Chromatid aberrations induced by *para*-aminosalicyclic acid (PAS), ethambutol (EMB) and capreomycin (CM) in human lymphocytes in vitro

Substance concentration (mg/ml) treatment time (h)	Number of cells analysed	Achromatic lesions (AL) % of cells	Achromatic No. per cell	Chromatid breaks (B′) % of cells	Chromatid No. per cell	Isochromatid breaks (B″) % of cells	Isochromatid No. per cell	Sum total of all breaking events per cell cell[c]
PAS, 0.153								
48	200[a]	10.00	0.125	3.50	0.035	2.00	0.020	0.075
24	200[a]	10.00	0.110	5.00	0.050	2.50	0.030	0.110
6.5	200[b]	9.00	0.115	6.50	0.070	2.00	0.020	0.110
EMB, 0.028								
48	200[a]	6.50	0.070	3.50	0.035	2.00	0.020	0.075
24	200[b]	7.50	0.080	4.00	0.040	2.50	0.025	0.090
6.5	150[b]	13.33	0.173	6.00	0.060	0.60	0.006	0.073
CM, 0.015								
48	100[a]	11.00	0.110	3.00	0.030	–	–	0.030
24	200[b]	5.00	0.060	4.00	0.040	1.50	0.020	0.080
6.5	200[b]	5.00	0.060	3.00	0.030	1.00	0.010	0.050

[a] 3 cultures. [b] 2 cultures. [c] Calculated from B′ = 1 and B″ = 2.

prophylaxis. The widespread application of antituberculosis drugs makes it very important to test their possible genetic hazards.

Microcultures (slightly modified from ref. [1]) were set up with the blood of a normal healthy man. *Para*-aminosalicyclic acid (PAS), ethambutol (EMB) or capreomycin (CM) were added 48, 24 and 6.5 h before culture stop (whole culture time 72 h). The following drug concentrations (mg/ml) were tested: PAS Na = 0.153; EMB dihydrochloride = 0.028; CM sulfate = 0.015. These concentrations are approximated from calculations based on the therapeutically single daily doses, being 150–200 mg/kg with PAS, 25 mg/kg with EMB and 15 mg/kg with CM. As can be seen from the Table, achromatic lesions (AL), chromatid breaks (B′) and isochromatid breaks (B″) were found. Chromatid translocations (RB′) were completely absent (for description of the aberration types mentioned see ref. [2]). The sum totals of all breaking events per cell (calculated from B′ = 1 and B″ = 2) range from 0.030 to 0.110. These values are not elevated over control values found in our laboratory ranging from 0.0250 to 0.1025 (ref. [3]). Irrespective of the cell cycle stages treated PAS, EMB and CM are inactive with our experimental conditions. Negative results have also been reported with other antituberculosis drugs. INH is inactive in the leukocyte test with concentrations up to 1.0 mg/ml and elevates the frequencies of chromatid aberrations only with the unphysiologically high concentration of 5.0 mg/ml[4]. Rifampicin (RMP) is inactive in the leukocyte test as well as in the *Drosophila* test[5]. Streptomycin (SM) exhibits a strong activity in inducing achromatic lesions (AL) in human chromosomes in vitro[6], an aberration type that should not be taken as an indicator of induced mutations. A prospective controlled clinical trial of patients with pulmonary tuberculosis to test possible chromosomal effects of INH, SM and PAS; INH, SM and EMB as well as INH, RMP and EMB in triple combinations in vivo is in progress.

Zusammenfassung. Die Antituberkulosemittel *Para*-aminosalizylsäure (PAS), Ethambutol (EMB) und Capreomycin (CM) zeigen keine chromosomenbrechende Aktivität an menschlichen Leukozytenchromosomen in vitro.

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Zur Klärung des «Trochophora»-Begriffes

Viele Larvenformen wirbelloser Tiere werden einfach als *Trochophora* oder als *Trochophora*-ähnlich etc. bezeichnet, was gewisse Verwandtschaftsverhältnisse vortäuscht und daher häufig sachlich verwirrt. Bei detaillierter Analyse zeigt sich aber vielfach nur eine ökologisch bedingte Übereinstimmung (Bewimperung; vergl. den Begriff «Wurm»), welche morphologisch jedoch keinen Aussagewert enthält[1,2].

Die charakteristische *Trochophora*-Larve ist nicht allein durch prae- wie postoralen Cilienkranz und durch Wimperschopf wie Telotroch, sondern durch Augen, rechtwinkeligen Darm, sich aushöhlende Mesodermbänder und besonders durch Solenocyten (Geisselzelle-Protonephridien, bei Coelomata) gekennzeichnet; sie kommt den meisten marinen Anneliden (Archiannelida, Polychaeta) wie Echiuriden zu (nicht aber den Myzosto-

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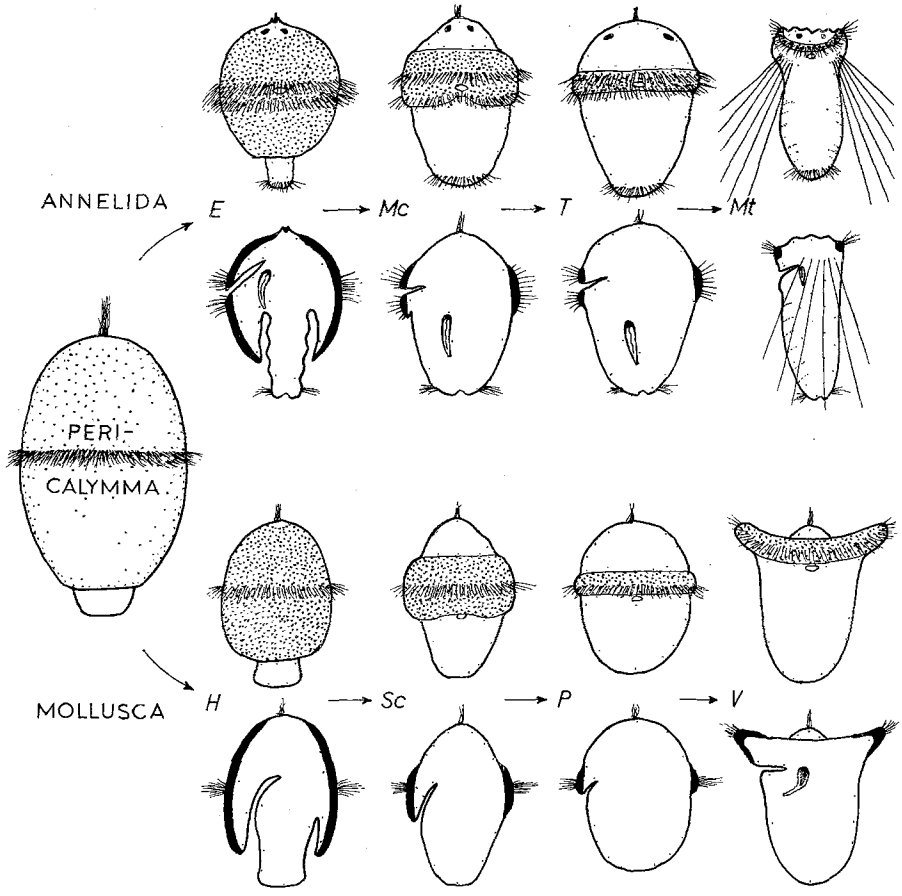

Entwicklung der Larvenformen bei Anneliden und bei Mollusken aus der *Pericalymma*-Larve, jeweils in Totalansicht (oben) und im schematisierten Längsschnitt (unten). E, Endolarve; H, Hüllglockenlarve (*Pericalymma*); Mc, *Metacalymma*; Mt, *Metatrochophora*; P, *Pseudotrochophora*; Sc, *Stenocalymma*; T, *Trochophora*; V, *Veliger*.

mida). Demgegenüber fehlen der ähnlichen Larve vieler Mollusken die Augen, die Solenocyten, meist auch der anale wie postorale Cilienkranz; das Mesoderm bildet zerfallende Streifen und die bei höheren Muscheln wie Süsswasser-Schnecken auftretenden Protonephridien sind als Osmoregulatoren (wie bei Mesenchymata/Acoelomata allgemein) stets Wimperflammen-Organe; die Larve muss daher als *Pseudo-Trochophora* bezeichnet werden («*Praeveliger*» bei[2]). Besondere Deutlichkeit gewinnen diese Verhältnisse jedoch dadurch, dass beide Larventypen convergent jeweils als vereinfachte *Pericalymma*-Larve (Hüllglocken-Typus, Testcell-type) festzustellen sind (Figur): Die grosszellige Hülle der Larve (Endolarve bei Annelida, Hüllglockenlarve bei Mollusca) wird zusehends zu einem breiten Wulst vermindert (*Metacalymma, Stenocalymma*) und schliesslich auf die Cilienkranz-Zellen eingeschränkt (*Trochophora, Pseudotrochophora*); convergent treten hieraus weitere Differenzierungen auf (*Veliger*; *Metatrochophora* und *Atrocha*, etc.). Im Anschluss an frühere Überlegungen war diese Entwicklungsreihe von CHANLEY[3] für die Muscheln, von SALVINI-PLAWEN[4] für die Solenogastres, von MINICHEV und STAROBOGATOV[5] für die Anneliden und schliesslich zusammenfassend allgemein festgestellt worden[1]. Es ergibt sich diesbezüglich daher eine gemeinsame Wurzel in der *Pericalymma*-Larve (Figur), deren primäre Differenzierung aus der MÜLLERschen Larvenform (*Protrochula*; Turbellaria-Polycladida) denkbar erscheint[1], und deren analoge Weiterentwicklung zu den acoelomat verbliebenen Mollusca bzw. zu den coelomaten Echiurida/Annelida schon durch das radiale (Mollusca) bzw. interradiale (Annelida) Blastomerenkreuz im Keim divergierend belegt ist.

Alle weiteren, als *Trochophora*-ähnlich bezeichneten Larven haben keine gesicherte Beziehung zum echten *Trochophora*-Typus. Die Larven der protanneliden Myzostomiden weichen zumindest durch den subanalen Caudalkörper sowie durch das Fehlen von Augen, Solenocyten und Coelombildung ab[6]. Die coelomaten Sipunculida entwickeln sich über eine *Pericalymma*- oder daraus vereinfachte *Trichosphaera*-Larve (die sog. «*Trochophora*» der Autoren, ohne Solenocyten u. a. m.) und weiter über die *Bentho-/Pelagosphaera*, mit verschiedenen Abkürzungen[1, 4, 7, 8]. Basal schliessen sich vielleicht die Phoronidea-*Actinotrocha*, oder die *Tornaria/Dipleurula*-Larven der deuterostomen Oligomera an, welche als Weiterdifferenzierung eines *Trochophora*-Typus von einer convergent vierten (!) Entwicklungsreihe aufgefasst werden können[1]. Die acoelomaten Kamptozoa zeigen wiederum eine primäre Gleitlarve[9, 10], wogegen die Nemathelminthes, wie

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Bryozoa und Brachiopoda schliesslich spezielle Larvenformen ohne morphologische Beziehungen zur *Trochophora* aufweisen.

Summary. The confusing term '*Trochophora*' is elucidated and restricted to a developmental stage of the Annelida and the protannelid Echiurida, derived convergently with the molluscan *Pseudo-Trochophora* from the common *Pericalymma*-larva. All other so-called *Trochophorae* cannot be termed as such, the name being morphologically misleading as in Sipunculida and other.

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Growth Stimulating Effects of the Bromophenol, Lanosol, on Red Algae in Axenic Culture

Brominated compounds in red algae have attracted attention since the thirties from chemical and pharmacological point of view[1]. The most frequently occurring compound is lanosol, 2,3-dibromo-4,5-dihydroxybenzyl alcohol, isolated from *Polysiphonia lanosa*[2]. Lanosol has been known as a highly toxic substance for bacteria and algae. MCLACHLAN and CRAIGIE[3] give a LD_{50} of 0.03 m*M* for unicellular marine algae. However, in nature lanosol seems to appear in the red algae mostly as a disulphate and this form does not inhibit growth of bacteria or fungi[4,5] nor growth of unicellular algae[3]. PROVASOLI[6] found lanosol influencing the morphology of *Ulva*. As many simple phenols, like 3-methylcatechol, 4-methylcatechol, and 3,4-dihydroxybenzoic acid, stimulate growth of axenically cultivated *Goniotrichum alsidii* (Zanard.) HOWE[7] lanosol and its sulphuric ester were tested on this alga as well as on *Polysiphonia urceolata* (Dillw.) Grev.

Lanosol[8] was isolated from *Polysiphonia brodiaei* (Dillw.) Grev. and its disulphate[2] from *Brongniartella byssoides* (Good et Wood.) Schmitz. The chemical preparations were made by Dr. P. SAENGER, Melbourne University, at his stay in our institute. The lanosol preparation contained a few percent of the corresponding aldehyde, and during storage it changed colour to dark brown and lost its activity. The methods for cultivation of the red algae and the arrangements for the experiments are described earlier[7,9]. The influence of the bromophenols on algal growth was tested in a range from 4 to 200 µmole/l. The growth of the algae on different concentrations is given in the Table as mean dry weight of algae from 6 parallel flasks, each flask containing 25 ml of the artificial seawater, ASP6 F[9].

In *Polysiphonia urceolata*, lanosol inhibited growth in a concentration of 0.2 m*M*/l, a result corresponding very well with the observation made by MCLACHLAN and CRAIGIE[3] on the red alga *Porphyridium sp.* Lower concentrations, however, strongly stimulated growth, giving an increase near to 90% at a concentration of 4×10^{-6} *M*. In *Goniotrichum alsidii* no inhibition was noted but the growth stimulation reached only 50%. The disulphate of lanosol was not only nontoxic but showed a pronounced growth stimulation in both species at the two highest concentrations tested.

At separation on a Sephadex column of seawater collected in the *Fucus-Ascophyllum* zone some fractions containing phenolic compounds were obtained which influenced growth and morphology of *Goniotrichum*[10]. The active substances in that experiment were not identified, but later it has been shown that *Polysiphonia brodiaei* exudes bromophenols, mainly lanosol in some form soluble in sea-water[11]. CHAN and MCMANUS[12] followed the bacterial flora on *Polysiphonia lanosa* and found the same richness from May to September, which could indicate that the nontoxic disulphate was exuded.

Analyses of bromophenols in Swedish red algae have shown that, whereas *Polysiphonia urceolata* contains

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Effects of lanosol and sulfonated lanosol on growth of the 2 axenically cultivated red algae, *Goniotrichum alsidii* and *Polysiphonia urceolata*. Sporelings of *Goniotrichum* and pieces of *Polysiphonia* threads used as inocula

Species investigated	Substances added	Mean dry weight of algae per 25 ml nutrient medium (mg)			
		Addition per l/µmole			
		0	200	40	4
Goniotrichum alsidii	2,3-dibromo-4,5-dihydroxy-benzyl alcohol (lanosol)	4.9 ± 0.9	5.6 ± 0.5[a]	7.5 ± 0.6[a]	7.2 ± 0.4[a]
	2,3-dibromo-4,5-dihydroxy-benzyl alcohol disulphate	4.9 ± 0.9	6.3 ± 0.2[a]	7.1 ± 0.6[a]	6.5 ± 1.0
Polysiphonia urceolata	2,3-dibromo-4,5-dihydroxy-benzyl alcohol	0.8 ± 0.2	0.2 ± 0	1.1 ± 0.1[a]	1.5 ± 0.2[b]
	2,3-dibromo-4,5-dihydroxy-benzyl alcohol disulphate	0.8 ± 0.2	1.5 ± 0.2[a]	1.1 ± 0.1[a]	0.8 ± 0.2

[a] Significantly different from control at $p < 0.05$. [b] Significantly different from control at $p < 0.01$, indicating growth stimulation. Inoculation time 28 days. 6 parallels in each series.