Résumé. La cardiolipine est le principal phospholipide dans *M. smegmatis* CDC. Il constitue le 45% du phospholipide total. Elle est suivie d'une quantité de phosphatidylinositolmannosides. Dans *M. phlei* ATCC 354 au contraire, ces derniers représentent le 46% du phospholipide total. Le monomannophosphoinositide y est présent en plus forte concentration que la cardiolipine.

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Aryl and Aniline Hydroxylases in Rat Nuclear Membranes after Pretreatment with Pregnenolone 16α-carbonitrile, Phenobarbital and Methylcholanthrene

The ability of pregnenolone 16α-carbonitrile, a potent catatoxic steroid, to protect rats against many toxicants[1], may be attributed to its capacity to induce mixed-function microsomal oxidases such as aryl hydrocarbon hydroxylase. The dual role of this enzyme system in either detoxification or in the formation of active intermediates in polycyclic hydrocarbon carcinogenesis has been established[2]. Pretreatment of rats with pregnenolone 16α-carbonitrile increases liver weight, stimulates smooth-surfaced endoplasmic reticulum proliferation in liver cells, and enhances NADPH cytochrome c reductase activity as occurs after phenobarbital treatment[3,4].

The inductive effect of pregnenolone 16α-carbonitrile in microsomes has been shown to be greater than that of phenobarbital but still less than the induction of the enzymes by methylcholanthrene[5]. However, the induction in microsomes of aniline hydroxylase, another inducible enzyme system, was the same for all 3 substances. KHANDWALA and KASPER[6] have recently demonstrated high levels of aryl hydrocarbon hydroxylase in the liver nuclei and nuclear membranes of methylcholanthrene-treated rats, but no differences were noted in the nuclei or nuclear membranes from phenobarbital-treated or control rats. Here we report that pregnenolone 16α-carbonitrile, which induced high quantities of aryl hydrocarbon hydroxylase and anilin hydroxylase in microsomes, as did methylcholanthrene and phenobarbital, was unable to induce the enzyme in purified nuclei and nuclear membranes.

For enzyme induction, male rats (WAG strain, C.E.S.A.L., Orleans, France) weighing 130–150 g were treated with phenobarbital, 3-methylcholanthrene, and pregnenolone 16α-carbonitrile in the following manner: 0.1% sodium phenobarbital was placed in the drinking water of 1 group of animals for 2 weeks, methylcholanthrene was injected into another group (20 mg/kg in 0.5 ml corn oil) once a day for 2 days, and a micronized suspension of pregnenolone 16α-carbonitrile in 2 ml water with a trace of Tween 80 was given per os to another group (50 mg/kg). The compound was administered at 8-h intervals (twice daily for 2 days and once on the 3rd day). Controls of injected rats received 0.5 ml corn oil only. The animals had free access to water and a standard diet. They were killed by decapitation 24 h after the last injection or last oral administration. The livers were excised quickly, chilled and weighed. The nuclei from rat liver were isolated (according to KASPER[4]) and the nuclear membranes were prepared by action of heparin on the nuclei as described by BORNENS[7]. The purity and integrity of both preparations were controlled by electron microscope studies.

Table I shows that, in the microsomes, methylcholanthrene and pregnenolone 16α-carbonitrile induce greater quantities of aryl hydrocharbon hydroxylase than phenobarbital. In the nuclei and nuclear membranes, however, neither pregnenolone 16α-carbonitrile nor phenobarbital induce the enzyme. On the other hand, methylcholanthrene, which induces 6 times more aryl hydrocarbon hydroxylase in the microsomes, induces 10 times more enzyme in the nuclei and 15 times more en-

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Table I. Specific activity of aryl hydrocarbon hydroxylase in the nuclei, the nuclear membranes and microsomes after induction by phenobarbital (PB), methylcholanthrene (MC) and pregnenolone 16α-carbonitrile (PCN)

Fraction	Treatment C	PB	MC	PCN
	(pMoles 3-hydroxy benzo(a)pyrene/30 min/mg protein)			
Nuclei	222 ± 48	202 ± 52 (−)[a]	2402 ± 342 (×10)	186 ± 42 (−)
Nuclear membranes	400 ± 120	416 ± 104 (−)	6956 ± 820 (×15)	422 ± 120 (−)
Microsomes	4200 ± 380	7450 ± 602 (×2)	26420 ± 2250 (×6.5)	12604 ± 620 (×3)

[a] The figures in parentheses show the degree of activation as compared to controls. Aryl hydrocarbon hydroxylase was determined by the method described by KINOSHITA and GELBOIN[2].

Table II. Aniline hydroxylase in the nuclei, the nuclear membranes and the microsomes after induction with phenobarbital (PB), methylcholanthrene (MC) and pregenolone 16α-carbonitrile (PCN)

Fraction	Treatment C	PB	MC	PCN
	(nMoles *p*-aminophenol/min/mg protein)			
Nuclei	0	0	0	0
Nuclear membranes	0	0	0	0
Microsomes	0.52 ± 0.02	0.75 ± 0.06	0.76 ± 0.07	0.76 ± 0.06

Aniline hydroxylase was determined according to the method of SHENKMAN et al.[9]

zyme in the nuclear membranes a s compared to controls. Although the 3 substances are strong inducers of microsomal aniline hydroxylase, they did not induce this enzyme in either the nuclei or nuclear membranes. These results support the suggestion that the intracellular controls regulating the nuclear membrane enzymes upon action of methylcholanthrene differ from those which control and regulate the microsomal hydroxylase[8,9].

All the known inducers of aryl hydrocarbon hydroxylase have been divided into 2 distinct categories represented by phenobarbital and methylcholanthrene[3,6]. Although microsomes from pregnenolone 16α-carbonitrile- and phenobarbital-pretreated rats have properties in common[10], differences have been noted[3,4], thus raising the possibility of a new category of inducers. In this comparative study of the 3 inducers, we were interested to know whether the nuclear hydroxylases show different specificities with substrates such as benzo(a)pyrene and aniline. Our results concerning the induction of nuclear aryl hydrocarbon hydroxylase show that pregnenolone 16α-carbonitrile is similar to phenobarbital and different from methylcholanthrene. All 3 inducers enhanced aniline hydroxylase activity to the same extent in rat liver microsomes, but the enzyme was not induced in the nuclei or nuclear membranes, as shown in Table II[11].

Although the nuclear membranes from phenobarbital and pregnenolone 16α-carbonitrile-pretreated rats show common aryl hydrocarbon hydroxylase activity, this does not preclude the possible differences in the metabolite profile of a benzo(a)pyrene substrate when incubated with these nuclei. RASMUSSEN and WANG[12] recently demonstrated the dependence of the specific metabolism of benzo(a)pyrene on the inducer of hydroxylase activity. Further study of the induction of these enzymes in the nuclear membrane is thus of great importance in benzo(a)-pyrene carcinogenesis.

Résumé. L'aryl hydrocarbure hydroxylase nucléaire répond seulement au méthylcholanthrène tandis que le phénobarbital et la prégnénolone 16α-carbonitril ne l'affectent pas. Les trois substances n'induisent pas l'aniline hydroxylase nucléaire. Cependant elles sont des puissants inducteurs de l'aryl et l'aniline hydroxylase microsomiale.

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The Relationship Between Metaphase Heterochromatin and Polytene Inversions in *Drosophila*

It has long been a cytogenetic problem as to the circumstances under which an extra heterochromatin arises[1–3]. Recently, BAIMAI[4] has reported a one-to-one correlation between an extra heterochromatic segment and chromosome inversions in chromosome 4 of *D. disjuncta*. The present paper reports a further study of this important problem. *Drosophila formella* Hardy and Kaneshiro, a picture-winged species from the island of Hawaii, is a member of the *D. hawaiiensis* group. This species exhibits chromosomal polymorphism for inversion $4j^3/k^3$. In particular, the proximal break-point of inversion $4k^3$ has apparently occurred within the area of centromeric heterochromatin[5]. The present work provides additional evidence which supports the previous finding of a relationship between heterochromatin as determined by mitotic metaphase and a given chromosome inversion as seen in the polytene chromosome. Such parallel chromosomal changes can only be detected in material where both types of tissue may be simultaneously examined. It is hoped that the advanced hypothesis will, if confirmed, be of some genetic and evolutionary interest.

Materials and methods. In this study, use was made of the laboratory stock No. M87G1 which was derived from an individual wild-caught female collected from Puuwaawaa, Northwest slope of Hualalai, Hawaii (about 1290 meters altitude) by Prof. H. L. CARSON in December, 1969. The stock has been maintained in the laboratory at

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