Effect of Weight on Mosquito (*Aedes aegypti* L.) Feeding[1]

Although extensive information is available with regard to how mosquitoes feed under various conditions[2], JONES and PILITT[3] recently reported that hungry females when glued to a pin-head and placed on a human arm would not take blood. The present note describes a new method for studying feeding behaviour in mosquitoes and shows that the act of restraint itself does not necessarily prevent feeding.

The Bangkok strain of *Aedes aegypti* (L.) was maintained at 80 °F and 80 ± 5% R.H. 3-day-old fasting females were lightly anaesthetized with nitrogen and small pieces of glass or metal of known weights were glued on to the dorsal surface of the thorax or abdomen, using either a rubber cement (Carter's) or Elmer's 'Glue-All'. After 1 h the females with known weights were placed in a small plastic cage which was inverted on a human arm for 15 min, and the number of females taking blood within this period was noted..

When weights of 1 to 5 mg were placed on the thorax, 8 out of 10 mosquitoes took blood in 15 min, and 2 landed on the arm but never attempted to feed. When 6 to 10 mg weights were placed on the thorax, 1 out of 12 females was able to take blood, whereas the others were unable to stand erect on their legs. Their legs were abnormally spread and the insects either fell to one side or were unable to lift the head into a feeding position. When 11 to 15 mg weights were added to the thorax, the mosquitoes stumbled and fell on their sides when they attempted to walk, and none attempted to feed. When weights were placed on the anterior part of the abdomen, the response of the mosquitoes was strikingly different in that all 10 females with 1 to 5 mg weights fed on blood, and 7 out of 10 females with 6 to 10 mg weights fed normally. With 11 to 15 mg of weights on abdomen, only 1 out of 5 females pierced deeply into the skin, but she did not take blood. In all cases where weighted females took blood, they did so to repletion – to stage 5 on the PILITT and JONES[4] scale of engorgement.

When most mosquitoes take blood, it is normally dispatched to the midgut[5]. To determine if weighting the female would alter this distribution, we dissected 26 weighted females which had taken blood. In most cases, the blood was found only in the midgut, but in 2 mosquitoes (which had 6 to 10 mg weights on the abdomen) blood was also found in the 2 dorsal diverticulae and in the crop.

It is evident from our work that an average female mosquito can pierce the human skin and take a normal amount of blood while bearing on the thorax an external weight which is twice her own weight. When weights are placed on her abdomen, she can feed with an external weight 3 to 5 times her own normal body weight. Possibly, the greater tolerance of weight on the abdomen is due to a shift in the fulcrum of the body. Heavier weights completely prevent orientation of the proboscis to a normal feeding angle and thus make piercing impossible.

Since JONES and PILITT[3] were unable to force-feed pinned mosquitoes, we decided to re-test whether restraint alone would inhibit feeding. With this in mind, we carefully glued one end of a long human hair to the thorax of a series of 20 mosquitoes and the other end into a glass capillary. When offered a human arm, all tethered females quickly landed, probed and pierced the skin and fed on blood to repletion, thus proving that restraint alone does not prevent normal feeding. The technique of glueing a mosquito to a hair allows one to leash the insect, and thus permits a whole variety of studies on its behaviour in general under truly controlled conditions.

Résumé. Les moustiques femelles *Aedes aegypti* L., alourdies par des poids placés sur leur thorax ou leur abdomen, peuvent encore se repaître normalement de sang humain. Sur le thorax, elles tolèrent 2 fois le poids de leur corps et 3 à 5 fois sur l'abdomen. Le fait d'être attachées ne restreint pas leur capacité d'absorber du sang. En expose le moyen de tenir des moustiques en laisse.

J. C. JONES[6] and B. V. MADHUKAR

Department of Entomology, University of Maryland, College Park (Maryland 20742, USA), 5 June 1974.

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Changes in the Concentrations of Elements in Liver After Partial Hepatectomy

The regenerating liver after partial hepatectomy constitutes a convenient model system for the study of growth control. Such studies are facilitated by the fact that the liver has a uniform structure, is composed of relatively few cell types and undergoes regular, controlled regeneration. The following report is concerned with the changes which occur in the concentrations of elements after $^2/_3$ liver resection. Neutron activation analysis was used to detect the elements.

Materials and methods. The experimental animals used were Sprague-Dawley rats (200 g, *Mus rattus*, Brunnthal-München). Animals were maintained in macrolon cages in climatised rooms (21 °C) under normal conditions of light/darkness. Water and food (Altromin® standard diet) were available ad libitum. According to a standard procedure[1], partial hepatectomy was carried out by removing the middle and left lobes of the liver ($^2/_3$ resection).

For determination of elements, the livers, which were removed without using metal instruments, were freeze-dried and irradiated for 1 h ($\varnothing = 2 \times 10^{12}$ $n/cm^{-2}/sec^{-1}$, Triga Mark I Reactor, DKFZ, Heidelberg). Na, K, and Mn could be directly determined by integration of the lines at 1360, 1520 and 845 keV respectively. After a few hours, the samples were treated with H_2O_2/H_2SO_4, and the elements, Na, K, P and Br were removed using a

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rapid process based on chelate extraction[2]. Cu and Zn were determined spectroscopically, from the 511 and 440 keV lines respectively, using a 40 cm³ Ge(Li)-detector. The error in analysis was less than 5%. A second sample was irradiated for 40 h and used for Fe determination and for a second Zn determination.

The incorporation of ^{3}H-thymidine (TdR) was measured by injection each animal i.p. with 100 µCi ^{3}H-TdR (spec. act. 25 Ci/m*M*) 1 h before sacrifice. A portion of each liver was frozen in liquid nitrogen immediately after removal. After thawing, the liver samples were homogenized and aliquots were pipetted on to round filter papers. The acid-insoluble incorporated radioactivity was measured in toluene scintillator using a liquid scintillation counter[3].

Results and discussion. Groups of 4 female rats were killed at various times after $^2/_3$ liver resection ($^1/_2$, 1, $1\,^1/_2$, 2, 3, 4, 5, 7, 10 days). 2 control animals were also killed at each time point. ^{3}H-TdR-incorporation and the concentrations of the elements Na, K, Zn, Cu, Fe, and Mn were determined as described under methods. The average values obtained are shown in Figure 1. ^{3}H-TdR incorporation follows the known pattern[3]: the maximum in incorporation (DNA synthesis) is reached after 24 h, and after 5 days the incorporation has returned to control values. The concentrations of the elements Na, K, Zn and Cu were also increased after partial hepatectomy, the maximum increases being 15% for Na, 20% for K and 100% for Zn and Cu. The elements Fe and Mn showed decreases in concentration after partial hepatectomy of 40% and 30% respectively. The differences observed between treated and control rats (without taking account of the time factor) and the differences in

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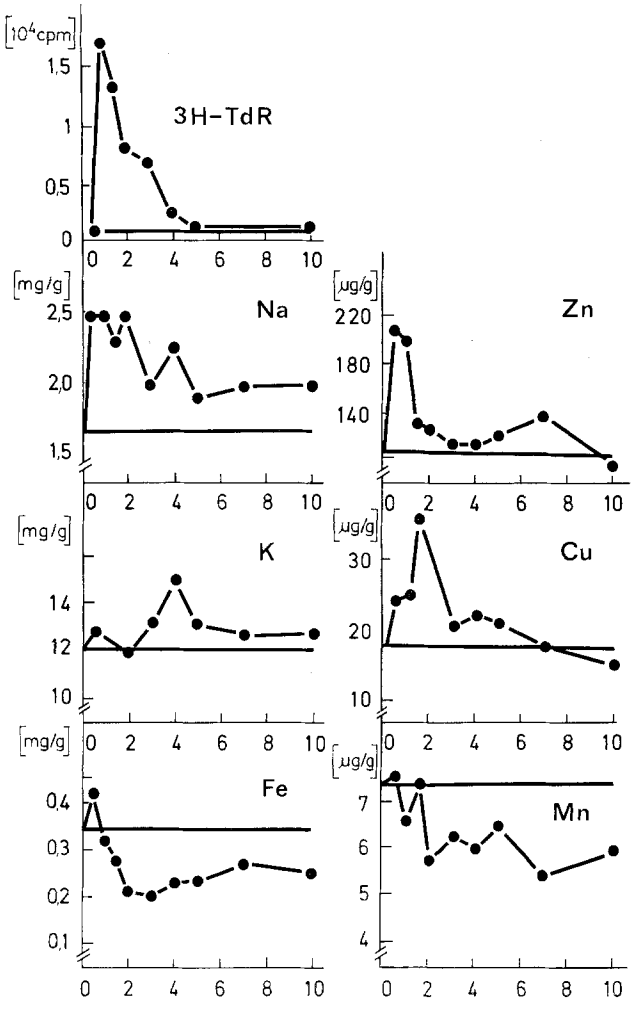

Fig. 1. ^{3}H-thymidine-(TdR)-incorporation (per 100 mg wet weight) and element concentrations in partially hepatectomized (curves) and untreated (straight lines) rats (Sprague-Dawley, 200 g, ♀). Average values from 4 or 2 animals. Abscissa: days after partial hepatectomy. Statistical analysis: Differences between partially hepatectomized and untreated animals (variance analysis): $K^{n.s.}$, Na^{+++}, Zn^{+}, Cu^{+}, Fe^{+}. Time-response in partially hepatectomized rats (variance analysis): K^{+}, Na^{+}, Zn^{+++}, Mn^{+}, Cu^{++}, Fe^{+}. Degree of significance: +++ > 99.9%; ++ > 99%; + > 95%.

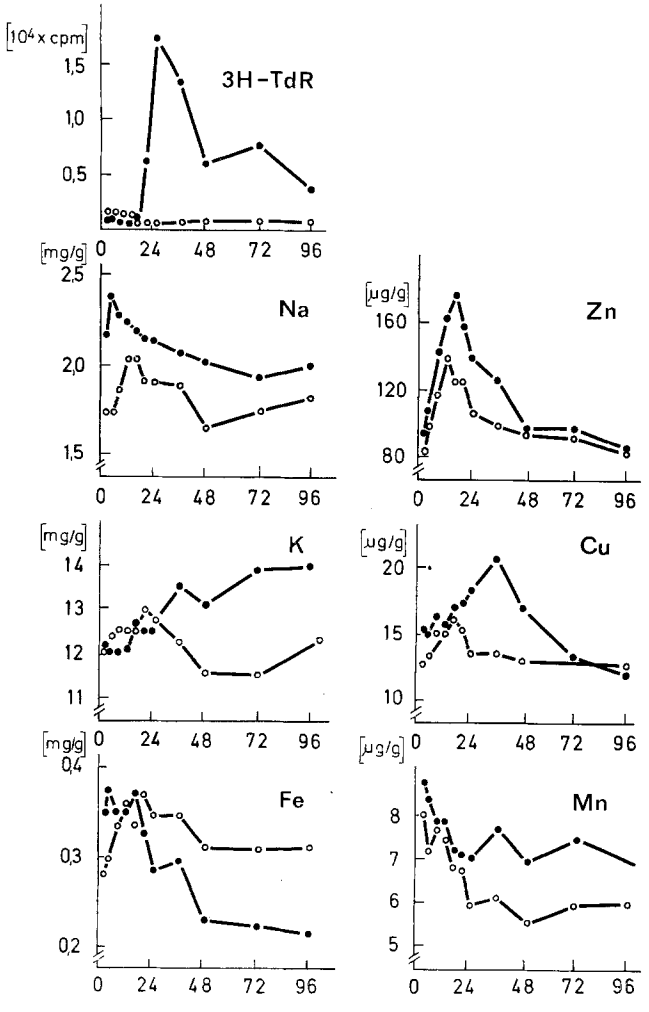

Fig. 2. ^{3}H-thymidine (TdR)-incorporation (per 100 mg wet weight) and element concentrations (mg/g or µg/g dry weight) in partially hepatectomized (•—•) or sham operated (○—○) Sprague-Dawley rats (200 g, ♂). Average values from 5 animals. Abscissa: h after operation. Statistical analysis: Differences between partially hepatectomized and sham operated animals (two-way variance analysis). Na^{+++}, K^{++}, Zn^{+++}, Mn^{+++}, Cu^{+++}, Fe^{++}. Time response: Na^{+++}, $K^{n.s.}$, Zn^{+++}, Mn^{+++}, Cu^{+++}, Fe^{+++}. Differences in time response between partially hepatectomized and laparotomized animals: Na^{++}, K^{+++}, Zn^{+++}, $M^{n.s.}$, Cu^{++}, Fe^{++}. Degree of significance: see Figure 1.

the temporal response observed with partially hepatectomized rats have been verified statistically by variance analysis.

A further experiment was carried out in order to answer the following questions: 1. Are the changes in element concentrations specific for liver regeneration or are they simply a result of the operation itself? 2. Can the very rapid changes in Na and Zn concentration observed after partial hepatectomy be correlated with the increase in DNA-synthesis (^{3}H-TdR incorporation)? 3. Are the changes observed the same for male and female animals?

110 male rats were randomly distributed into 2 groups, of which one was partially hepatectomized and the other sham operated (laparotomized). 2, 4, 8, 12, 16, 20, 24, 36, 48, 72 and 96 h after the operation, 5 rats from each group were killed and the ^{3}H-TdR incorporation and the concentrations of elements were determined. The average values obtained are shown in Figure 2. ^{3}H-TdR incorporation increases sharply after 16 h in partially hepatectomized rats, reaching its maximum around 24 h. No increase in incorporation was observed with laparotomized rats. The earliest change in element concentrations in partially hepatectomized rats is observed for Na, which reaches a maximum as rapidly as 4 h after the operation. The increase in Zn concentration also precedes the onset of DNA-synthesis. In both cases, the increases observed in partially hepatectomized rats are appreciably higher than in the corresponding laparotomized rats. For this reason, the elevated Na and Zn concentrations can only be partially attributed to a non-specific effect of the operation. In particular, the very early increase in Na concentration after partial hepatectomy could be of importance for liver regeneration. This early increase has also been reported by other authors[4].

A clear increase in K concentration between the 2nd and 4th days was observed only in partially hepatectomized animals. In addition, the large increase in Cu concentration after 36 h is only seen in hepatectomized rats. Shamoperated animals showed only a small increase in Cu concentration during the first day after the operation. The increase in copper concentration is temporally correlated with the increase in ^{3}H-TdR incorporation and therefore appears to be related to liver regeneration. It is noteworthy that in earlier investigations on various transplanted tumours, we demonstrated that changes in Cu concentration in blood plasma could be correlated with ^{3}H-TdR incorporation in the tumours[5,6]. The concentration of Fe in partially hepatectomized rats shows a clear decrease after 2 days. A similar decrease is observed for Mn. However, in laparotomized rats, this effect is even more pronounced. This observation can probably be explained by the overlapping of 2 different effects. During liver regeneration it could be expected that the Mn concentration is increased. (The microbodies were more numerous in regenerating liver – and Mn is located in the microbodies[7,8]). However, owing to the strong effect of the operation (see Figure 2), a small decrease in Mn concentration is observed rather than the expected increase.

For the elements Mo, Co, Rb, Se, As, Mg and Cs, no significant differences were observed which could be related specifically to partial hepatectomy. In addition, no fundamental differences in element concentrations could be detected between male and female rats.

Zusammenfassung. Während der Leberregeneration nach einer Teilhepatektomie lassen sich mit der Neutronenaktivierungsanalyse unspezifische und spezifische Erhöhungen bzw. Erniedrigungen der untersuchten Elemente nachweisen. Spezifische Erhöhungen finden sich bei Cu, Na, K, Zn, eine spezifische Erniedrigung bei Fe.

M. Volm, J. Schumacher, K. Wayss and H. Wesch

Deutsches Krebsforschungszentrum, Institut für experimentelle Pathologie und Institut für Nuklearmedizin, Kirschnerstrasse 6, D–6900 Heidelberg (Federal Republic of Germany), 15 July 1974.

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Leukocytes and Prostaglandins in Acute Inflammation

Since the importance of prostaglandins (PG's) as mediators in acute inflammation was described[1–3], the source of these substances has remained a matter of speculation. Some have proposed the leukocytes as the cells responsible for the release of the inflammatory PG's (PGE_1, PGE_2 and $PGF_{2\alpha}$) because both these cells and the PG's appear early in the inflammatory tissue, and therefore inflammatory exudates rich in leukocytes, mainly polymorphonuclear (PMN), are often also rich in PG's[4–6]. In addition it has been found that suspensions of PMN-rich peritoneal exudates incubated together with bacteria release PGE_2 and $PGF_{2\alpha}$ in vitro[7]. On the other hand, it has been shown that acute inflammations can be elicited in leukopenic animals, and that therefore the release (if any) from these cells of PG's may not be relevant[8], but – to our knowledge – PG's have not been measured during leukopenic inflammation.

We observed two symptoms of inflammation; nociception and temperature-rise, to appear before PMN or even monocytes can be seen at the site of inflammation in the avian microcrystal arthritis[9]. Therefore we reasoned that

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