THE FEEDING BEHAVIOUR OF THE CLEAR-WINGED GRASSHOPPER *Camnula pellucida* (Scudder) (ORTHOPTERA:ACRIDIDAE) WITH SPECIAL REFERENCE TO THE CHEMOTACTIC INFLUENCE OF SOME ORGANIC CONSTITUENTS FOUND WITHIN FOOD PLANTS

A Thesis

Presented to

the Department of Entomology

Faculty of Agriculture and Home Economics

The University of Manitoba

Winnipeg

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

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May 1959

ABSTRACT

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THE FEEDING BEHAVIOUR OF THE CLEAR-WINGED GRASSHOPPER *Camnula pellucida* (Scudder) (ORTHOPTERA:ACRIDIDAE) WITH SPECIAL REFERENCE TO THE CHEMOTACTIC INFLUENCE OF SOME ORGANIC CONSTITUENTS FOUND WITHIN FOOD PLANTS

The feeding behaviour of the first instar nymph of the clear-winged grasshopper *Camnula pellucida* (Scudder) reveals that chemical stimuli, primarily gustatory, play a dominant role in regulating feeding behaviour as observed in the field. Various plants, which were reported to be acceptable to *C. pellucida*, were extracted, and these extracts were found to be palatable. Using chromatographic techniques, particularly attractive fractions of Lee wheat were isolated and some constituents of these fractions were identified. Pure chemicals were then used in an attempt to duplicate these results. Of these, the following elicited varying degrees of response when tested singly: α-alanine, γ-amino butyric acid, L-asparagine, L-aspartic acid, L-cystine, glycine, hydroxy-L-proline, L-serine and D-sucrose. The amino acids were active at lower concentrations but did not elicit as strong a feeding response as did sucrose.

The combination of the pure chemicals - alanine, serine and γ-amino butyric acid, which had been identified in a palatable fraction of wheat extract, resulted in a synergistic effect. Summation occurred

when this combination was tested with the addition of sucrose.

There was a general decline in palatability at highest concentrations. This was also observed in the choice tests involving 0.02 and 0.5 Molar sucrose, of these two the higher concentration (0.5 Molar) was rejected. However, an anomaly existed in the choice tests involving 0.02 and 0.5 Molar fructose, in that preference was shown for the higher concentration (0.5). In choice tests between sucrose and fructose, comparing 0.02 molar solution of each, a preference was shown for sucrose.

All chemicals and extracts were presented on an ingestible substrate consisting of Japanese elder pith discs of uniform diameter and thickness. These were presented in a dry, brittle state and it was found that feeding was not enhanced by moistening the discs.

ACKNOWLEDGEMENTS

I am grateful to my adviser Dr. A. J. Thorsteinson, Professor of Entomology, for suggesting this study, and for his constant encouragement and most valued direction throughout its entirety.

I would like to thank Mr. L. G. Putnam, Co-ordinator of Grasshopper Research, for his fine co-operation in supplying necessary materials.

This investigation was made possible by my wife, and the help of an Extra Mural Research Grant provided by Canada Research Branch, Department of Agriculture, Ottawa, sponsored by Mr. L. G. Putnam, Co-ordinator of Grasshopper Research, Field Crop Insect Laboratory, Saskatoon.

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INTRODUCTION

> That which the palmerworm hath left hath the locust eaten; and that which the locust hath left hath the cankerworm eaten; and that which the cankerworm hath left hath the caterpillar eaten.
>
> Joel 1:4

This display of distinct food preferences by insects was observed during Biblical times, but the impact of vegetation as food and the mechanisms through which it operates in the ecology of insects, was not understood until more recent times.

Pfadt (1949) stressed the significant role played by various species of food plants in regulating both diet and quantity eaten by grasshoppers. He observed that grasshoppers were attracted equally to preferred and unpreferred plants, however, only biting on a preferred plant resulted in feeding. He suggested that this differential feeding had a chemotactic basis.

The present study was undertaken to explore some of the stimuli, which appear to be chemotactic and primarily gustatory, governing the host plant relationship of grasshoppers.

Review of the Literature

Studies of the effect of vegetation on grasshopper distribution has had two emphasis. One view has stressed the physical requirements and the modifying effect of vegetation on the microclimate: presented by Strohecker (1937). Urquhart (1941) and Cantrall (1943).

The other view was presented by Pfadt (1949). He felt that the differential in the abundance of grasshoppers is due in part to the variation in the nutritive values of plants and the difference in the quantities eaten. He stressed the latter particularly, because of the positive correlation he found between food preference on the one hand, and survival, growth and egg production on the other.

Ball (1936) reported that many grasshoppers were monophagous or oligophagous. Isley (1938) recognized the importance of various food plants in the distribution of many species of grasshoppers.

Differential feeding of grasshoppers was studied by Brunson and Painter (1938), Smith (1939), Jacobson and Farstad (1941), McBean (1948), and Misra (1956).

The differential effect of various species of plants on fecundity, development and survival was reported by Drake _et al_, (1945), Brett (1947), Smith, Handford and Putnam (1952), Barnes (1955), Pickford (1958) and Misra (1956).

The above studies reveal, (a) that the absence of proper food plants may be a limiting factor in the distribution of grasshoppers, and (b) that many grasshoppers e.g. _Melanoplus mexicanus_ (Sauss.) and _Camnula pellucida_ (Scudd.) which were considered general feeders show predilections and are selective in their feeding behaviour, and (c) that food selection may be based on taste.

The gustatory influence of plant constituents was shown in a number of studies as reviewed by Dethier (1947) (1953) and Thorsteinson (1953) (1956) (1960).

In 1905 Grevillius found that larvae of Euproctis chrysorrhoea (L) could be induced to feed on plants other than its host, the chickweed Stellaria, when these were covered with a paste containing a constituent of chick weed.

In 1910 Verschaffelt observed that the range of acceptable host plants for the larvae of Pieris rapae (L.) and P. brassicae (L.) was related to the distribution of the mustard oil glucosides among plants.

In 1936 Raucourt and Trouvelot working with Leptinotarsa decemlineata (Say) tested the palatability of potato extracts in an attempt to isolate the constituents that induce feeding on the host. They showed that consumption of leaves was proportional to the concentration of an unidentified substance. Chauvin (1945) (1952) improved on their methods for testing plant extract palatability and isolated an attractive fraction.

Dethier (1941) found that the feeding of Papilio spp. was profoundly influenced by essential oils.

Thorsteinson (1953) studied the chemotactic basis of the host specificity of the diamondback moth Plutella maculipennis (Curt.) and P. brassicae (L.). He extended the experiments of Verschaffelt (1910) and refined his techniques, making possible an analysis of the factors involved.

The chemotactic significance of biologically important saccharides and their importance in regulating feeding is well known in many insects (see Dethier (1947) (1953)). However, the gustatory effect of organic nitrogen compounds has been less extensively reported, and the influence of the amino acids in particular has had relatively little support.

The effect of amino acids on feeding was investigated by von Frisch (1930) (1934), Thorpe _et al_, (1947), Skaife (1955) and Beck and Hanec (1958).

Thorpe _et al_, (1947) and Crombie and Darrah (1947), found that substances present in food plants elicit two responses in wire-worms _Agriotes_ spp.; aggregation and biting. Wireworms react to chemicals borne in the soil water but do not react to atmospheric odour. Aspartic acid, asparagine, glutamic acid and glutamine elicited aggregation, whereas biting was evoked by fats and polypeptides. Sugars alone elicited both aggregation and biting.

Beck (1956a) (1956c) found the larvae of _Pyrausta nubilalis_ (Hubn.) were very sensitive to sugars, and that the orientation of the insect was influenced by the concentration of sugar within the tissues of the corn plant. Beck (1956b) reported a bimodal response to glucose, sucrose and fructose.

Thorsteinson and Procter[+], studying the feeding behaviour of _L. decemlineata_ found that the larvae responded well to 0.1 Molar sucrose.

[+] unpublished data

Fructose, however, was refused at all concentrations. They found that alanine and γ-amino butyric acid stimulated feeding at 0.004 Molar concentration.

Beck and Hanec (1958) showed that the feeding duration of the European corn borer was increased by amino acids, notably L-alanine, DL-α-amino-n-butyric acid, L-serine and L-threonine. A negative effect on feeding was shown by L-tryptophane, L-arginine, β- alanine.

Thorsteinson and Jay+ found that D-glucose, D-fructose, D-maltose, D-galactose, L-inositol, Melibiose, and D-raffinose elicited excellent feeding responses from Camnula pellucida (Scudder).

Thorsteinson (1958) working with Chorthippus longicornis (Latreille) reported on the feeding response to asparagine, betaine, monosodium glutamate and to some water soluble vitamins.

Maltais and Auclair (1957) stated that varieties of cultivated peas susceptible to the pea aphid contained more nitrogen and less sugar than resistant varieties.

Auclair, Maltais and Cartier (1957) reported that susceptible varieties of cultivated peas contained a higher concentration of free and total amino acids than did resistant varieties. They suggest that lower concentration of amino acids in resistant varieties affected the rate of growth and reproduction adversely. In view of what is known of the gustatory effects of amino acids the above suggests that preference may be operating.

An examination of the literature on insect feeding behaviour leads us to conclude that insects possess sensitive receptors capable

+ unpublished data

of delicate discrimination among a diverse array of substances. The reactions to these substances both pure and compound enables us to understand food selection as we observe it in nature.

Materials and Methods

This study is based on the premise, which is supported by observation in the case of the grasshopper and by experiments in the case of various insects e.g. wireworms, that feeding behaviour can be interpreted in terms of chemotactically active constituents of food plants. Therefore in our investigation of gustatory behaviour both extracts of plant material and pure chemicals which are known to be present in plants, were subjected to testing.

Insect

Camnula pellucida (Scudd.) was utilized in all tests. In survey tests involving extracts of various species of grasses and weeds, the responses of *Melanoplus mexicanus* (Sauss.) and *Melanoplus bivittatus* (Say) were also studied, but the latter results will not be reported here.

C. pellucida (Scudd.) undergoes diapause while in the egg stage. To promote the resumption of development, cold treatment is required. Putnam+ (1956) suggested that the eggs be stored at 40°F. after being mixed with moistened sand. The top layer of sand should be kept moist to prevent the eggs from drying.

Five first instar nymphs were used in each replicate. The nymphs were allowed to feed on lettuce prior to testing and were thus not in a starved condition. To protect the grasshopper from the effect of possible insecticidal residues a culture of *Drosophilia* spp. was maintained for bioassays of the food.

\+ personal communication

Cages

The rearing cages used were patterned after those described by Hunter-Jones (1956) with modifications developed at the Saskatoon laboratory, (Putnam, 1956)+.

Temperature

Tests by Thorsteinson and Jay+ showed that optimum feeding on sucrose centered on 25°C., and this temperature was maintained in our incubators during tests. Pfadt (1949) conducted his food preference tests at temperatures ranging from 24° to 27°C.

Temperatures within the cages were held at 25°C. for several hours prior to feeding. Chapman (1955) (1957) noted that the amount of feeding in the tests reflected the conditioning effect of the temperature a few hours prior to testing.

Time

Tests were run overnight, for a period of eighteen hours, in an incubator and under conditions of total darkness.

Substrate

The method used to investigate the regulation of feeding involved the use of plant extracts and pure chemicals applied to an ingestible substrate. The technique, developed by Raucourt and Trouvelot (1936) was improved upon by Chauvin (1945) and Thorsteinson (1955).

Japanese elder pith was sectioned and the resulting discs measuring 240 microns in thickness were stamped out with a die to produce a uniform area and a discrete edge. The discs were then

+ unpublished data

extracted with 80% ethanol, dried and clipped into the split end of short wooden sticks cut from wooden medicinal applicators. Substances to be tested were applied to the discs.

The wooden applicator sticks were fastened with wax to the floor of a plastic dish (diameter 7 cm., depth 1.5 cm.) which had been previously lined with waxed filter paper.

All the chemicals tested were presented on a substrate of Japanese elder pith discs which were in a dry and brittle state. There was no enhancement of feeding as a result of moistening the disc, cf., Misra (1956), Chapman (1957) in the discussion later.

<u>Survey of Extracts</u>

The present study was developed in several stages. Initially ethanol extracts of the following grasses and weeds were surveyed for palatability.

Poaceae - grass family

Agropyron cristatum (L.) Gaertn.	Crested wheat-grass
A. elongatum (Host.) Beauv.	tall wheat-grass
A. intermedium (Host.)	intermediate wheat-grass
A. smithii (Hydb)	Western couch-grass
A. trachycaulum (Link) Malte	slender wheat-grass
Festuca elatior L.	Fescue
Poa compressa L.	Canada bluegrass
P. pratensis L.	Kentucky bluegrass
Triticum vulgare L. var. Lee	Lee wheat

Cichoriaceae - chicory family

Sonchus spp.	sowthistle
Taraxacum officiale Web	dandelion

50 gms. of fresh leaf tissue were boiled in 250 mls. of 80% ethanol and then macerated in a blendor. The crude extract was cooled, filtered and evaporated to 10 mls. Japanese elder pith discs were then treated and fed to C. *pellucida*, M. *mexicanus* and M. *bivittatus*.

The Lee wheat extract was separated into fractions by the use of paper chromatography (for method see Lederer and Lederer (1957), Block, Durrum, Zweig (1958)). The fractions were eluted from the chromatographic paper and each was tested for palatability. See fig. 1 for the results. Identification of plant constituents was accomplished by comparing Rf values in a variety of solvents and by chromogenic detection; see Dent (1948), Thompson *et al*, (1951), Lederer (1957), Block, Durrum, Zweig (1958), Feigl (1956). Pure chemicals were subsequently used in an attempt to simulate the feeding response produced by the plant extract or one of the active fractions.

In subsequent work with Lee wheat the crude extract was cooled, filtered, evaporated, and the residue was taken up with the water. Chlorophyll was removed by washing with benzene. The benzene was removed by means of a separatory funnel and the extract was saturated with lead acetate and filtered. The precipitate formed was treated with H_2S and filtered. Palatability was tested at each stage listed to ascertain the active constituents and to discard those that were inactive. The clear filtrate was singular in that it elicited an excellent feeding response.

This clear filtrate was applied to Whatman 3 MM filter paper and developed in a solvent of pyridine, isobutanol, NH_4OH (4:1:2).

To test the fractions for palatability it was found convenient to divide the chromatogram on the basis of the banding visible under ultra violet radiation.

Tests

Each test consisted of one or more chemicals made up into a dilution series whose members were one fifth the molar concentration of each preceding one, and whose range was 5.0×10^{-1} Molar to 6.4×10^{-6} Molar.

Each concentration in the series was replicated at least three times. Replicates generally contained two discs, a treated disc, and an untreated disc.

See table 1 and table 2 for a review of chemicals tested and for results.

Controls

Each test dish contained one negative control, consisting of an untreated disc, for every treated disc.

Every test included a positive control consisting of three replicates containing one disc treated with 0.02 sucrose and one untreated disc. In early experiments, C. Jay and the author found that _C. pellucida_, _M. mexicanus_ and _M. bivittatus_ responded consistantly well to sucrose. Optimum feeding centered on 0.02 Molar concentration.

Choice Tests

All the tests were, in a sense, choice experiments, in that the insects chose between treated and untreated discs. However, in the following experiments a choice was given between discs treated with, a) various chemical concentrations b) different chemicals.

1) Fructose: Tests were run comparing 0.02 Molar with

(a) 1.0 Molar fructose See fig. 20 Column d
(b) 0.5 Molar fructose See fig. 20 Column a

Each test dish consisted of the two concentrations plus an untreated negative control.

2) Sucrose: Tests were run comparing 0.02 Molar with

(a) 0.1 Molar sucrose See fig. 19 Column a
(b) 0.004 Molar sucrose See fig. 19 Column b
(c) 0.5 Molar sucrose See fig. 20 Column c

3) Sucrose and Fructose:

The insect had a choice of 0.02 Molar fructose and 0.02 sucrose. See fig. 20, column b.

Results and Discussion

Feeding results of the survey tests using *C. pellucida*, *M. mexicanus* and *M. bivittatus* show that plant extracts were differentially selected by each species, and that there was an interspecific variation in their choice, (Jay and Tauber)+.

The details of these survey tests will not be considered here; however, it should be noted that an acceptable plant resulted in an acceptable extract. An anomaly existed in the case of *A. elongatum* which was rated by Misra (1956) as being low in acceptability but which was found to be readily acceptable as an extract.

Chromatography

The pyridene, isobutanol, NH_4OH (4:1:2) solvent mixture used to fractionate the Lee wheat extract was very useful because it separated the amino acids and the carbohydrates quite well.

The chromatogram on which the plant extract was loaded and developed was divided into fifteen horizontal bands, based on the banding produced under ultra violet radiation. This method of division led to less waste and gave a more satisfactory result than the divisions produced on the basis of chromogenic detection of carbohydrates and amino acids.

Feeding

Although some feeding was elicited by nearly all bands, it was found that three were especially active; see fig. 1. One band contained the amino acids, serine, alanine and γ-amino butyric acid. The second

+ unpublished data

band contained one fluorescent and one reducing substance. The third band contained two fluorescent and a reducing sugar.

Controls

Many investigators have noted the general acceptance of sucrose by various insects, see Dethier (1953). In initial experiments, C. Jay and the author found that the feeding response of C. pellucida (Scudd.) was consistent and at an optimum at 0.02 Molar concentration. Therefore this was adopted as a standard for comparison and as a positive control.

The negative control consisting of an untreated disc was seldom eaten by the grasshoppers and never to any appreciable extent.

Pure Chemicals

Feeding results on pure chemicals are summarized in table 1 and table 2.

It is interesting to note that alanine, γ-amino butyric acid and serine, which were present in a fraction of the extract that elicited an excellent feeding response (see fig. 1), were not very effective when the chemicals were tested singly in pure form, see figures 2, 3 and 12. However, in dual combinations and particularly when the three were combined, they elicited a very good response, see figure 17.

In general the responses to single amino acids were not as strong as responses to single sugars; see figures 7, 8, 11 and 13. However, amino acids elicited feeding at lower concentrations, e.g. hydroxy-L-proline, figure 10. This fact was also reported by Thorpe et al, (1947), in regard to the biting response of wireworms.

When alanine, serine and γ-amino butyric acid were combined with sucrose summation occurred, see figure 18. This combination was far superior to any combination of sugars tested; e.g., fructose plus glucose; fructose plus glucose plus sucrose; fructose plus glucose plus sucrose plus maltose. Thorsteinson and Jay (1958)+ found that fructose, glucose and maltose elicited very good responses when tested individually.

+ unpublished data

Table 1

Results of single substances tested for feeding response

Organic Nitrogen Compounds

Figure	Active	Inactive
2	L-alanine	β-alanine
3	γ-amino butyric acid	L-arginine
4	L-asparagine	L-cysteine
5	L-aspartic acid	L-glutamic acid
6	L-cystine	L-glutamine
9	glycine	L-Histidine
10	Hydroxy-L-proline	L-isoleucine
12	L-serine	L-leucine
		L-lysine
		L-Methionine
		L-Phenylalanine
		L-Proline
		L-Threonine
		L-Tryptophane
		L-Tyrosine
		L-Valine

Figure	Carbohydrates
13	D-Sucrose
7	D-Fructose
8	D-Glucose
11	D-Maltose

Table 2

Results of combined substances tested for feeding response

	Active amino acids	Active sugars	
Figure	2 substances	2 substances	Figure
16	L alanine + γamino butyric acid	fructose + glucose	20
15	L alanine + serine		
14	γ amino butyric acid + serine		
Figure	3 substances	3 substances	
17	L alanine + γ-amino butyric acid + serine	fructose + glucose + sucrose	
Figure	4 substances	4 substances	
18	L alanine + γ-amino butyric acid serine + sucrose	fructose + glucose + maltose + sucrose	

<u>Choice experiments</u>

Fructose: Tests in which the grasshoppers could choose between (a) 0.02 molar and 0.5 molar, (b) 0.02 molar and 1.0 molar, showed that there was consistently greater feeding on the higher concentration: see figure 20 column a and d.

This result is difficult to explain in view of the optimum feeding response centering on 0.02 molar when each fructose concentration was presented singly: see figure 7.

<u>Sucrose</u>: This anomaly does not occur at high concentrations in the case of sucrose. Grasshoppers having a choice of (a) 0.1 molar and 0.02 molar, (b) 0.02 molar and 0.004 molar, consistently preferred the highest concentration presented: see figure 19.

However, a choice of 0.02 molar and 0.5 molar sucrose resulted in greatest feeding at the lower concentration: see figure 20 column b. This is in keeping with results obtained when only single concentrations were presented to the grasshoppers and there was a falling off in feeding at 0.5 molar: see figure 13.

<u>Insect</u>

Diapause development of the <u>C. pellucida</u> eggs occurred at 40°F. The author found that optimal hatching occurred after cold treatment for fifty days.

All grasshoppers were allowed to feed on lettuce leaves before being used on the tests. Pfadt (1949) found that there was no evidence of conditioning of the grasshoppers to a particular food.

Tests were conducted to detect any residual effect of chromatographic solvent on feeding. The results were negative.

All insects used were taken from the field at various locales. No attempt was made to eliminate variation by inbreeding or by selection of experimental insects, e.g. feeders and non-feeders. Thus we feel that our results reflect the variability and deviation in gustatory perception occurring in the field.

Time and Temperature

Tests were run overnight, in the dark, in an incubator at 25°C. It is of interest that Chapman (1957), working with the red locust, reports that there was no evidence of feeding at night, even at a favourable temperature.

The temperature within the cages was held at 25°C. for several hours prior to running the feeding tests. Chapman (1955) (1957) noted that the amount of feeding in the tests reflected the conditioning effect of the temperature a few hours prior to testing.

Succulence and Toughness

Many investigators have stressed the importance of succulence and toughness as factors in the selection of food.

Chapman (1957), working with the red locust, suggests that food preference is based on moisture content and toughness of the leaf. He made no attempt to investigate chemotactic affinities.

Misra (1956) who worked with C. pellucida, is attached to the theory that succulence is the most important factor in food preference, but he sees that other factors of a chemical nature sometimes prevail. He tries to resolve these two ideas, and states anthropocentrically that physical factors are unimportant if the plant is nutritionally unfavourable. Misra shows confusion in the face of his findings that food preferences are not rigidly and serially correlated to nutritional needs. He appears to lose sight of the fact that the manifestation of the "botanical sense" by the insect is due to the insect and plant evolving together, and is not a result of their being tightly hitched together. Any "nutritional mistake" by the insect is not necessarily a reflection on the sensitivity of its gustatory perception.

Painter's (1951) observations dispose of the toughness, moisture content theory as the basis of food preference. He reports that grasshoppers in Kansas ate the corn, chewed the bark from certain trees and the labels from stakes in sorghum nurseries, but only nibbled at the sorghum leaves.

Our tests with _C. pellucida_ showed that feeding was the same for dry and brittle discs as it was for moist, pliant ones.

The experimental tests using extracts and pure chemicals confirm the significance of chemical constituents of the plants as chemotactic regulators of grasshopper feeding. The tests support Pfadt's (1949) observation that selection has a chemotactic foundation, and that choice of plant is primarily a matter of taste.

<u>Figure 1.</u> The Feeding Behaviour of <u>Camnula pellucida</u> (Scudd) on Active Plant Fractions separated by paper chromatography

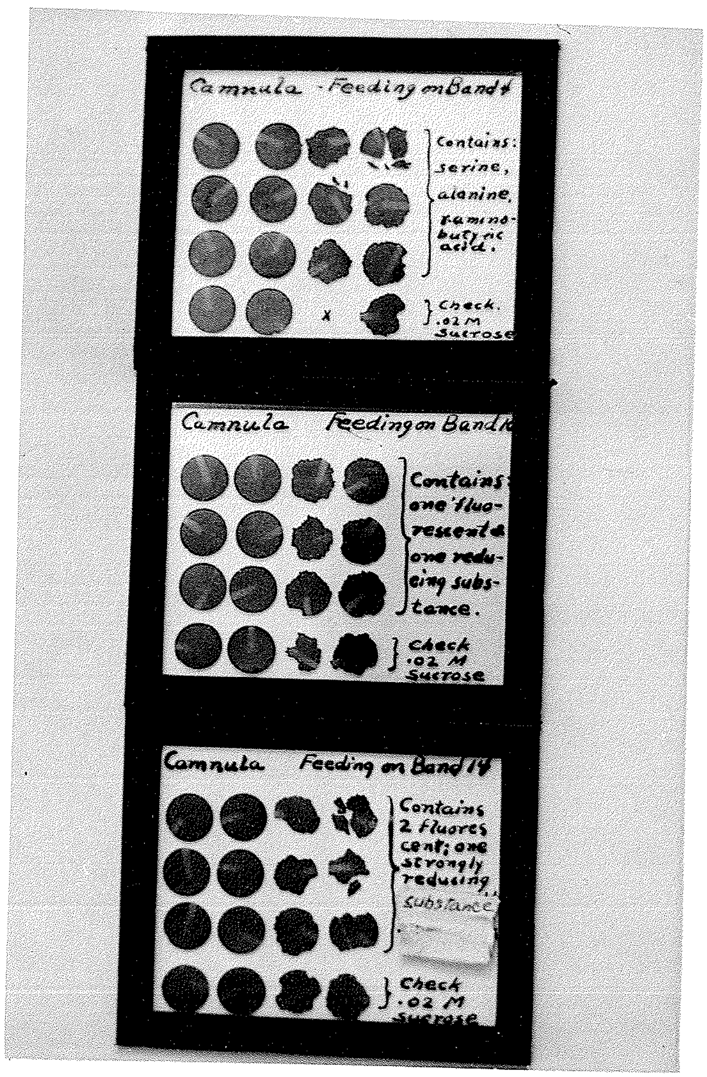


Figure 2. The feeding of *Camnula pellucida* (Scudd.) on L-Alanine

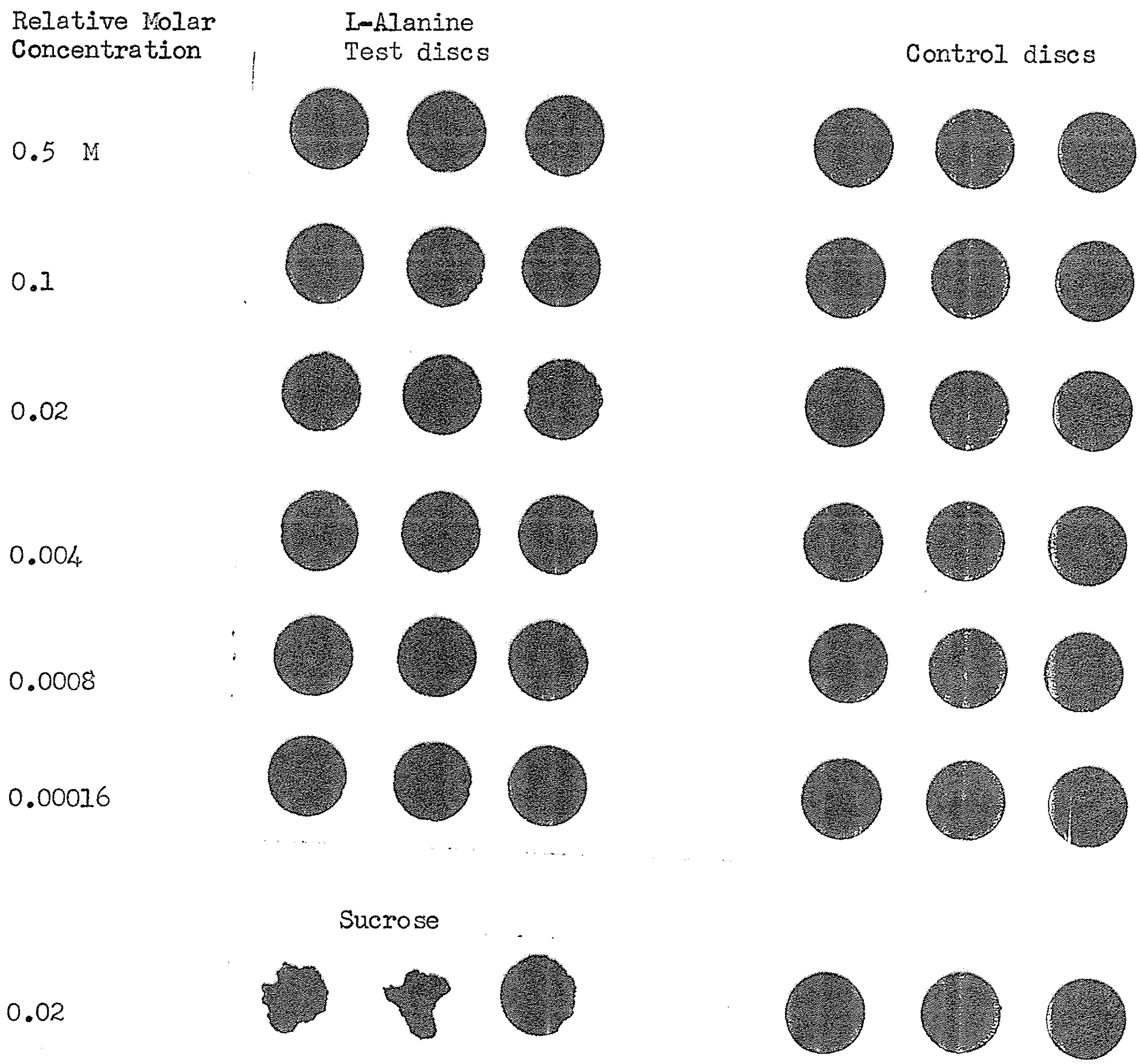


Figure 3. The feeding of Camnula pellucida (Scudd.) on γ-Amino Butyric Acid

Relative Molar Concentration	γ-Amino Butyric Acid Test discs	Control discs

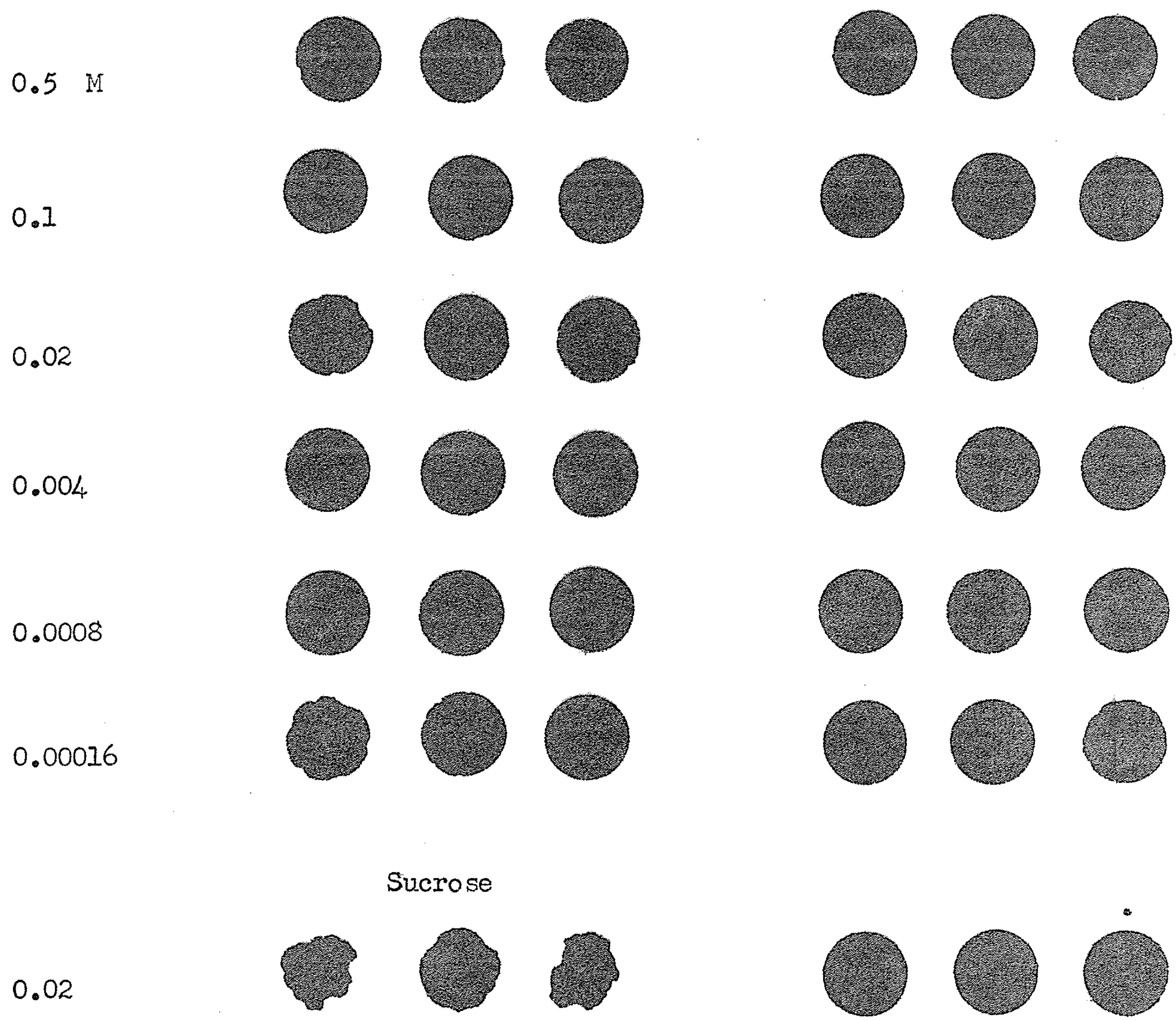


Figure 4. The feeding of Camnula pellucida (Scudd.) on L-Asparagine

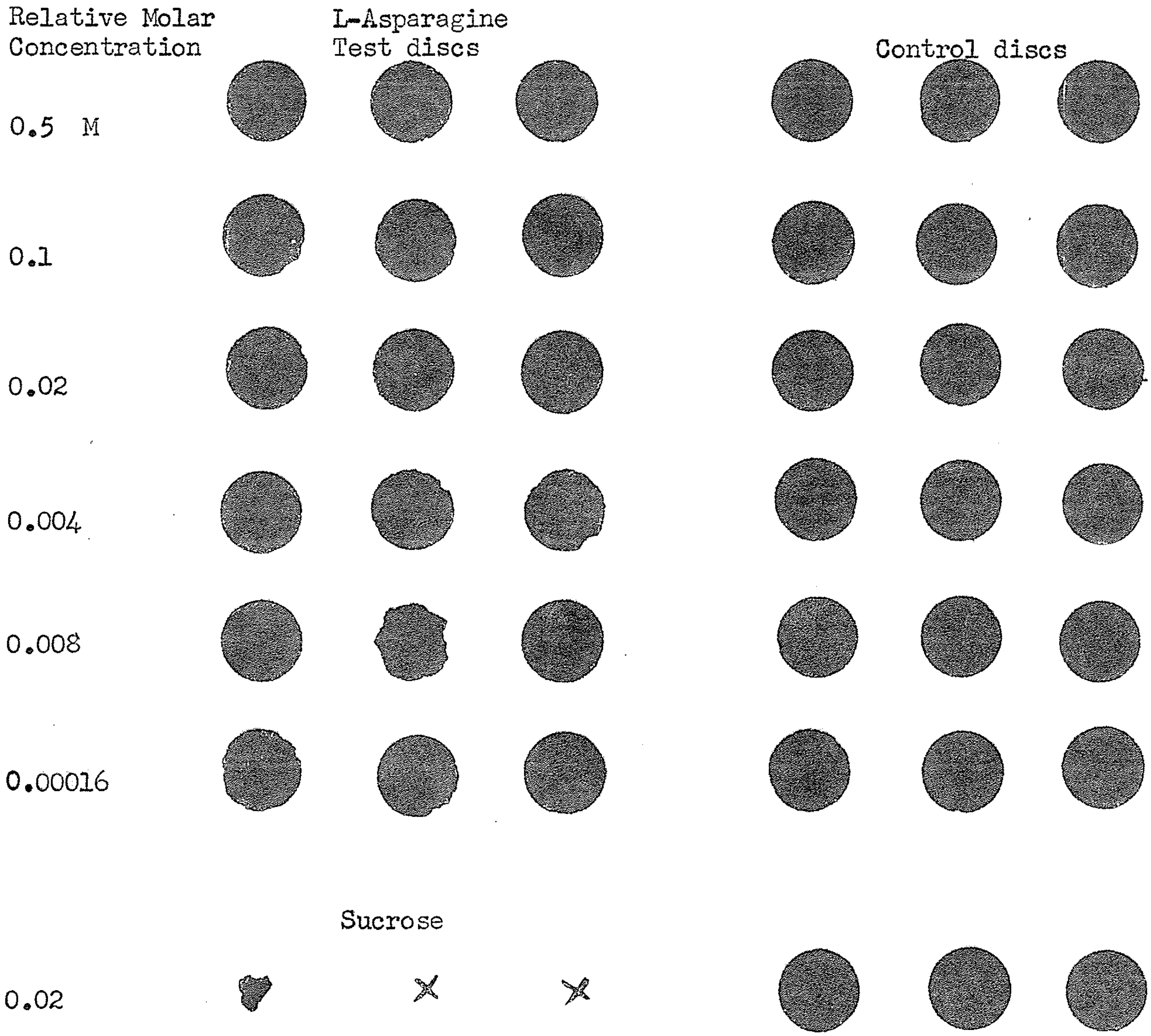


Figure 5. The feeding of <u>Camnula pellucida</u> (Scudd.) on L-Aspartic Acid

Relative Molar Concentration | L-Aspartic Acid Test discs | Control discs

0.5 M

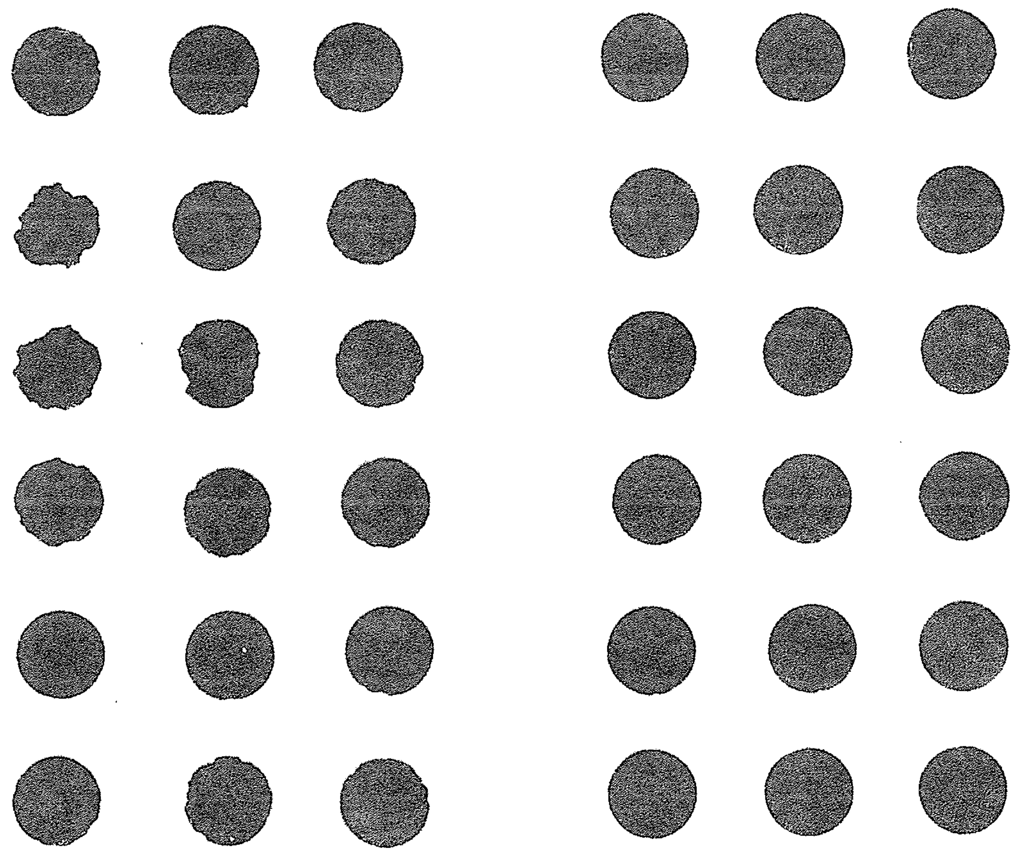
0.1

0.02

0.004

0.0008

0.00016



Sucrose

0.02

Figure 6. The feeding of Camnula pellucida (Scudd.) on L-Cystine

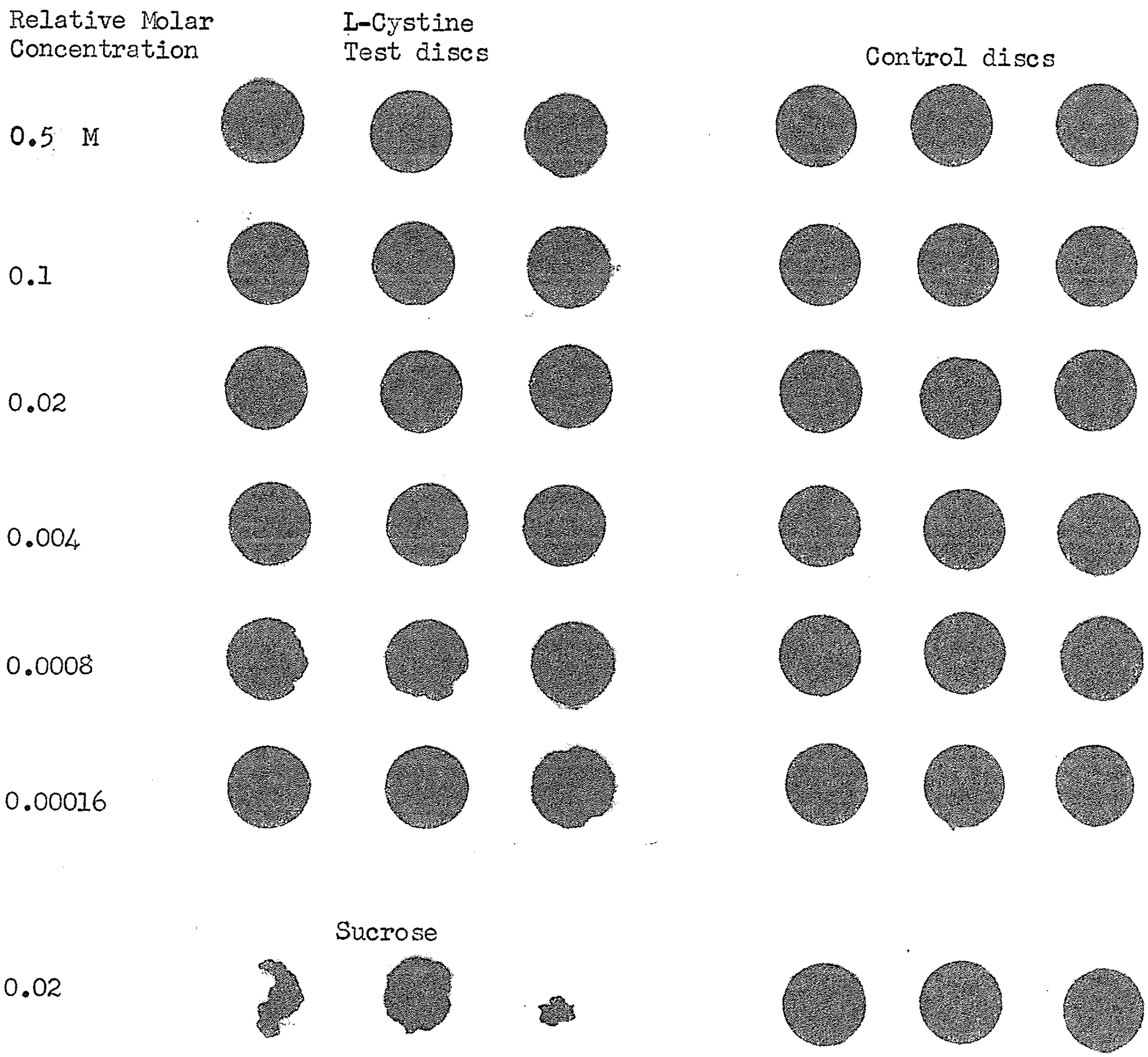


Figure 7. The feeding of <u>Camnula pellucida</u> (Scudd.) on D-Fructose

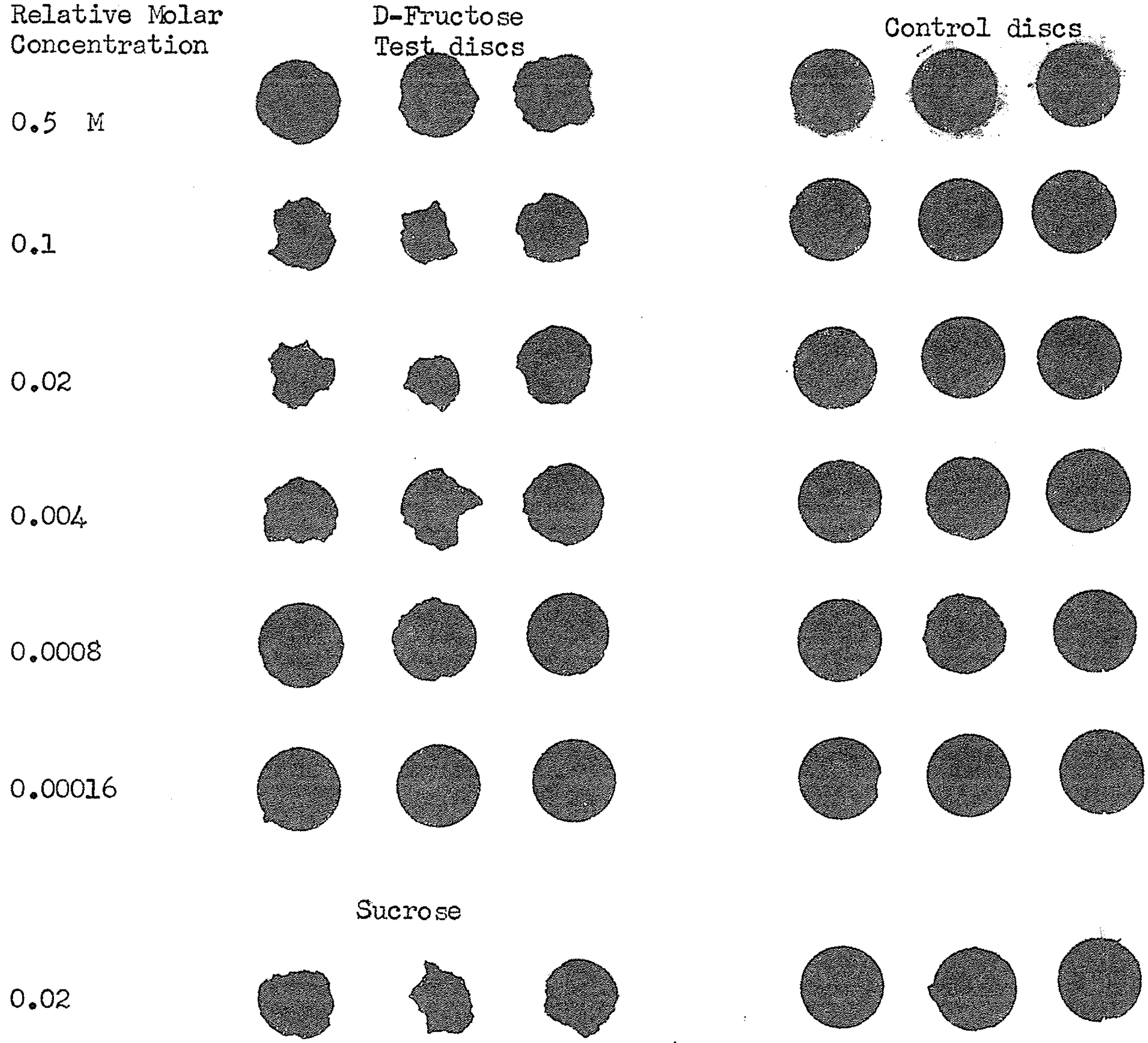


Figure 8. The feeding of <u>Camnula pellucida</u> (Scudd.) on D-Glucose

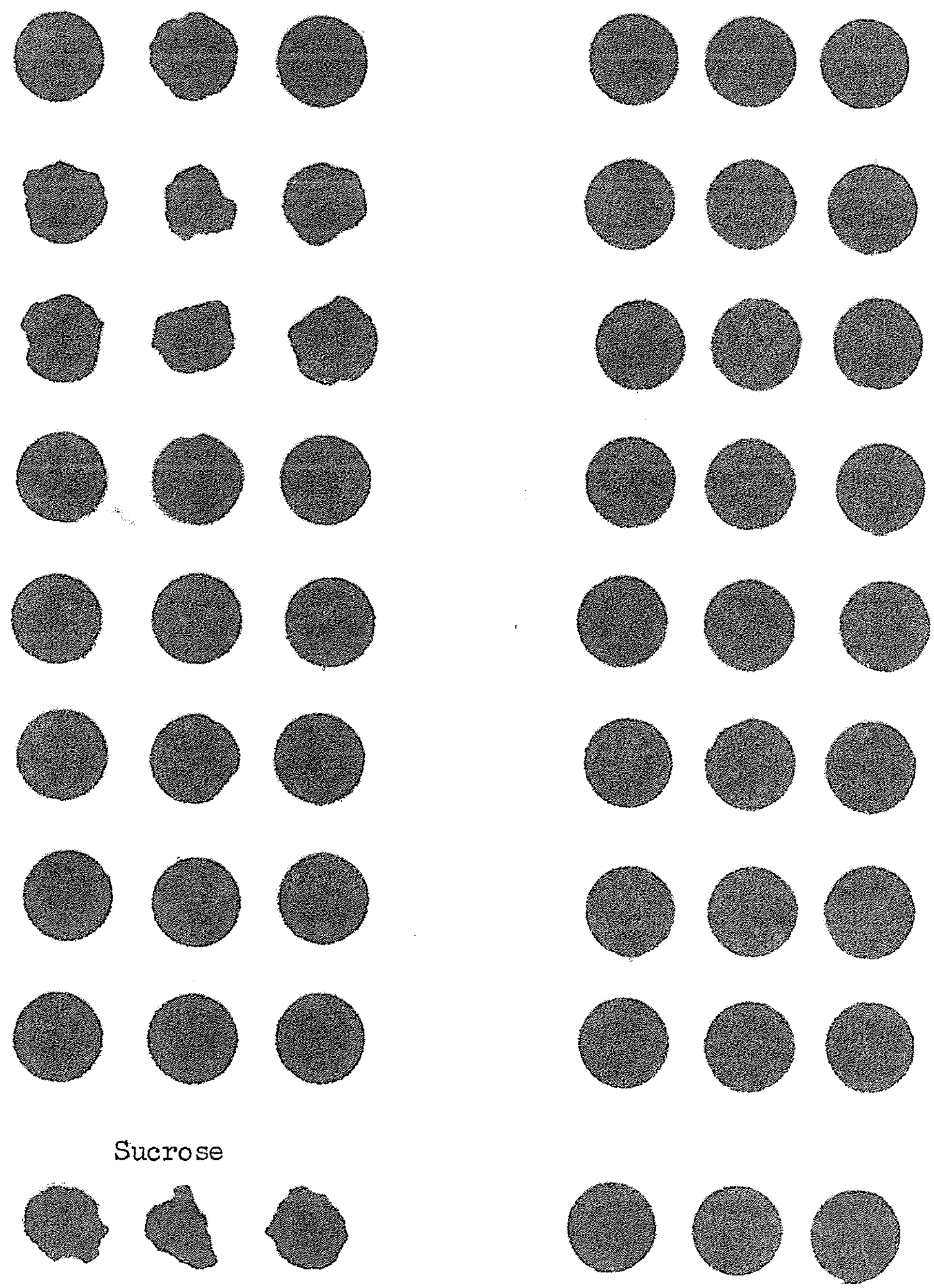


Figure 9. The feedong *Camnula pellucida* (Scudd.) on Glycine

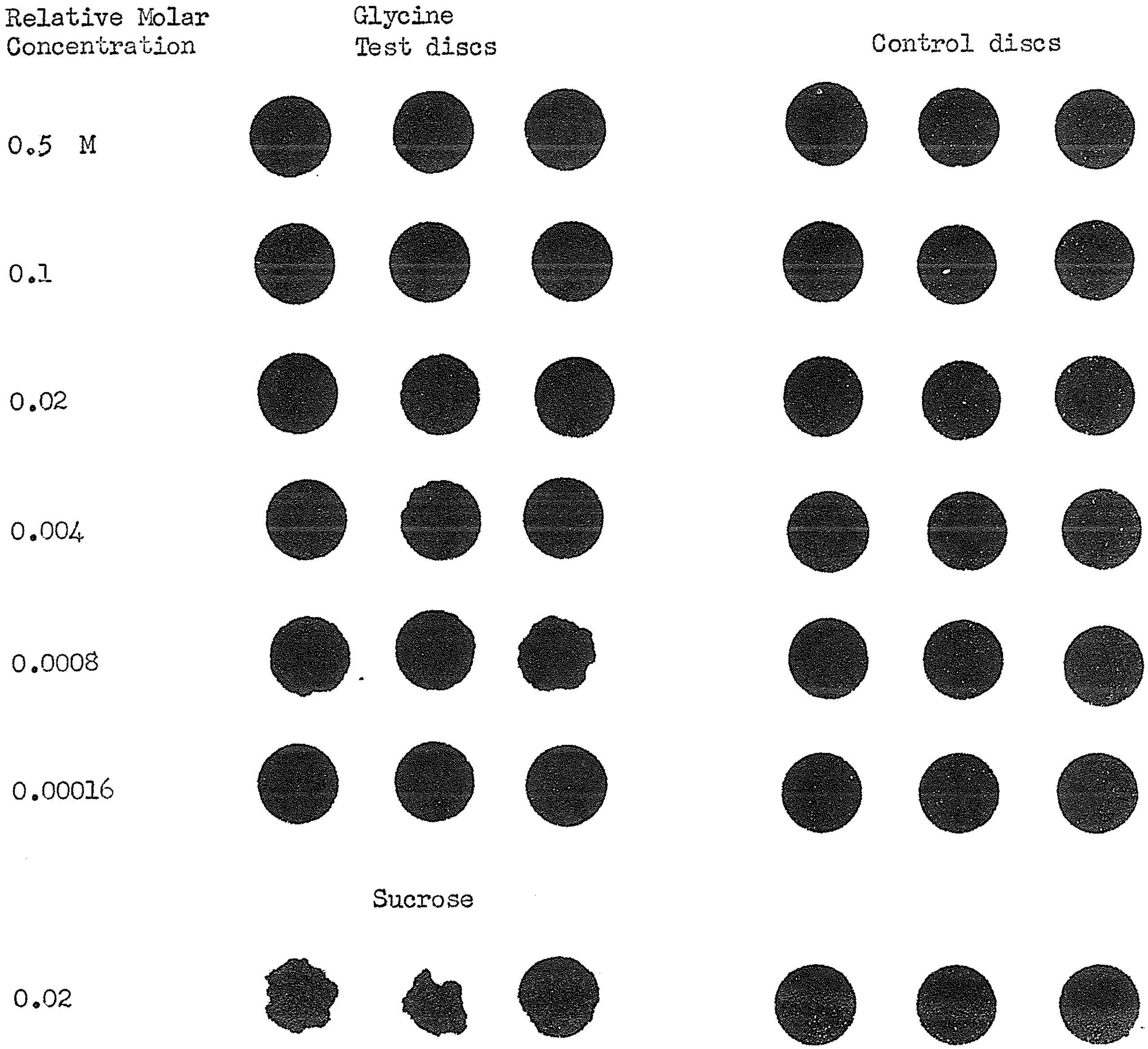


Figure 10. The feeding of <u>Camnula pellucida</u> (Scudd.) on Hydroxy L-Proline

Relative Molar Concentration — Hydroxy L-Proline Test discs — Control discs

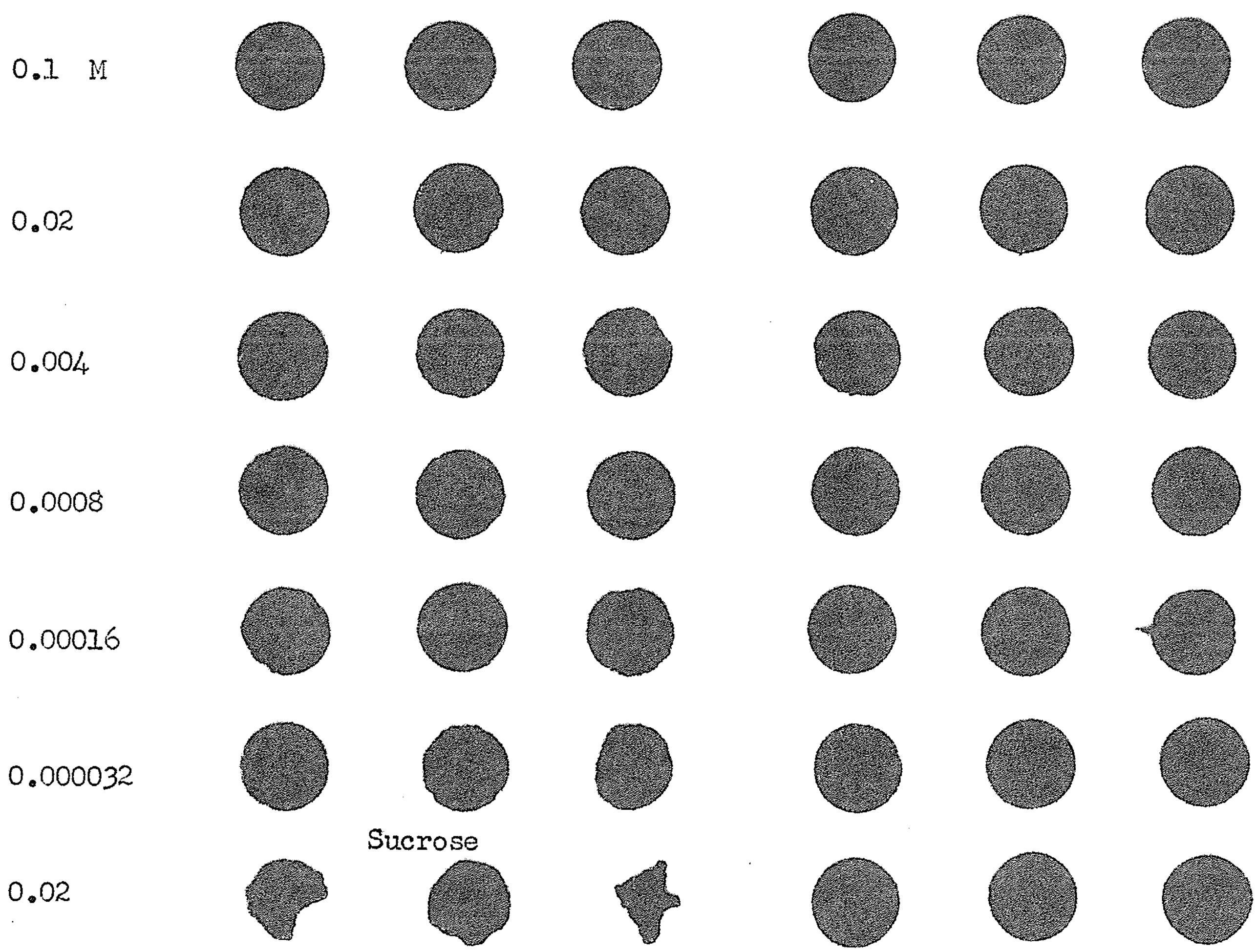


Figure 11. The feeding of <u>Camnula pellucida</u> (Scudd.) on D-Maltose

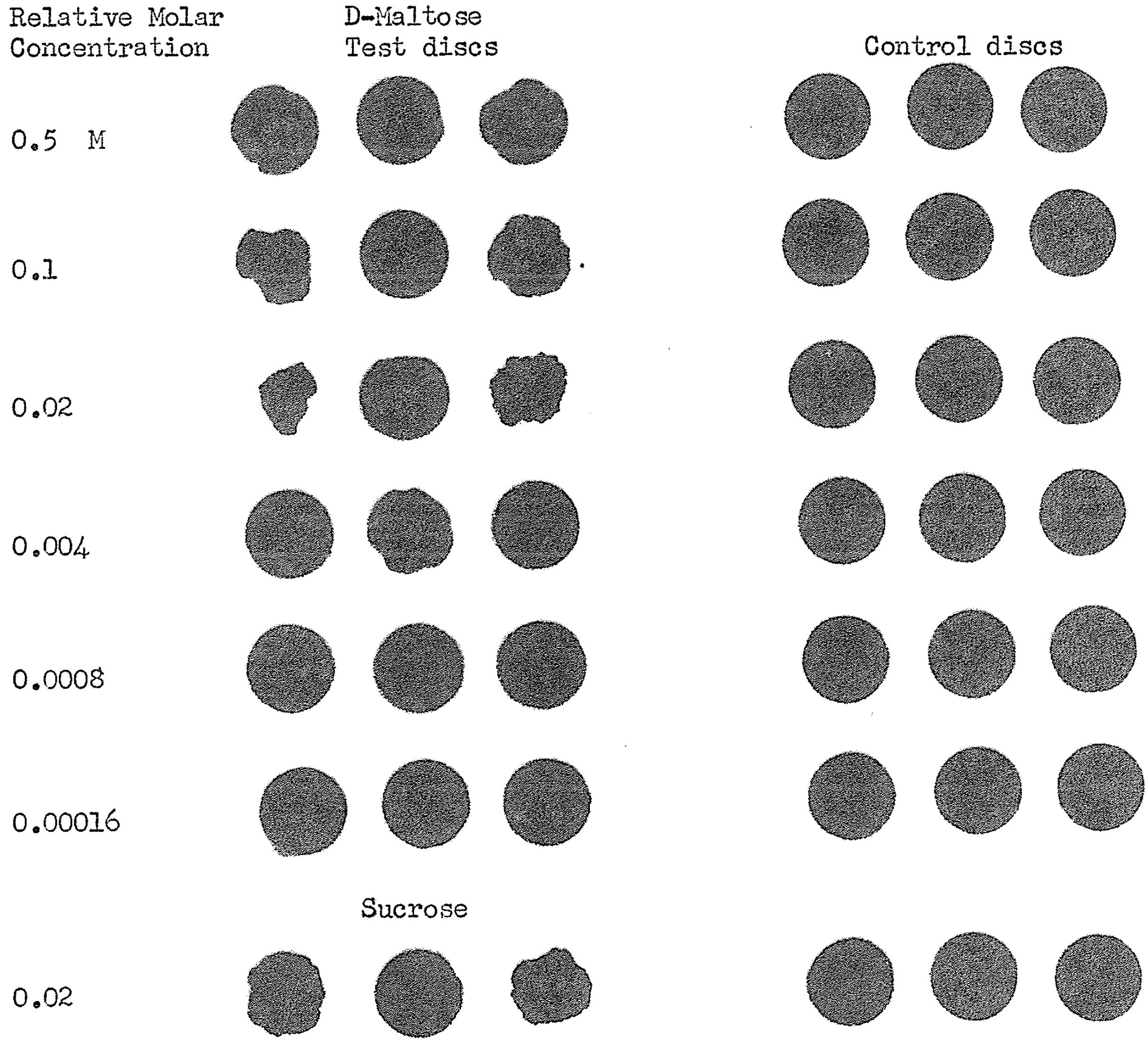


Figure 12. The feeding of <u>Camnula pellucida</u> (Scudd.) on L-Serine

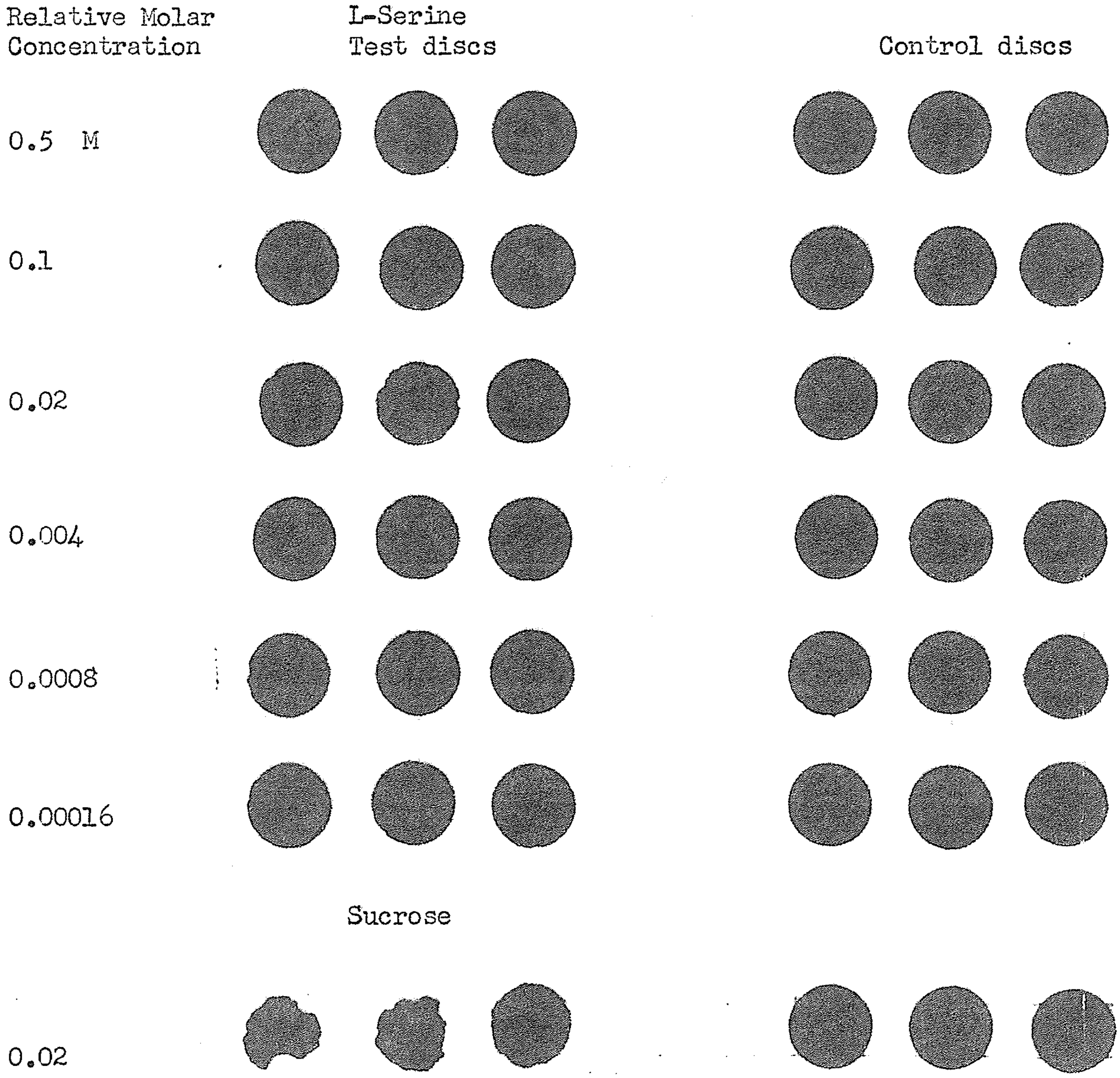


Figure 13. The feeding of <u>Camnula pellucida</u> (Scudd.) on D-Sucrose

Relative Molar Concentration | D-Sucrose Test discs | Control discs

0.5 M

0.1

0.02

0.004

0.0008

0.00016

Sucrose

0.02

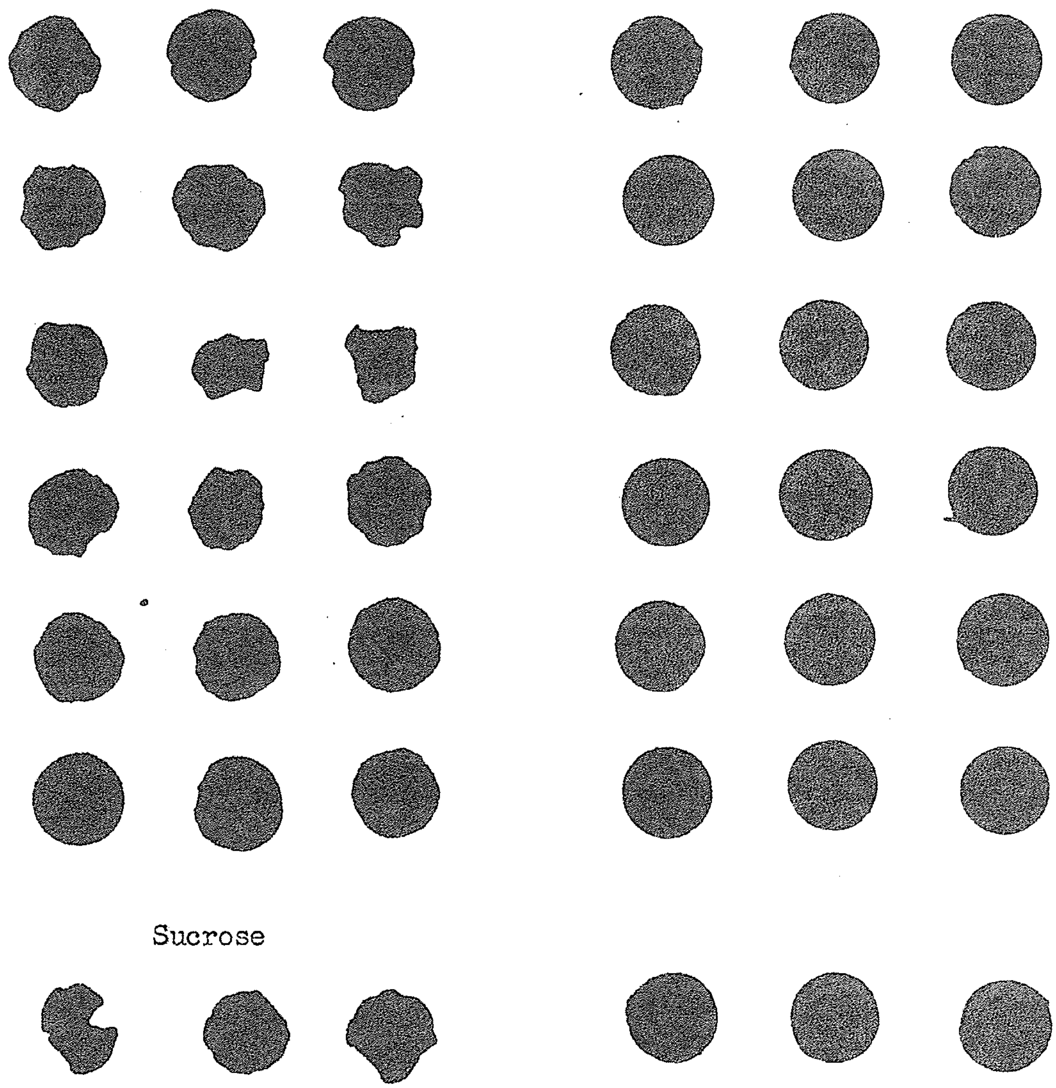


Figure 14. The feeding of <u>Camnula pellucida</u> (Scudd.) on Serine + γ-Amino Butyric Acid

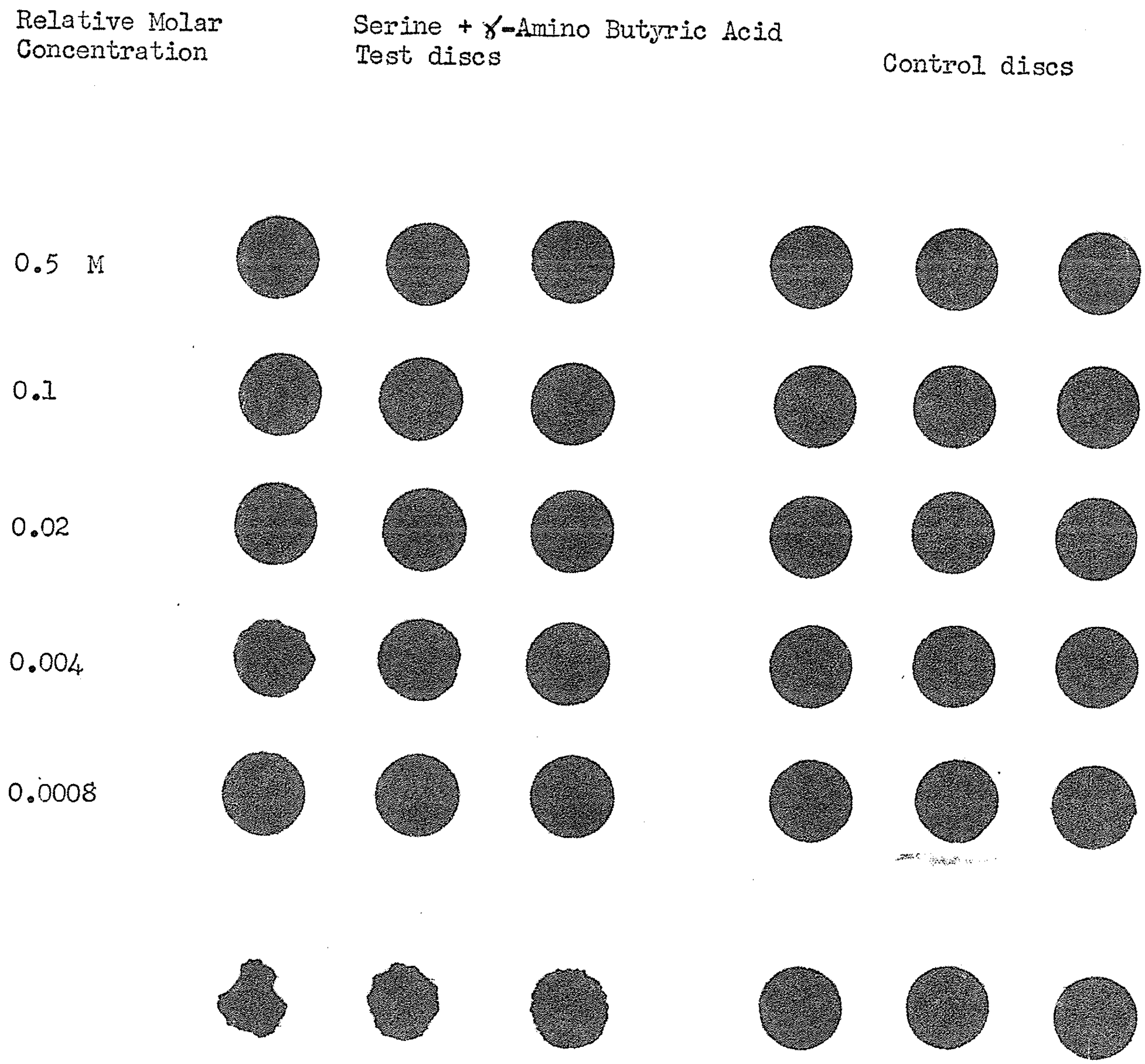


Figure 15. The feeding of Camnula pellucida (Scudd.) on γ-Alanine + Serine

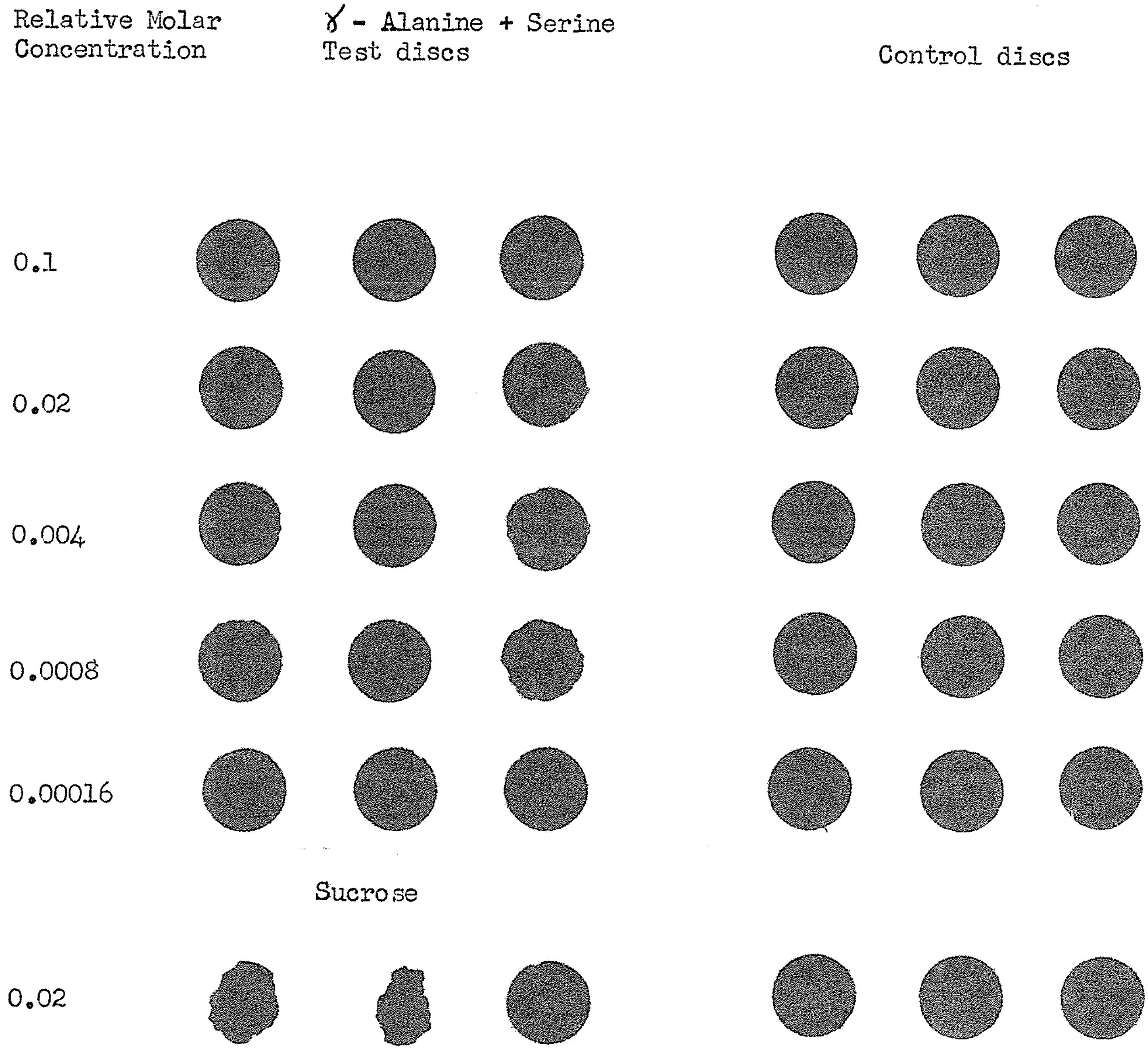
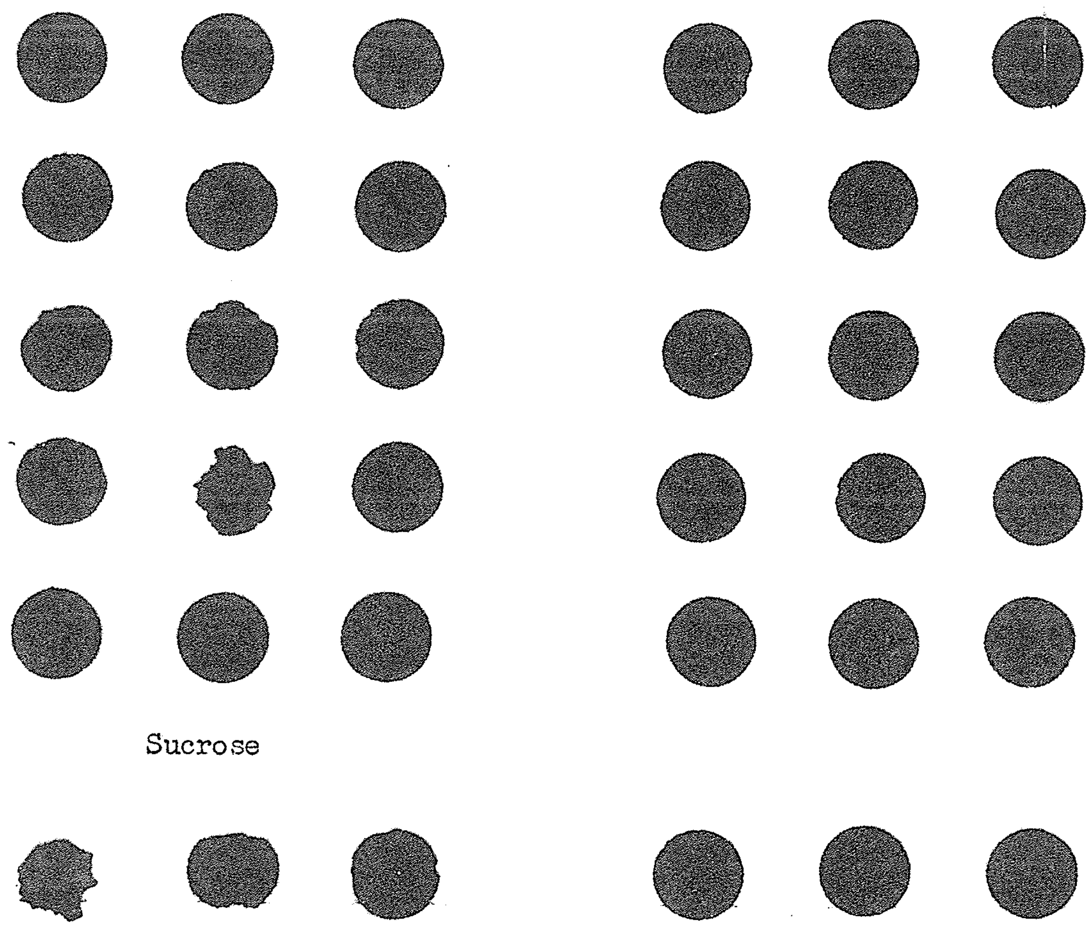


Figure 16. The feeding of Camnula pellucida (Scudd.) on Alanine + γ-Amino Butyric Acid

Relative Molar Concentration | Alanine + γ-Amino Butyric Acid Test discs | Control discs



0.1

0.02

0.004

0.0008

0.00016

Sucrose

0.02

Figure 17. The feeding of <u>Camnula pellucida</u> (Scudd.) on Alanine, Serine γ-Amino Butyric Acid

Relative Molar Concentration	Alanine, Serine, γ-Amino Butyric Acid Test discs	Control discs

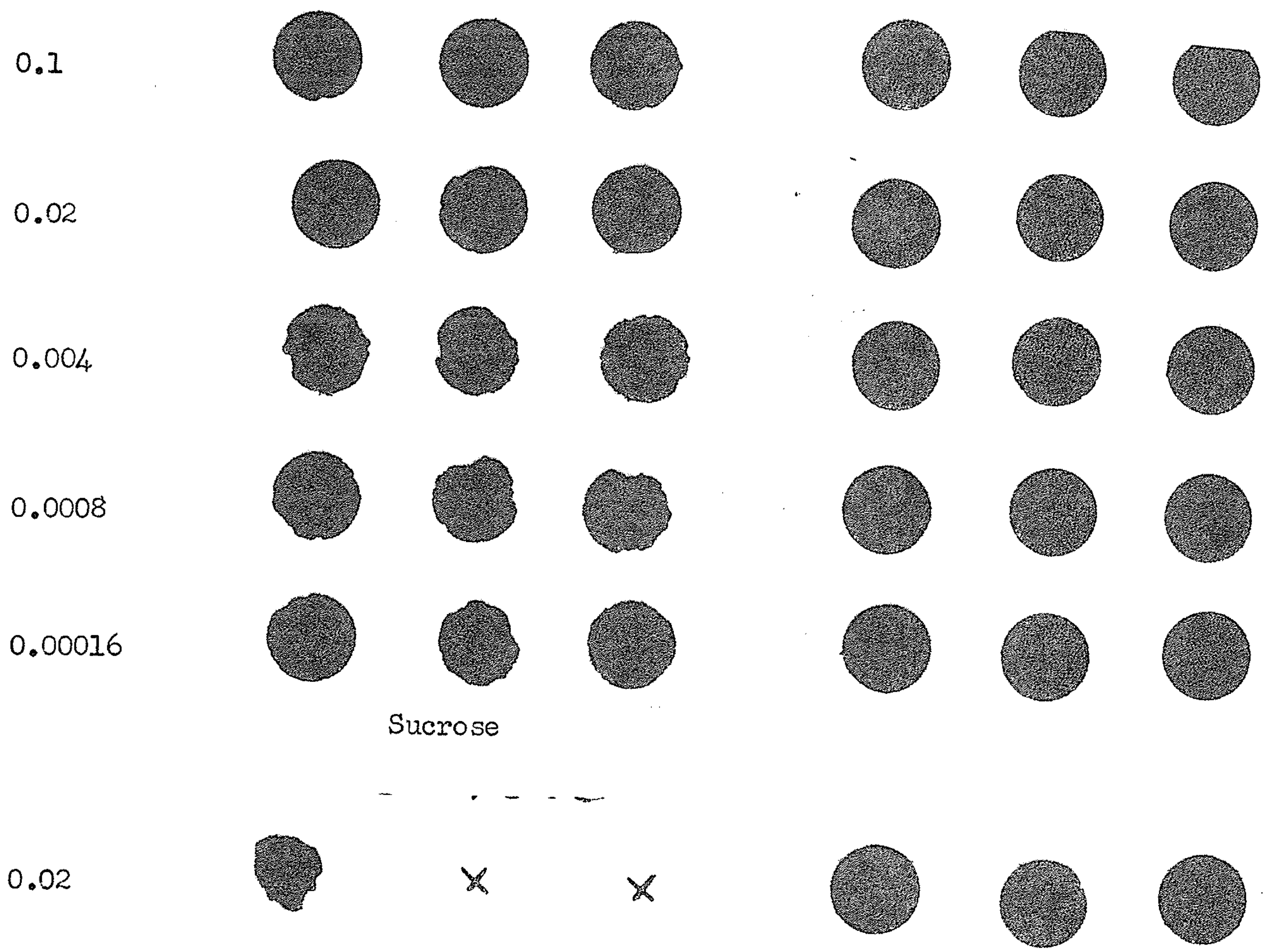


Figure 18. The feeding of <u>Camnula pellucida</u> (Scudd.) on Sucrose, Alanine, Serine, γ-Amino Butyric Acid

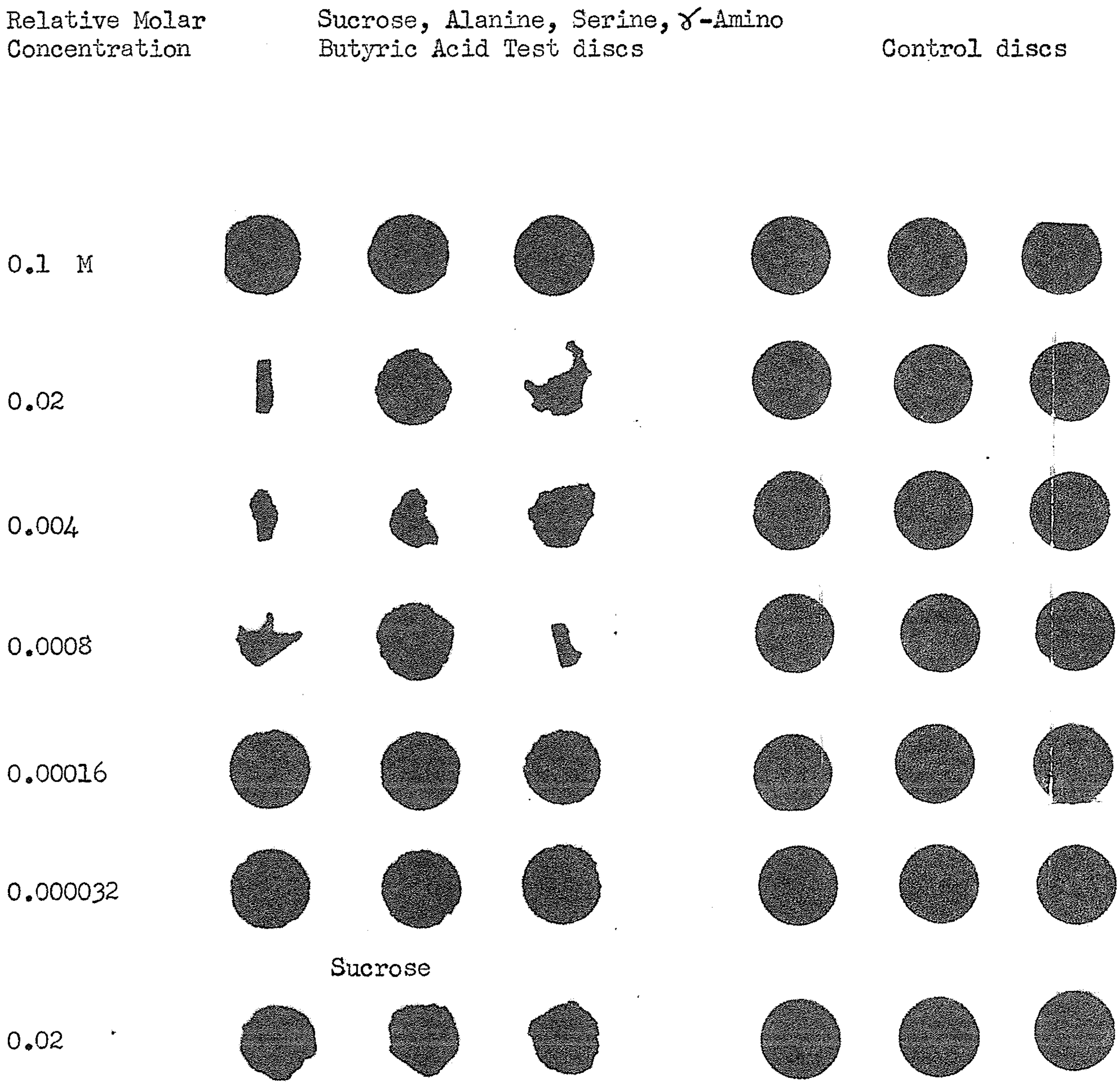


Figure 19. The feeding of Camnula pellucida (Scudd.) on paired molar concentration of D-Sucrose

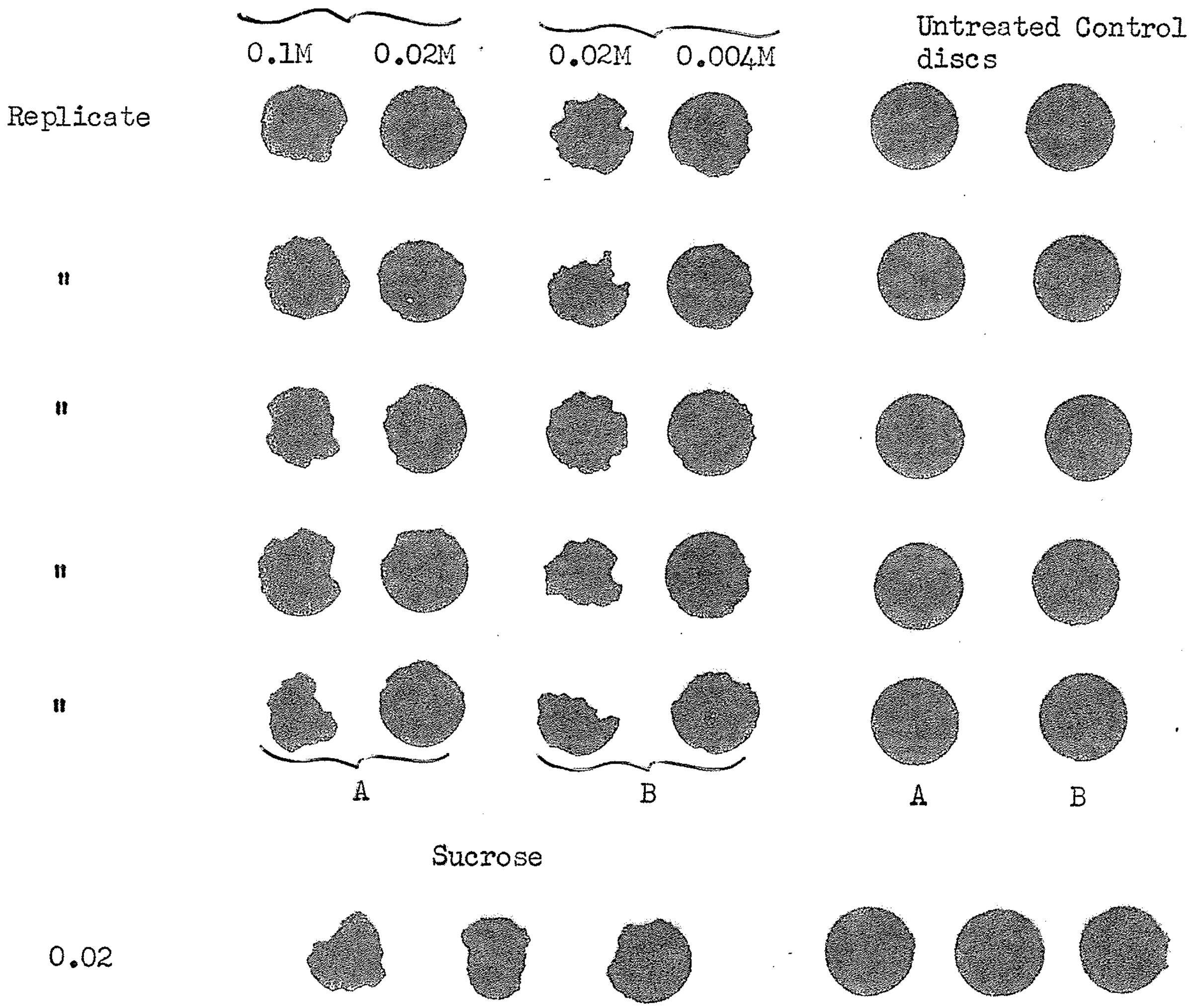
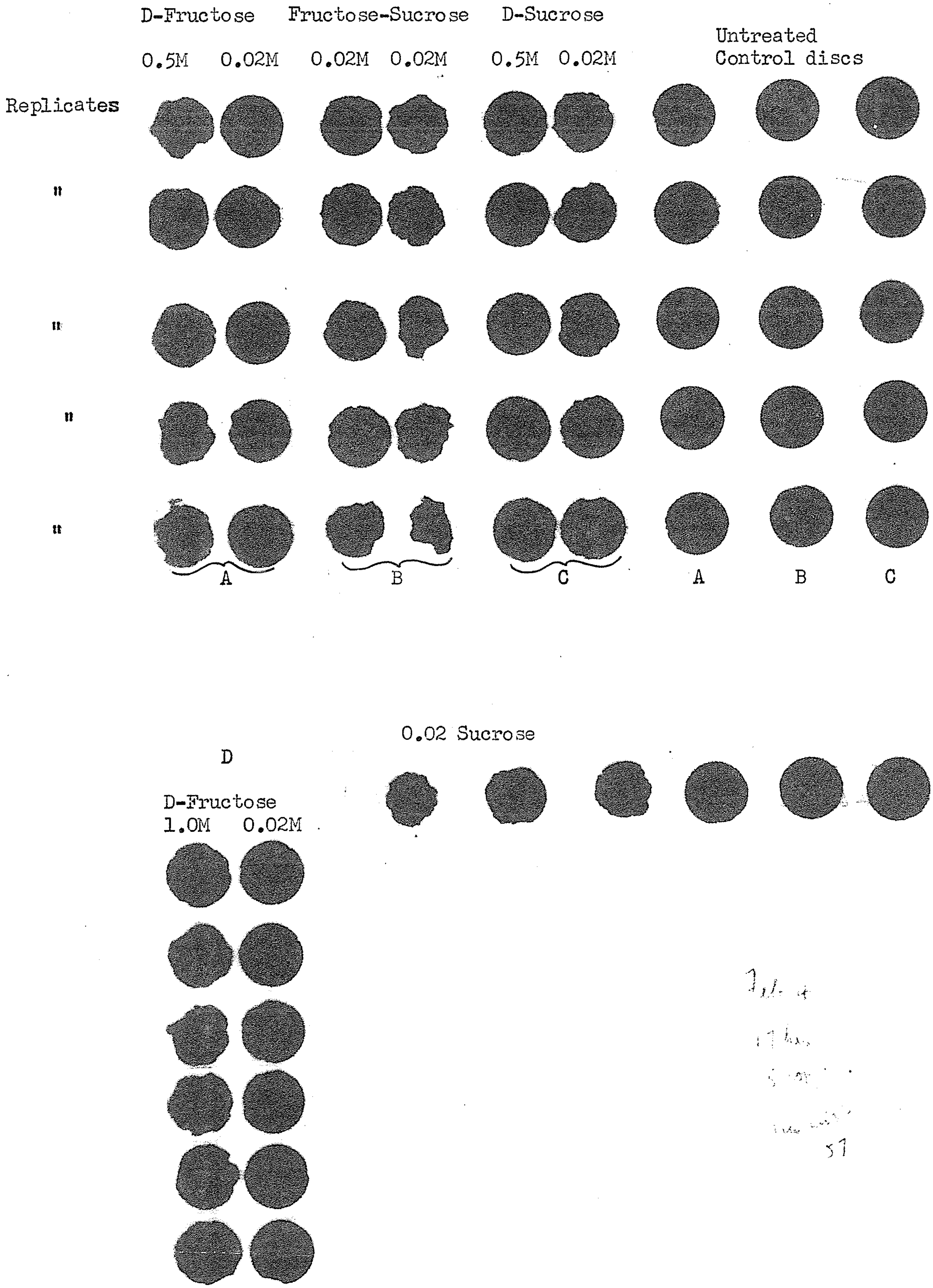


Figure 20. The feeding of Camnula pellucida (Scudd.) on various combinations of Fructose and Sucrose



Summary and Conclusions

A study was made of the gustatory response of *Camnula pellucida* (Scudder) to some organic nitrogen compounds and certain carbohydrates, since it was observed that selection of grasshopper food plants might be made on the basis of taste.

Substances were presented to the grasshopper on a substrate consisting of Japanese elder pith discs of equal diameter and thickness. Tests were run overnight in an incubator at 25°C.

Positive controls within each replicate consisted of 0.02 Molar sucrose.

The discs, with substances absorbed, were presented in a dry and brittle state. Feeding was not improved by moistening.

The investigation progressed in several stages. Alcoholic extracts of plants were tested for feeding response. Feeding on the various plant extracts showed that an acceptable plant resulted in a palatable extract. Lee wheat leaf extracts were broken down into fractions by the use of chromatography, and these fractions were subjected to palatability tests.

There was a striking difference in the amount of feeding on the various chromatographic fractions. A fraction shown to contain α-alanine, serine and γ-amino butyric acid, was found to be very palatable.

An attempt was made to duplicate the feeding response elicited by plant extracts and fractions thereof, by feeding pure chemical compounds, singly, in combination, and in both cases in a series of dilutions. It was found that feeding responses were elicited to varying degrees by α-alanine, γ-amino butyric acid, L-asparagine, L-aspartic acid, L-cystine, glycine, hydroxy-L-proline, serine and sucrose.

Feeding responses to single amino acids were not as striking, in total amount eaten, as were responses to saccharides such as sucrose. Amino acids elicited feeding at notably lower concentrations.

The combination of α-alanine, serine and γ-amino butyric acid, resulted in very good feeding. When these substances were combined with sucrose summation occurred.

A characteristic reaction in all single substances tested was that feeding fell off at high concentrations, e.g., 0.1 Molar to 0.5 Molar. This fact may explain the non feeding of grasshoppers on sorghum and sugar beets, which contain a high concentration of sugar.

This response involving single substance tests was supported by tests in which the grasshoppers were given choices of two concentrations. In the case of sucrose feeding, the grasshoppers chose 0.02 Molar in preference to 0.5 Molar. An anomaly existed in the case of fructose where the higher concentration was preferred.

Work with various insects show that the chemical constituents of plants have a significant role in feeding behaviour. Observations of grasshopper habits testify to the fact that they are attracted equally to preferred and non preferred plants, but that only biting on a preferred plant results in feeding. Since the natural food of insects contains many chemical compounds, e.g. free amino acids, it is possible that some of these substances have an active chemotactic role in the regulation of feeding. This idea was subjected to tests, by using plant extracts and pure chemicals. We conclude from the results obtained that the regulation of feeding behaviour as seen in the field, may be explained in terms of the grasshopper reacting to chemical stimuli, primarily gustatory.

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