umn replaced the Hyflo Super-Cel filtration column, and normal rat tissues treated in the same manner as the test tissues were used instead of the hexane "blank" in colorimetric determination. It was assumed that "total" gossypol content of tissues reflects intake of the "free" gossypol of the diet since it has been demonstrated that only the "free" form of gossypol is absorbed and is responsible for the toxicity (*10*).

The concentrations of gossypol in these tissues (Table 1) clearly reflect the toxicity symptoms and growth performance data. The highest concentrations were observed in the group fed the uninoculated cottonseed, which, in turn, produced the most dramatic toxicity symptoms. The reduced toxicity of the inoculated cottonseed appears to be due in part to reduction of free gossypol and in part to lower toxicity of residual free gossypol. Although it would appear that the extra feed intake could have afforded protection for the residual gossypol, additional studies using Leghorn cockerels have demonstrated that the intake of equal dietary levels of free gossypol obtained before and after fungal growth results in no demonstrable toxicity for the gossypol remaining after fungal growth. Furthermore, tissue gossypol accumulation, feed intake, body weight change, and gross symptomatology for these cockerels confirm the observations made with rats.

Such detoxication may have industrial application provided a nontoxic fungus could be chosen to prepare the fermented cottonseed meal; or, perhaps a cell-free fungal extract, lacking toxic factors, might be prepared which would serve as the feed additive used to detoxify the gossypol of cottonseed.

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Chromatin and Histones: Binding of Tritiated Actinomycin D to Heterochromatin in Mealy Bugs

Abstract. *The degree of actinomycin D binding to DNA in chromatin is dependent upon the state of repression of chromatin. Living cells bind three times more tritiated actinomycin to euchromatin than to genetically inactive heterochromatin. Extraction of histone results in a general increase in tritiated actinomycin binding and in a ratio of the uptake in heterochromatin to that in euchromatin approaching unity.*

We describe here the uptake of tritiated actinomycin D in genetically active and inactive chromatin in both living cells and cells from which the histones have been extracted. The work was done on the cells of the male mealy bug *Planococcus citri* (Homoptera, Coccoidea). Many of the cells in this organism contain condensed interphase chromatin in the form of five readily visible heterochromatic chromosomes (H, the paternally derived haploid set). The five heterochromatic chromosomes are late replicating (*1*) and genetically inactive (*2*), the block occurring in DNA-directed RNA synthesis (*3*). The five euchromatic chromosomes (the E chromosome set) are the genetically active chromosomes.

Premeiotic gonial cells within whole testes, which readily incorporate tritiated uridine (*3*), were incubated with tritiated actinomycin D (Schwarz; specific activity, 3.38 c/mmole). Living cells and cells previously fixed and squashed were used, the former to study the uptake of actinomycin D in vivo and the latter to determine the degree of actinomycin D binding to DNA after alteration of the relation between the DNA and its associated proteins.

Unfixed living testes were incubated in 0.2 ml of tritiated actinomycin D in Ringer solution for 2 to 4 hours. Care was taken to prevent residual labeled material from being taken up passively after fixation. The testes were flooded with unlabeled Ringer solution for ½ hour to dilute the ratioactivity and wash the unbound tritiated actinomycin D out of the tissue prior to fixation. The testes were first transferred to slides previously coated with gelatin and then squashed in either 45 percent acetic acid or 10 percent neutral buffered formalin. Tissue squashed in formalin was fixed in formalin for an additional 5 minutes. All slides were washed in distilled water and dried in air. Actinomycin D (0.05 μg/ml) will suppress at least 90 percent of the RNA synthesis in premeiotic testis cells after a 2-hour incubation (*3*). In this series of experiments, we used not less than 0.74 μg/ml of tritiated actinomycin D.

Table 1 (items B and C) shows a comparison of living testes which were incubated with 5 μc/ml of tritiated actinomycin but were subject to different fixation techniques after squashing. The data indicate that the amount of labeled actinomycin retained in the euchromatin after formalin fixation is seven times greater than that retained after acetic acid fixation. Camargo and Plaut (*4*) suggest that 45 percent acetic acid will break hydrogen bonds and that this may account for differences in retention of the tritiated actinomycin D. Nevertheless, it is significant that the ratio of uptake in euchromatin to that in heterochromatin remained the same regardless of fixative. The relatively low uptake in the live cells (Table 1, item A) probably resulted from the use of trititiated actinomycin D with a low specific activity in the early experiments.

The relative difficulty with which tritiated actinomycin D was bound to the condensed, heterochromatic chromosome set can be observed in Table 1 and in Fig. 1A. The ratio of uptake of tritiated actinomycin D into the euchromatic and heterochromatic chromosomes of living cells was 3.2–3.4 to 1. Since the amount of DNA is the same in these two sets of chromosomes (*5*), these results indicate that heterochromatin has fewer available binding sites for actinomycin D than euchromatin.

In the second series of experiments, tissue was squashed and fixed prior to incubation according to a procedure

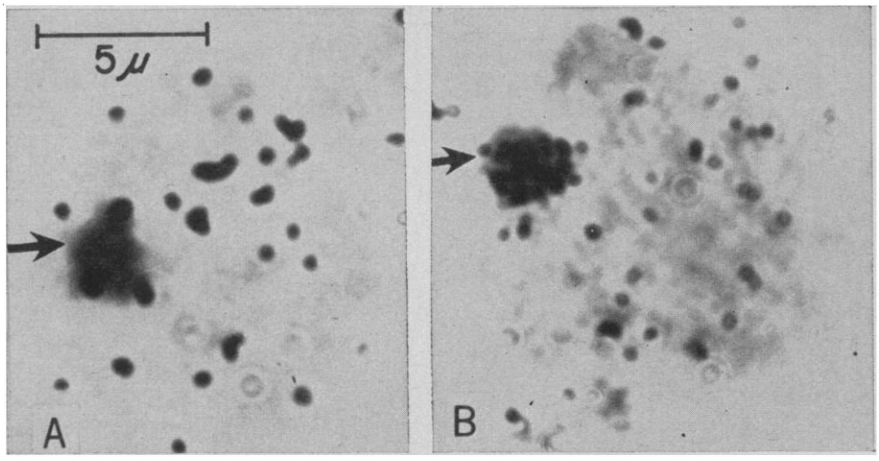

Fig. 1. Tritiated actinomycin labeling. Gonial interphase; heterochromatic set, arrow. (A) Exposure for 4 hours at 50 μc/ml in vivo, acetic acid fixation; heterochromatic set takes up less label than euchromatic set. (B) Exposure for 1 hour at 2 μc/ml after acetic acid fixation and extraction; label over euchromatic set and heterochromatic set about the same.

modified from Ebstein (*6*). Testes were squashed on slides covered with albumin which had been previously treated with unlabeled actinomycin D to prevent nonspecific binding of tritiated actinomycin D to the slide. After removal of the cover slip, testes squashed in 45 percent acetic acid were either treated with 0.5*N* H_2SO_4 for 30 minutes at 0°C or transferred directly to distilled water and washed for 2 minutes before air drying. Testes squashed in 10 percent neutral buffered formalin were fixed in formalin and washed in distilled water before air drying. Air-dried slides were placed in a moist chamber, and two drops of tritiated actinomycin D were placed directly over the tissue on the slide. The slides were incubated for 1 hour at room temperature, flooded with distilled water, washed, and air-dried. Control slides, treated in the same manner, were immersed in deoxyribonuclease (Worthington DPFF, 160 μg/ml of 0.01*M* phosphate, 0.003*M* $MgCl_2$, *p*H 6.2) for 2 hours at 37°C after being dried in air and before incubation in the actinomycin D. This deoxyribonuclease treatment completely inhibited uptake of tritiated actinomycin D. The autoradiographic procedure used in all experiments has been described (*3*).

With cells fixed and squashed before incubation, optimum saturation was achieved with tritiated actinomycin D after 1 hour. The average number of grains over euchromatin in cells extracted with acetic acid before incubation was approximately two times the number of grains in formalin-fixed and approximately 15 times the number of grains in cells fixed in acetic acid incubated in vivo. Dick and Johns (*7*) have reported that after treatment for 30 seconds with 45 percent acetic acid, 50 percent of the total histone was extracted from calf thymocyte nuclei. Extraction of the remainder of the histone with 0.5*N* H_2SO_4 (Table 1, item F) resulted in a further increase in uptake of tritiated actinomycin D into the euchromatin. It appears that extraction of histone with acid increased the ability of actinomycin D to bind with double-stranded DNA in both heterochromatin and euchromatin.

Fixing proteins with formalin tends to precipitate them *in situ*. This phenomenon is employed to keep the histones intact so that alkaline-fast-green reaction may be applied (*8*). Uptake of tritiated actinomycin D (5 μc/ml) after fixation with formalin was five times less than after extraction with acetic acid and approximtely two and a half times less than in testes incubated in vivo before formalin fixation (Table 1). These data suggest that alteration of chromatin-bound protein reduces the ability of actinomycin D to bind to the DNA helix. Some of the observed reduction may also be the result of formalin denaturation of DNA itself (*9*).

When premeiotic testis cells were extracted with 45 percent acetic acid before incubation, the heterochromatin takes up relatively more actinomycin D than the heterochromatin of live incubated cells [ratio of uptake of E to H, 1.2–1.3 to 1 (Table 1)]. The capacity to bind actinomycin D, of the repressed heterochromatin relative to that of the euchromatin, was increased by acid extraction. However, cells fixed in formalin prior to incubation (Table 1, item G) incorporated relatively even less tritiated actinomycin D into heterochromatin than living cells did (ratio of E to H, 8.4 to 1).

The lysine-rich histones are the principal histones removed with acetic acid extraction (*7*). In our system, cells squashed in acetic acid, in comparison to formalin-fixed cells, showed a substantial, visible reduction in the alkaline–fast green reaction for histone. A solution of 0.5*N* H_2SO_4 removes all of the histones and completely eliminates the alkaline–fast green reaction. Furthermore, in cells treated with deoxyribonuclease after extraction with H_2SO_4, chromatin structure was completely removed.

It has been suggested that the lysine-rich histones are responsible for condensed chromatin or heterochromatin (*10*). Although the absolute uptake of tritiated actinomycin D increased in tissues extracted with 0.5*N* H_2SO_4, after extraction with acetic acid and before incubation with actinomycin D, the ratio of uptake of E to H remained the same (Table 1).

Table 1. Uptake of tritiated actinomycin D (AMD-^{3}H) in the gonial cells of male mealy bugs (*Planococcus citri*); E, euchromatin; H, heterochromatin; S.E., standard error; HAC, 45 percent acetic acid.

Item	Fixation	AMD-^{3}H (μc/ml)	Incubation	Cells (No.)	Grains/cell H (Av. No. ± S.E.)	Grains/cell E (Av. No. ± S.E.)	Ratio of grains E/H
				Live			
A	HAC	50	4	506	4.35 ± .12	14.0 ± .65	3.2
B	HAC	5	2	90	0.92 ± .05	3.17 ± .02	3.4
C	Formalin	5	2	36	6.94 ± .24	23.25 ± .92	3.4
				Extracted and squashed			
D	HAC	5	1	16	37.4 ± 2.0	45.4 ± 3.0	1.2
E	HAC	2	1	134	9.20 ± .61	11.70 ± .23	1.3
F	HAC + 0.5*N* H_2SO_4	2	1	50	14.1 ± 1.17	19.60 ± .46	1.4
				Fixed and squashed			
G	Formalin	5	1	88	1.17 ± .06	9.80 ± .12	8.4

Desai and Tencer (*11*), who used trypsin digestion of nucleoproteins, also concluded that histone interferes with the ability of tritiated actinomycin D to bind to DNA. It has been suggested (*12*) that most of the gene sites in metabolically active interphase chromatin are repressed as a result of histone binding. Such repression would account for the observed increase in the binding sites available in mealy bug euchromatin after acid extraction.

These findings taken as a whole suggest that the degree of actinomycin D binding to chromatin is related to the degree of repression of the chromatin and that removal of histone increases the capacity of repressed, condensed chromatin to bind actinomycin D.

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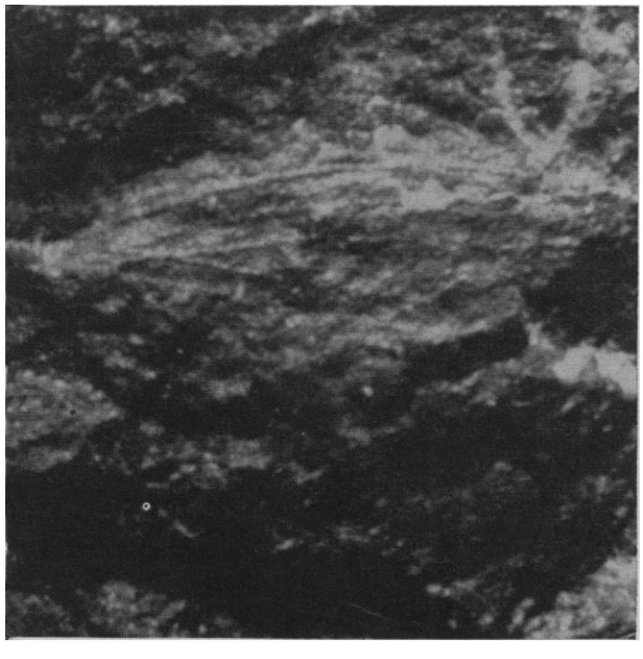
Permian Insect Wing from Antarctic Sentinel Mountains

Abstract. *A homopterous insect wing was found in micaceous graywacke from the Polarstar Formation, Sentinel Mountains. The unusual venation is reminiscent of family Stenoviciidae known from the Permian and Triassic of Eastern Australia and elsewhere. This first documented account of Paleozoic insects in Antarctica bears on drift questions.*

A Permian insect wing was found (*1*) in micaceous graywacke from the Polarstar Formation of the Antarctic Sentinel Mountains. The fossil fragment was collected from the east slope of Polarstar Peak near the 2000-meter contour. Fossil spoor (trails and burrows) and plant debris (*2*) are encountered sporadically almost to the 1800-meter contour [Newcomer Glacier Sheet, 1960, or simplified version of same (in *3*)], but are most dense immediately above and below 2000 m. This dense fossil-spoor zone is a probable correlate of another such zone that was sampled (*4*) at a different exposure of the Polarstar Formation; and it may be placed well below the *Glossopteris* beds (*3*, and field observations).

Although a fossil insect from the *Glossopteris* beds of Theron Mountains has been mentioned (*5*) the specimen was never described and was subsequently lost. Thus, this is the first firm example of Gondwana Paleozoic insect distribution in Antarctica that is pertinent to continental drift theory. Permo-Carboniferous insects are known from South America, Falkland Islands, West Africa, South Africa, and Australia (*6*). A possible transantarctic migratory route was suggested for Gondwana insects; sparcity of reports does not preclude their widespread occurrence in the area during the Paleozoic.

The fossil (*7*) consists of a basal portion of a small wing (Fig. 1) from which the clavus was detached. The length of the fragment (about 3.5 mm) indicates a complete wing of about 5.5 mm. The fact that the insect wing was preserved in this slightly metamorphosed rock is considered to indicate that the wing was relatively heavily sclerotized—it could be compared in texture with the fore wing of many Recent Homoptera. The straight clean break that forms the posterior margin of the preserved portion of the wing is considered to indicate that the clavus became detached along this line before fossilization of the wing—the clavus



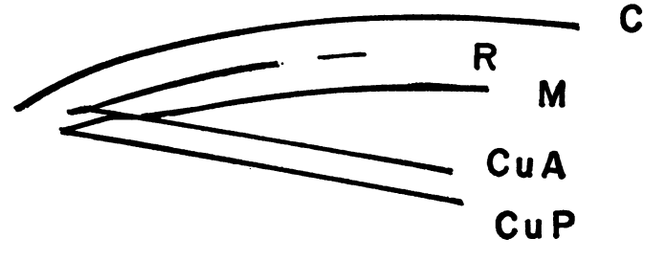


Fig. 1 (left). Portion of fossil wing of a Permian homopterous insect from the Polarstar Formation, Sentinel Mountains, Antarctica (× 20) [E. F. Riek].

Fig. 2 (above). Line drawing of wing venation as seen in Fig. 1. Costa, *C*; radius, *R*; media, *M*; and cubitus, *Cu* (× 20) [E. F. Riek].