hyperphagia. The normal sibling controls ranged in age between 18 and 32 years. Five were females and 2 were males. None of the 7 control subjects were obese, hyperphagic or diabetic. Between 1000 and 1200 h a 15 ml venous blood sample was collected into a heparinized plastic syringe. Blood samples were centrifuged in a refrigerated unit within 5 min of collection and the plasma frozen at −20 °C until the time of assay. Ir-β-ep was extracted from plasma by use of the talc extraction procedure of Inturrist et al.[5]. The mean recovery of β-ep standards from plasma rendered endorphin free is 93.6% ± 12.8 (SD). Beta-lipotropin (β-LPH) is not converted to β-ep during the extraction[5]. Estimates of ir-β-ep in plasma extracts was obtained by use of the radioimmunoassay kit (NEK-003) supplied by New England Nuclear. The lower limit of sensitivity is 5-pg/ml of authentic β-ep and the intraassay coefficient of variation is 5%. β-LPH has a 50% (molar) cross-reactivity with the antiserum. β-LPH and β-ep, the predominant endorphins in human plasma, are present in a 2:1 β-LPH to β-ep molar ratio[6,7]. The table shows that no significant difference was found in the range or mean values of ir-β-ep in the plasma of P-L-W patients compared to their normal siblings. Dent et al.[8] have shown a diurnal variation in the plasma ir-β-ep of normal volunteers with levels remaining constant during 1000–1800 h at mean value of approximately 5.6 fentamoles/ml (19.0 pg/ml). Thus our single sample taken between 1000 and 1200 h is probably representative of the ir-β-ep levels during a major portion of the daytime. Our results suggest that if changes in ir-β-ep occur in the hypothalamus or pituitary of P-L-W patients they are not reflected peripherally as a change in basal plasma ir-β-ep.

Recently a deficit in pancreatic polypeptide has been discovered in P-L-W patients[9]. Pancreatic polypeptide is not an opioid peptide and does not contain the enkephalin sequence characteristic of many of the opioid-like peptides. However, endorphin-like immunoreactivity has been reported to be localized to the pancreatic-polypeptide containing cells of the lizard by immunohistochemical methods[10]. It will be of interest to learn whether alterations of these opioid and nonopioid peptides are associated with the symptoms of the Prader-Labhart-Willi syndrome.

Plasma ir-β-endorphin in Prader-Labhart-Willi Syndrome (P-L-W) patients and normal siblings

	Age (years)	Sex	Plasma ir-β-endorphin (pg/ml)
P-L-W			
Patient No.			
1	20	M	13
2	28	M	10
3	17	F	6
4	22	F	25
5	12	F	18
6	16	F	12
mean	19.2	-	14
± SE	2.5	-	3.0
Siblings			
1	18	M	14
2	20	F	20
3	19	F	17
4	32	F	9
5a	22	F	5
5b	19	M	31
6	27	F	13
mean	22.4		15.6
± SE	2.3		3.8

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Expression of H-Y antigen in the sex-change fish *Coris julis*[1]

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Summary. In the sex reversal from females to secondary males, in *Coris julis*, the H-Y antigen seems to be one sex change factor. The gonadal cells of primary and secondary males are H-Y antigen positive, the gonadal cells of females H-Y antigen negative.

In the order Perciformes, sex reversal, especially from female to male, is a common phenomenon. In the species *Coris julis* L. (Labridae, Teleostii) some adult females turn, during the months of September and October, into secondary males. This change is not only reflected in their outer appearance, but also in the morphological characteristics of the gonads as well as the animals' behavior[3–7].

The total chromosome number of *Coris julis* was found to be 48 in all individuals. Karyotype analyses revealed that females and secondary males have 10 metacentric and 38 acrocentric chromosomes, while primary males, on the other hand, exhibit 11 metacentrics and 37 acrocentrics.

On cytogenetic grounds, 2 kinds of females could be distinguished in *Coris julis*. Our earlier observations suggest

that only one of them undergoes sex inversion because the identical karyotype was found in secondary males. It is assumed that only this type of female contains 'male' germ cells, as observed in histological preparations of the gonads[4].

Both types of females as well as primary and secondary males take part in reproduction. All produce functional germ cells[4]. Both kinds of males were observed participating in the spawning act. Through the sex inversion of some of the females into secondary males, which produce functioning female-determining sperm, the ratio of females to males is substantially increased in the next generation.

Environmental factors, i.e. physical and social interactions between individuals, as well as genetic factors, are regarded as influencing this sex reversal[4,6-12].

Material and methods. For cytogenetical investigations and H-Y antigen-typing the animals (females, primary males and secondary males) were transported live from Elba (Italy) to Basel in thick plastic bags containing some water and pure oxygen.

In order to study the cytogenetical aspects of the sex change, fibroblasts were cultured according to a method described previously[4]. The sex of the animals (both kinds of males and females) was determined by studying the gonads.

Antisera to H-Y antigen were prepared in 8-12-week-old female inbred Lewis rats by i.p. injection of 15-20 million spleen cells from male inbred Lewis rats. Four weekly doses were given. After a 5-week interval 2 further, weekly, doses were administered.

Blood was taken 10 days after the last immunization. The antisera were inactivated at 56 °C for 30 min and absorbed with an equal volume of human female AB Rh+ erythrocytes for 1 h at 4 °C and female rat spleen cells. Before use, the antisera were stored at −60 °C.

The presence or absence of H-Y antigen on animal cells was determined using a cytotoxic test described by Fellous et al.[13].

1 vol. gonadal cells from each of 2 females, 1 primary, and 1 secondary male were mixed with 2 vols of antiserum, diluted 1:1 with PBS. These were incubated twice for 60 min on ice. After spinning down the cells, the antiserum was transferred in 5 different dilutions (1 µl per hole) on micro-titreplates. 1 µl of Raji-cells (3500 cells per µl) was added as H-Y antigen positive target cells and incubated for 60 min at ambient temperature. Subsequently, 3 µl of appropriately diluted rabbit complement (equal volume of complement and PBS with 17% human female AB serum) was added and incubated for an additional 60 min at ambient temperature. 1 µl of trypan blue solution was added and lysed Raji-cells counted.

Results and discussion. Our H-Y antigen analyses of *Coris julis* showed that gonadal cells of primary and secondary males are positive and gonadal cells of females H-Y antigen negative (fig.). From these preliminary results it seems that H-Y antigen negative somatic cells of the female gonads might become H-Y antigen positive during sex inversion. However, this requires corroborative evidence from investigations on the females' gonads at the beginning of and during the sex change. Until now, for technical reasons, it has not been possible to study non-gonadal somatic cells, but we are planning to do so as soon as new material is available.

H-Y antigen is a membrane component which is normally present only in male tissue and is assumed to be the testis-determining factor[14,15]. Pechan et al.[16] have shown that among male teleosts, *Xiphophorus maculatus, Haplochromis burtoni* and *Orizias latipes* are H-Y antigen positive. Also, Müller and Wolf[17] have studied the effect of antisera obtained from Lewis rats to H-Y antigen in fish species with a known XX/XY mechanism of sex determination *(Lebistes reticulatus* and *Xiphophorus helleri).* They found that the H-Y antigen was expressed only by the males of the heterogametic sex.

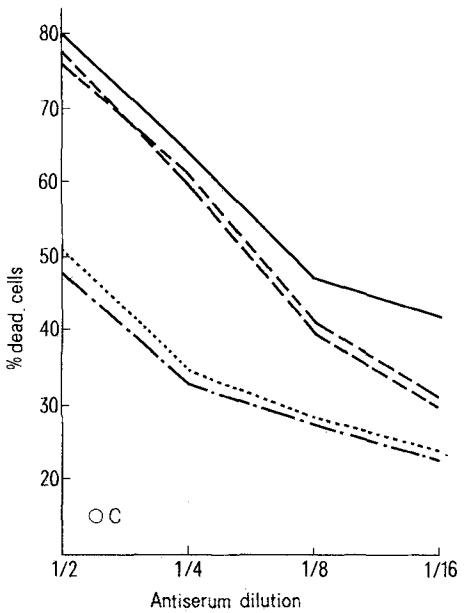
In their study of fish from low evolutionary groups, *Isospondyi* and *Ostariophysi*, the same authors found that both sexes show the same degree of H-Y antigen expression. There remains the questions as to when, on the evolutionary scale, the H-Y antigen is limited to the male sex, and whether it always plays the major role in the differentiation of male fish gonads. Müller and Wolf[17] surmise that H-Y antigen was expressed in both sexes in the lower vertebrates and only in the higher vertebrates did it exert its effect in the gonads of the heterogametic sex. What part H-Y antigen plays in hermaphroditism, especially in spontaneous sex reversal in fish, is not yet known.

Studies on the protogyne hermaphroditic fish species (from females to males) show that during natural sex changes there is a marked fall in the level of oestrogen and a corresponding rise in the level of androgens. However, androgen-synthesis is not the primary cause bringing about sex change but rather a secondary effect[18].

Experimental treatment with testosterone of *Anthias squamipinnis*[10] and *Coris julis*[19] can produce a sex change, whereas administration of testosterone to *Monopterus albus*[18] does not produce sex reversal.

A common finding in the gonads of some protogyne hermaphroditic fish are germ cells of the opposite sex[8,20].

The change from females to males often takes place surprisingly quickly. *Coris julis* females complete their sex change within a few weeks. During this time the female gonads degenerate and the inactive germ cells, within them, develop into male germ cells in the newly organized testes. These germ cells are stimulated to grow and develop into mature spermatocytes. At the same time, the other male gonad specific cells, such as sertoli and Leydig cells, develop from the remnants of the original ovary and the external appearances alter, and the fish develops a lateral, orange, 'zig-zag' band and a blue spot, characterizing the secondary males[3-5].



H-Y antigen expression in *Coris julis* L. Cytotoxicity of anti-H-Y antiserum to Raji-cells after absorption with gonads cells of 2 (----) females, (....) 1 primary male and (-.-.-) 1 secondary male (—) Unabsorbed antiserum. O, Complement control.

Conclusions. We believe that some change in the endocrine system (gonadotropin levels) and probably the phenomenon of H-Y antigen expression in the female fish influence the initiation of sex inversion, from female to male in *Coris julis.*

Reinboth[21] discusses a controlling mechanism maintaining the fish in the female sex until the beginning of the sex-reversal. On the receipt of 'impulses' (environmental or genetical factors) the role of the 'control' is altered and development of the new sex occurs. The location of this 'control' is perhaps in the CNS.

Genetic factors determine and control the development of the gonads as well as the age of sex-maturation. Possibly they also determine sex reversal in *Coris julis.* This control mechanism brings about changes in the pituitary and in the gonads as well as in H-Y antigen expression. The CNS, which also controls social interrelations between individuals may, on the one hand, be directed by these genetically determined control mechanisms, on the other hand it might influence them. Environmental factors, primarily social relationships, may play a major role in the initiation of the sex-reversal process in *Coris julis.*

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Effect of specific and nonspecific immune complexes on injury of intact hepatocytes in vitro[1]

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Summary. Immune complex (IC)-mediated cytotoxicity of hepatocytes was investigated in vitro, using antibodies against specific and nonspecific target cell surface membranes. The results indicated that neither specific, nor nonspecific IC evoked significant lysis of intact hepatocytes. Thus, the IC are unlikely to play an important role in the immune-associated injury of normal hepatocytes in acute and chronic liver diseases.

Sera from patients with hepatitis B virus (HBV)-induced acute and chronic liver diseases contain HBV-specific immune complexes (IC)[2], as well as antibodies to liver membrane antigens[3]. Circulating immune complexes have also been demonstrated in patients with primary biliary cirrhosis[4] and alcoholic hepatitis[5]. Multiple nonspecific bacterial and dietary antigens, their antibodies, and IC originating from the gastrointestinal tract may appear, when the function of hepatic reticuloendothelial system is diminished, such as seen in chronic liver disease[6]. Furthermore, immunoglobulins (Ig), as well as IC, are also present in the livers[7,8]. In vivo fixed IgG is demonstrable on hepatocytes from animals with experimentally produced chronic active hepatitis (CAH) and from patients with CAH[9]. Interestingly, complement (C′) is found in vitro to be fixed on liver cells from patients with acute B-viral hepatitis[10]. Despite extensive studies of the induction of hepatocyte damage in vitro by cell-mediated immune mechanisms[9], such as cytotoxic lymphocyte-mediated hepatocytolysis in CAH[11], it remains unclear whether in vivo antibodies, C′ and IC injure liver parenchymal cells in acute and chronic liver diseases. Thus, in the present study, we examine the possible role of these humoral products in the pathogenesis of hepatocyte damage in vitro.

Materials and methods. Rat hepatocytes were isolated by collagenase perfusion[12]. The medium used in these experiments was RPMI 1640 and 10% heat-inactivated fetal calf serum, supplemented with streptomycin, penicillin, fungizone, 10 mM HEPES and 2 mM L-glutamine (Grand Island Biologicals, Grand Island, N.Y., USA). Six antisera (Ab) were produced through rabbits; anti-human liver-specific protein (LSP)[13], anti-rat hepatocyte (RH)[14], anti-Chang cell[14], anti-T cell (JM-T)[14], and anti-Null cell (NALM −16)[14] (both of the last 2 cell lines are those of human leukemia cells), and anti-sheep red cell (SRBC) hemolysin (Cappel Laboratories, Cochranville, PA, USA). Human liver tissues for the preparation of human LSP were obtained within 4 h after death of 2 cases, who did not have any liver injury macroscopically and histologically. The antisera were all heat-inactivated (56 °C for 45 min). A titer of anti-human LSP was determined by passive hemagglutination (1:6400)[15]. Titers of the other antisera were measured, using antibody-dependent cell-mediated cytotoxicity (optimal dilutions, 1.2×10^3 to 4×10^3). The specificity of each Ab was also preliminarily tested by indirect immunofluorescence; each had the cell membrane antibody against each corresponding cell type, including the anti-LSP which expressed its antigen-binding capacity on