Hecker's Compound A_1 (1.5 mg/100 ml acetone) was similarly applied to both groups 3 times a week for the next 20 weeks, by which time the initiated-promoted group were developing multiple papillomas. With the above dosage, there was no ulceration at any time, merely a slight epilation over the painted area. Mice were killed at intervals of 3–4 weeks and the entire treated area was fixed immediately in Baker's calcium-formol, blocked in paraffin, cut at 8 μ and stained with toluidine blue at pH 2.0 for mast cells.

The results fully support the hypothesis, previously expressed[11,15], that a mast-cell reaction in some way reflects the changes occurring in the upper dermis during the promotion phase of carcinogenesis: the connective tissue core of the first small papilloma to appear at 12 weeks was filled with mast cells. However, a further control was made by repeating the above experiment after adding to the Compound A_1 0.0005% sodium selenide, an antioxidant which, as SHAMBERGER and RUDOLF[16] find, nullifies completely the promoting effect of crude croton oil. No mast-cell reaction now occurred in either initiated or non-initiated skin and no tumours emerged.

Mast cells and anti-carcinogens. For a long time it was believed that epidermal cancer can be caused by 'chronic irritation' alone. Now it seems more probable that prolonged mechanical irritation is merely a contributory factor in carcinogenesis: a co-carcinogen. The effects of chemical irritants are more complex. Many years ago BERENBLUM, investigating various forms of chronic irritation on the tarred skin of mice, found, unexpectedly, that certain chemical 'irritants' are, in fact, anti-carcinogens. The first to be discovered was mustard gas[7]. Subsequently, CRABTREE[8] showed that many halogenated compounds behave in this way. Recently, a yet more powerful anti-carcinogen has been found in ethyl phenyl propiolate[9,10].

Since carcinogenic hydrocarbons and promoters stimulate an epidermal hyperplasia in the intact skin, it might have been anticipated that the anti-carcinogens would have an opposite effect, inhibiting the growth of the epidermis. This is certainly not so. Anti-carcinogens provoke an epidermal hyperplasia as do carcinogenic hydrocarbons and the non-carcinogenic promoters. It is when we examine conditions in the underlying dermis, beginning at the basement membrane, that clear morphological differences are encountered between the effects of pro- and anti-carcinogenic agents on mouse skin. Prolonged treatment with the anti-carcinogen, ethyl phenyl propiolate, provokes an epidermal hyperplasia, with associated epilation and destruction of sebaceous glands, as does a typical carcinogenic hydrocarbon. But instead of subepithelial fibrillary collagen and a progressive mast-cell reaction, there develops instead a distinct broadening and stabilization of the basement membrane zone. The hyperplastic epidermis now rests firmly on a base of poorly refractile connective tissue: this is sometimes so thick as to be visible on gross examination of the tissue block. Such mast-cell reaction as develops lies deep to this.

Thus, by refining the experimental conditions, step by step, from painting the skin with crude coal tar to the use of pure initiators, promoters and anti-carcinogens, at low dosage, we arrive at a point at which at least 1 morphological feature differentiates pre-cancerous (or co-carcinogenic) skin from hyperplastic normal skin (or skin treated with an anti-carcinogen). This is the basement membrane zone, consisting of the basement membrane itself and the adjacent thin band of refractile collagenous dermis. An index of the trend towards cancer is the gradual and progressive development of a mast-cell reaction within this zone. The fact of such a reaction is now established: its significance remains to be determined[17].

Zusammenfassung. Wenn auf der Haut der Maus eine Karzinogenese mit minimalen Dosen eines reinen Initiators und eines reinen Promoters (Co-karzinogen) induziert wird, tritt eine Mastzellreaktion auf. Diese Reaktion erreicht ein Maximum unter den Papillomen. Sie kann durch Applikation eines Antikarzinogens verhindert werden.

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[16] R. J. SHAMBERGER and G. RUDOLPH, Experientia *22*, 116 (1966).

[17] My thanks are due to Dr. F. L. ROSE, F.R.S., for preparing a sample of ethyl phenyl propiolate, and to Professor E. HECKER for his generous gift of 'Compound A_1'. I am also most grateful to Dr. D. M. SHEPHERD, University of Dundee, for his continued help. This research programme has been supported by a grant from the Scottish Hospital Endowments Research Trust.

Role of Symbiotes in Tanning of Termite Cuticle

In a recent study on the role of symbiotes in termites, *Reticulitermes assamensis* Gardner, it was found that the flagellate symbiotes in the hind-gut of workers, as in *Zootermposis angusticollis*[1], die shortly before each ecdysis and the recently moulted and defaunated workers regain their infections by the solicitation of proctodael 'food', which contains active protozoa, from their non-moulting associates. But, when the freshly moulted defaunated workers were prevented from reinfection, they displayed abnormal symptoms. They became less active and their abdomens were seen to be smaller and slightly flattened. The length of the time required for abnormalities to appear depends on the kind of food fed after ecdysis, the more decayed the wood, the longer it is before any abnormalities appear. In 2 or 3 days after this first symptom was noticed the abdomen became still more flattened. Death occurred, in some instances in less than 15 days, and in a few cases after 20 days, the longest being 30 days. It was striking to notice that the defaunated workers were lighter in colour than the re-infected ones which became deep amber coloured 5 days after ecdysis. Similarly when the workers were artificially defaunated by exposing to 45 lb of O_2 for 1.5 h, they moulted, but they failed to become deep amber coloured as the normal ones. This recalls the report of SCHNEIDER[2] in *Sitophilus*

[1] W. L. NUTTING, Biol. Bull. mar. biol. Lab., Woods Hole *110*, 83 (1956).

[2] H. SCHNEIDER, Naturwissenschaften *41*, 175 (1954).

granarius and RICHARDS and BROOKS[3] in *Blatella germanica* and *Rhodnius prolixus* that the aposymbiotic members of these insects are lighter coloured.

Histochemical examination of the cuticle of defaunated worker termites shows that the epicuticle comprises an outer lipoid and inner lipoprotein layers. The outer region of the procuticle is fuchsinophil with Mallory's triple stain, while the inner region shows affinity to aniline blue. But in the normal individuals, the procuticle is differentiated into outer amber coloured exocuticle and inner blue staining endocuticle. These observations may indicate that the lighter colour of the defaunated forms is due to the absence of exocuticular formation.

Application of histochemical tests like Millon's and Hg/nitrite on the procuticle of lighter coloured forms shows that the outer fuchsinophil region is rich in protein containing phenyl groups. Phenol oxidase also exists in the same region as evidenced from the positive reaction to 'Nadi' reagent. Comparison of the colorimetric estimation of phenols of acid hydrolysates of the cuticle of defaunated and normal individuals by using Folin and Ciocalteu's reagent[4] gave a ratio of 0.6:1.0. In view of these observations, it may be inferred that the failure of exocuticle formation may be due to inadequate supply of tanning precursors to form the substrate of the cuticle[5]. This is further confirmed by the fact that incubation of the lighter coloured cuticle in 3,4-dihydroxyphenylalanine (DOPA), dopamine and catechol results in acquisition of amber colour as in the normal ones.

A point of interest is that such defaunated forms when re-infected by solicitation of proctodeal droplets containing symbiotic protozoa from the normal non-moulting (intermoult) workers of *R. assamensis*, became active and amber coloured. Examination of the cuticle of such re-infected members shows that the procuticle is differentiated into outer amber exocuticle and inner blue staining endocuticle. Besides, the amount of phenols obtained from the acid hydrolysates is almost equal to that of the normal ones.

BRUNET[6,7] reported that protocetachuic acid which is the tanning precursor may be synthesized by 2 independent routes: (a) directly from tyrosine or (b) from glucose. Of the 2 metabolic paths, the latter is known to occur in microorganism and plants and that the glucose pathway to aromatic compounds in *B. germanica* and *R. prolixus* is a result of the activity of symbiotic microorganisms. The role of symbiotes in wood-eating termites like *R. flavipes* has been shown to digest cellulose resulting in glucose production[8] and supply of proteins and vitamins[9,10]. In the light of these facts, it is reasonable to presume that dietary aromatic compounds in *R. assamensis* are insufficient and must be supplemented by symbiotic products before an adequate amount of phenolic pigments can be synthesized[11].

Résumé. Chez la termite *Reticulitermes assamensis* Gardner la cuticule des ouvriers aposymbiotics est de couleur plus claire que celle des individus normaux. Ceci est dû à l'approvisionnement inadéquat des précurseurs de tannage, en l'absence de la formation de l'exocuticule. La réinfection des formes aposymbiotiques par des symbiotes cause l'acquisition de la couleur ambrée semblable à celle des individus normaux. Il est probable que les composés aromatiques du régime alimentaire des termites sont dus aux symbiotes.

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[3] A. G. RICHARDS and M. A. BROOKS, A. Rev. Ent. *3*, 37 (1958).
[4] T. E. HUGES, J. exp. Biol. *36*, 363 (1959).
[5] R. DENNELL and S. R. A. MALEK, Proc. R. Soc. B *144*, 545 (1956).
[6] P. C. J. BRUNET, Nature *199*, 212 (1963).
[7] P. C. J. BRUNET, Endeavour *26*, 68 (1967).
[8] R. E. HUNGATE, J. Elisha Mitchell scient. Soc. *62*, 9 (1946).
[9] L. R. CLEVELAND, Biol. Bull. mar. biol. Lab., Woods Hole *46*, 178 (1924).
[10] E. C. HENDEE, Science *77*, 212 (1934).
[11] Acknowledgments: Thanks are due to Prof. Dr. G. KRISHNAN, Department of Zoology, University of Madras for his guidance. Comments of Prof. Dr. P. KARLSON, Department of Physiology, University of Philips, West Germany, are gratefully acknowledged.
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Enzymhistochemische Untersuchungen am Subfornikalorgan der Ratte

Das Subfornikalorgan (SFO) hat in letzter Zeit vermehrt Aufmerksamkeit auf sich gezogen. Ausführliche licht- und elektronenmikroskopische sowie histochemische Studien wurden durchgeführt[1-5]. Experimentelle Untersuchungen[6-9] haben den Gedanken aufkommen lassen, dass das Organ Rezeptorfunktionen[6-10] (z.B. im Dienst der Osmoregulation) ausübt; endgültige Beweise stehen jedoch noch aus. Vorliegende Mitteilung soll weitere Beobachtungen über experimentell erzeugbare Veränderungen am SFO bekanntmachen.

Wir haben erwachsene Wistarratten eigener Zucht (ca. 200 g) 7, 10, 12 und 14 Tage dursten lassen und anschliessend das Diencephalon färberisch-lichtmikroskopisch (Kresylechtviolett) und enzymhistochemisch (unspezifische Esterase, Glucose-6-Phosphat-Dehydrogenase, NADH, NADPH) untersucht. Hierbei zeigt sich, dass die Parenchymzellen des SFO der Dursttiere gegenüber Normaltieren an Grösse zunehmen; Kern und Cytoplasma sind gleichmässig betroffen. Ferner kommt es durch Durst zu einer bemerkenswerten Aktivitätssteigerung der G-6-DH (Figur 1); die kräftigste Reaktion ist nach 10 Dursttagen zu beobachten. Betroffen ist vor allem das Cyto-

[1] K. AKERT, H. D. POTTER und J. W. ANDERSON, J. comp. Neurol. *116*, 1 (1961).
[2] K. H. ANDRES, Z. Zellforsch. *68*, 445 (1965).
[3] H. HOFER, in *Primatologia* (S. Karger, Basel und New York 1965), vol. 2, Teil 2.
[4] Y. NAKAJIMA, T. R. SHANTA und G. H. BOURNE, Histochemie *13*, 331 (1968).
[5] A. WEINDL, Z. Zellforsch. *67*, 740 (1965).
[6] K. DIERICKX, Naturwissenschaften *50*, 163 (1963).
[7] H. LEGAIT und E. LEGAIT, C. r. Ass. Anat. *43*, 502 (1956).
[8] N. PACHOMOV, Dt. Z. NervHeilk. *185*, 13 (1963).
[9] M. PALKOVITS, Naturwissenschaften *53*, 336 (1966).
[10] CH. PILGRIM, Experientia *23*, 943 (1967).