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Water loss in *Periplaneta americana* (L.) with the application of certain insecticides

(Blattariae: Blattidae)

With 1 textfigure

So far, very little work has been done on this aspect of the mode of action of insecticides, which needs further investigation. Ingram (1955), has reported that the insects treated with pyrethrum loose greater weight than the controls, and that, this loss in weight is due to the loss of body water. Wigglesworth (1941) reported that during first day, the loss of weight in the Pyrethrum treated insects is sometimes rather greater than in the normal. He further reported that, this is due to the spasmodic muscular contractions during the early stages of poisoning. The „spasmodic muscular contractions“ has been interpreted by Ingram (1955), as the hyperactivity. Buck & Keister (1949), concluded with DDT treated Blowflies (*Phormia regina* Mg.), that DDT and dry air enhances water loss, and, DDT enhances loss of both metabolic and reserve water. They also concluded that the loss of water is not the primary cause of death.

In the series of present experiments, we have made an attempt to determine the amount of water loss per gram of the body weight in the insecticide treated insects under both hyperactive and seized hyperactive conditions. The insecticides were applied in decisive lethal doses, so as to give a cent percent knockdown within an hour, and the insects passed in to the moribund condition directly from this stage. This was done to observe the maximum amount of water loss in insects in the first two hours. Actually, in such an acute poisoning, the first two hours are the most vital period, as the insects reach the moribund condition within this time.

Material and Methods

Male and female cockroaches (*Periplaneta americana* [L.]) were used in equal numbers in these experiments and were anesthesized with CO_2. The insecticides were applied topically on the pronotum with the help of a micro-applicator in known quantities. Air, dried by passing through the calcium chloride tubes with the help of a filter pump, and then passed over the cockroaches in order to absorb any water droplets or wetness from the surface of the body and the same air was again passed through another calcium chloride tube in order to collect the water. Water droplets could be seen adhering to the inside surface of the calcium chloride tube. This procedure left the cockroaches almost dry and thus enabled

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us to obtain the correct weight after experimentation. The treated and control cockroaches were weighed before and after running the experiment for two hours,

To study the hyperactivity as a cause of weight loss, following experiment was devised, to find out, whether weight loss in the insecticide treated insect was mainly due to the (hyperactivity) spasmodic muscular contractions or it was independent of the increased activity. Cockroaches anesthesized by CO_2 were treated and were run for two hours in an ice-bath container, the temperature of which was regulated at 1 °C to 2 °C; and air pre-cooled to 1 °C was passed through the calicum chloride. This cooling process inhibited the hyperactivity and the normal body activity was reduced.

Observations

Average body water loss of the various groups of exeprimental and control insects have been arranged in a tabular form. From the table it is clear that in all the cases, insects treated with any of the insecticides loose a greater amount of water, than those run under normal and controls conditions. In case of normal and control experiments, the amount of water loss was almost the same, except the slight variation. In certain cases the treated cockroaches did not show the droplets on the body surface, but definitely gave the wet appearance. The degree of wetness differs from insecticide to insecticide, and, is in direct relation with the amount of weight loss per gram of the body weight. This is contrary to the normal and control experiments, wherein, we do not find any visible presence of droplets or the wetness on the surface of the roaches. To study the hyperactivity causing

Table 1
Detail of treatments and water losses in *Periplaneta americana* (L.)

Treatment	Water loss per gram body wt.	Average percentage of water loss as calculated from the total body water	Average percentage of water loss as calculated from the body weight	S. E. ± of the percentage of water loss	Dose of insecticides in r/gm
Normal	0.0126	2.0	1.26	0.246	—
Control	0.0130	2.17	1.30	0.208	—
*Control	nil	—	nil	—	—
Aldrin	0.0200	3.33	2.00	0.290	151.4
*Aldrin	0.0185	3.08	1.85	—	151.5
Dieldrin	0.0210	3.50	2.10	0.206	143.8
Sevin	0.0240	4.00	2.40	0.197	115.1
Lindane	0.0250	4.16	2.50	0.285	143.9
Endrin	0.0260	4.33	2.60	0.301	144.4
p-p. DDT	0.0304	5.06	3.04	0.297	185.4
Methoxychlor	0.0320	5.33	3.20	0.320	138.2
Phosdrin	0.0334	5.56	3.34	0.370	160.9
**Dimecron	0.0390	6.50	3.39	0.356	138.9
Nuvan	0.0401	6.68	4.01	0.280	134.0
Paration	0.0446	7.43	4.46	0.299	109.0
Allethrin	0.0504	8.40	5.04	0.307	116.2
*Allethrin	0.0332	5.53	3.32	—	116.2

Temperature 20 °C. Each experiment run five times with 3 male and 3 female adult cockroaches.

* After arresting the hyperactivity and general body activity at 1 to 2 °C.

** Systemic insecticide, used tropically.

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weight loss in insects, as has been reported by WIGGLESWORTH (1941), the hyperactivity and the normal body activity were inhibited by cooling the cockroaches; and then the water loss percentage was calculated. It was found that though the percentage of water loss was reduced under inhibited conditions, yet it is very high when compared to the control experiment, where, it is almost nil. The results have been shown through Table 1 and a histogram (Fig. 1).

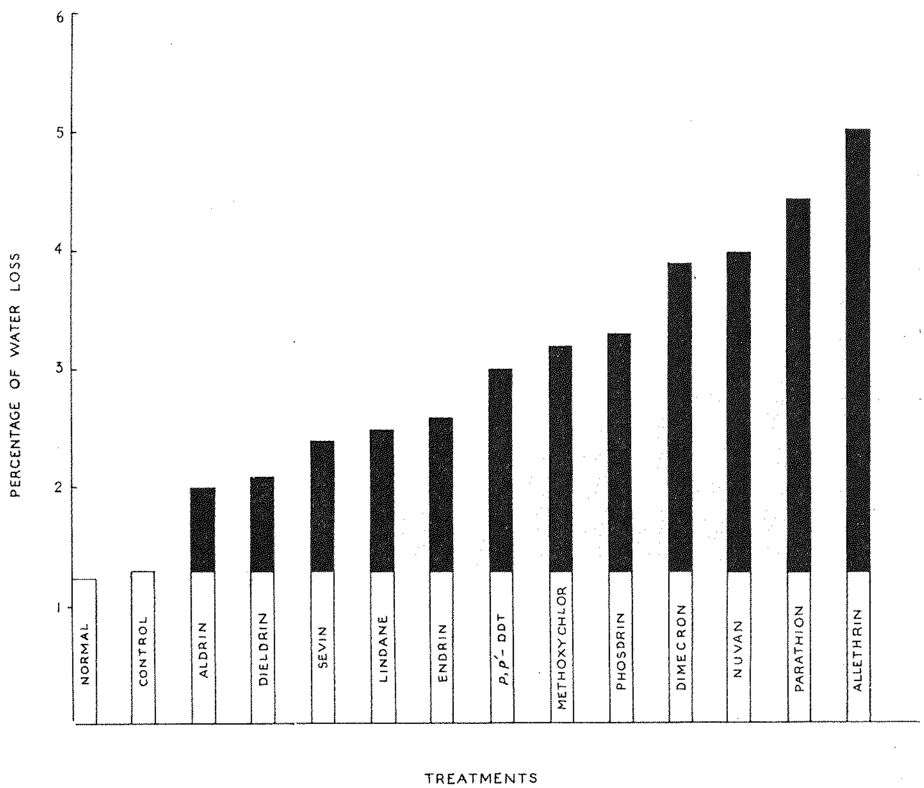

Fig. 1. Histogram showing the comparative water loss percentage after treatment with different insecticides in *Periplaneta americana* (L.)

Discussion

MELLANBY (1935) has reported three possible ways, in which water might be evaporated from an insect's body, exluding excretion. 1. Through the general surface of the body wall; 2. from tracheal system; and 3. partly from body surface, and partly from the tracheal system. He further states that, the rate of evaporation of water from any system in still air is governed by two principal factors. 1. The area of the surface from which the evaporation takes place, and 2. difference between the vapour pressure at the surface and the vapour pressure of the air.

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JORDAN (1927) and GUNN (1933) states that the insect keep their spiracles closed most of the time, only opening them sufficiently to obtain enough O_2, in order to conserve water and that most of the water evaporated is lost through the spiracles. MELLANBY (1934b) concluded that in insects with mechanism for closing the spiracles, the rate of water loss under the mixtures of gases (such as containing 5% CO_2) was 2 to 7 times than that in dry air. In insects which could not close their spiracles, the rate of loss of water was practically the same under all conditions.

DEWITZ (1890) states that the CO_2 passes readily through the chitin, and that the insects get rid of some of that gas through their integument. BUDDENBROCK & ROHR (1923) quoted from MELLANBY (1935) suggests that water vapour may pass from the insect's body in a similar manner. Further experiments by MELLANBY (1934b) supported the view that the integument is nearly water tight, even in the case of insects possessing thin cuticles such as the flea larvae and cloth moth larvae. The water evaporated from their body is lost mainly through the tracheal system. RAMSAY (1935) has shown that at temperatures above 30 °C, the body surface of the cockroach becomes more permeable to water, due to a phase change, probably melting of a layer of some fatty or waxy substance which occurs naturally on the surface of the insect. JORDAN (1927), BUXTON (1932), MELLANBY (1934b), and RAMSAY (1935) believe that water loss in insects takes place through the spiracles, but WIGGLESWORTH (1941) found contrary results, where spiracles remained closed in *Rhodnius* with Pyrethrum poisoning. BEAMENT (1945), WIGGLESWORTH (1945), and RICHARDS (1953) held the view that the water is lost through the integument, but is controlled by the waxy protective layer on the surface of the integument. In these experiments it was observed that the visible droplets or the wetness as the case may be, depending on the insecticide, are found all over the body excluding; wings, antenna, and legs, and are not localized to the areas of the treatment, which suggests that the water is lost through the general body surface, as well as through the other openings, e. g., mouth, anus, respiratory openings etc., etc.

ROBINSON (1928) has shown that there is some relation between the percentage of water in insect's food, and the percentage of water in its body. He also finds that those insects containing a low percentage of water in their bodies have a high percentage of ,,bound water". But MELLANBY (1935) gave contradictory views, that the surface area, together with the dryness of the atmosphere governs the rate of loss of water, the total amount of water present has no effect. As long as there is any water available for evaporation the rate at which that water is lost will remain unaffected, whether, there is much or little solid matter or much or little bound water.

MELLANBY (1932c) reported that the bedbug loses water more rapidly in dry air than in moist, but, in proportion to the saturation deficiency, the rate of loss is greatest in air which is 90% saturated. BUXTON (1931, 1932) has hypothesized that the rate of evaporation of water from insects is usually governed by the saturation deficiency of the air. Nevertheless, the hypothesis has been questioned

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by a number of workers, JANISCH & GHABN (1933) and MAERCKS (1933) who states that the water loss is frequently governed by relative humidity.

BUXTON (1930), GUNN (1933), and MELLANBY (1932b, 1932c, 1934a) reported that at one temperature and several humidities, the rate of loss of water in most cases is proportional to the saturation deficiency. MELLANBY (1935) stated that, whenever the internal temperature of an insect's body is different from that of the surrounding air, the evaporation of water will be affected. Insect's body temperature may be raised by the absorption of radiation (BUXTON, 1924) or by the effects of muscular energy (BACHMETJEW, 1901) and cooled by evaporation of water (NECHELES, 1924). But, when after arresting the hyperactivity the treated and non-treated insects were run at 1 °C, it was observed that, the water loss is almost absent in the non-treated insects and is significantly high in the treated insects (see Table 1). The body is already cool in the treated and non-treated roaches and, hence, there is no loss of water in the non-treated insects. But, the loss of water in the treated insects suggests, that this loss of water through evaporation has not been carried out in order to cool the body, but is the direct action of insectidices. Further, the reduced percentage of water loss has been attributed to the reduced efficacy of insecticides under chilled conditions or, perhaps due to the want of hyperactivity or both.

GUNN (1933), MELLANBY (1932b, 1932c), and INGRAM (1955) found that metabolism alone caused practically no change in the weight in insects. GUNN (1933), and MELLANBY (1932b, 1932c) concluded that, provided the insects did not excrete, the loss in weight during starvation was all due to loss of water. INGRAM (1955) reported that any change in weight could be interpretated as due almost entirely to loss of water by other routes, especially with treated flies. Present authors are in agreement with the views of GUNN (1933), MELLANBY (1932b, 1932c), and INGRAM (1955) and hence, weight loss has been interpreted as the water loss.

WIGGLESWORTH (1941) reported the loss in weight in the treated *Rhodnius*, and he attributed this to the hyperactivity. But in the present experiments, it was found that after reducing the hyperactivity and the general body activity to almost nil, the Allethrin and Aldrin treated cockroaches loose a significantly high percentage of water from the body (see Table 1), when compared to the controlled cockroaches run under the identical conditions, where it is almost nil. Hence, it has been concluded that hyperactivity can not be, presumably, a cause of water loss in treated insects.

These experiments were conducted to throw some light on the cause of death of insects, but no positive evidence were obtained. It has been concluded that the action of insecticides causes the loss of body water from the surface, which proves that the whole body from within has been affected by the poison. Further, the loss of water alone can not be the only cause of death, and does not rule out the possibility of histochemical changes that are taking place within the insect's body which are also to a very large extent responsible for the tissue degeneration

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and death. However, loss of water helps the insects to die earlier and for the insecticide to act more acutely since it gets concentrated inside the body.

Conclusions

With the above observations we have arrived at the following conclusions.

1. In the above experiments, where the conditions of temperature and saturation deficiency being constant throughout, the authors find that the insecticide treated insects do not follow the ,,saturation deficiency law" of Mellanby (1935). However, it appears that with the treatment of insecticides, a disruption in morphological or physiological or both states sets in, and thus, the rate of water loss is not proportional to the saturation deficiency of air.
2. Different insecticides are supposed to act in different ways and cause different type of physiological reactions, and hence, we find variation in the amount of water loss in treated insects.
3. Weight loss has been interpreted as water loss, and water loss takes place through the general body surface; and is not localized to the areas of treatment.
4. Treated insects behave hyperactive, but this hyperactivity is not the only of water loss.
5. Reduction in the percentage of water loss under the seized hyperactive condition has been attributed perhaps to the reduced efficacy of insecticides under chilled conditions or perhaps may be due to the want of hyperactivity or both.
6. Loss of water, alone can not be the only cause of death, but it certainly enhances the process of death.
7. With the loss of water probably insecticides act more acutely, due to the concentration of body fluids.

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Summary

Water loss in cockroaches was calculated with twelve insecticides, running normal and control experiments. Various details of the treatments and results obtained have been shown through a table and a histogram. It has been concluded that weight loss has been caused by the water loss, and further water loss alone can not be the only cause of death, but it certainly enhances the process of death. Control and treated experiments were run with Allethrin and Aldrin poisoning after arresting the hyperactivity and the general body activity under chilled conditions. The results obtained are contrary to Wigglesworth's views, and it was established that hyperactivity is not the only cause of water loss in treated insects.

Zusammenfassung

Im Verlaufe von normalen Untersuchungen und bei Bekämpfungsexperimenten mit zwölf Insektiziden wurde der Flüssigkeitsverlust bei Schaben festgestellt. Verschiedene Einzelheiten der Behandlung und die erzielten Resultate werden in einer Tabelle und

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einem Histogramm erläutert. Es wird gefolgert, daß der Gewichtsverlust durch den der Flüssigkeit verursacht wird und weiterhin, daß der Flüssigkeitsverlust allein nicht die einzige Ursache für das Absterben sein kann, aber daß er sicherlich den Sterbeprozeß beschleunigt. Die Experimente wurden mit den Giften Allethrin und Aldrin durchgeführt, nachdem die Hyperaktivität und die allgemeine Körpertätigkeit durch Abkühlung gehemmt worden war. Die erzielten Ergebnisse stehen im Gegensatz zu denen von WIGGLESWORTH, indem festgestellt wurde, daß die Hyperaktivität nicht die einzige Ursache des Flüssigkeitsverlustes bei behandelten Insekten darstellt.

Резюме

В ходе обычных исследований и опытов по борьбе с насекомыми при помощи двенадцати инсектицидов была установлена потеря влаги тараканами. Подробности обработки и результаты отражены в таблице и гистограмме. Делается вывод, что потеря веса обусловлена потерей влаги, и что потеря влаги не может быть единственной причиной отмирания, но вероятно, она ускоряет процесс отмирания. Эксперименты проводились с ядами аллетрин и алдрин, после чего гиперактивность и общая деятельность тела сокращались путем охлаждения. Полученные результаты противоречат результатам WIGGLESWORTH, в которых утверждается, что гиперактивность не является единственной причиной потери влажностн у обработанных насекомых.

References

BACHMETJEW, P., Experimentelle entomologische Studien vom physikalisch-chemischen Standpunkt aus. Mit einem Vorwort v. Prof. AUG. WEISMANN. **1**, Temperaturverhältnisse bei Insekten. Leipzig, X & 160 pp.; 1901.

BEAMENT, J. W. L., The cuticular lipoids of insects. Journ. exp. Biol., **21**, 115—131; 1945.

BUCK, J. B. & KEISTER, M. L., Respiration and water loss in adult blowfly, *Phormia regina*, and their relation to physiological action of DDT. Biol. Bull., **97**, 64—81; 1949.

BUXTON, P. A., Heat, moisture, and animal life in deserts. Proc. R. Soc. London, (B) **96**, 123—131; 1924.

—, Evaporation from the meal-worm (*Tenebrio*: Coleoptera) and atmospheric humidity. Proc. R. Soc. London, (B) **106**, 560—577; 1930.

—, The law governing the loss of water from an insect. Proc. ent. Soc. London, (A) **6**, 27—31; 1931.

—, Terrestrial insects and the humidity of the environment. Biol. Rev., **7**, 275—320; 1932.

DAVIS, M. E. & EDNEY, E. B., The evaporation of water from spiders. Journ. exp. Biol., **29**, 571—582; 1952.

DEWITZ, H., Einige Beobachtungen, betreffend das geschlossene Tracheensystem bei Insektenlarven. Zool. Anz., **13**, 500—504; 1890.

EVANS, A. C., Studies on the influence of the environment on the sheep blow-fly *Lucilia sericata*, MEIG. I. The influence of humidity and temperature on the egg. Parasitology, **26**, 366—377; 1934.

GUNN, D. L., The temperature and humidity relations of the cockroach (*Blatta orientalis*). I. Desiccation. Journ. exp. Biol., **10**, 274—285; 1933.

INGRAM, R. L., Water loss from insects treated with Pyrethrum. Ann. ent. Soc. Amer., **48**, 481—485; 1955.

JANISCH, E. & GHABN, A. A. A. E., Über den Einfluß der Luftfeuchtigkeit auf die Entwicklung junger Seidenraupen. Arb. biol. Reichsanst. Berlin-Dahlem, **20**, 245—257; 1933.

JORDAN, H., Die Regulierung der Atmung bei Insekten und Spinnen. Bericht über eine in niederländischer Sprache erschienene Dissertation von E. H. HAZELHOFF. Ztschr. vergl. Physiol., **5**, 179—190; 1927.

DOI: 10.21248/contrib.entomol.14.3-4.525-532

Maercks, H., Der Einfluß von Temperatur und Luftfeuchtigkeit auf die Embryonalentwicklung der Mehlmottenschlupfwespe *Habrobracon jaglandis* Ashmead. Arb. biol. Reichsanst. Berlin-Dahlem, **20**, 347–390; 1933.

Mellanby, K., The influence of atmospheric humidity on the thermal death point of a number of insects. Journ. exp. Biol., **9**, 222–232; 1932a.

—, The effect of atmospheric humidity on the metabolism of the fasting mealworm (*Tenebrio molitor* L., Coleoptera). Proc. R. Soc. London, (B) **111**, 376–390; 1932b.

—, Effects of temperature and humidity on the metabolism of fasting bed-bug (*Cimex lectularius*), Hemiptera. Parasitology, **24**, 419–428; 1932c.

—, The influence of temperature and humidity on pupation of *Xenopsylla cheopis*. Bull. ent. Res., **24**, 197–202; 1933.

—, Effects of temperature and humidity on the clothes moth larva, *Tineola biselliella* Hum. (Lepidoptera). Ann. appl. Biol., **21**, 476–482; 1934a.

—, The site of loss of water from insects. Proc. R. Soc. London, (B) **116**, 139–149; 1934b.

—, The evaporation of water from insects. Biol. Rev., **10**, 317–333; 1935.

Necheles, H., Über Wärmeregulation bei wechselwarmen Tieren. Ein Beitrag zur vergleichenden Physiologie der Wärmeregulation, Arch. ges. Physiol. Berlin, **204**, 72–86; 1924.

Pierce, W. D., A new interpretation of the relationships of temperature and humidity to insect development. Journ. Agric. Res. Washington, **5**, 1183–1191; 1916.

Ramsay, J. A., The evaporation of water from cockroach. Journ. exp. Biol., **12**, 373–383; 1935.

Richards, A. G., The penetration of substances through the cuticle. In: Roeder, K. D., Insect Physiology. New York, p. 42–54; 1953.

Robinson, W., Water conservation in Insect. Journ. econ. Ent., **21**, 897–902; 1928.

Wigglesworth, V. B., A theory of tracheal respiration in insects. Proc. R. Soc. London, (B) **106**, 229–250; 1930.

—, The extent of air in the tracheoles of some terrestrial insects. Proc. R. Soc. London, (B) **109**, 354–369; 1931.

—, On the function of so-called „Rectal Glands“ of insects. Quart. Journ. micr. Sci., London, N.S. **75**, 131–150; 1932.

—, Permeability of insect cuticle, Nature, **147**, 116; 1941.

—, Transpiration through the cuticle of insects. Journ. exp. Biol., **21**, 97–114; 1945.

DOI: 10.21248/contrib.entomol.14.3-4.525-532