terfering fluorescent compounds were removed by silica-gel chromatography, by the procedure (*10*) for isolation of aflatoxin M_1. Aflatoxin P_1 was found in this fraction, free of interfering compounds (fraction 5).

The chloroform-soluble fractions (1, 3, 4, and 5) were subjected to thin-layer chromatography (*11*). When viewed under ultraviolet light, all the fractions were found to contain a yellow-green fluorescent compound with an R_F value different from authentic aflatoxins B_1, M_1, or the hemiacetal derivative of aflatoxin B_1. Autoradiography on x-ray film revealed a single radioactive spot that coincided with the fluorescent substance.

On the basis of this evidence, we concluded that all of the fractions contained a common metabolite, present in fraction 2 principally as a glucuronide and to a lesser extent as a sulfate. In order to accumulate sufficient quantities of this substance for chemical identification, three male rhesus monkeys weighing 3.0 to 4.4 kg were dosed once weekly for 10 weeks with aflatoxin B_1 (0.2 mg per kilogram of body weight). Urine was collected for 24 hours after each injection, and pooled samples were subjected to extraction and purification as above.

The isolated compound had ultraviolet absorption maxima (in ethanol) at 267 and 362 nm; in ethanol-sodium hydroxide, the spectrum showed shifts in the maxima to 298, 337, and 420 nm. The mass spectrum resembled that of aflatoxin B_1, offset by 14 mass units, and indicated that the compound had a molecular weight of 298. Methylation of the metabolite with diazomethane produced a product having mass spectrum, ultraviolet spectrum, and thin-layer chromatographic behavior identical with those of an authentic sample of aflatoxin B_1. All of these properties are consistent with the conclusion that the compound is the phenol produced by demethylation of aflatoxin B_1. The structure of aflatoxin P_1 is shown in Fig. 1.

The existence of this metabolic product was predicted by our earlier observations on the metabolism of methoxy-labeled aflatoxin in the rat (*12*), which indicated that *O*-demethylation was a major route of metabolism of the toxin. The reason other investigators have not found aflatoxin P_1 in urine is probably that only a small fraction of the total amount in urine is present in unconjugated, chloroform-soluble form. Previous investigators have concerned themselves almost exclusively with that fraction of urinary extracts containing chloroform-soluble materials.

Our experiments indicate that aflatoxin P_1 represents at least 60 percent of the urinary aflatoxin derivatives; of this, about 50 percent is present as glucuronide, 10 percent as sulfate, and 3 percent as unconjugated phenol. Together, these represent more than 20 percent of an injected dose of aflatoxin B_1. Aflatoxin M_1 (4-hydroxyaflatoxin B_1), the only other known metabolite, accounted for only 2.3 percent of the dose administered to these monkeys.

Aside from their value in interpreting species differences in response, these findings have potential use in estimating aflatoxin exposures of human populations. At present, epidemiologic surveys dealing with relations between liver cancer and aflatoxin in foods rely on dietary surveys that involve systematic collection of food samples, analysis for aflatoxin content, and calculation of ingestion by estimation of food intake. Such surveys yield only semiquantitative information on group (rarely individual) exposure, and are expensive to apply to large populations.

If aflatoxin P_1 is a urinary metabolite of aflatoxin B_1 in man and its excretion is quantitatively related to aflatoxin B_1 intake, then exposure to the toxin could be estimated by analysis of urine samples. Such analysis has been attempted, with amounts of aflatoxin M_1 in urine being used as the index of ingestion (*5*), but the low recovery (1 to 4 percent) prohibited quantitative estimation of toxin intake. If man excretes aflatoxin P_1 in quantities comparable to those excreted by the monkey, this approach may be epidemiologically feasible.

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29 September 1970

Linkage Group–Chromosome Correlation in *Culex tritaeniorhynchus*

Abstract. *Linkage groups of a culicine mosquito,* Culex tritaeniorhynchus, *have been assigned to their respective chromosomes by genetic and cytologic observations of radiation-induced aberrations. Linkage group I is assigned to the smallest chromosome, linkage group II to the submetacentric chromosome, and linkage group III to the metacentric chromosome.*

A few translocations have been reported for two species of mosquitoes belonging to the subfamily Culicinae—*Culex pipiens* and *Aedes aegypti* (*1*). These studies are important because translocations and other chromosomal aberrations are generally associated with a high degree of sterility and thus are potentially useful as a method of genetic control. None of the above translocations have been identified with particular chromosomes of the karyotype. There has not yet been a successful assignment of linkage groups to their respective chromosomes in any culicine mosquito. Even sex has not been identified with a particular chromosome. This is somewhat surprising since these mosquitoes have only three pairs of chromosomes; thus, one would think that the assignment of linkage groups to chromosomes would be comparatively easy. With this thought in mind we exposed 130 males to approximately 3000 rad of gamma radiation from a ^{60}Co source in an attempt to produce a series of radiation-induced aberrations on genetically marked chro-

Table 1. Comparison of genetic observations and the associated chromosomal aberrations as seen in the testes and ovaries of the 62 lines of *Culex tritaeniorhynchus*. Abbreviations: (1), small chromosome only; (1-2L), small chromosome and short arm of submetacentric chromosome; (1-2R), small chromosome and long arm of submetacentric chromosome; (1-3), small chromosome and metacentric chromosome.

Genetic observations	Cytologic observations					
	(1)	(1-2L)	(1-2R)	(1-3)	"Normal" karyotype	Complex rearrangement
No crossing-over go^+ to M	15					1
Linkage Rs to sex		3			3	4
Linkage go^+ to Rs		7			1	1
Sex-linked sterility			10	15	2	

mosomes. The culicine mosquito selected for this study was the principal vector of Japanese encephalitis—*Culex tritaeniorhynchus*, for which some excellent mutant strains are available. Sex in *C. tritaeniorhynchus* and the other two species appears to be determined by a single pair of alleles for which the males are heterozygous, m/M, and the females homozygous, m/m (*2*). There are no dimorphic sex chromosomes in either sex. However, all three pairs of chromosomes can be distinguished cytologically. The smallest pair which is easily distinguished from the other two by its size (4.6 to 6.0 μm) is metacentric. The remaining pairs are equal in length (7.4 to 10.3 μm) but can be differentiated by the position of their centromeres (*3*). One pair is submetacentric (unequal arms) and the other is metacentric (Fig. 1A).

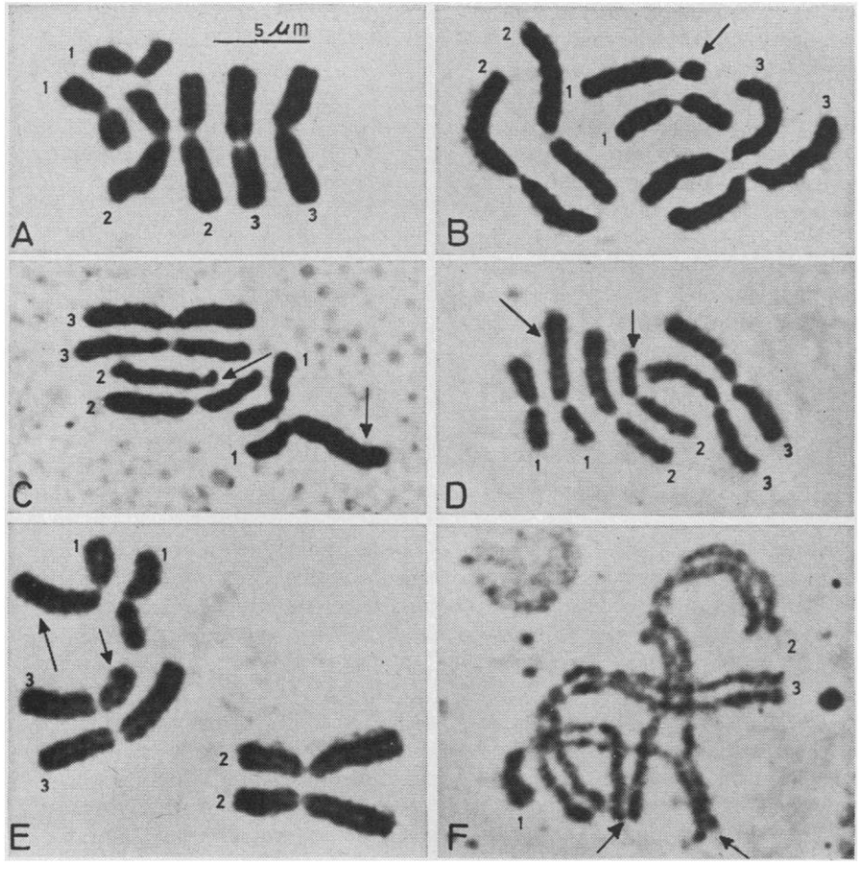

Fig. 1. Chromosomes from testes. Arrows point to chromosome or chromosomes involved. (A–E) Metaphase; (A) normal complement; (B) change in centromere position due to a pericentric inversion for smallest chromosome (1); (C) reciprocal translocation between chromosome 1 and short arm of submetacentric chromosome (2L): (D) reciprocal translocation between chromosome 1 and long arm of submetacentric chromosome (2R); (E) reciprocal translocation between chromosome 1 and metacentric chromosome (3). (F) Prophase chromosomes showing reciprocal translocations that involve segments of equal length to produce a karyotype that appears "normal" in metaphase configuration.

The experiments were designed to produce aberrations large enough to be detectable genetically and cytologically. We had hoped (i) to produce large inversions in linkage group I so that we could identify the sex chromosomes and (ii) to produce specific translocations between the sex chromosomes and the autosomes. For linkage group I, the markers used, golden (*go*) and sex (*M*), represent the extreme ends of the genetic map (25 to 27 map units) (*2*) as it is presently known. The only other marker used, red spotted eye (*Rs*), a conditionally codominant gene, is on linkage group II (*4*). Sixty-three lines that showed a characteristic sterility without exception or displayed new linkage relationships for eight generations (or did both) were saved. Although we found cases of autosome-autosome translocations, none of these were saved because we did not have appropriate markers to identify the phenotypes which carried the translocations.

The genetic observations could be grouped into four distinct types. Three involved new linkage relationships: nearly complete suppression of crossing-over between *go* and sex, *Rs* linked to sex, and go^+ linked to *Rs*. The fourth type showed no new linkage relationship, but was characterized by high sterility (33 to 70 percent) which was linked to sex and go^+. The cytologic observations for 62 of these strains are summarized in Table 1.

Sixteen lines were isolated in which there occurred complete or nearly complete suppression of crossing-over between *go* and *M*. Fifteen of these lines showed 27 to 37 percent sterility. Cytologic examination of testes revealed that the two larger pairs of chromosomes did not deviate from the description of the normal complement given above. However, the smallest pair displayed a striking dimorphism—both chromosomes were equal in total length but one was metacentric and its homolog was clearly submetacentric (Fig. 1B). This change in the position of the centromere strongly suggests the induction of a pericentric inversion. Examination of anaphase configurations did not show fragments or chromatid bridges. This is expected, because single crossovers within a pericentric inversion produce duplications and deficiencies but not the dicentric and acentric chromosomes characteristic of single crossovers within a paracentric inversion. Moreover, inversion loops involving the centromeres were found

in the prophase configurations of the smallest pair of chromosomes. The remaining line, which was characterized by 70 percent sterility, showed a complex series of aberrations involving the smallest pair and the long arm of the submetacentric chromosome. These observations strongly suggest that the two markers, *go* and *M*, are located on the smallest pair of chromosomes and that the centromere is between them. This chromosome has been designated as chromosome 1.

Nineteen isolated lines showed new linkage relationships involving *Rs*, a marker in linkage group II, and either sex or *go*+ (markers on linkage group I). Ten of the lines clearly showed exchanges between one homolog of the smallest pair (chromosome 1) and the short arm of one of the submetacentric chromosomes (Fig. 1C). Four lines were characterized by normal karyotypes, whereas five involved more complex aberrations.

Of the remaining 27 lines with no new linkage arrangements, 25 had cytologically detectable exchanges between chromosome 1 and either the long arm of the submetacentric chromosomes (Fig. 1D) or the metacentric chromosomes (Fig. 1E). The karyotypes which appeared normal probably represent reciprocal translocations involving nearly equal chromosomal segments. This was confirmed by cytological observations of prophase configurations which clearly showed chromosomal exchanges (Fig. 1F).

Except for these "normal" karyotypes, whenever *Rs* showed new linkage relationships with the markers on chromosome 1, there was a cytologically detectable exchange involving one homolog of the smallest pair of chromosomes and one of the short arms of the submetacentric pair. Conversely, in the lines characterized by sex-linked sterility but showing no new linkage relationships, the cytological observations showed an exchange between the smallest chromosomes and either the long arm of the submetacentric chromosomes or an arm of the metacentric chromosomes. These observations suggest that *Rs* is located on the short arm of the submetacentric chromosome. Therefore, we have assigned linkage group II to the submetacentric chromosome. The shorter of the two arms has been designated as 2L and the longer as 2R. In two earlier reports (*2*, *3*) the submetacentric pair was arbitrarily given the number 3 and the metacentric pair number 2; therefore, this designation should be reversed. Since we have tested linkage groups I and II and have assigned them to the small metacentric and the submetacentric chromosomes, respectively, it is not unreasonable to conclude that linkage group III, although not tested here, is associated with the large metacentric chromosome.

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9 September 1970

Sleep Stage and Personality Characteristics of "Natural" Long and Short Sleepers

Abstract. *A group of college freshmen that typically slept 5½ hours or less per night and a group that typically slept 9½ hours or more did not differ significantly on a battery of personality, scholastic, and medical measures. Compared with control subjects not selected on a sleep length criterion, electroencephalograph recordings of the short sleepers contained reduced amounts of stage 2 and rapid eye movement sleep.*

We reported earlier on the intrasleep characteristics of a group of "natural" long and short sleepers (*1*). This report discusses certain psychological and physical characteristics of another sample of "natural" long and short sleepers and presents additional electroencephalograph (EEG) data on a portion of these short sleepers.

For two successive years (1968 and 1969), entering freshmen at the University of Florida were asked to designate which of several categories best described the length of their typical sleep period during the previous year. Table 1 presents the results of these questionnaire responses for individuals 18 years old or under. There is a significant chi-square associated with these data ($\chi^2 = 27.14$; $P < .001$). The proportionately larger number of females (or smaller number of males) in the 5½- to < 6½-hour sleep category contributed most substantially to this finding.

An attempt was made to contact all subjects who reported sleeping less than 5½ or more than 9½ hours per night, and the following numbers of available subjects were interviewed: short sleepers: 13 (1968), 12 (1969); long sleepers: 21 (1968), 37 (1969). On the basis of these interviews, 3 short sleepers and 26 long sleepers were eliminated from the investigation. Subjects were withdrawn from the sample if they had failed, in the opinion of the interviewer, to maintain a pattern of long or short sleep during the previous year or if they had significantly modified their sleep length under the impact of entering college and, hence, gave evidence of a lack of stability of the sleep pattern. The shortening of sleep length upon entrance to college accounts primarily for the considerably higher

Table 1. Sleep length category response frequencies, with expected values given in parentheses.

Sleep length (hours)					
< 5½	5½ to < 6½	6½ to < 7½	7½ to < 8½	8½ to < 9½	> 9½
		Men			
32	157	661	1160	297	82
(26)	(192)	(697)	(1112)	(285)	(77)
		Women			
17	198	630	899	230	61
(23)	(163)	(594)	(947)	(242)	(66)