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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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-

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plasma der Parenchymzellen des SFO. Das Enzym, das bei Kontrolltieren diffus im Cytoplasma erscheint, gewinnt nach Durst gute Lokalisierbarkeit. Die Aktivität der anderen von uns untersuchten Enzyme wird durch Durst nicht beeinflusst. Im Gegensatz zum SFO vermindert Durst die Aktivität der G-6-DH im Plexus chorioideus (Figur 2). In den Nuclei supraopticus und paraventricularis hypothalami kommt es durch Durst zu der bekannten starken Aktivitätssteigerung für G-6-DH[10].

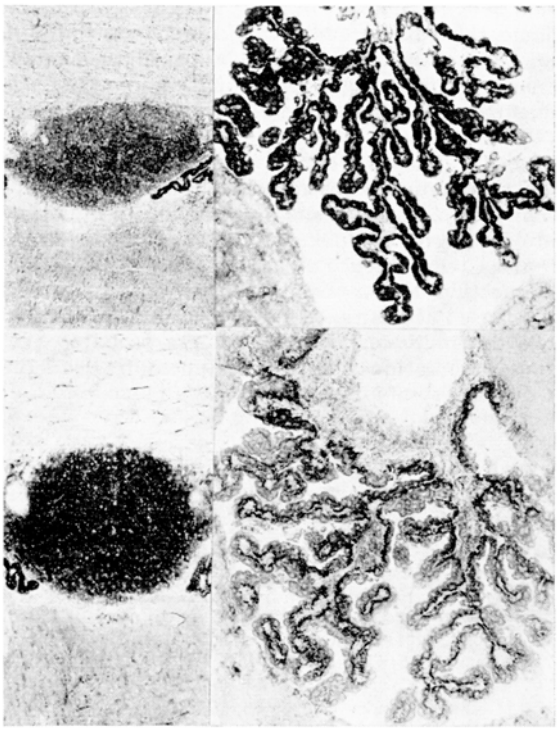

Fig. 1a. SFO, Ratte. Kontrolle. G-6-DH. Fig. 1b. SFO, Ratte. 10 Tage Durst. G-6-DH. Fig. 2a. Plexus chorioideus, Ratte. Kontrolle. G-6-DH. Fig. 2b. Plexus choriodeus, Ratte. 10 Tage Durst. G-6-DH.

Die vorgelegten Befunde bestätigen die bereits von anderen Autoren beobachtete osmotische Empfindlichkeit des SFO. Darüber hinaus machen sie wahrscheinlich, dass Durst eine starke Aktivierung der synthetischen Zellleistungen im SFO hervorruft. Offenbar wird der Pentose-Phosphat-Zyklus im SFO stark mobilisiert. Offenbleiben muss, welches Ziel hiermit in den Parenchymzellen des SFO verfolgt wird. Bemerkenswert ist in diesem Zusammenhang, dass insbesondere endokrine, aber auch andere sekretorisch tätige Zellen über einen aktiven Pentose-Phosphat-Zyklus verfügen[11]. Auffällig ist an unserem Material ferner das gleichartige Verhalten von SFO und neurosekretorischen Zellen. Möglicherweise unterstützen unsere Beobachtungen die Vorstellungen über funktionelle Beziehungen zwischen diesen beiden diencephalen Strukturen[6,7].

Summary. In the rat, thirst leads to an increase of glucose-6-phosphate-dehydrogenase activity in the subfornical organ, and to a decrease in choroid plexus.

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Effect of Temperature on in situ Feulgen Reaction with Schiff Reagent at Less Acid pH

In an earlier publication[1] it has been shown that the concentration of DNA-Feulgen is increased in hydrolysed tissue sections when staining is carried out with Schiff reagent whose pH is raised by a dilute solution of sodium hydroxide. It has also been noticed by DUTT[2] that Schiff reagent at less acid pH causes some non-specific reaction when used at 32 °C but does not cause non-specificity when staining is carried out at 5 °C. The purpose of the present investigation is to find out the effect of different temperatures that help to cause optimum stainability in hydrolysed tissue section by Schiff reagent at less acid pH.

The Schiff reagent was prepared according to DE TOMASI[3] with pararosaniline (C. I. No. 42500) manufactured by National Aniline Division, New York, USA. The material used in this investigation consisted of kidney of a male Indian water buffalo (*Bubalus bubalis* L.) that was fixed overnight in 10% neutral formalin, washed thoroughly in tap water and then preserved in 70% alcohol. Paraffin sections, 10 μ in thickness, were used throughout. Sections were hydrolysed in 1 *N* HCl at 60 °C for 7 min, rinsed in distilled water and then stained with Schiff reagent whose pH was raised from the initial value of 2.3 to 4.0 by the addition of a 0.2 *M* aqueous solution of borax. Staining of these sections was carried out at different temperatures, viz. 5°, 18° and 25 °C for 50 min at each temperature. All slides were stained and processed simultaneously. Following staining, slides were bleached with the usual bleaching solution for 15 min with 3 changes of 5 min each. Later they were dehydrated through grades of alcohol, cleared in dimethylaniline and then mounted in DPX, manufactured by the British Drug Houses, London. The amount of DNA-Feulgen in arbitrary units was determined by a microspectrophotometer of the Pollister type[2]. For measurement of the amount of DNA-Feulgen, whole nuclei from the periphery as well as from the centre of the section were selected. The 2-wave-length method of ORNSTEIN[4] and PATAU[5] was followed, the wave-lengths being 560 and 500 nm. DNA values were calculated according to MENDELSOHN[6].

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