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The antifertility action of α-chlorohydrin: metabolism by rat and boar sperm[1]

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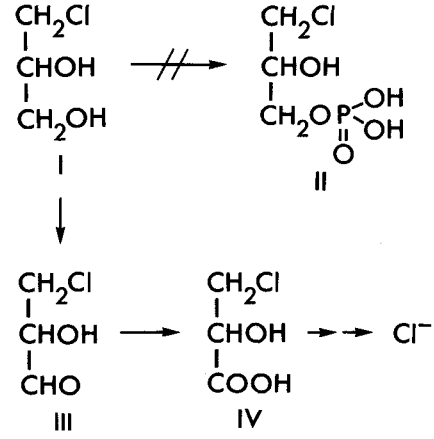
Summary. The male antifertility agent α-chlorohydrin (**I**) is metabolized by rat and boar sperm to β-chloroactaldehyde (**III**), β-chlorolactate (**IV**) and Cl^- and not to the proposed active metabolite, α-chlorohydrin-l-phosphate (**III**). It is proposed that β-chlorolactaldehyde is produced intracellularly by a specific enzyme and that this is the metabolite responsible for the species-specific antifertility activity of α-chlorohydrin.

The current theory[3] for the mechanism of action of α-chlorohydrin (3-chloropropan-1,2-diol, **I**) as a male antifertility agent is that, upon entry into sperm, it is converted by glycerol kinase to α-chlorohydrin-l-phosphate (**II**). This metabolite is proposed to inhibit glyceraldehyde-3-phosphate dehydrogenase and triosephosphate isomerase thereby inhibiting glycolysis and resulting in a decreased production of ATP. When ejaculated, the sperm are morphologically normal but have such diminished motility that fertilization can not be achieved.

Our studies into the metabolism of α-chlorohydrin by rat and boar sperm have led us to question this hypothesis, for when sperm were incubated with ^{36}Cl-α-chlorohydrin, ^{36}Cl-α-chlorohydrin-l-phosphate[4] could not be detected. Furthermore, glycerol kinase[5] was found to be unreactive towards α-chlorohydrin, a result which has recently been confirmed by Brooks[6]. It is known that α-chlorohydrin per se is not the inhibitory agent of sperm glycolysis as a period of pre-incubation is required before an inhibitory effect is evident in vitro[7]. On the basis that this indicates the production of an active metabolite of α-chlorohydrin, we have investigated the comparative metabolisms of ^{36}Cl-α-chlorohydrin by rat, boar and rabbit sperm, and by isolated kidney tubules of each species.

Results. Sperm obtained from the cauda epididymides of mature boars within 1.5 h, or adult rats or rabbits within 0.5 h of sacrifice, were washed with phosphate-buffered saline[8] (PBS) (pH 7.4) and centrifuged. This washing procedure was repeated twice. In a typical experiment, a suspension of rat or boar sperm in PBS (5 ml, containing 20–40 mg protein ml^{-1}) was incubated at 34 °C with 1 mM fructose and 100 μM ^{36}Cl-α-chlorohydrin[9] for 2 h, then centrifuged. When assayed by TLC (silica gel G plates developed in chloroform:methanol 7:3 followed by radiochromatogram scanning), the supernatant was found to contain ^{36}Cl-α-chlorohydrin (R_f 0.55) and trace amounts of $^{36}Cl^-$ (< 5%) as the only radioactive constituents. The pellet was suspended in PBS (1 ml), the cells disrupted by sonic oscillation (20 kHz for 2 min) and centrifuged. When this supernatant was examined by TLC, 4 radioactive components were detected. Three of these were identified as described previously[10]; they were Cl^- (R_f 0.10), β-chlorolactate (**IV**) (R_f 0.25) and α-chlorohydrin. The 4th component had a polarity between the latter 2 compounds at R_f 0.47 and was base labile, indicating that it possessed the α-halohydrin structure. When the supernatant was treated with 2,4-dinitrophenylhydrazine (2,4-DNP) reagent[11], extracted with ethyl acetate and the extract examined by TLC, the compound at R_f 0.47 was absent but was replaced by a ^{36}Cl-2,4-DNP derivative at R_f 0.66, corresponding to authentic[10] β-chlorolactaldehyde-2,4-DNP. There was no metabolism of α-chlorohydrin by rabbit sperm or by suspensions of isolated kidney tubules of the boar, rat or rabbit.

Glycolytic inhibition studies were performed in conventional Warburg flasks with 1 ml cell suspensions containing D-[U-^{14}C]fructose (1 mM) and α-chlorohydrin (10 mM). Sperm suspensions (20–40 mg protein ml^{-1}) or isolated kidney tubules[12] (4–15 mg protein ml^{-1}) were incubated at 34 °C and 37 °C, respectively, for 1 h and the $^{14}CO_2$, trapped in 2 M NaOH, was assayed by established procedures[8]. The inhibition of $^{14}CO_2$ production was found to be 91% with boar sperm and 67% with rat sperm. There was no effect on



The metabolism of α-chlorohydrin (**I**) in rat and boar sperm. The phosphorylated derivative (**II**) is not produced, instead oxidation gives β-chlorolactaldehyde (**III**), β-chlorolactate (**IV**) and Cl^-.

the production of $^{14}CO_2$ by rabbit sperm or by kidney cells obtained from rat, boar or rabbit.

Discussion. The isolation of β-chlorolactaldehyde (**III**), by means of its 2,4-DNP derivative, as a primary metabolite of α-chlorohydrin indicates that rat and boar sperm are capable of oxidatively metabolizing α-chlorohydrin via the aldehyde (**III**) to β-chlorolactate (**IV**). That rabbit sperm and isolated kidney tubules of the rat, boar or rabbit do not carry out this conversion is interesting since there is a direct correlation with the ability to oxidize α-chlorohydrin and an inhibitory effect on glycolysis.

We propose that the metabolite of α-chlorohydrin which inhibits glycolysis is not α-chlorohydrin-1-phosphate (**II**) but is β-chlorolactaldehyde (**III**), produced by an enzyme with α-chlorohydrin dehydrogenase activity. If this enzyme is present in certain cells (such as rat or boar sperm) it would explain why α-chlorohydrin is not a general inhibitor of glycolysis and why it has species-specificity as an antifertility agent. (α-Chlorohydrin has antifertility activity in the rat and the boar but is ineffective in the rabbit[3]). Mechanistic considerations also favour the presence of an aldehyde group in an inhibitor of glyceraldehyde-3-phosphate dehydrogenase and triosephosphate isomerase as both enzymes require this functional group for active site attachment[13]. The α-chlorohydrin dehydrogenase activity of boar sperm and the synthesis of β-chlorolactaldehyde are being investigated.

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An unusual case of a complex heterozygote presenting no taxonomical problem in *Chelidonium majus* L. (Papaveraceae)

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Summary. Chelidonium majus shows a closed ring of 12 chromosomes at meiosis. The maintenance of this translocation heterozygosity is assured by a balanced combination of lethal alleles. The fertility is estimated to be 60–70%. In spite of its genetic system and its wide distribution, the genus is monospecific.

Permanent translocation heterozygosity is rather rare, being well known especially in *Rhoeo spathacea*[1] and in a few diverse genera of the family Onagraceae[2]. In some of these genera, namely *Calylophus, Gaura, Gayophytum* and *Oenothera* and in *Rhoeo* also, all the chromosomes are united at meiosis in one giant circle[3]. In some species, entire populations consist of permanent, true-breeding hybrids, since balanced lethal combinations of alleles have been developed which inhibit the union of similar gametes. Another way in which translocation heterozygosity is maintained was recently described: a sex-linked translocation system in Viscaceae[4] and in termites[5–7].

Here, we report a case of permanent translocation heterozygosity involving the entire chromosome set in *Chelidonium majus* L. (Papaveraceae). This plant is widespread (Asia, Europe and North Africa). It is used in traditional medicine against warts. Nagao and Sakai[8] observed in this species from Japan a closed ring of 10 chromosomes (2n = 10). The arrangement on the meiotic metaphase plate was usually zig-zag. They also observed 68.9% of empty pollen and 71.8% of empty seeds. They explained this sterility by the aberrations occurring in meiotic divisions, but gave no information on the breeding behavior.

In our case, the plants studied (from 2 Belgian populations, collected at Thon and Marche-les-Dames in the Meuse valley-Vouchers at the national herbarium of Bruxelles-BR-Tilquin, 421 and 422) are cleistogamous, the anthers bursting over the receptive stigma as much as 24 h or more before the flower opens (anthesis). The dehiscence of the buds is stimulated by the growth of the fertilized ovary.

Furthermore, our observations are quite different. The somatic chromosome number is 2n = 12 instead of 10. All the chromosomes are also united at meiosis in one ring (figure, 1 and 2). At metaphase I, the ring shows a regular alternate orientation. Counts in anaphase I and telophase I confirm the regular numerical disjunction. Occasionally a laggard is visible in telophase I, but this is probably due to the squash technique.

All the products of male meiosis seem to develop into normal pollen grains (figure, 3 and 4) instead of 69% of empty pollen. Furthermore, we did not observe empty seeds. The mature capsules are well filled, given the appearance of full fertility. But in examining the young capsules, we could nevertheless observe aborted ovules distributed at random among the developing ovules. The fertility is now estimated at 60–70%. We plan extensive further analyses of the fertility. Indeed, the maintenance of heterozygosity must be assured by a balanced lethal system which involves a 50% reduction in seed set. 2 kinds of lethality are possible: the zygotic and the gametic. If the departure from 50% fertility is significant and confirmed, then lethality should be of the gametic type, probably associated with a system of megaspore competition known as the Renner effect[3] and found in some *Oenothera*. This system is the more elaborate one and restores full fertility. Basically, the functional megaspore in each ovule is that