frequency in the electric basic potentials, described by Kao[5] in myometers of pregnant rats in the same stage of gestation.

The simple puncture made in the amniotic sac, without withdrawing the fluid, provoked only an increase in wave frequency (up to 7/min), the mean pressure value remaining stable (figure 1, A).

After fluid withdrawal (0.04 ml) from the amniotic cavity, the record baseline fell and a series of irregular waves began, similar in frequency to those caused by simple needle introduction but about twice as high in amplitude (figure 1, B). The reintroduction of the fluid previously withdrawn brought about an immediate decrease in both frequency and amplitude of pressure waves. Further subtraction of the amniotic fluid (0.04 ml) in the same sac determined a new contraction whose amplitude and frequency waves were similar to those recorded during the first amniocentesis (figure 1, C).

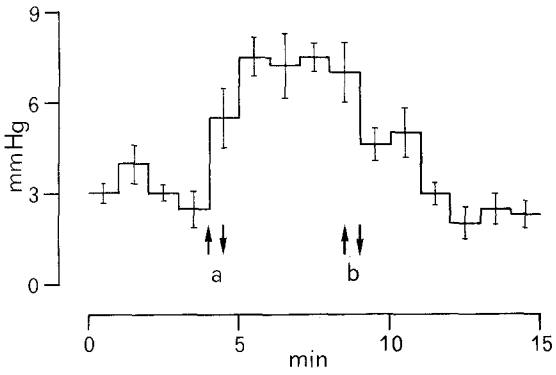


Fig. 2. Increase of endouterine pressure following amniocentesis, in Wistar rats at 15th day of gestation. Mean values of endouterine pressure for successive 1-min periods. Vertical bars give the SE of respective mean values. Between arrows, a, amniocentesis (0.04 ml); b, previously withdrawn fluid reintroduction. Abscissa: time; ordinate: endouterine pressure.

In 35 withdrawal and reintroduction tests, mean values of uterine pressure were obtained for successive 1-min periods and their SE evaluated (figure 2).

According to the results of the present research, amniocentesis induces an increase of mean endouterine pressure. This effect cannot be related to mechanical injuries, because they provoke only a transitory increase in pressure wave frequency, while an increased mean pressure value has never been observed. Admittedly, this increase in mean pressure ought to be determined by fluid withdrawing.

In previous works, fetal malformations have been observed when fluid withdrawing performed with amniocentesis was permanent. In fact, immediate reintroduction of fluid was able to eliminate the pathological effect. Needle puncture alone was also unable to induce malformations[4].

These observations support the hypothesis that fetal alterations are brought about by amniocentesis considered as volume reduction, which could provoke, through the increment of endouterine pressure, the histologically observed haemorrhagic processes and the fetal malformations. The mechanism involved in such a pathological event is not clear. The teratogenetic effect of fluid reduction as a nutritive element does not appear to play a role in this pathogenesis.

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## Sex chromosome aberrations involving loss and translocation of tumor-inducing loci in *Xiphophorus*[1]

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*Summary.* Karyotypic changes involving a deletion and a translocation of certain sex-linked tumor-inducing loci of the platyfish, *Platypoecilus (Xiphophorus) maculatus,* have been investigated. The effects of these chromosome aberrations on tumor formation and viability of the fish are discussed.

Several well-investigated melanophore spot pattern determining loci, including Sd (spotted dorsal fin) and Sr (stripe sided body), involved in melanoma formation, have been located on the sex chromosomes of the platyfish, *Platypoecilus maculatus*[2-4]. In addition to the melanophore pattern loci, certain pterinophore determining loci are also located on the sex chromosomes. In a certain strain of platyfish, the X-chromosome carries the Sd locus and a pterinophore locus Dr (red dorsal fin), while the Y-chromosome carries Sr and another pterinophore locus Ar (red anal fin)[5]; the females are genotypically X-Dr Sd, X-Dr Sd, while the males are X-Dr Sd, Y-Ar Sr. Spot-specific melanomas develop on the fish of appropriate genotype probably due to enhanced expression of Sd (on the dorsal fin) or Sr (on the body side), following deletion or impairment of their controlling genes through selective matings or exposure to irradiation[6]. Genetic and phenotypic analysis has revealed the presence of several aberrations of the sex chromosomes of the platyfish[5], which have occurred in several different strains, and these are maintained in a balanced state in this laboratory. So far these sex chromosome abnormalities have not been cytologically characterized in terms of the size of the chromosome fragment involved and the position of the translocated material. In this report we examine the cytological nature of a deletion involving the Sd locus, and an X-Y translocation involving the Sr locus, and the genetic consequences of these sex chromosome aberrations on the tumor-potential and viability of the platyfish.

Cells for the chromosome spreads were derived from a relatively simple short-term fin explant culture method[7]. The explants were grown on a cover-glass in a culture medium containing medium 199 and calf serum (5:1, by volume), plus penicillin and streptomycin. Chromosome spreads were obtained by treating the cells, following a growth period of 48–72 h, with 0.04 mg/ml colchicine for 3 h, a 30-min treatment with a hypotonic (0.85% sodium citrate), fixation in cold methanol:acetic acid (2:1), air drying the cover-glass with the cells, a short treatment in

0.05% trypsin, and finally staining the chromosomes with Giemsa. At least 6 cells with a modal configuration were analyzed in each genotype.

Karyotypic analysis of the normal platyfish has revealed (figure 1) that the X-chromosome is metacentric, while the Y is acrocentric and nearly half the size of the X. The 23 pairs of autosomes are all acrocentric, as reported earlier[8]. The metacentric X-chromosome showed 2 major Giemsa (G) bands on each arm (figure 2, a), while the Y-chromosome exhibited only 2 major bands (figure 2, b). Analysis of the Sd deletion (females X-Dr $Sd^{del}$, X-Dr $Sd^{del}$, and males X-Dr $Sd^{del}$, Y-Ar Sr) has revealed a loss of one major terminal G-band of the X-chromosome (figure 2, c), which may represent a deficiency of up to a quarter of the X-chromosome. Considering the fact that the size of the X-chromosome in an average metaphase plate is about 2 µm, the Sd chromosome fragment lost due to deletion is estimated to be 0.3–0.5 µm in length. Clearly the deletion represents a large piece of the X-chromosome, which may carry many loci other than Sd. The genetic consequences of the deletion involving the Sd locus are that neither females (X-Dr $Sd^{del}$, X-Dr $Sd^{del}$) nor males (X-Dr $Sd^{del}$, Y-Ar Sr) exhibit the black spot on the dorsal fin. Nor do these animals develop melanomas on the dorsal fin following exposure to a chemical carcinogen[9]. Also, the hybrids between the Sd deletion platyfish and the swordtail, *Xiphophorus helleri*[5], behave in this way. On the other hand, loss of the Sd chromosome fragment leads to significant increase in the pteridines determined by the Dr locus in the platyfish and its hybrids to swordtail[10]. It is suggested that the Sd deletion segment may also carry modifiers or regulating genes that control the expression of Dr, and their loss along with Sd leads to enhanced expression of Dr. Alternatively, the product of the Sd gene may, in some way, be inhibitory to the expression of Dr, and loss of Sd may lead to enhanced expression of Dr. In the light of these observations, it would appear that Dr and Sd loci are located on the same arm of the X-chromosome, but their precise linkage relationship cannot be specified. The effect of the Sd deletion segment on all life processes of the platyfish has not been fully investigated. However, this rather large deficiency of the X-chromosome in the homozygous condition in the female or in the hemizygous state in the male apparently has no detectable effect on viability.

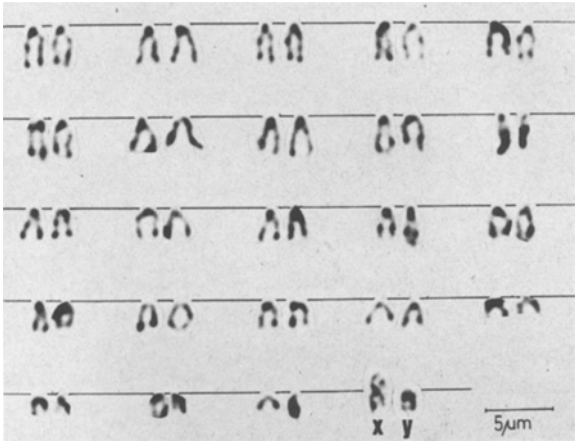


Fig. 1. Karyotype from a platyfish male showing 23 pairs of acrocentric autosomes, a metacentric X-chromosome and an acrocentric Y-chromosome (last pair).

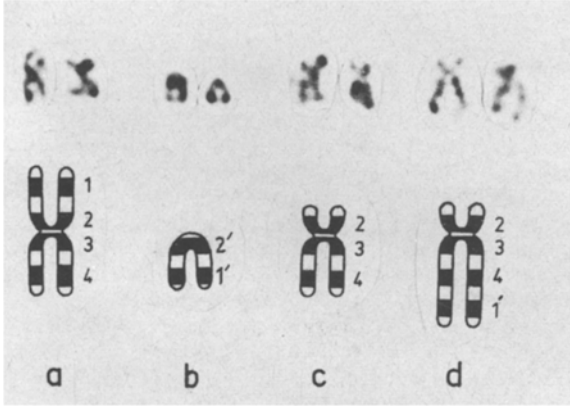


Fig. 2. Normal and aberrant sex chromosomes of platyfish (top row; the chromosomes in each pair are derived from 2 different animals), and their schematic diagrams (bottom row). *a* Normal metacentric X showing 4 G-bands; *b* normal acrocentric Y showing 2 G-bands; *c* Sd deletion X-chromosome lacking a terminal G-band 1, and *d* Sd deficient X which lacks G-band 1, with an Sr translocation from the Y-chromosome showing an extra terminal G-band 1′ on the normal arm. It appears that Sd is located in or near the terminal G-band 1 and Dr in or near the G-band 2 of the X, while Sr is located in or near the terminal G-band 1′ and Ar in or near the G-band 2′ of the Y.

The 2nd chromosome abnormality involves an interchange between the X- and the Y-chromosome, in which a fragment carrying the Sr locus from the Y has been translocated onto the Sd-deficient X-chromosome. It was previously suggested[5] that the Sr locus is translocated next to the Dr locus on the Sd-deficient X-chromosome. However, karyotypic analysis has revealed that the Sr fragment is most likely translocated to the end of the normal arm of the X-chromosome, and not to the deficient arm, since there is an additional major G-band on the normal arm (figure 2, d). The phenotypic consequences of this translocation are as follows. Since in the translocation stock the Sr locus is present in the male (X-Sr Dr $Sd^{del}$, Y-Ar Sr) as well as the female (X-Sr Dr $Sd^{del}$, X-Sr Dr $Sd^{del}$), both sexes are stripe-sided. Although the translocation females are stripe-sided, they do not exhibit the anal red, Ar, expression, indicating that the region of the terminal G-band translocated from the Y carries only the Sr but not the Ar locus. Earlier studies have suggested[6] that Sr expression is controlled by a linked regulating gene. In the present situation, introduction of the Sr locus through translocation into the female (which normally neither has Sr, nor its controlling gene) does not lead to melanoma development. Thus it would appear that the controlling gene, which presumably governs the expression of Sr, is translocated along with Sr and therefore must be closely linked to it. This is confirmation of a previous result[6]. Further, the Sr translocation does not affect the viability of either sex.

In the framework of a recent genetic model on carcinogenesis[11], the tumor-inducing loci, such as Sd or Sr, may be designated as Tu loci. Their expression is presumably controlled by a system of tissue-specific regulating genes (R-genes), which may be nonlinked and/or linked to the Tu. Impairment or deletion of the R-genes could conceivably release the Tu genes from the restraint of suppression, and trigger a chain of events which eventually lead to onset of tissue-specific neoplasms. On the other hand, loss of Tu genes would lead to loss of potential for the formation of neoplasms.

Specific chromosome abnormalities have been found to be associated with chemically[12–14] and virally[15] induced animal tumors, and with certain human neoplasms[16–18]. We have considered the possibility[19] that these human neoplasms and animal tumors, associated with specific chromosome aberrations, might arise as a result of a change in state, or a deletion, of tissue-specific R-genes which control Tu ex-

pression in those tissues. Thus, impairment or deletion of R-genes, on the one hand, presumably leads to neoplatic development, whereas loss or impairment of the Tu genes in an animal or man, on the other hand, would lead to loss of tumor-inducing potential in a specific tissue; and the loss of Tu genes might represent a potential mechanism involved in reversal from hereditary tumors to normal state.

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## Activation de l'adénylate cyclase et ses répercussions sur le taux intracellulaire d'AMP cyclique dans les cellules KB infectées produisant du virus Sendaï infectieux sous l'influence de la trypsine

## Adenylate cyclase activation and its effects on intracellular cAMP in infected KB cells during trypsin-induced infectious Sendaï virus production

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*Summary.* KB cells infected by Sendaï virus can produce infectious virus if they are trypsinated twice over 24 h. Adenylate cyclase activity in infected KB cells is higher and more strongly activated by trypsin than that of control cells, but intracellular concentration of cAMP is the same, except during a short time after trypsinations, especially after the second trypsination which causes infectious virus production. During this short time, intracellular cAMP is slightly higher in infected cells. This miseffect of adenylate cyclase activation on intracellular cAMP concentrations might be related to an increased cell permeability caused by trypsin.

Il est connu depuis les travaux de Homma[1-3] puis de Scheid et Choppin[4] que l'infection des cellules en culture par le virus Sendaï, n'entraine pas la production de virus directement infectieux pour les autres cultures de cellules où pour l'embryon de poulet, parce que, dans ces conditions, la protéolyse d'une glycoprotéine de la membrane externe du virus - étape indispensable à la maturation du virus infectieux - ne se produit pas spontanément comme c'est le cas lorsque le virus est cultivé sur embryon de poulet.

Nous même en 1964[5] avions montré que, dans des conditions appropriées, des traitements par la trypsine des cellules KB infectées par du virus Sendaï, provoquaient la production d'une importante quantité de particules virales infectieuses.

Le fait que, d'une part, la présence des antigènes viraux entraine des changements morphologiques[6] au niveau de membrane plasmatique des cellules où est localisée l'adenylate cyclase et que, d'autre part, Ryan[7] puis nous même[8] aient montré que la trypsine activait l'adénylate cyclase des cellules traitées, nous a conduit à étudier les variations d'activité de cet enzyme et ses répercussions au niveau des taux intracellulaires d'AMP cyclique (cAMP) au cours des étapes, conditionnées par les trypsinations, qui permettent d'obtenir une importante quantité de virus infectieux à partir de cellules KB infectées mais initialement non productrices de virus sous forme infectieuse.

*Matériel et methodes.* Détermination de l'activité de l'adénylate cyclase (pmoles cAMP/min/mg protéine). Après rinçage des tapis cellulaires avec du milieu Tris 25 mM, saccharose 0,25 M, $MgCl_2$ 1 mM, pH = 7,6, les cellules sont décollées par agitation de billes de verre et broyées au Potter, puis l'activité est déterminée selon une méthode adaptée de celle de White et Zenzer[9] que nous avons précédemment décrite[8,10]. Elle est dans tous les cas, proportionnelle au temps d'incubation avec l'ATP jusqu'à 20 min et à la concentration en protéine jusqu'à 1 mg.

Dosage du cAMP. Après rinçage des tapis cellulaires 3 fois avec NaCl 9‰, le cAMP est extrait par 2 ml d'acide trichloracétique à 5%. Les cellules grattées et détachées sont éliminées par centrifugation 10 min à 7000 × g. 6 traitements par 4 volumes d'éther saturé d'eau évaporés sous azote permettent d'éliminer l'acide. Les extraits aqueux lyophylisés sont remis en suspension dans $KH_2PO_4$ 200 mM, $MgCl_2$ 100 mM et caféine 100 mM, pH = 6,6 pour être dosés en fonction d'une gamme d'étalonnage établie, lors de chaque dosage, par dilution isotopique selon la méthode de Gilman[11]. Le rendement d'extraction, calculé avec ($^3$H)-cAMP comme étalon interne est 80±3%.

*Résultats.* La figure met parallèlement en évidence les variations d'activité de l'adénylate cyclase et du taux intracellulaire de cAMP. On peut remarquer tout d'abord que l'adénylate cyclase des cellules infectées a, dans l'ensemble, une activité un peu plus forte que celles des cellules témoins alors que l'on n'observe pas de différence dans les taux intracellulaires de cAMP à l'exception d'une période de quelques heures qui suit les trypsinations. On peut