time relation in fluctuations of corticosterone and TP would coincide.

Figure 1B shows that the circadian rhythm of TKT is not appreciably altered after adrenalectomy. There appears to be a lowering of both the maximum and minimum points in the curve; however, this is not statistically significant. Our experiments indicate that adrenal hormones do not play a major role in determining the circadian rhythmic pattern of TKT. Our results are in agreement with the findings of Wurtman and Axelrod (*7*) who also studied the circadian variation of rat liver TKT. Our findings imply that caution is necessary before assuming that a hormone, capable of inducing an enzyme when administered in pharmacological amounts, is the primary physiological inducer of the enzyme. The natural inducer or inducers of TKT remain to be determined.

It has been shown that TKT activity is increased after starvation, whereas TP activity is decreased (*8*). In man, starvation produces significant increases in glucagon (*9*). Studies in this laboratory have shown that glucagon can act as an inducer of TKT activity, but not as an inducer of TP activity, independently of the pituitary adrenal axis (*4*). Thus, it is possible that glucagon may be one of the hormones involved in the control of the circadian rhythm of TKT.

The other liver transaminase studied, PPT, does not show any circadian variation. This enzyme is not significantly changed in 4 to 6 hours after glucocorticoids and glucagon (*4*), the period at which the maximum increase in both TP and TKT activities is obtained. Thus, there appears to be a relation between an enzyme's short-term inducibility by exogenous inducing agents and its natural circadian variation.

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Gynogenesis and Triploidy in the Viviparous Fish Poeciliopsis

Abstract. *Three all-female strains of the viviparous fish* Poeciliopsis *occur in the Río Fuerte of Sinaloa, Mexico.* Poeciliopsis lucida, *a bisexual species, provides sperm for these monosexual forms which I designate as Cx, Cy, and Cz. Form Cy is a triploid that when test-mated to males of various species produces all-female, triploid offspring devoid of paternal characters. Both Cx and Cz are diploid and express characteristics of both parents.*

Poeciliopsis lucida (order Cyprinodontiformes, family Poeciliidae) is a viviparous fish of northwestern Mexico. Associated with it is an all-female form designated as Cx. Males of *P. lucida* provide sperm for both the *P. lucida* females and the monosexual Cx form. This mating triangle occurs in all three rivers inhabited by *P. lucida*, namely, the Ríos Mocorito, Sinaloa, and Fuerte (*1*). On the basis of external morphology there is little difference in the Cx-*lucida* complex from one river system to the next. Hybridization experiments with distantly related species of *Poeciliopsis* now reveal that the monosexual form in the Río Fuerte actually consists of at least three distinct all-female types, Cx, Cy, and Cz. All of these produce exclusively female progeny when mated to males of *P. lucida* in the laboratory and apparently rely mainly on *P. lucida* sperm for fertilization in nature. Other species of *Poeciliopsis* that occur in the Fuerte are *latidens, monacha,* and *prolifica* (*1*).

Form Cx has been studied most extensively. After 16 laboratory generations from matings of Cx with *P. lucida* giving rise to over 800 offspring, the first male has yet to be produced. Form Cx mates successfully with the males of nine other species of *Poeciliopsis.* As long as the paternal species is one that is closely related to *P. lucida*, the offspring are all females; but when males are from other species groups, progeny of both sexes are produced. Sex ratios vary, however, from one mate combination to another. Since all offspring from Cx females exhibit characteristics of both parents, regardless of the father, they must be considered true hybrids and not the product of parthenogenesis or gynogenesis. Paternal chromosomes of the F_1 generation, however, are not transmitted through the ova; only those characteristics of the female germ line pass to the next generation (*2, 3*).

One species particularly useful for tracing the flow of genetic material in crosses with monosexual forms is *P. latidens*, which is boldly marked with black bars and spots and has dense black pigment about the genitalia. These characters are in stark contrast to the uniform olive coloration and sparse genital pigment of the monosexual forms and *P. lucida.* Other equally contrasting traits are found in the shapes of the jaws, lips, and teeth. When Cx and *P. latidens* are crossed (Fig. 1), the F_1 generation has fewer spots than *P. latidens* and no bars; in other characters it is more or less intermediate (*3*).

Stocks of Cz and Cy perpetuated in the laboratory by matings with *P. lucida* males were thought to be Cx until individuals were mated to *P. latidens.* Form Cz, instead of giving birth to progeny of both sexes, as Cx does, produced only males. In matings to Cz, Cy, *P. lucida*, and *P. latidens* these males failed to reproduce; they probably are sterile. Although males from matings of Cz with *P. latidens* have a few weakly expressed *P. latidens* traits, they also have characteristics of *P. monacha*, another species that lives in the Fuerte. This suggests that Cz has had a hybrid origin that involved *P. monacha.* The reproductive mechanism that results in these all-male progeny has not been determined. The third all-female form, Cy, when mated to *P. latidens*, produces only female offspring morphologically indistinguishable from the mother. It is with these that this investigation is primarily concerned.

The original laboratory stocks of Cy and Cz are each descendants of

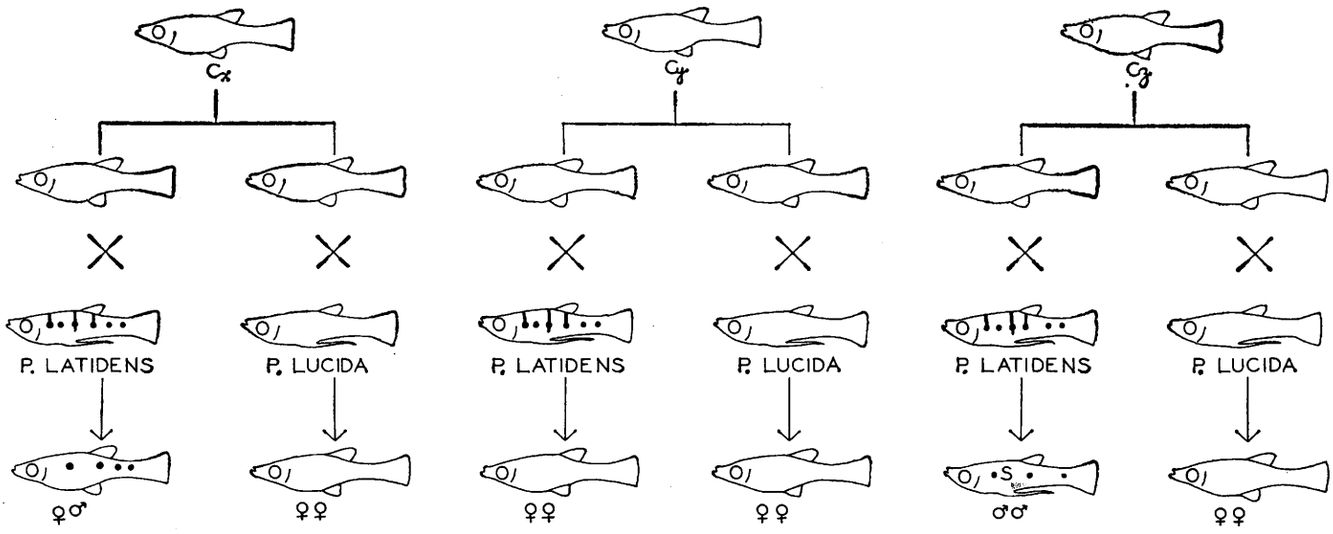

Fig. 1. Progeny types produced by the monosexual forms Cx, Cy, and Cz when mated to males of *Poeciliopsis latidens* or *P. lucida*. (*S*) Sterile male with a black wedge above the gonopodium, a characteristic of *P. monacha*.

single, wild females. Nonetheless, these lines represent successful strains that appear to be a significant proportion of the natural population rather than anomalous isolates of Cx. Females of *P. lucida* can be distinguished from the monosexual forms by their longer, more slender genital papillus and by a band of black pigment that surrounds the anterior margin of the anus; this band is absent in the all-female forms. As an example of the proportion of monosexuals to *P. lucida* in natural populations, one collection contained 74 adult females of *P. lucida* and 174 adults of the monosexual forms. Since, as yet, I am unable to distinguish among the three all-female forms, mating tests were used to acquire some notion of their proportions. Thirteen lines of Río Fuerte all-females were established in the laboratory and perpetuated by mating them to *P. lucida* males. Females from each line were mated to males of *P. latidens*: (i) Six lines produced only female progeny identical to the mother and thus were Cy; (ii) five produced only males and were Cz; and (iii) two produced offspring of both sexes typical of Cx. Although the presence of Cy and Cz in the Ríos Mocorito and Sinaloa has not been verified, both apparently are well established in the Río Fuerte.

To assess the consistency with which Cy produces only female offspring, the lines of Cy identified by the *P. latidens* mating test were maintained in the laboratory for various lengths of time by matings with males of *P. lucida*. Two lines were kept for two generations, two others for three generations, one for 14 generations, and one for 16 generations. Over 400 young

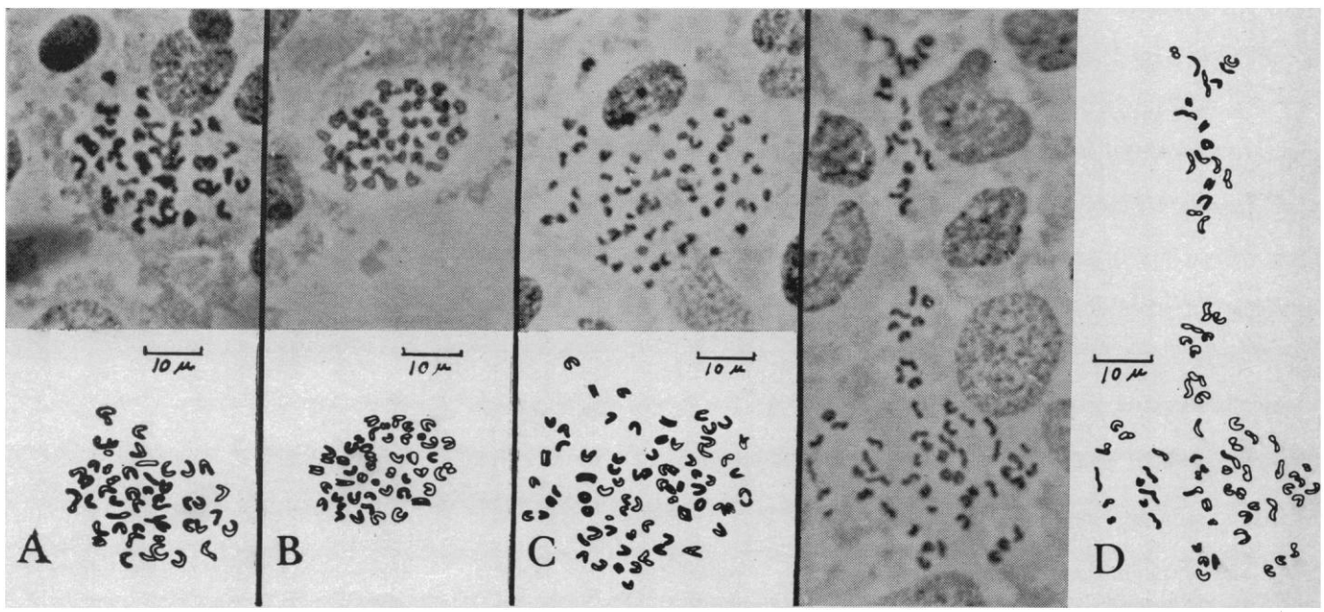

Fig. 2. Mitotic figures in epithelial cells of (A) *Poeciliopsis lucida*, diploid number 48; (B) *P. latidens*, diploid number 48; (C) Cy, triploid number 72; and (D) offspring of Cy mated with *P. latidens*, triploid number 72.

Table 1. Progeny produced from crosses of Cy with *P. latidens*. Stocks of Cy (up to 15 generations) used in the crosses were maintained in the laboratory by matings with males of *P. lucida*. All of the F_1 offspring produced were females. In all, 15 females were tested, and 134 females were produced.

Generation of female	Different lines represented	Females tested (No.)	Female progeny produced (No.)
F_1	4	4	40
F_2	2	2	17
F_3	1	1	5
F_4	1	1	14
F_{11}	2	4	29
F_{12}	1	1	13
F_{15}	1	2	16

were reared to sexual maturity; all were females. The mechanism that results in all-femaleness in Cy thus is not capricious.

To demonstrate that successive generations of *P. lucida* matings do not alter the matroclinous inheritance of Cy, females of the F_4, F_{11}, F_{12}, and F_{15} generations were mated to males of *P. latidens*. Each produced exclusively female offspring none of which showed the slightest trace of *P. latidens* characteristics (Table 1). Two F_1 females were also backcrossed to *P. latidens*. One produced 22 young and one 7 young, all morphologically identical to their mothers and their grandmothers.

The consistent failure of *P. latidens* characters to appear in the offspring from matings of Cy with *P. latidens* suggests that Cy reproduces gynogenetically. The sperm simply stimulate the ova to develop; no gametic fusion occurs. Thus, Cy would resemble another poeciliid, *Poecilia formosa*, in which sperm from *Poecilia latipinna* or *P. sphenops* provides the stimulus for development. Progeny of *P. formosa* are all identical to the mother (*4*).

The possibility was considered that union of Cy and *P. latidens* gametes did not occur because of the distant relationship of Cy and *P. latidens*. If so, Cy could have two modes of reproduction depending on the kind of sperm provided. With *P. latidens* sperm, gynogenesis would be the rule but with *P. lucida* sperm, true hybrids possessing chromosomes from both parents might occur as in Cx and Cz. Although *P. lucida* has no marker genes that can be used to establish paternity in the offspring, F, an undescribed species closely related to *P. lucida* has a suitable marker in the form of a black spot at the posterior base of the dorsal fin. *Poeciliopsis lucida* and F are sufficiently similar to produce completely fertile hybrids, the viability of which is only slightly impaired. When clear-finned females of either Cx or *P. lucida* are mated to spot-finned F males, the spot is transmitted to all of the young (*2*). The spot is also passed on to progeny of Cz mated with F. In a cross between the clear-finned Cy and a spot-finned F, however, the spot was not transmitted to the F_1 generation: all 14 of the young produced in this cross were clear-finned females. Thus the proximity of relationship between Cy and its various mates does not alter the mode of reproduction.

Apparent absence of gametic fusion suggested examination of the chromosome number of Cy. Chromosomes were counted in gill epithelium from *Poeciliopsis latidens*, *P. lucida*, Cy, and offspring of Cy mated with *P. latidens* injected with colchicine after the technique of McPhail and Jones (*5*). Preparations were made from two individuals each of *P. latidens*, *P. lucida*, and offspring of Cy and *P. latidens*, and two individuals from each of the two lines of Cy maintained in the laboratory. From each preparation at least three cells were counted that had chromosomes sufficiently spread to leave no doubt about the accuracy of the determination. Numerous other cells were assessed to within one or two chromosomes.

Both *P. latidens* and *P. lucida* have a diploid number of 48, whereas the two Cy lines and offspring of matings of Cy with *P. latidens* were clearly triploids with a chromosome number of 72 (Fig. 2). *Poeciliopsis* Cy is thus the first naturally occurring population of triploid fish to be reported. The only other vertebrates known to have reproducing triploid populations are gynogenetic salamanders of the genus *Ambystoma* (*6*) and parthenogenetic lizards of the genus *Cnemidophorus* (*7*).

The means by which Cy undergoes meiosis and produces fertile triploid eggs, as they presumably are, is not known. In gynogenetic and parthenogenetic diploid forms, the diploid chromosome complement might be maintained by suppression of one of the meiotic cleavages, reentry of the second polar body, or suppression of the first mitotic cleavage (*8, 9*). None of these pathways is applicable to apomictic triploids. It is more likely that triploidy in Cy is maintained as in the *Ambystoma* all-females (*10*) and in the gynogenetic, triploid spider beetle, *Ptinus* (*11*); in these, early in oogenesis the triploid chromosome number is elevated to a hexaploid level by an endomitotic division. Subsequent divisions result in triploid eggs that require only the stimulus of sperm penetration in order to develop. There is no evidence, in fact, that diploid gynogenetic *Poecilia formosa* (*12*) and diploid parthenogenetic lizards, genus *Lacerta* (see *13*), do not also maintain their chromosome number by endomitosis.

The origin of naturally occurring monosexual vertebrates has not been satisfactorily resolved. It has been demonstrated that application of temperature shock or chemical agents at the proper time can alter meiosis and result in the production of apomictic haploids, diploids, and various polyploids (*8, 14*). Nevertheless, those who have studied natural populations of all-female *Poecilia* and *Ambystoma* have championed a theory of hybrid origin (*4, 6*). Their arguments are based on the fact that the monosexual forms are morphologically intermediate between bisexual species presumed to be their parents. Evidence that triploid vertebrates can be produced by hybridization is presented in the experiments of Rasch *et al.* (*15*); they mated the gynogenetic, diploid *P. formosa* to *P. vittata*. As judged by measurements of nuclear DNA, the offspring were triploid. Similarly, the diploid, parthenogenetic lizards of the genus *Lacerta* occasionally hybridize with bisexual lizards and produce triploid progeny (*13*). Triploid hybrids of *Poecilia* and *Lacerta* were sterile, however.

The origin of monosexuality in *Poeciliopsis* also seems best explained by hybridization. Forms Cx, Cy, and Cz have certain basic traits in common that differ from those of *P. lucida*, the mate of all three strains. Other species, *P. monacha* in the Río Fuerte and *P. viriosa* in the Río Mocorito, could easily have provided these morphological differences. The best hypothesis I can offer at this time is that Cx and Cz arose through hybridization of either *P. monacha* or *P. viriosa* with *P. lucida*. Both all-female

forms are diploid, however, and are not known to produce other than haploid eggs. The peculiar meiotic process of these hybrids is probably delicately balanced and more susceptible to temperature shock than nonhybrid fish are. Suppression of a cleavage stage and the production of diploid eggs followed by fertilization with *P. lucida* sperm is the most likely origin of the triploid Cy. Once the triploid chromosome complement is acquired, meiosis is probably preceded by endomitosis, the population henceforth being sustained by gynogenesis from triploid eggs. This explanation is similar to that offered for the origin of the triploid *Ambystoma* (*16*), except that in *Poeciliopsis* the intermediate hybrid stage is still available in the form of diploid all-female strains.

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Delayed Hypersensitivity in Man: A Correlate in vitro and Transfer by an RNA Extract

Abstract. *The migration of sensitized human monocytic-phagocytic cells from lymph-node tissue-culture suspensions was regularly and specifically inhibited by purified protein derivative or histoplasmin. Moreover, when nonsensitive cells were incubated with an RNA extract from lymph nodes of donors sensitive to purified protein derivative, histoplasmin, or both, the migration of these cells was specifically inhibited by these antigens. Ribonuclease inactivated the RNA extract.*

Tests in vitro of delayed hypersensitivity have thus far depended on the inhibition of migration of sensitive cells (*1*) or on the appearance of transformed cells (mitogenesis) (*2*) by specific antigen. Although the correlation with skin reactions is high, there is no direct evidence that these phenomena in vitro are manifestations of delayed hypersensitivity. The technique of inhibition of migration in capillary tubes (*3*, *4*) offers advantages in experimental animals, but it has not been successfully used for man.

Using an inoculum of living cells, Landsteiner and Chase, and Chase (*5*) described the successful passive transfer of tuberculin hypersensitivity from sensitive donor animals to normal recipients. Lawrence, using a cell-free extract of disrupted leukocytes (*6*), has demonstrated that delayed hypersensitivity can be transferred in vivo in man. This "transfer factor" apparently has the following properties: (i) it is unaffected by ribonuclease, deoxyribonuclease, or trypsin; (ii) it is stable at −20°C for 5 months or at 25° to 37°C for 6 hours; and (iii) it is dialyzable and is separated by Sephadex G-25 (its molecular weight being less than 10,000). Recently a factor isolated in the same manner as "transfer factor" has been reported to transfer sensitivity in vitro, as judged by the mitogenesis assay for delayed hypersensitivity (see *7*).

I now report that the capillary-tube technique can be used with human lymph-node cells in tissue culture to devise an in vitro correlate of delayed hypersensitivity in man. I also report that a cell-free extract containing RNA can transfer delayed hypersensitivity in vitro as judged by this technique. The extract containing RNA reported here differs from "transfer factor" described by Lawrence (*6*) and by Fireman (*7*); but it is similar to that used by Fishman (*8*) and Cohen (*9*) in experimental animals to convert nonimmune cells into antibody-forming cells.

My study included (i) patients with active tuberculosis, (ii) patients with positive skin tests for purified protein derivative (PPD) or histoplasmin, and (iii) normal controls with negative skin tests. Human lymph nodes were obtained by biopsy or at the time of thoracic surgery and were placed in iced Earle's solution, until they were used for tissue culture. The lymph nodes were minced with a scalpel, forced through an aluminum-nickel No. 40 screen, and washed three times in Earle's solution. The separated cells were resuspended in TC 199 (Difco Laboratories), a tissue-culture fluid, containing 10-percent patient's serum and 10-percent fetal calf serum; they were then placed in siliconized tissue-culture bottles for 72 hours. The bottles were gently agitated, and the cell suspensions thus obtained contained both a monocytic-phagocytic "macrophage" type cell population (approximately 25 percent; ability to engulf Fe particles) and a lymphocyte-like population with early transformation (approximately 65 percent), which is similar to cell populations used by other investigators (see *10*).

The monocytic-phagocytic cell suspension, containing approximately 10^5 cells per milliliter, was placed in a sterile capillary tube and centrifuged at 500 rev/min for 5 minutes to obtain a cell pellet. The capillary tube was broken at the cell-fluid interface, placed in a Sikes-Moore tissue-culture closed system chamber and fastened with sterile silicone. To each chamber was added approximately 1 ml of tissue culture medium TC 199 modified to contain lymph-node donor's serum (10 percent), fetal calf serum (10 percent), and fresh-frozen glutamine (0.5 mg/ml). The chambers and contents were incubated for 24 hours at 37°C. The chambers were then placed in a Zeiss projection microscope, and the area of cell migration from the capillary tube was measured by planimetry. The percentage of migration in the presence of antigen is defined as the migration index (M.I.) (*1*), or the ratio of average area of migration with antigen to the average area of migration without antigen times 100.

Figure 1 shows the area of migration and migration index obtained when cell