would appear that—when the results of the wet and dry season surveys are considered for different age-groups—the prevalence rate of *P. malariae* in Karamoja district may vary between 14.4% and 19.8%. Figures for the 0-4-year age-groups varied between 19.2% and 23.9%. It must be admitted that the prevalence rate of *P. malariae* infection in Karamoja district was one of the highest recorded in the malariometric surveys carried out in fourteen districts in Uganda.

The Effect of Protein Sources and of the Frequency of Egg Collection on Egg Production by the Housefly (*Musca domestica* L.)

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An efficient and therefore economical method of rearing the housefly, *Musca domestica* L., is of importance for biological assays whether large or small numbers are required. Although a considerable volume of literature has been published on rearing houseflies—a good review of which has been published by Sawicki & Holbrook [a]—several major aspects have either been overlooked or have been treated insufficiently to make efficient housefly rearing possible. Sawicki & Holbrook noted that few variations from a basic milk diet have been used for adult flies. The present paper is a report of an investigation of two aspects of housefly rearing—the source of adult protein and optimum frequency of egg collections. Recently Robbins et al.[b] found that both casein and yeast hydrolysate contain feeding stimulants for adult female houseflies. Our experiments have shown that high fecundity is obtained when the adults are fed yeast paste.

Materials and methods

All results were obtained in triplicate with the WHO insecticide Reference Strain WHO/IN/*Musca domestica*/1. Females of this strain were reported to produce between 234 and 369 eggs each [c] in contrast with 500-800 eggs per female reported for other strains by Sawicki & Holbrook.[a] The adult fly cages measured 25 cm × 20 cm × 20 cm, the frame being constructed of 10-gauge Tobin bronze welding rod with a loose Terylene cover (52 holes/cm²). An oviposition site consisted of a clear plastic cup 8 cm in diameter by 4 cm deep with an opaque yellow lid in which 10 holes roughly 1.2 cm in diameter had been melted. A preliminary experiment indicated that yellow lids were slightly better for oviposition sites than green and considerably better than red or blue. Each cup contained cotton wool (5 g) covered by organdie and saturated with 60 ml of a 5% solution of full-cream powdered milk.

Newly laid eggs, being hydrophobic, tended to remain in clumps, as reported by Sawicki & Holbrook.[a] To overcome this, eggs were washed off the organdie into flasks in which they were vigorously shaken. It appears likely that this clumping of eggs has caused some of the disagreement in the literature as to the numbers of eggs/0.1 ml, the range being from 500 [d, e, f] to 750.[g] Various authors [h, i, j] suggest 700/0.1 ml and the numbers estimated in the present paper are based on this figure.

Eggs were measured in millilitres in modified pipettes of 0.78 cm internal diameter, a system differing only slightly from that suggested by Wilkes et al.[k] The tip of each pipette was cut off back to the

[a] Sawicki, R. M. & Holbrook, D. V. (1962) *Pyrethrum Post*, **6**, 3-18.

[b] Robbins, W. E., Thompson, M. J., Yamamoto, R. T. & Shortino, T. J. (1965) *Science*, **147**, 628-630.

[c] WHO unpublished information.

[d] Cox, A. J. (1944) *Soap sanit. Chem.*, **20**, No. 6, pp. 114-117.

[e] Louw, B. K. (1964) *Bull. Wld Hlth Org.*, **31**, 529-533.

[f] West, L. S. (1951) *The housefly*, Ithaca, N.Y., Comstock Publishing Co.

[g] Smith, A. G. & Harrison, R. A. (1951) *N.Z. J. Sci. Tech.*, *B*, **33**, 1-4.

[h] Basden, E. B. (1947) *Bull. ent. Res.*, **37**, 381-387.

[i] McKenzie, R. E. & Hoskins, W. M. (1954) *J. econ. Ent.*, **47**, 984-992.

[j] *Soap Blue Book* (1965) New York, MacNair-Dorland, pp. 233-235.

[k] Wilkes, A., Bucher, G. E., Cameron, J. W. M. & West, A. S., Jr (1948) *Canad. J. Res.*, *D*, **26**, 8-25.

point where no constriction remained and a 500-ml funnel was fused to its upper end. To allow water to drain while retaining eggs, copper gauze (72 mesh), attached to a short length of polyethylene tube by melting the end of the latter, was placed over the bottom of each pipette (Fig. 1).

Eggs suspended in water ready for seeding on to larval medium were drawn into a 0.47-cm diameter glass tube by suction from a syringe connected to the tube by a polyethylene tube (Fig. 2). The same system, modified slightly by replacing the glass tube with an accurate pipette, is used extensively in this laboratory for handling toxic substances used in biological assays. When the eggs had settled, 3 drops were placed into each of a number of 10 cm × 12 cm × 12 cm jars. Each jar contained 270 g of larval rearing medium made up as follows: bran, 110 g; full-cream milk powder, 20 g; Nipagin M[l] (sodium combination), 0.55 g; water, 140 ml. The average weight of 100 pupae reared under these conditions ranged from 2.03 g to 2.35 g with an average of 870 pupae/jar. Experiments indicated that the water content of the larval medium was extremely important. When the milk content was increased beyond that shown above larval growth was inhibited, possibly owing to a decrease in the temperature of the medium. The addition of finely milled maize increased neither larval survival nor pupal weight.

FIG. 1
APPARATUS FOR MEASURING VOLUME OF HOUSEFLY EGGS

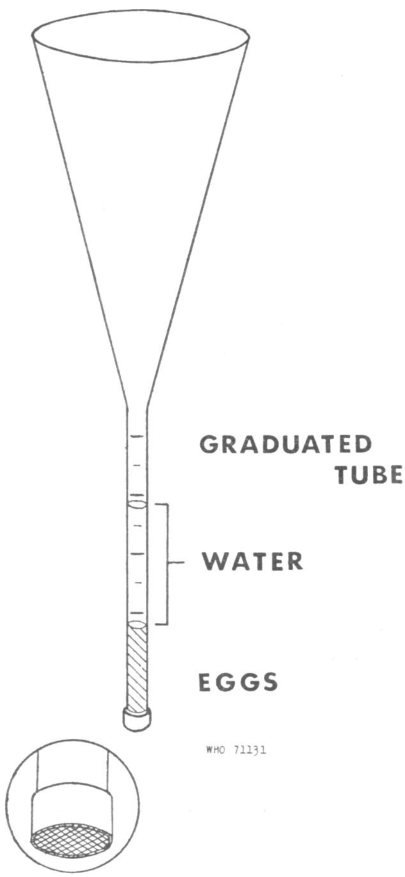
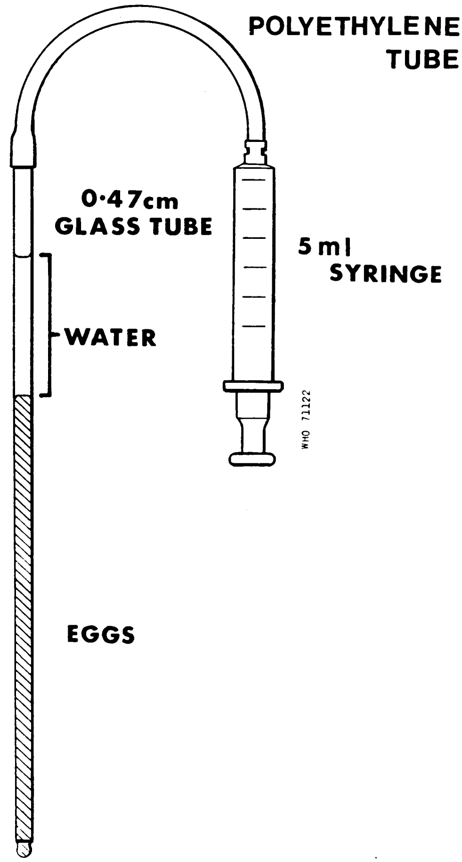


FIG. 2
APPARATUS FOR SEEDING HOUSEFLY EGGS ON TO LARVAL MEDIUM



[l] A product of Nipa Laboratories, Pontypridd, Glamorgan, United Kingdom.

Food was supplied externally to the cage, as described by Osborn & Shipp [m] except for proteins in powder form. Sucrose, in the form of loaf sugar, was placed on top of the cage cover. Water, when supplied, sucrose and proteins were available throughout the cohort's life-span unless otherwise stated. Each cage of flies was supplied with 5.6 cm^2 of sucrose with an equal surface area of protein source whether in liquid, paste or powder form. Diets in which sucrose and protein were combined offered 11.2 cm^2 of the mixture. Water was provided in a plastic half-pound butter-box with a Kleenex wick folded over the lid and supported in close contact with the floor of the cage.

Adult flies of the same age were obtained by removing all pupae from emergence cages each day. As this method resulted in a wide range of adult densities it was abandoned after the initial experiments involving various protein sources. Thereafter pupae, measured by volume, were placed in cages and allowed to emerge over 2 days, resulting in adult densities of approximately 650-700 per cage.

Eggs were collected every second day in the protein experiments, beginning on the fifth or sixth day of adult life and continuing until the 20th day, although most eggs were laid by the 16th day. In the first two protein experiments described, oviposition sites were placed in cages at 16.30 hours and removed at 09.00 hours the following morning. This resulted in eggs hatching or dehydrating before measurement thereby producing errors in estimation of egg production. In the remaining experiments this was overcome by collecting eggs for 4 hours only, beginning at 09.00 hours.

All stages of the life-cycle were reared in one room kept at 26.5°C and a relative humidity of approximately 60%.

Effect of protein sources on housefly fecundity

First experiment. Initially 11 protein diets were compared for their effect on fecundity. External feeding prevented flies from drowning in liquid diets as these could be reached only by the extension of a proboscis through the cage cover. However, this mode of supplying food necessitated constant refilling of liquid diets to ensure that it was always available. Table 1 shows the composition of most of the diets. For some, however, further explanation may be useful: the Peet-Grady diet contained 5% spray-dried non-fat milk solids and 2% granulated sugar; [j] the egg-mixture diet (Gahan's [n] diet) contained powdered egg, skim milk and granulated sugar in the ratio of 1: 6: 6; and the 4 Kraylift pastes contained Kraylift yeast [o] and icing sugar in the ratios of 1:3, 2:3, 3:1 and 4:0 respectively, moistened to a paste with 5% glycerol.

[m] Osborn, A. W. & Shipp, E. (1965) *J. econ. Ent.*, **58**, 1023.

TABLE 1

ADULT FOODS AND THEIR EFFECT ON HOUSEFLY FECUNDITY IN FIRST EXPERIMENT

Diet	Average no. of eggs/female
Peet-Grady (liquid) [a, b]	234
Skim milk 5 % [b]	280
Skim milk 5 %	262
Cream milk 5 %	288
Cream milk 5 % [c]	118
Egg mixture (powder) [a]	146
Skim milk (powder)	90
Kraylift paste 25 %	166
Kraylift paste 50 %	204
Kraylift paste 75 %	286
Kraylift paste 100 %	266

[a] Loaf sugar not supplied.
[b] Water boxes not supplied.
[c] Protein offered only on 3rd, 4th and 5th days of adult life.

In this experiment adult densities varied considerably. The proportions of eggs hatching varied from 97% on the 8th day to 93% on the 16th day. Wilkes et al.,[k] studying their wild strain, reported 90% and 89% hatch of eggs laid by females of the same respective ages. None of the milk diets, either in powder or in liquid form, was significantly better for egg production than the 75% Kraylift yeast paste. Liquid milk diets required daily attention and because of the labour involved were not suitable for efficient housefly maintenance. Milk powder required less attention but flies fed on it did not produce sufficient quantities of eggs to warrant further investigation. The egg powder in Gahan's diet was in a dilute concentration and because the concentration of yeast in the Kraylift paste series indicated

[n] Gahan, J. B. (1963) In: *Proceedings of the US Department of Argriculture Planning and Training Conference for Insect Nutrition and Rearing*, Beltsville, Md., US Department of Agriculture, p. 48.

[o] Kraylift yeast is a product of Kraft Foods Pty Ltd, Australia.

TABLE 2
ADULT FOODS AND THEIR EFFECT ON HOUSEFLY FECUNDITY IN SECOND EXPERIMENT

Diet	Average no. of eggs/female
Peet-Grady (liquid) [a, b]	258
Egg mixture (powder) [a]	168
Egg powder	394
Egg paste 75 %	248
Egg-Kraylift paste	392
Kraylift paste 75 %	366

[a] Loaf sugar not supplied.
[b] Water boxes not supplied.

greatly increased fecundity with increased percentages of protein, it was decided to try more concentrated forms of egg powder.

Second experiment. Using the results of the first experiment as a guide, 6 protein diets, which are listed in Table 2, were chosen and tested for their effect on housefly fecundity. The Peet-Grady diet was chosen as the standard milk diet because of its widespread use. The 75% egg paste was made in the same way as the 75% Kraylift yeast paste, by substituting egg powder for the yeast, while the egg Kraylift paste contained egg powder, yeast and icing sugar in the ratio of 3:3:2 moistened with 5% glycerol.

The egg mixture, 75% egg paste and Peet-Grady protein diets produced 168, 248 and 258 eggs per female respectively and thus were not considered adequate for maximal housefly egg production. The pure egg powder was satisfactory, producing 394 eggs per female, but required more attention than the pastes as it needed changing every second day. The most satisfactory proteins offered were the 75% Kraylift yeast paste, which averaged 366 eggs per female, and the egg-Kraylift paste, with 392 eggs per female; both were applied to the external surface of the cage when they were stocked with pupae. In these diets additional protein was required only once or twice during the cohort's life-span.

Third experiment. A final group of 3 protein diets, which are listed in Table 3, was tested as before, the aims being to determine whether Kraylift would be as satisfactory in powder form as it is in a paste, whether another cheap yeast could be substituted for Kraylift, and whether Fleischmann's yeast hydrolysate type M[p] would be as suitable for increasing housefly fecundity as it is for increasing tephritid fecundity.[q] All diets provided water and loaf sugar in addition to protein. A 75% Torula[r] paste produced only 188 eggs per female and was therefore not suitable, possibly because it desiccated readily. Kraylift yeast as a powder similarly did not induce high housefly fecundity, although in paste form in the two previous experiments it had done so. Similar results had been obtained with non-fat milk powder and a 5% milk solution (see " First experiment "). In both cases the powder forms were significantly less suitable than the respective pastes. Therefore not only was the protein type of importance, but also the state in which it was offered. Preliminary experiments had shown that milk pastes were unsuitable because they desiccated rapidly, hardened and became unattractive for further feeding. Sawicki & Holbrook [a] reported early deaths of flies fed on a milk paste made from powdered milk and sucrose.

TABLE 3
ADULT FOODS AND THEIR EFFECT ON HOUSEFLY FECUNDITY IN THIRD EXPERIMENT

Diet	Average no. of eggs/female
Kraylift powder	186
Torula paste	188
Fleischmann's paste	226

Flies fed on Fleischmann's yeast hydrolysate type M produced an average of 226 eggs per female; thus a paste made from this yeast moistened with a 5% glycerol solution was not satisfactory for houseflies.

Optimum frequency of egg collection for maximum oviposition

To determine the optimum frequency of egg collection oviposition sites were offered in one of four ways—daily, or every second, third or fourth day. The results are recorded in Table 4, from which it will be seen that houseflies produce more eggs if

[p] Fleischmann's yeast hydrolysate type M is a product of Standard Brands Inc., New York, N.Y., USA.

[q] Hagen, K. S. & Finney, G. L. (1950) *J. econ. Ent.*, **43**, 735.

[r] Torula yeast is a product of Waldhof, Mannheim, Germany.

TABLE 4
EFFECT OF FREQUENCY OF EGG COLLECTIONS ON EGG PRODUCTION

Interval between egg collections (days)	Volume of eggs (ml)		
	On oviposition sites	On water containers	Total
1	23	1.2	24.2
2	23.1	1.3	24.4
3	8.5	8.2	16.7
4	7.1	9.8	16.9

oviposition sites are available at least every second day than if less frequently. Under these conditions a far greater proportion of the eggs are laid in the containers provided for the purpose, with only 5.3% of the total eggs being wasted. In comparison with collections made on alternate days, there is no significant increase in fecundity or percentage of eggs laid in oviposition sites when daily collections are made; therefore, the former is more efficient in that it involves only half the labour.

Conclusions

High fecundities, equal to or better than those obtained on milk-based diets, have been achieved with yeast pastes. Provision of protein in this form considerably reduces the labour required for housefly maintenance.

Egg collection on alternate days was found to be optimal. The density of adults in these experiments was approximately one fly per cubic inch.

The Relationship between the Antigenic Structure of the Pomona Serogroup of Leptospiral Serotypes and their Circulation in Particular Species of Animals in the USSR*

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In the modern serological classification parasitic leptospires are represented by 114 independent serotypes (in 1966). In view of the large number of types in this group of organisms the question arises how serological types are formed among them. Gsell [a] put forward the hypothesis that new serotypes of leptospires may be formed by adaptation to a new species of animal host. Anan'in [b] also suggests that the adaptation of leptospires to various species of animals leads to a change in their antigenic structure. Roth et al.[c] explain the antigenic differences of leptospiral strains in the Tarassovi serogroup,[d] isolated from opossums, as due to the presence *in vivo* of an antigenic mutation directed towards the formation of an independent serotype.

It should be emphasized that as long ago as 1945 Terskih [e] drew attention to a difference in biological properties among the causal agents of leptospiroses belonging to one and the same serotype. In particular, leptospires of the *grippotyphosa* serotype circulating in cattle in the south of the European part of the USSR are more pathogenic (causing severe disease in human beings with manifestations of jaundice) than leptospires of the same serotype cir-

* The information reported in this article was given in a paper read at the Ninth International Congress on Microbiology, Moscow, 24-30 July 1966.

[a] Gsell, O. (1949) *Rev. méd. Suisse rom.*, **69**, 613.

[b] Anan'in, V. V. (1964). In: *Discussion, Second International Symposium on Leptospirae and Leptospirosis in Man and Animals, Lublin 1962*, Warsaw, Part I, p. 159.

[c] Roth, E. E., Moore, M., Greer, B., Newman, K., Adams, W. A. & Sanford, G. E. (1963) *Zoonoses Res.*, **2**, 91-104.

[d] Previously called the Hyos serogroup, and renamed Tarassovi by the Taxonomic Subcommittee on Leptospira of the International Committee of Bacterial Nomenclature, after S. I. Tarassov, who first isolated a leptospiral strain in this group in 1938.

[e] Terskih, V. I. (1945) [*Leptospiral diseases of humans and animals*], Moscow, Medgiz.