

thick). The 3 double locules (compartments or cavities) each have 2 rows of seeds (about 10–12 in a row). The brownish-gray pulp is dried to a light fibrous mass. The seeds are light brownish-gray, flattened (15–18 mm long, 10–12 mm broad, and 3–4 mm at edges), and have a depressed area in the center of each side. A detailed characterization of the tissue, as well as the taxonomic status of the plant was described by SWINGLE³, who reported that the species was introduced to the United States through the Division of Plant Exploration and Introduction, Bureau of Plant Industry. SWINGLE also stated that 1000 tons of the green fruits were delivered every year to the drying sheds at Kweilin (Kwangsi Province). The fruits lost much weight in drying and were then carefully packed in boxes and shipped to Canton, where most of the crop was used, but large numbers were also exported to Chinese communities outside China.

Folk medicine valued them as household remedies for colds, sore throats, and minor stomach and intestinal troubles. Memory from the author's own childhood experience that the cooked broth of this fruit tasted both very sweet and bitter prompted the author to investigate the constituents which gives rise to such taste qualities.

The sweet principle can be extracted by water from either the fibrous pulps or from the thin rinds⁴ of Lo Han Kuo. 50% ethanol was also found to be a good extractant. In general, the rinds afforded a more easily purified extract. The pulps or rinds (or both) were suspended with the extractant and stirred in a Waring blender. The suspension was centrifuged to afford a very dark brown extract. Initial fractionation was effected by ultrafiltration through an Amicon PM-10 membrane filter. The concentrated colored filtrate was then passed through a column of Sephadex G-25. The sweet tasting fractions, well separated from the bitter fractions, were found to give little or no UV-absorption by the monitoring spectrophotometer (Uvicord II). These fractions were lyophilized and examined by thin layer chromatography (ethyl acetate: methanol: water, 5:3:1, v/v). A major component was revealed by spraying and heating the plates with 50% H₂SO₄. It had an R_g (relative to glucose) value of 0.67. Stevioside, the sweetener from *Stevia rebaudiana*, under the same conditions, have an R_g value of 1.1, while glycyrrhizin (the free acid form) had an R_g value of 0.27.

It was later found that if an aqueous extract of the fruit was passed through an Amberlite XAD-2 resin (Rohm and Haas Company), the sweetener was retained by the resin, but easily eluted with 50% ethanol. Both the Sephadex G-25 and Amberlite XAD-2 treated

materials still showed several spots on TLC. The major component at R_g 0.67 could be isolated by preparative layer chromatography for organoleptic evaluation and chemical studies.

The sweetness of Lo Han sweetener was accompanied by a lingering taste described as licorice-like, somewhat similar to that of stevioside, glycyrrhizin, and the dihydrochalcones⁵. The potency was estimated to be about 150 times sweeter than sucrose, but more accurate determination has to be made when a larger quantity of the pure sweetener is available. The sweetener appears to be stable in boiling water for 5 h, as shown by tasting and by TLC.

Preliminary toxicological studies on a lyophilized extract with sweetness intensity equal to that of ammonium glycyrrhizinate showed that the LD₅₀ was in excess of 10 g/kg mice⁶, which is not surprising in view of the common household usage of the dried fruits.

Chemical and spectroscopic studies indicate the sweetener component to be a glycoside of a triterpenoid. Structural elucidation is underway and will be the subject of subsequent communication⁷.

Zusammenfassung. Die getrockneten Früchte, Lo Han Kuo, von *Momordica grosvenori* Swingle, enthalten einen Süss-Stoff, der mit Hilfe von Dünnschicht-Chromatographie abgesondert wurde.

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³ W. T. SWINGLE, J. Arnold Arboretum 22, 198 (1941). I am grateful to Dr. SHIU YING HU of Harvard University for supplying this reference.

⁴ The paper by SWINGLE³ noted the intense sweet taste of Lo Han Kuo pulps, but no mention was made of the taste of its rinds. It is quite noteworthy that the rinds also contain the sweetener.

⁵ R. M. HOROWITZ and B. GENTILI, J. agric. Food Chem. 17, 696 (1969).

⁶ Male albino mice (Charles River Laboratory) weighing 19–24 g were dosed orally with aqueous solutions of the lyophilized extracts at a volume of 0.3 ml/10 g body weight and were kept for observation for 1 week. A very crude extract (without any processing) as well as the Sephadex G-25 treated extract were tested with 10 animals per group. None of the mice died. At a dose of 15 g crude extract per kg mice, the animals exhibited a mild sedation and some diarrhea; however, these effects were transient as all animals appeared normal within 30–60 min. I thank P. HOLMES and A. PRITCHARD for these results.

⁷ The technical assistance of J. WEINBERGER is appreciated.

The Glair Glands and Oosetae of *Austropotamobius pallipes* (Lereboullet)

The origin and function of the glair exuded by the crayfish just prior to egg laying is obscure. In this connection a number of studies have been made of spawning in the European¹, Australian and American crayfishes, but not of *A. pallipes*, the endemic British species.

In mid-September, sexually mature females of *A. pallipes* are conspicuous by the presence of creamy-white patches on the pleura, and sterna of the abdomen, and on the pleopods and uropods, but never on the telson. A closer examination of these cream coloured areas, using the scanning electronmicroscope, discloses the presence of numerous pores in the overlying integument (Figure 1a). These pores are grouped together in roughly circular

patches, presenting the appearance of 'pepper-pot' tops (Figures 1b, c and d). Observations in the field show that it is through these pores that the glair is exuded (Figure 1e), and the cream colouration is due to the very large groups of glair glands underneath the areas of perforated integument. On the pleopod the pores occur in much smaller groups (Figure 1f).

In *A. pallipes* the distribution pattern of the pores is constant; they are very numerous on the anterior faces of the pleura, less so on the anterior faces of the protopodites, and first segments of the endopodites and

¹ Z. MALACZYNSKA-SUCHCITZ, Bull. Soc. scient. Lett., Poznan, 13 B, 39 (1956).

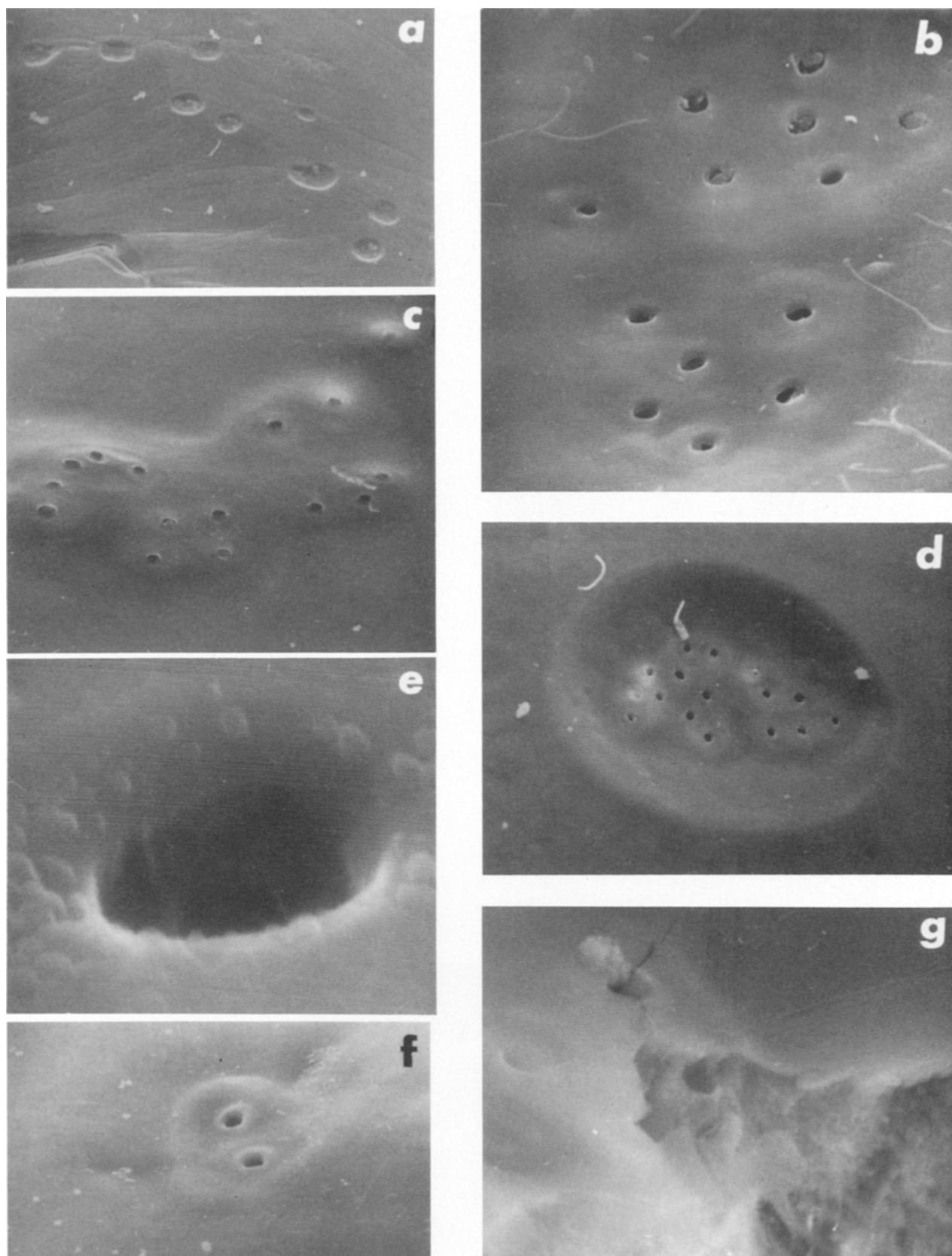


Fig. 1. a) The pleural surface of abdominal segment 3, showing the glair gland pores. $\times 73$. b) A group of pores from the pleuron of segment 4. $\times 2.2K$. c) A group of pores on the sternum of segment 4. $\times 1.08K$. d) Pores in the sternum of segment 3, near the base of a pleopod. e) A single pore of a glair gland on the pleuron. $\times 22K$. f) Typical pore group on the endopodite of a pleopod. $\times 2.15K$. g) Sagittal cut through the integument showing the pore and its attendant duct and gland. $\times 3.05K$.

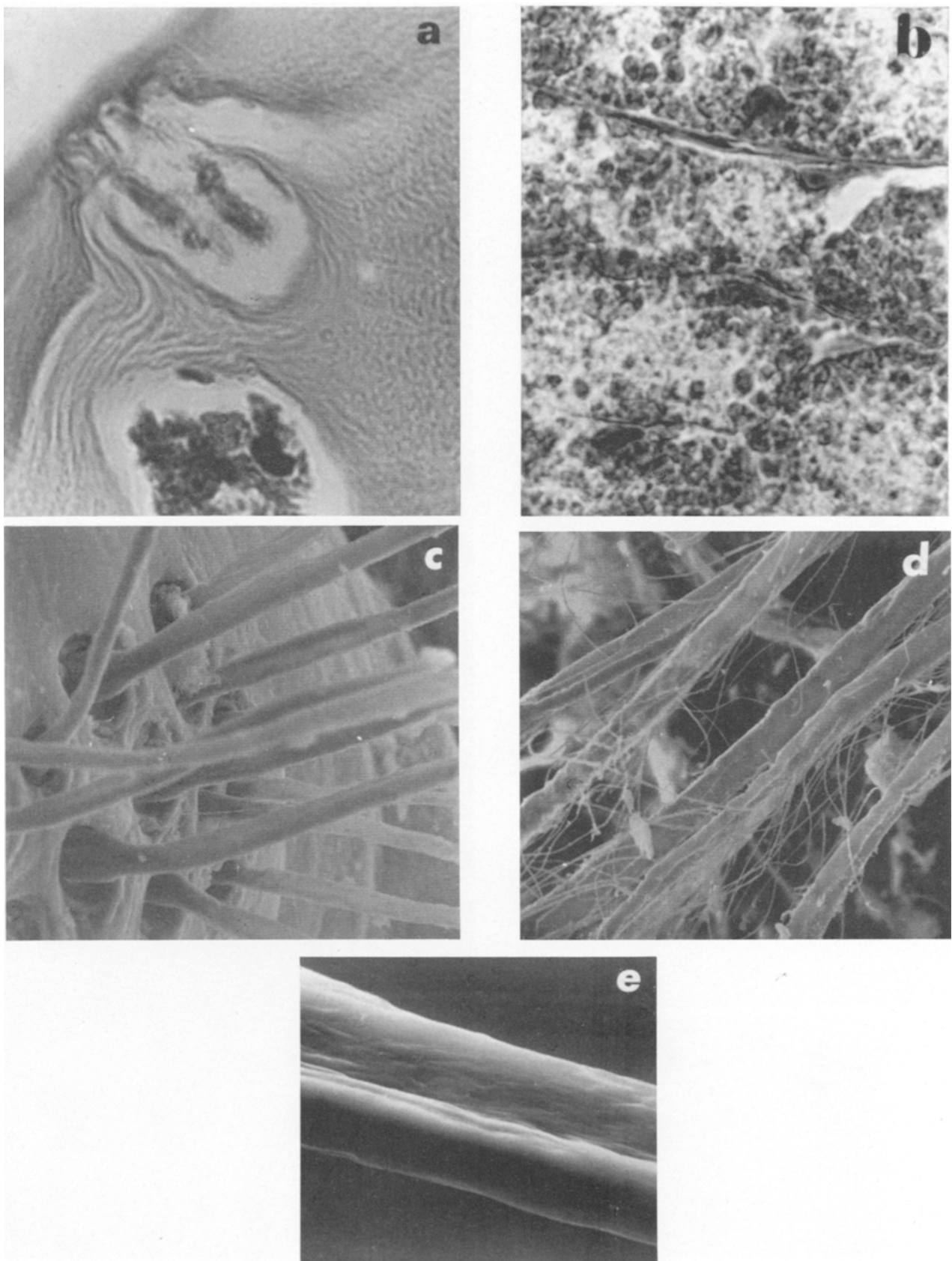


Fig. 2. a) T.S. through the integument in the region of a group of pores. $\times 500$. b) T.S. through the underlying glair glands showing the large nuclei and ducts. $\times 500$. c) The proximal end of a group of oosetae from the pleopod of a mature female of *A. pallipes*. $\times 620$. d) The distal ends of a group of oosetae from the sternum of *A. pallipes* showing the flattened shaft and delicate setules. $\times 620$. e) A high power view of the proximal part of the shaft of an ooseta; note the grooved nature of the shaft at this level. $\times 2.6K$.

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 Eiablage dienen, befinden sich ebenfalls an diesen Stellen. Dies sind sekundäre sexuelle Charakteristika, die eng mit dem Legen und der Ablage der Eier verbunden sind.

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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 claim¹⁻³. 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 respiration⁴ and to catalyze drug detoxification⁵.

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 membranes⁸ and affects mitochondrial functions⁹; 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 mitochondria¹⁰.

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

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² R. W. SWICK and C. A. BAUMAN, *Cancer Res.* 17, 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. SCHWARZ, *Ann. N.Y. Acad. Sci.* 203, 45 (1972).

¹⁰ C. K. CHOW and A. L. TAPPEL, *Lipids* 7, 518 (1972).