of the mouse, amount of food in the digestive system, etc. These conditions seemed to have more effect on the time than the size or weight of the animal. Mice weighing between 15 to 20 grams seemed to react about the same; above 20 grams, a slightly larger dose is required; the sex of the mouse apparently makes no difference, males and females reacting similarly. So far, in our experience we have found no adverse after-effects from the use of this drug.

The concentration of the solution recommended by the manufacturers has been found, for practical purposes, to be a little too great. It is much easier to administer accurately a less concentrated solution; and this is quite an item when a very small excess of a highly concentrated solution will cause death. The most satisfactory solutions were found to be 1 grain Nembutal dissolved in 5 cc or 10 cc of water, and of these two, the writer prefers the latter as being a little more convenient for accuracy.

The peripheral nerves apparently react to Nembutal more slowly than the central nerves. This drug will, however, produce complete anesthesia in mice in from 3 to 15 minutes, depending on the amount given. The following table gives the dosage, average time to anesthetize and average recovery time, as experienced in this laboratory. The time limits given in the table represent conditions both when the administering of the drug was, and when it was not followed by an operation (such as vasectomy).

Nembutal	Weight	Anesthetized	Recovery	Lethal
1/16 gr.	15–20 gm.	2– 4 min.	None	15–15 min.
1/25 gr.	15–20 gm.	6– 8 min.	3½– 6 hrs.	1 death in 67 min.
1/33 gr.	15–20 gm.	6–10 min.	50–80 min.	
1/40 gr.	15–20 gm.	6–10 min.	45–90 min.	
1/50 gr.	15–20 gm.	8–12 min.	40–70 min.	
1/66 gr.	15–20 gm.	8–15 min.	10–25 min.	
1/100 gr.	15–20 gm.	Partial		
1/100 gr.	Repeated	10–15 min.	50–120 min.	

Dosages of 1/20 gr. and over are generally lethal in mice weighing less than 20 grams; larger dosages than this result in death in a few minutes preceded by convulsions. For average purposes, the most satisfactory dosage is 1/50 grain dissolved in .2 cc of water and administered intraperitoneally. Since respiration is lowered by the administering of the drug, and at times it is quite slow, it might be necessary under cooler climatic conditions to take precautions to keep the mouse warm, especially after an operation; in our laboratories, however, we have not found this necessary.

There is considerable satisfaction in the use of Nembutal for anesthesia in mice: the results are prolonged, there is no need of watching the anesthetic, there are no after-effects, and death seldom results where normal dosages are administered. We have not recorded a single death in hundreds of cases where the dosage did not exceed 1/50 grain.

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AN EFFICIENT MEDIUM FOR REARING HOUSEFLIES THROUGHOUT THE YEAR

THE rearing of houseflies (*Musca domestica* L.) throughout the year for use in various lines of research has developed largely in the last decade. Bacteriological, biological and more especially insecticidal studies have made the need urgently felt for the rearing of houseflies, even during the winter months. Glaser[1, 2] (in 1923 and 1924) reported on his trials for rearing flies throughout the year. He was able to breed flies from the middle of April to the middle of December, but it was not found possible to rear them on his culture medium (fresh horse manure) during the remainder of the year. Further studies (1927)[3] showed that the addition of small amounts of a bakery yeast suspension made possible the rearing of flies throughout the year. Fresh horse manure was found to be deficient in this respect during the winter months of the year. Grady (1928)[4] developed the technique of rearing large numbers of flies for daily insecticidal tests, and by the use of Glaser's method he was able to rear flies continuously throughout the year. Hockenyos (1931)[5] found that by the addition of hog manure to horse manure (1 part of the former to 3 or 4 parts of the latter) with yeast as a supplemental food, he could produce a more satisfactory medium for breeding houseflies than with horse manure alone. More nutriment per unit for the larvae was apparently provided by this mixture.

The use of horse manure, or mixtures of it with hog manure, as a rearing medium has several distinct disadvantages. For one, it is not always available to most laboratories. Then, too, it is rather disagreeable to handle. Most important, however, is the fact that frequently a species of red mite, parasitic on the housefly, is brought in on horse manure. This mite attacks the adult housefly on the underside of the thorax and abdomen between the segments, and also between the coxae of the legs, as well as at the place of attachment of the wings. Five to 40 or more mites are sometimes found on one housefly, and of course the presence of these mites precludes the use of such

1 *Jour. Exp. Zool.*, 38, 383–412, 1923.
2 *Jour. Econ. Ent.*, 17, 486–96, 1924.
3 *Jour. Econ. Ent.*, 20, 432–33, 1927.
4 *Jour. Econ. Ent.*, 21, 598–604, 1928.
5 *Jour. Econ. Ent.*, 24, 717–725, 1931.

parasitized flies for insecticidal tests. Once this mite is established, it is exceedingly difficult to eradicate. Thorough treatment with steam of all apparatus and media is necessary, and one is forced to start entirely new cultures.

In studies made for the rearing of large numbers of houseflies continuously throughout the year, it was found that a wheat bran-alfalfa meal mixture, supplemented with small amounts of yeast and diamalt (a commercial product composed of a large percentage of malt sugar) provided a very satisfactory larval medium. The materials that make up this rearing medium are readily available to any laboratory. They are clean and easy to handle. Most important, no parasitic mites are present on them. Furthermore, it appeared that this medium contained more nutriment per unit than a horse manure medium, and with it, larger numbers of flies could be reared. The formula which was found most satisfactory is given as follows:

Wheat bran	3½ lbs.	Mix thoroughly
Alfalfa meal	1¾ lbs.	
Water	5000 cc	Mix thoroughly together
Yeast suspension (prepared from 1 lb. baker's yeast to 2 liters of water. This stock suspension should be kept on ice and used as needed)	300 cc	
Diamalt (a product of the Fleischmann Yeast Co., containing a large percentage of malt sugar)	25 cc	

Add the water mixture to the bran mixture and stir thoroughly. The housefly eggs may be added immediately. The quantity of yeast suspension necessary can probably be lessened considerably by preparing the entire mixture one day before use, so that the yeast cells will have time to multiply. Flies are attracted to and readily oviposit on this medium and small amounts of it can be used in the breeding cages for this purpose. The housefly eggs can then be added to the larval mixture, and the jars containing it can be incubated at 90° F. Fresh diluted milk appeared to be a satisfactory food for the adult houseflies.

With this larval medium houseflies in large numbers have been successfully reared at Ames, Iowa, throughout the year. During the winter of 1931 and 1932, F. L. Campbell and Sullivan, of the U. S. Bureau of Entomology at Washington, D. C., have used it with very good results.

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SPECIAL ARTICLES

NOTE ON THE GROUP SPECIFIC SUBSTANCE OF HORSE SALIVA

IN previous work on the so-called Forssman hapten, active fractions were prepared from alcoholic extracts of horse kidney which were richer in carbohydrate than the known cerebrosides, thus suggesting that the specificity of the substance may be due to carbohydrate groupings[1] (not improbably in a combination with lipoids). Evidence pointing in the same direction was afforded by investigations[2] on the Forssman substance of bacilli which behaves similarly to, and could not be separated from the precipitable substance consisting largely of carbohydrates. Closely related to these investigations are those on the human group substance A whose relationship to the Forssman substance has been demonstrated by Schiff.[3] From human serum of group A individuals, Brahn and Schiff[4] obtained a serologically active preparation, which on hydrolysis yielded sugar, and quite recently Freudenberg, Eichel and Dirscherl[5] reported briefly on an active substance from the same source, of an elementary composition resembling that of a polysaccharide, and strongly reducing Fehling's solution after hydrolysis.

Continuing the studies quoted above, horse saliva was investigated, which, as Schiff[4] has shown, reacts with immune sera specific for the human group substance A. In these preliminary experiments an active

1 Landsteiner and Levene, *Jour. Immunol.*, 14, 81.

2 Brahn and Schiff, *Deut. Med. Wschr.*, 1930, p. 1207; Kurt Meyer, *Ztschr. Immunitätsf.*, 68, 69; Landsteiner and Levine, *Jour. Immunol.*, 22, 75.

3 *Kl. Wschr.*, 1924, 679; Schiff and Adelsberger, *Ztschr. Immunitätsf.*, 40, 335.

4 "Ueber die gruppenspezifischen Substanzen," etc., Jena, G. Fischer, 1931, p. 102. Another substance reacting with anti A immune sera and rich in carbohydrate was found by these authors in commercial pepsin (*Kl. Wschr.*, 1932, p. 1592).

5 *Naturwiss.*, 1932, p. 658.