·0	-2·77	-6·71	-8·30	-12·00	-11·41	-13·29	-16·40													
1504 mg | kg	5	Average weight	1412	1388	1288	1323 (3)	1415 (2)	1340 (2)	1110 (1)							Above values are based on weight of three birds per treatment							
		% Change	0·0	-3·11	-8·78																		

Numbers in parentheses indicate the number of birds.

Figure 2
Percent change in weight of Fayoumi hens during treatment



a) Effect on general appearance and behavior. All control birds appeared normal and active throughout the treatment with the exception of one which appeared slightly inactive and gradually reduced its feed intake. At the beginning of the treatment all treated birds appeared active. Their food and water consumption was nearly normal except for a slight reduction on the first 2-3 days of treatment. It was noticed that after receiving one or two doses of Rabon the birds appeared disturbed but as soon as they started drinking enough water they began to appear quite normal. The birds which started drinking water a little later were more inactive and lethargic. A few days later they did not show any difference in behavior and general appearance from other comparable birds. One bird receiving 200 mg/kg was very inactive, droopy and stopped feeding and drinking. The bird excreted mucus mixed with blood and died after receiving only three doses.

b) Effect on weight. The average weight of birds for each treatment is presented in Table 5 and percentage change in weight for each treatment is graphically presented in Figure 3.

Birds receiving 25 and 50 mg/kg did not lose any weight and their weight fluctuated very close to that of the control birds. The birds receiving 100 and 200 mg/kg lost a maximum of 4.4 and 7.5 percent of their weights respectively. Toward the end of the second half of the treatment period, these birds began regaining their weight and by the end of the

Table 5.--Effect of oral doses of Rabon on average weight of Comet hens.

Treatment mg/kg	Average wt. (gms) and percentage change	Pre-treatment	Treatment						Post-treatment				
			Days after first treatment						Days after last treatment				
			1	2	3	4	5	6	1	2	3	4	5
Control	Weight	2060	2080	2060	2060	2080	2070	2060	2170	2160	2160	2160	2160
	% change	0	+1.0	0	0	+1.0	-0.5	0	0	-0.5	-0.5	-0.5	-0.5
25	Weight	2032	2080	2080	2030	2030	2030	2020	2010	2000	2000	2020	2010
	% change	0	+0.4	+0.4	-0.1	-0.1	-0.1	-0.6	0	-0.5	-0.5	+0.5	0
50	Weight	2090	2090	2100	2090	2090	2090	2090	2030	2020	2010	2010	2000
	% change	0	0	+1.0	0	0	0	0	0	-0.5	-1.0	-1.0	-1.5
100	Weight	2052	2060	2030	1990	1960	1980	1980	1910	1910	1930	1950	1990
	% change	0	+0.4	-1.1	-3.0	-4.5	-3.5	-3.5	0	0	+1.1	+2.1	+4.2
200	Weight	2154	2065	1978	2020	2015	2050	2088	2150	2170	2170	2180	2190
	% change	0	-4.1	-8.2	-7.2	-6.5	-4.8		0	+0.9	+0.9	+1.4	+1.9

Percentage change based on the group average of the preceding five days pre-treatment weights.

Figure 3

Percent change in weight of Comet hens during treatment

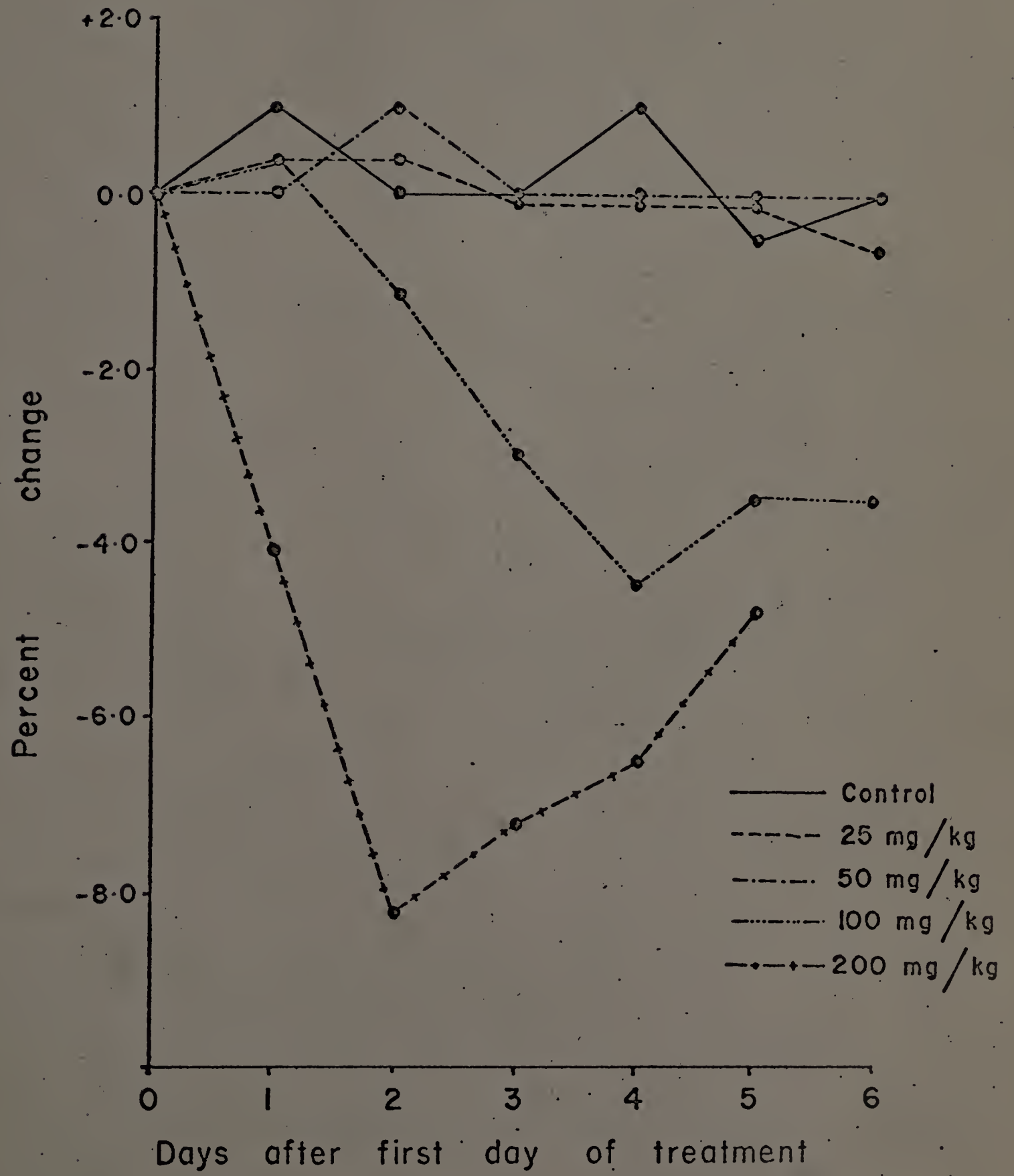
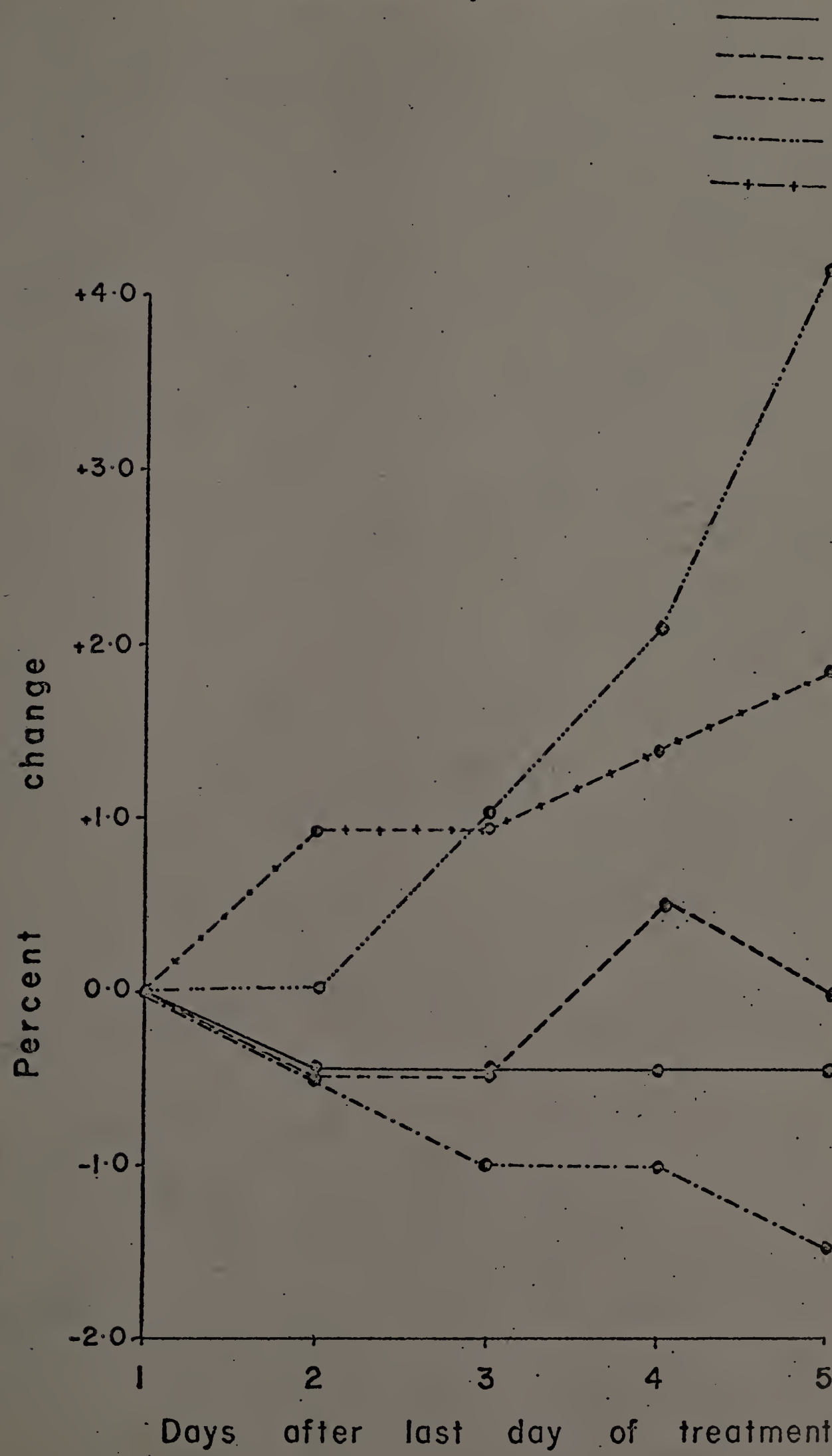


Figure 4

Percent change in weight
of Comet hens
after last day of treatment



treatment period they had practically regained their initial weights.

Post-treatment records on weights show a general trend of gain in weight by birds which received 100 and 200 mg/kg. But the control birds and those receiving 25 and 50 mg/kg maintained their weights very close to their initial weight (Figure 4).

c) Effect on water consumption. The data on average water consumption are presented in Table 6 and the percent reduction in water consumption is graphically presented in Figure 5.

A decrease in water consumption was noticed on the second day of treatment and on the third day a considerable drop was observed. However, this decrease was found in the control as well as treated birds. There was not much difference between water consumption by control birds and those receiving 25 and 50 mg/kg, as each of them had a reduction of 25-30 percent in water consumption.

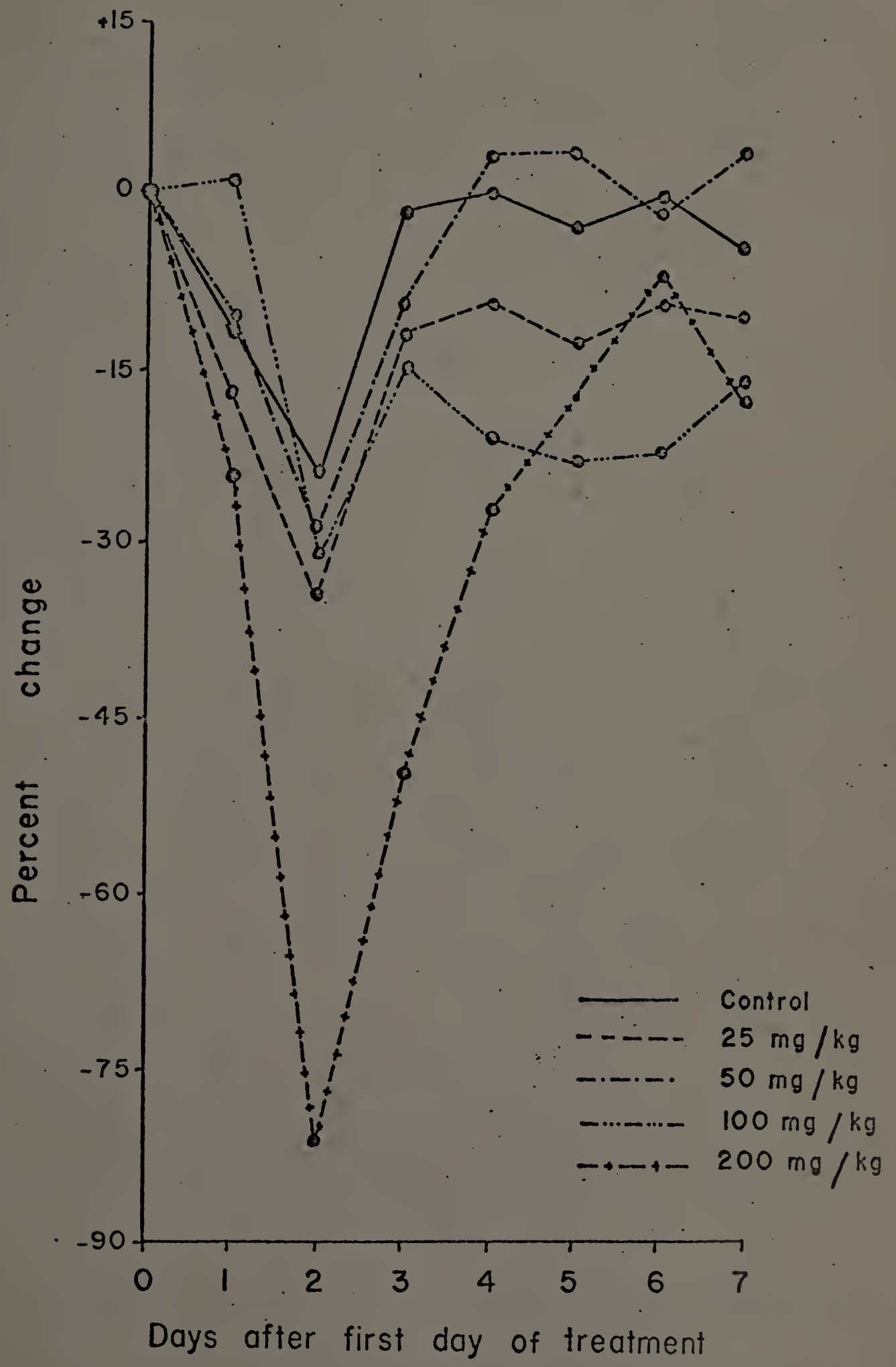
Table 6.-- Effect of oral doses of Rabon on average water consumption of Comet hens

Treatment mg/kg	No. of Birds	Average consumption and percent change*	Average Water Consumption in Milliliters							
			Pre-treatment	Treatment						
				Days after first treatment						
				1	2	3	4	5	6	7
Control	5	Consumption	228	202	173	225	229	234	229	208
		% change	0	-11.4	-24.1	-1.3	+0.4	-2.6	+0.4	-4.4
25	6	Consumption	236	196	153	208	214	206	215	212
		% change	0	-17.0	-35.2	-11.9	-9.3	-12.7	-8.9	-10.2
50	6	Consumption	203	182	143	185	210	211	200	211
		% change	0	-10.3	-29.6	-8.9	+3.4	+3.9	-1.5	+3.9
100	6	Consumption	238	241	163	204	188	184	185	200
		% change	0	+1.3	-31.5	-14.3	-21.0	-22.7	-22.3	-16.0
200	4	Consumption	236	179	45	119	171	196	221	195
		% change	0	-24.2	-81.0	-49.6	-27.5	-17.0	-6.4	-17.4

*Percentage change based on the group average of the preceding 5-day pre-treatment water consumption records.

Figure 5

Percent change in water consumption of Comet hens. during treatment



d) Record of weight of manure. A record of manure weight was maintained as an index of food consumption. It appears that birds receiving 200 mg/kg ate very little on the first 3-4 days of treatment but gradually started eating more, and by the end of treatment their feed consumption became approximately normal (Table 7).

e) Effect on egg laying. The control birds and the birds receiving 25 and 50 mg/kg did not show any decrease in egg production, except one control bird which appeared sick and did not lay throughout the treatment. Remaining birds on above treatments stayed in production throughout the treatment and post-treatment period. A marked effect was noticed on egg laying of birds that were fed 100 mg/kg. These birds became very irregular in laying and some of them stopped laying for a few days (Table 8a).

At the 200 mg/kg dosage egg production ceased in all the birds 3 days after the beginning of treatment but these birds came back into production after a lapse of ten days.

The analysis of egg production data (Table 8b) indicated a significant difference between the treatments. The relation between the treatments has been shown in Figure 6. The difference between birds within treatments was also significant. No significant difference was found between the days of treatment. However, the interaction of days and treatment was significant.

Table 7.--Average weight of manure collected from Comet hens during and after treatment.

Treatment mg/kg	No. of Birds	Average weight of manure in grams												
		Days after first day treatment												
		Treatment period						Post-treatment period						
		1	2	3	4	5	6	7	8	9	10	11	12	13
Control	5	75.8	64.0	72.8	82.4	88.6	91.8	83.4	112.0	126.0	139.3	99.6	114.0	109.0
25	6	102.6	89.5	90.0	109.8	113.0	107.2	101.2	115.0	122.5	132.0	119.7	140.5	118.0
50	6	79.8	76.0	80.0	88.3	95.5	92.0	90.5	101.7	104.5	95.5	86.2	103.0	86.0
100	6	85.8	77.5	88.6	70.8	81.0	92.5	85.0	97.0	90.0	112.7	93.7	97.2	93.0
200	4	62.6	43.2	36.4	25.8	46.2	68.7	82.0	134.5	151.5	127.5	105.5	123.5	97.5

Figure 6

Actual and Predicted regression of egg production of Cornet hens

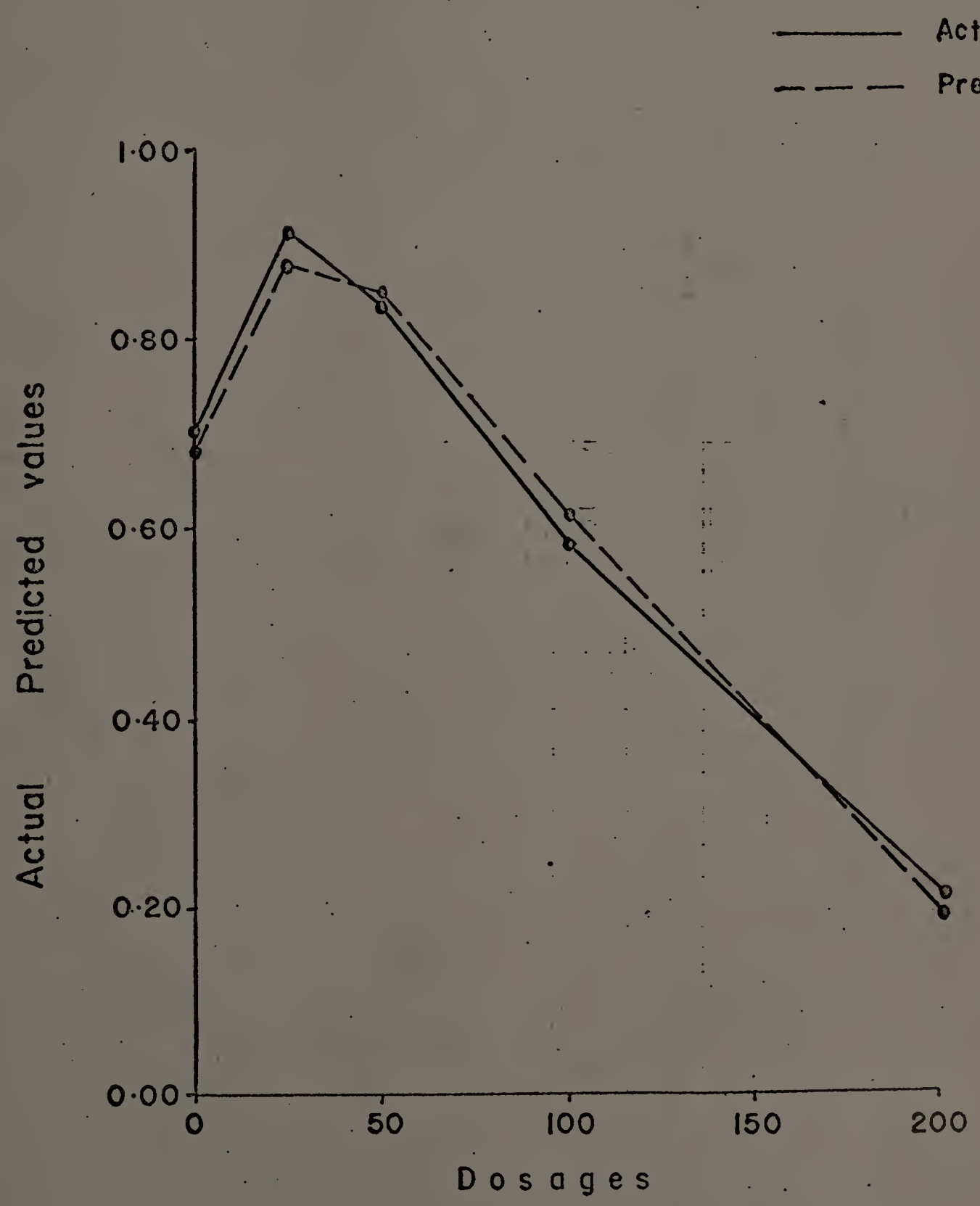


Table 8a.--Effect of oral doses of Rabon on egg production of Comet hens.

Treatment mg/kg	No. of Birds	Percent egg production																	
		Pre-treatment Period (Days)				Treatment Period (Days)							Post-treatment Period* (Days)						
		1	2	3	4	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Control	5	80	60	80	80	20	80	80	80	60	80	60	80	66	100	66	100	66	66
25	6	83	83	66	83	66	100	66	100	100	100	100	100	100	75	75	100	100	100
50	6	100	83	100	66	66	83	66	100	83	83	100	100	100	100	75	75	50	100
100	6	83	83	83	66	83	33	83	100	16	50	66	33	25	25	25	0	50	50
200	4	75	75	75	50	75	25	50	0	0	25	0	0	0	0	0	0	0	50

*Number of birds in post-treatment period is two less than starting number.

Table 8b.--Analysis of variance for egg production data.

Source	c.f.	S.S.	M.S.	F*	
Treatments (T)	4	11.0895	2.7724	9.05*	(4,22)
Linear regression	1	5.7977	5.7977	18.92**	(1,22)
Quadratic	1	5.1326	5.1326	16.75**	(1,22)
Birds within treatments (B:T)	22	6.7434	0.3065	2.45*	(22,154)
Days (D)	7	1.0324	0.1475	1.18	(7,154)
D x T.	28	8.8531	0.3162	2.53*	(28,154)
DB:T	154	19.2395	0.1249		

*At 95 percent level

B. Recoveries of Residues in Spiked Samples

i) Recoveries. As mentioned in the previous section in the chromatographic cleanup column two different combinations of silicic acid and water were used. Also the solvent mixtures to elute the column were prepared in two ratios of dichloromethane and hexane. In one case, 20 percent water added to silicic acid and 4:1 mixture of solvents were used as compared to 14 percent water added to silicic acid and 3:1 solvent mixture in the other.

To test the efficiency of the procedure 20 g. samples of each tissue were spiked with 0.5 ppm of Rabon and cleaned for gas chromatography. The best recoveries were obtained at a range attenuation combination of R1-A4 or R1-A2 as indicated on the aerograph control dials, depending on the sensitivity of the machine. The recovery data are presented in Table 9. The calculations are based on peak heights.

Table 9.--Percent recoveries of Rabon from body tissues and egg yolk of hens (0.5 ppm Rabon added).

Tissue	Percent Recovery	
	20 percent water added in silicic acid and 4:1 solvent mixture	14 percent water added in silicic acid and 3:1 solvent mixture
Fat	78.4	79.3
Liver	60.8	96.2
Leg	97.5	92.7
Breast	114.4	95.2
Egg yolk	92.7	88.5

ii) Sensitivity of method. At the range and attenuation setting of R1-A4 or R1-A2 depending on the sensitivity of the machine, 0.2mg of Rabon gave a good recorder response. Depending on the sample size and by measuring smaller peaks, the presence of 0.008 ppm of Rabon can be detected easily. The sensitivity can be increased by using more concentrated extracts or by operating the machine at more sensitive attenuation settings, but these manipulations increase the degree of interferences in blank as well as fortified samples.

C. Residue in Tissues and Egg Yolks

i) Experiment I. No residues were found at the levels of Rabon used in this experiment. Tissues analyzed included leg and breast muscles, fat, liver and egg yolks.

ii) Experiment II. Tissues collected from birds slaughtered 24 hours after the last day of treatment period were analyzed for residues and the data are presented in Table 10.

Residues were present in fat of all birds treated with Rabon. No residue was found in liver, leg and breast muscles of birds that received 94 and 188 mg/kg dosages. Considerable residues were detected in all the above mentioned tissues of birds that were fed 326 and 752 mg/kg of Rabon. A very high amount of residue was detected in leg muscles of bird No. 12. The averages of residues for each treatment are graphically presented in Figure 7.

Table 10.--Residues in tissues of Fayoumi hens receiving continuous oral doses of Rabon.

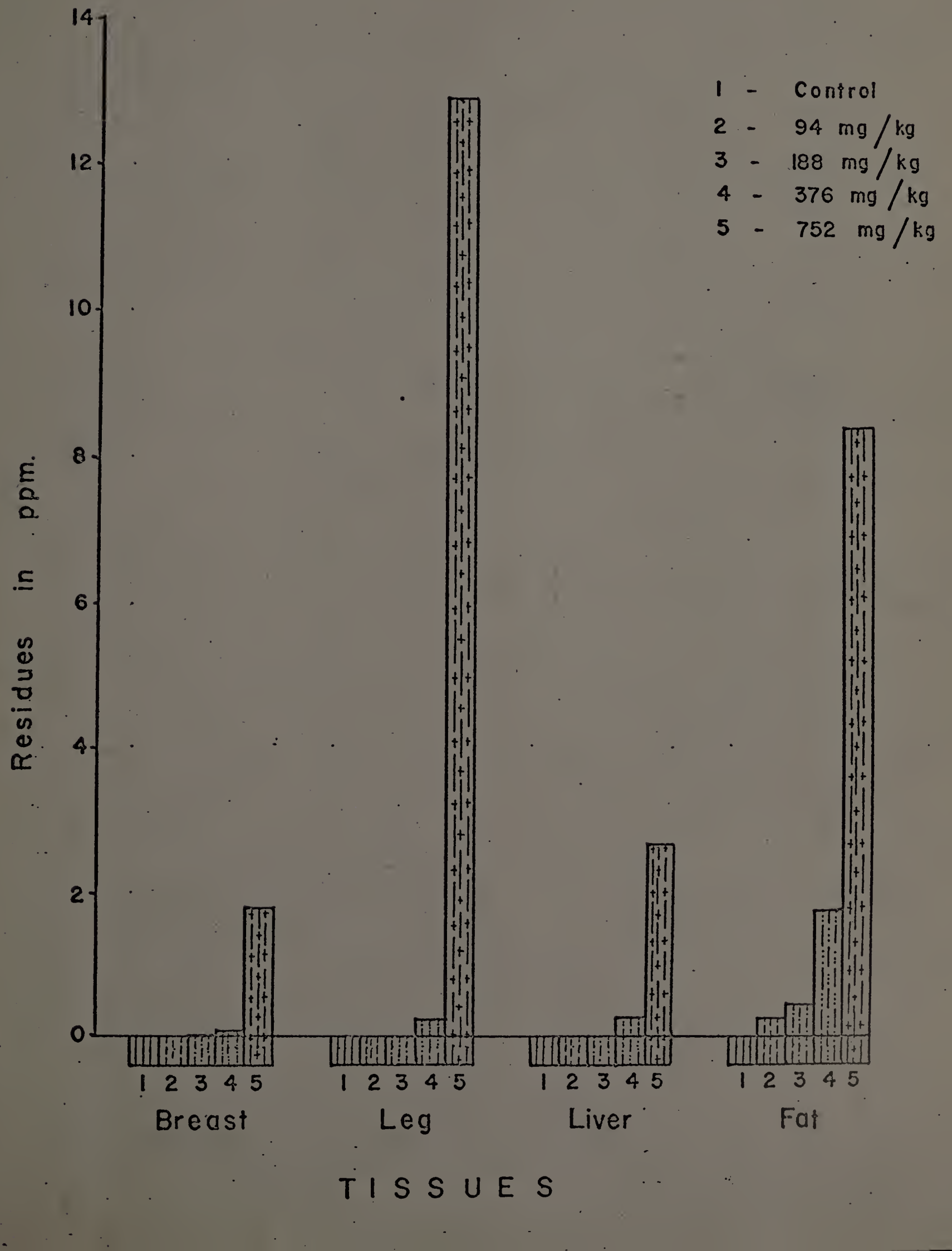
Treatment	Bird #	Residues in ppm			
		Fat	Liver	Leg	Breast
Control	26	ND*	ND	ND	ND
	16	ND	ND	ND	ND
	Average	ND	ND	ND	ND
94 mg/kg	30	0.326	ND	ND	ND
	27	0.189	ND	ND	ND
	Average	0.257	ND	ND	ND
188 mg/kg	18	0.439	ND	ND	ND
	3	**	ND	ND	ND
	Average	0.439	ND	ND	ND
326 mg/kg	28	2.610	0.207	0.404	0.165
	20	0.859	0.299	0.007	0.047
	Average	1.739	0.253	0.205	0.106
752 mg/kg	12	10.048	2.235	24.920	3.008
	11	6.845	3.015	0.962	0.572
	Average	8.446	2.625	12.941	1.790

*ND=Not-detectable.

**Went to dryness, experimental error.

Figure 7

Average residue in tissues of Fayoumi hens



iii) Experiment III

a) Residues in tissues. The analysis of tissues collected from birds slaughtered 24 hours after the last day of treatment indicated that residues of Rabon were present in fat of all the treated birds, but no detectable amount of residue was found in liver, leg and breast muscles of any bird in any treatment. It was also observed that Rabon was completely eliminated within seven days after termination of treatment. At the time of the second slaughter no detectable Rabon was present in the fat of any bird at the given treatment level, nor was any present in the fat tissues at the time of the third slaughter. All fat tissues were likewise free of Rabon.

The residue data are presented in Table 11 and the averages of residues in fat collected from first slaughter, for each treatment are presented in Figure 8.

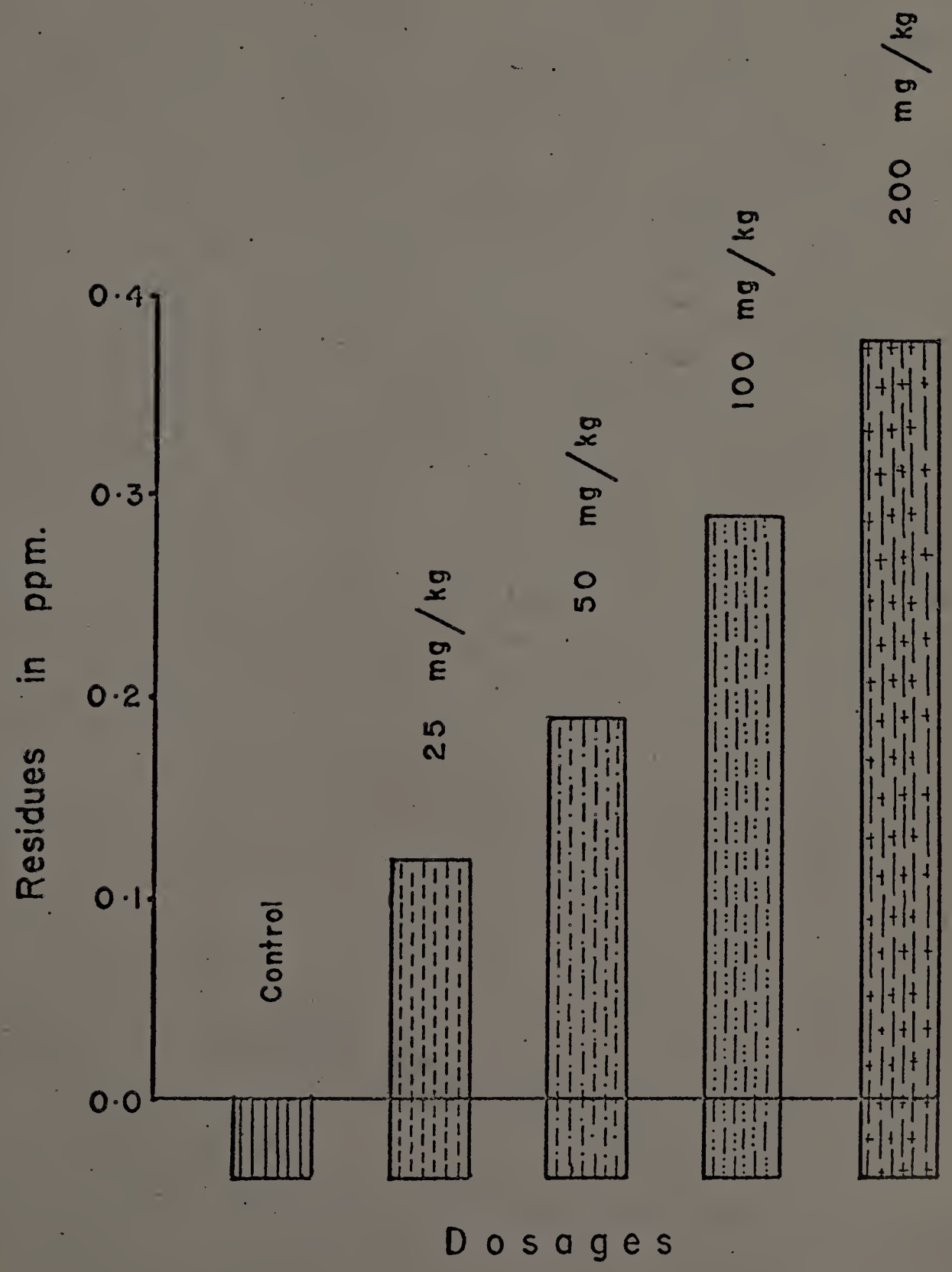
Table II

Residue of Rabon in tissues of Comet hens

Treatment mg/kg	Bird No:	Residue in ppm			
		Fat	Liver	Leg	Breast
		First slaughter (24 hours post treatment)			
Control	97	ND	ND	ND	ND
	29	ND	ND	ND	ND
	Average	ND	ND	ND	ND
25	2	0·082	ND	ND	ND
	26	0·152	ND	ND	ND
	Average	0·117	ND	ND	ND
50	15	0·211	ND	ND	ND
	21	0·169	ND	ND	ND
	Average	0·190	ND	ND	ND
100	11	0·265	ND	ND	ND
	24	0·310	ND	ND	0·018
	Average	0·288	ND	ND	0·009
200	8	0·354	ND	ND	ND
	13	0·406	ND	ND	0·007
	Average	0·362	ND	ND	0·003
		Second slaughter (seven days post treatment)			
Control	6	ND	-	-	-
	22	ND	-	-	-
25	12	ND	-	-	-
	27	ND	-	-	-
50	3	ND	-	-	-
	16	ND	-	-	-
100	5	ND	-	-	-
	9	ND	-	-	-
200	1	ND	-	-	-
	17	ND	-	-	-
		Third slaughter (fourteen days post treatmant)			
Control	28	ND	-	-	-
25	18	ND	-	-	-
	19	ND	-	-	-
50	20	ND	-	-	-
	23	ND	-	-	-
100	10	ND	-	-	-
	30	ND	-	-	-
200					

Figure 8

Average residue in Fat tissue of Comet hens



b) Residues in egg yolk. It was found that residue appeared in egg yolk two days after administering the first dose and disappeared to non-detectable amounts 5 days after the last day of treatment except in those eggs which were laid after a prolonged egg laying inhibition period (i.e., 200 mg/kg group). The first egg laid after the inhibition period contained lower residue than the last egg laid by the same bird just prior to inhibition (Table 12).

Residues appeared in eggs collected from all the birds that were fed Rabon with the exception of eggs collected from birds which received 25 mg/kg. In this group, no residue was found in eggs from certain birds, whereas in others the residues detected were mostly below the sensitivity of method. Also these traces of residues were present in the egg laid on the first 3-4 days after first day of treatment and gradually disappeared by the end of the treatment period.

The data indicates considerable variation of residues in eggs collected from birds in the same treatment.

Table 12

Residue of Rabon in egg yolks of Comet hens

Treatment mg/kg	Bird No.	Residues in ppm													
		Days after first treatment													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
		Treatment period								Post treatment period					
Control	6	-	-	ND	-	ND	ND	ND	-	-	-	-	-	-	-
	7	-	ND	-	ND	-	-	-	-	-	-	-	-	-	-
	22	ND	-	-	-	-	-	-	ND	ND	-	-	-	-	-
	28	-	ND	ND	ND	ND	ND	ND	-	ND	ND	ND	ND	-	-
25	2	ND	0·022	0·010	0·008	ND	ND	ND	-	-	-	-	-	-	-
	12	-	-	0·006	0·026	-	-	ND	-	-	-	-	-	-	-
	16	-	ND	-	ND	-	-	ND	-	-	ND	-	-	-	-
	19	-	-	ND	ND	ND	ND	-	ND	ND	-	ND	-	-	-
	26	-	0·003	-	0·010	-	-	0·007	-	-	-	-	-	-	-
50	3	ND	-	0·015	0·013	0·014	ND	ND	ND	-	-	-	-	-	-
	16	-	0·022	-	0·070	-	-	0·029	-	0·014	-		-	-	-
	21	ND	0·018	-	-	0·004	-	ND	-	-	-	-	-	-	-
	23	-	ND	0·030	0·029	0·027	0·026	0·007	0·008	ND	-	ND	-	-	-
100	9	-	0·035	0·026	-	0·052	0·060	0·070	-	-	0·016	ND	-	ND	-
	10	-	ND	-	-	-	0·055	-	0·061	-	-	-	-	-	ND
	24	ND	-	0·067	0·316	0·314	0·141	0·290	-	-	-	-	-	-	-
	30	ND	0·246	0·190	-	0·150	-	-	-	0·181	-	-	ND	-	ND
200	1	-	0·054	-	-	0·217	-	-	-	-	-	-	-	-	0·020
	17	-	0·030	-	-	-	-	-	-	-	-	-	-	0·023	-
	25	-	0·030	-	-	-	-	-	-	-	-	-	-	-	-

ND= Not detectable
Figures rounded to three decimal places

DISCUSSION

A. Treatment of Birds

i) Methods of treatment. Two methods were used to to administer the insecticide to hens. They include the use of gelatin capsules and a plastic tube and funnel.

In the first experiment, gelatin capsules were utilized. The appropriate amount per bird was weighed and placed in capsules. The principal advantage of this method was that one person could hold the bird and administer the capsule. A glass rod was helpful to force the capsule into the oesophagus. Capsules of size 00 were well accepted by the birds. However, since this size capsule would not hold over 100 mg. of Rabon 75 WP, for subsequent experiments it would have been necessary to use about 32 capsules to provide a dosage of 1504 mg/kg, the highest dosage in Experiment II, and four capsules to provide a dosage of 200 mg/kg, the highest dosage in Experiment III. This would require much time to fill the capsules and also to administer the capsules to birds. In an attempt to overcome this problem large capsules of size 000 were used as relatively fewer were needed to contain the appropriate dosages. It was apparent that the birds did not tolerate the large capsules. Therefore, capsules were not used in Experiments II and III.

Due to the disadvantages of capsule technique, a second method was developed. This consisted of preparing a suspension of Rabon in water. A plastic tube, attached to a funnel, was inserted in the oesophagus extending down to the crop. The slurry was then poured into the funnel and through the tube to the crop. This method proved to be superior and faster. No spillage or other loss of insecticide occurred. Its only disadvantage was that it required the assistance of a second person to hold the bird.

ii) Gross appearance of hens during treatment. In Experiment I the birds which were fed 30 mg/kg Rabon appeared normal throughout the treatment. In Experiment II, birds were fed massive dosages. It was found that all birds receiving 1504 mg/kg dosage died after receiving 6 doses. Thus an approximate total of 9024 mg/kg was needed to cause 100 percent mortality. Birds receiving 752 mg/kg survived longer but 60 percent of them died after receiving 13 doses. Thus an approximate total of more than 9776 mg/kg would be required to kill 100 percent of the birds in this treatment. The chronic LD_{50} for 6 days was 1064 mg/kg. Sherman et al. (1967) reported an acute oral LD_{50} of 2528 mg/kg for chicks. They found no mortality when the chicks were fed a ration containing 800 ppm of Rabon for a period of 2 weeks. Therefore, it appears that birds receiving comparatively lower amounts of Rabon could metabolize or eliminate more than those receiving higher dosages.

Birds receiving 376 mg/kg survived throughout the treatment indicating that a total of 4968 mg of Rabon administered at a rate of 376 mg/kg/day over a period of 13 days was not lethal to Fayoumi hens. But in Experiment III in which Comet pullets were used, one of the birds receiving 200 mg/kg/day died after receiving only 3 doses. This bird received a total of only 600 mg/kg Rabon. The above amount is very low compared to the amounts fed in Experiment II where none of the birds that received 4968 mg/kg, died. It was observed during Experiment III that the bird which died was not drinking. During all three experiments any bird which started drinking sufficient water within the first 2-3 days of treatment showed normal activities sooner than did those which started drinking sufficient water later. Therefore, it would seem likely that greater amount of water aids the birds in diluting and excreting the insecticide.

The data from Experiment II indicated that birds fed Rabon at a rate of 94 mg/kg/day gained a maximum of 4.2 percent in weight. But in Experiment III birds receiving 100 mg/kg dosage lost 4.4 percent of their weight. From the above variations it would be difficult to arrive at any conclusion whether or not amounts less than 100 mg/kg would cause any reduction in weight of birds.

From the above discussions it would seem possible to infer that a difference in susceptibility to Rabon between different breeds of hens may exist. It also appears from the observations on mortality and weight change discussed above that the Fayoumi

breed was less susceptible to the insecticide as compared to White Leghorn or Comet breeds. However, more data would be required to support this conclusion.

iii) Egg production. No information was obtained on egg production in Experiment II since all the birds went out of production before administering the insecticide. Most of the data on egg production was available from Experiment III. Birds receiving 25 and 50 mg/kg dosages did not show any decline in egg production and were very regular in laying. Birds receiving 100 mg/kg became very irregular, except for one of these birds which was drinking and eating normally and which laid regularly throughout the treatment. Some of the birds in this group stopped laying for a few days. Dosages of 200 mg/kg completely inhibited egg laying for a period of 10 days. Therefore, it appears that the critical range of dosages which inhibit egg production lie between 100 and 200 mg/kg dosages. The data on egg production is not sufficiently complete to permit any hypothesis to be drawn about the mechanism of egg inhibition. It might be reasoned that an inhibition of enzyme or hormone systems would be likely because most organophosphate insecticides have enzyme inhibitory power. Another possibility could be that the treatment caused the birds to stop eating and drinking and this in turn caused egg inhibition. However, Callahan et al. (1967) in work with Ronnel, observed that inhibition was not due to reduction in food and water consumption, but that other mechanisms such as a disturbance of enzyme or hormone systems were involved.

B. Analytical Procedure

A highly purified extract is needed for gas chromatography. This factor is complicated by a myriad of interfering compounds, especially lipids. In the present work, the extraction procedure for Zytron from poultry tissues and cleanup procedure for Rabon from cattle tissues was combined. As described previously, this combined procedure yielded a very acceptable and usable extract. This extract was comparatively better when 14 percent water added to silicic acid and 3:1 solvent mixtures were used in cleanup column than when 20 percent water added to silicic acid and 4:1 solvent mixture were used. The extract prepared by the latter combination was good only when single samples were to be injected into the gas chromatograph but when more samples were involved, the sensitivity of the detector was gradually reduced and more time was needed to standardize the machine. This combination is economical as it requires comparatively smaller amounts of chemicals.

However, for analyzing more samples the former combination (14 percent water added to silicic acid and 3:1 solvent mixture) is recommended. The disadvantages of this procedure are the length of time and amount of chemicals needed. A series of 5 or 6 samples, the maximum number conveniently handled at one time, took 8 to 10 hours of work to prepare the extract for injection into gas chromatograph. This does not include the time necessary for redistilling the solvents.

This method proved to be sensitive enough to determine the presence of 0.008 ppm of Rabon. Any residue less than that represents the traces of Rabon. Sensitivity of 0.002 ppm has been reported by Ivey et al. (1968) in cattle tissues. Beroza and Bowman (1966) reported a sensitivity of 0.02 ppm in corn extract.

C. Residues in Tissues and Egg Yolks

i) Residues in tissues. In Experiment I no residue could be detected in any tissues. In Experiments II and III a considerable amount of residues was found, in fat tissues collected from all the treated birds. No residues could be found in any tissues other than fat of any bird receiving up to 200 mg/kg dosage. Therefore, it appears that Rabon has more affinity for fat, probably because of presence of Chlorine molecules. These observations agree with those of Ivey et al. (1968) where almost all the residues were found in fatty tissues of cattle sprayed with Rabon.

Residues could be detected in tissues other than fat, from birds which received 376 and 752 mg/kg dosages. It is likely that residues present in liver, leg and breast muscles were due to fat in these tissues.

Ivey et al. (1968) detected a maximum of 0.1 ppm residues of Rabon in fat tissues of cattle one week after the treatment. But in the present work on hens, the residue was completely eliminated from fat, irrespective of amount of Rabon administered, within a week after the last day of treatment.

ii) Residues in Egg Yolks. Residues appeared in eggs collected on the third or fourth day of treatment. This irregularity is probably due to variance in ovarian cycle of birds. Ova which were mature when the insecticide was administered would contain lower residues than ova undergoing vitellogenesis directly following the treatment.

No residues were found in egg yolks of some of the birds receiving 25 mg/kg dosage whereas traces were detected in eggs collected from other birds in the same group. These trace amounts were present in the eggs laid during the first 3-4 days after administering the first dose and gradually disappeared by the end of treatment. It appears that birds did not metabolize or eliminate Rabon initially but later on they developed pathways to metabolize or eliminate some of the Rabon present in the system.

As mentioned above, no residues could be detected in yolks from some of the birds receiving 25 mg/kg dosage. With other dosages eggs from some of the birds had higher residues then others receiving the same treatment. Also, in eggs from some birds, the residue disappeared to non-detectable amounts a few days earlier than others receiving the same treatment. Therefore, it could be concluded that 25 mg/kg is the border line amount which may or may not leave any residue in egg yolks, but dosages higher than that would leave detectable amount of residues in egg yolks. It could also be concluded that different birds metabolize or eliminate Rabon at different rates.

By the fifth day after the last day of treatment, eggs from all levels of treatment had undetectable residues. The quantity of residue did not seem to be related to the quantity of Rabon present in fat tissues. It appeared that there was a certain amount of Rabon deposited in the egg yolks and beyond that no higher amount was deposited, irrespective of the amount administered.

SUMMARY AND CONCLUSIONS

This investigation concerned the analysis of edible tissues and egg yolks of poultry in order to determine the presence of Rabon. The analytical method used was gas chromatography.

An analytical procedure for extraction and cleanup of tissues and egg yolk was developed. Recovery studies were made on fat, liver, leg and breast muscles and egg yolks fortified with Rabon. Tissues and egg yolks collected from birds fed Rabon were analyzed.

The method of extraction and cleanup is very satisfactory and 79 to 96.7 percent of 0.5 ppm Rabon added to various tissues could be recovered. The method is sensitive enough to determine the presence of 0.008 ppm of Rabon using a gas chromatograph equipped with electron capture detector.

Three experiments were conducted using White Leghorn hens in Experiment I, Fayoumi hens in Experiment II, and Comet pullets in Experiment III. In the first experiment Rabon 75 WP was filled in gelatin capsules and force fed to birds. In the other two experiments the insecticide was fed through a plastic tube placed directly into the crop of birds.

Detailed studies were made on the effects of various dosages of Rabon on weight fluctuation of and egg production by birds. Observations were also made on the effect of treatments on water consumption by birds in Experiment III.

It was found that the toxicity of Rabon is fairly low to hens. When dosage fed to birds did not exceed 50 mg/kg/day no inhibition of egg production resulted. However, a dosage of even 25 mg/kg resulted in detectable amounts of residue in fat tissues, but not in liver, leg and breast tissues, and any traces present in these tissues may be due to its affinity for fat present therein. Traces of Rabon less than the sensitivity of the method were deposited in egg yolks of birds fed 25 mg/kg dosage. Dosages of 50, 100 and 200 mg/kg resulted in a considerable amount of residues in egg yolks. Rabon was metabolized in or eliminated from the body of birds within a period of 7 days, and eggs laid after this period did not contain any detectable amount of residue.

From the above observations and discussions it is concluded that:

1. the toxicity of Rabon is fairly low for hens;
2. Rabon did not inhibit egg production when fed to hens in quantities less than 100 mg/kg;
3. it has high affinity for fatty tissues and amounts of 25 mg/kg did leave residues in fat;
4. amounts up to 200 mg/kg did not leave any residues in liver, leg and breast muscles;
5. Rabon was metabolized or eliminated from the body tissues within a period of seven days and eggs laid after that did not contain Rabon residues.

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