exopodites of the pleopods. There are large numbers of pores on the sterna of the abdomen, particularly around the bases of the pleopods and uropods. It is interesting to note that all the pores face anteriorly – facing the openings of the two oviducts. This siting of the pores is particularly significant when considering the arched condition of the abdomen during spawning – forming the egg chamber. The openings of the glair glands are quite characteristic and differ from the pores of the integumental glands, which never occur as groups. A hand section through a pore region, viewed under the scanner, shows clearly, well developed ducts leading into the glair glands (Figure 1g). Transverse sections through the pore region (Figure 2a) shows that the ducts emerging from the glair glands merge to form a roughly spherical chamber within the integument and it is in these chambers that glair is stored until exuded. The ducts ramify through the entire gland (Figure 2b), leading finally into the integumentary chambers. It is this mass of glair, in the large numbers of chambers within the ventral integument, that gives the creamy colour to the female abdomen in September. Glair glands and pores first appear in the females of *A. pallipes* in the second September of their lives. Pores appear in the integument after the final moult before spawning. So these pores and glands could be looked upon as belatedly appearing secondary sexual characteristics. Also developed early in the life of the female are the oosetae, setae specialized for egg attachment. These oosetae (Figures 2c and d) are found on the pleopods and sterna of females near the glair glands and their openings, increasing in number as the crayfishes grow larger.

Proximally, the oosetae are smooth (Figure 2e), with a pronounced groove in the shaft; it may be that part of the glair moves up the shaft to play some role in the attachment of the eggs. Distally the oosetae are flat in section, bearing very fine setules (Figure 2d) and it is these setules which become intimately attached to the eggs. After egg laying, the glands persist until late July becoming inconspicuous following an early August moult, which takes place when the hatchlings have become totally independent. Soon after the glands start developing again in preparation for another spawning.

Zusammenfassung. In der Deckhaut des sexuell gereiften Weibchens von *A. pallipes* treten Porengruppen auf. Diese Poren überlagern die Schleimdrüsen, die während des Laichens grosse Mengen Schleim produzieren. Die Poren und Drüsen befinden sich auf dem Unterleib und den Pleopoden; die Oosetae, die zur Eiablagerung dienen, befinden sich ebenfalls an diesen Stellen. Dies sind sekundäre sexuelle Charakteristika, die eng mit dem Legen und der Ablage der Eier verbunden sind.

W. J. Thomas and E. Crawley[2]

Department of Biological Sciences, University of London, Goldsmith's College, New Cross, London SE14 6 NW (England), and Zoology Department, University College, Gower Street, London (England), 16 September 1974.

[2] Acknowledgments. To the Central Research Fund of the University of London who provided the financial assistance for this research work.

The Effect of Dietary Vitamin E on Glutathione-Induced Liver Mitochondrial Swelling from Mice Treated with 3'-4-Methyl-4-Dimethylaminoazobenzene

The action of Vitamin E as an inhibitor of chemical carcinogenesis has been under dispute for several years, with evidence appearing both supporting and opposing the claim[1–3]. These reports suggest that lipid peroxidation results from the stresses of chemical carcinogens and that vitamin E as a lipid anti-oxidant might confer protection. Vitamin E has also been shown to reduce the depressing effects of the carcinogen urethane on mitochondrial respiration[4] and to catalyze drug detoxification[5].

Studies of the early stages of carcinogenesis as induced by 3'-methyl-4-dimethylaminoazobenzene (3-Me-DAB) considered the large amplitude swelling and contraction of rat liver mitochondria[6,7]. The degree of swelling and contraction decreased markedly after 4 weeks of carcinogen feeding, after which time volume changes returned to near normal levels. Further, it was found that if the carcinogen was fed for up to 4 weeks and then discontinued, the incidence of tumours after a 7-month incubation period was low. However, if the 3-Me-DAB was fed for 5 weeks and then discontinued, the later incidence of tumours rose sharply. Therefore the 4-week swelling and contraction minimum is correlated with the irreversible induction of cancer after the 5-week feeding period.

Vitamin E is accumulated by mitochondrial membranes[8] and affects mitochondrial functions[9]; however, there is no information concerning the large amplitude swelling of liver mitochondria from Vitamin E-deficient animals. Various substances influence swelling and contraction of isolated liver mitochondria; glutathione is of particular interest in view of recent involvement of vitamin E and the glutathione peroxidase system associated with the mitochondria[10].

Methods and materials. 1. Care and feeding of animals. Male albino mice (Canadian Breeding Farms and Laboratories Ltd.) at the weaning stage were divided into 2 groups and fed ad libitum a low vitamin E diet (Nutritional Biochemical Co.), or the same diet with vitamin E supplemented in the amount of 200 units DL-α-tocopherol acetate per kg food. This feeding regime was continued for 7 weeks after which time vitamin E deficiency was determined with a hydrogen peroxide red blood cell

[1] K. Narayan, Experientia *26*, 840 (1970).
[2] R. W. Swick and C. A. Bauman, Cancer Res. *11*, 948 (1951).
[3] D. Harman, Cancer Res. *17*, 125 (1969).
[4] A. Smith, R. Roy, C. Scott and I. Waheed, Experientia *29*, 601 (1973).
[5] M. Carpenter, Ann. N.Y. Acad. Sci. *203*, 81 (1972).
[6] J. C. Arcos, J. B. Mathison, M. J. Tison and A. M. Mouledoux, Cancer Res. *29*, 1288 (1969).
[7] J. C. Arcos, G. W. Griffith and R. W. Cunningham, J. Biophys. Biochem. Cytol. *7*, 49 (1960).
[8] J. Lucy, Ann. N.Y. Acad. Sci. *203*, 4 (1972).
[9] K. Schwarlz, Ann. N.Y. Acad. Sci. *203*, 45 (1972).
[10] C. K. Chow and A. L. Tappel, Lipids *7*, 518 (1972).

haemolysis test[11]. From the 8th to 20th week 3-Me-DAB was added to the diets of half the animals in both the vitamin E-deficient and supplemented groups yielding 0.06% 3-Me-DAB (w/w).

2. Mitochondrial swelling and contraction. Percentage liver mitochondrial swelling and contraction was determined, as described elsewhere[6]. Mitochondria were prepared at various times following initiation of 3-Me-DAB feeding over a 90-day time course.

Results and discussion. No distinct swelling minimum was observed in mice on the 3-Me-DAB diet as was previously reported by ARCOS[6] in rats. There was, however, a slight decrease in the swelling of mitochondria from 3-Me-DAB mice in comparison to their respective controls (Table I). It has been shown that 3-Me-DAB interferes with the biochemical integrity of mouse liver mitochondria in vitro[12]; however, one can reconcile this apparent lack of effect of the carcinogen on mitochondrial swelling. It has been found that 3-Me-DAB acts by binding to specific sites on the rat liver mitochondria[6,13], and these sites may be fewer or not of suitable conformation. It is also possible that a swelling minimum occurs after the 90-day sampling time[6].

The 3-Me-DAB-fed mice displayed slightly decreased swelling ($p < 0.10$). There was no significant interaction between vitamin E and 3-Me-DAB treatments as indicated by two-way analysis of variance.

The most dramatic difference in mitochondrial swelling was observed between mice on vitamin E-deficient diets and their counterparts on vitamin E-supplemented diets. Glutathione-induced swelling of mitochondria was greatly reduced in the vitamin E-deficient mice. As swelling is a membrane-linked function[14], the mitochondrial membrane was undoubtedly the site affected by vitamin E deficiency. Outer and inner membranes isolated from the liver mitochondria of ducks maintained on a vitamin E-deficient diet were found to have a critical loss of unsaturated fatty acids resulting in specific structural damage[15]. Another study showed that liver homogenates from vitamin E-deficient rats had a lipid peroxidation level 4.5 times greater than normal[16]. The antioxidant function of vitamin E in the prevention of lipid peroxidation would appear to be involved in the previous study. Mitochondria of rats on vitamin E-deficient diets have also been found to be hypertrophied[17] and to have herniations of the outer membranes[18]. Vitamin E deficiency in rats has also been shown to cause loss of respiratory control in liver mitochondria which was attributed to changes in membrane structure[19].

No significant differences in mitochondrial contraction were observed (Table II). Other studies have shown that mitochondria swollen with reduced glutathione failed to contract upon the addition of ATP[20]. This effect was traced to the detachment of a protein from the mitochondria by the glutathione. Contracting mitochondria in our study were probably influenced by this phenomenon, resulting in the masking of any dietary effects on degree of contraction.

In summary, no discrete swelling minimum occurred in glutathione-induced mitochondrial swelling in mice fed 3-Me-DAB for periods up to 12 weeks in contrast to rats which display a swelling minimum at 4 weeks under similar conditions. Vitamin E status has little effect on the slight swelling decrease caused by 3-Me-DAB, and vitamin E deficiency caused a marked decrease in the ability of liver mitochondria to swell in the presence of glutathione.

Résumé. On a mesuré l'effet de la vitamine E sur l'induction du gonflement causé par le glutathion sur les mitochondries isolées du foie de souris qui avaient été nourries de 3-Me-DAB pendant 12 semaines. Le 3-Me-DAB n'a pas influencé le gonflement des mitochondries; l'absence de vitamine E a produit une réduction du gonflement.

Table I. Percentage swelling of mouse liver mitochondria in the presence of 7×10^{-3} *M* glutathione

	Vitamin E supplemented	Vitamin E supplemented with 3-Me-DAB	Vitamin E deficient	Vitamin E deficient with 3-Me-DAB
N	6	13	7	13
$\bar{X}$	81.6%	77.5%	72.5%	71.1%
S.D.	3.14%	3.39%	4.30%	4.41%

Two-Way analysis of variance: F(Vit. E) = 30.7, $p < 0.005$; F(3-Me-DAB) = 3.15, $p < 0.10$; F(interaction) = 0.90, $p > 0.25$.

Table II. Percentage contraction of swollen mouse liver mitochondria in the presence of 5×10^{-3} *M* ATP and 3×10^{-3} *M* $MgCl_2$

	Vitamin E supplemented	Vitamin E supplemented with 3-Me-DAB	Vitamin E deficient	Vitamin E deficient with 3-Me-DAB
N	6	13	7	13
$\bar{X}$	45.7%	48.3%	48.4%	48.6%
S.D.	2.01%	3.44%	3.72%	2.95%

Two-Way analysis of variance: no significant differences.

J. A. BLOCK and R. M. ROY

Department of Biological Sciences, Sir George Williams University, 1455 de Maisonneuve Boulevard West, Montreal 107 (Quebec H3G 1M8, Canada), 19 August 1974.

[11] C. S. ROSE and T. GYORGY, Am. J. of Physiol. *168*, 414 (1952).
[12] P. EMMELOT, Z. Krebsforsch *64*, 35 (1960).
[13] T. YAMADA, M. MATSUMOTO and H. TERAYAMA, Exp. Cell Res. *29*, 153 (1963).
[14] D. GREEN, *Energy and the Mitochondria* (Academic Press, New York 1970).
[15] J. VOS, I. MOLENAAR, M. SEARLE-VAN LEEUWAN and F. A. HOMMES, Acta Agric. Scand. Suppl. *19*, 192 (1973).
[16] W. DJACZENKO, L. CERULLI, P. CIMMINO and E. GARACI, Riv. Biol., Perugia *62*, 463 (1969).
[17] U. REIDE, Beitr. Path. *145*, 24 (1971).
[19] A. WAKIZAKA and I. YOH, J. Vitam. *17*, 32 (1971).
[19] Y. Y. YEH and R. M. JOHNSON, Arch. Biochem. Biophys. *159*, 821 (1973).
[20] A. LEHNINGER, *The Mitochondrion* (W. A. BENJAMIN, New York 1964).