peak; however, the assay used was not able to show which of them possessed the hemagglutinating activity. Both electrophoretic patterns together were equivalent to the whole coelomic fluid electropherogram. Colored protein bands were not visible in the gel after electrophoresis of the 3rd peak, probably owing to the very low protein concentration.

The pooled fractions of each peak were also investigated with an immunoserum against the whole coelomic fluid. IEP (fig. 2,a) showed that several antigens of differing electrophoretic mobility were contained in the coelomic fluid and characterized each chromatographic component. The fractions eluted with dextran blue, mainly consisting of electrophoretically slow proteins of intermediate mobility, were found in the 2nd peak; 3 distinct anodal immunoprecipitates were typical of the 3rd peak. ID (fig. 2,b) of these reactants confirmed that several immunologically distinguishable components were contained both in the fractions eluted with the dextran blue (at least 3 immunoprecipitates) and in those eluted with thyroglobulin (at least 2 immunoprecipitates). Three very thin arcs, difficult to see, represent the antigenic composition of the low molecular weight fraction. By SDS-PAGE, 2 protein chains, 68,000 and 30–35,000 daltons respectively, were found in the fractions of both the 1st and 2nd peak of the A_{280} profile. The latter also showed molecules of 185–200,000 and 115–130,000 daltons but we do not know whether they are large molecular chains or derivatives produced as a result of treatment during the SDS-PAGE procedure. The 3rd peak showed 2 protein chains of about 90,000 and 68,000 daltons.

The relationship between protein subunits and hemagglutinins was investigated by absorption experiments. Coelomic fluid preparations were absorbed with an equal volume of packed RE to eliminate the anti-RE hemagglutinins. After centrifugation, the supernatant was studied in SDS-PAGE. The electropherogram, compared with that of a non-absorbed sample, showed an evident reduction of the 68,000 and 30–35,000 dalton bands.

The coelomic fluid, frozen at −20 °C for 4–5 weeks, and then passed through a Bio-gel column, apparently lost most of the light molecular weight molecules and no hemolytic activity was found in the fractions of the 3rd chromatographic component. The largest hemagglutinins were sensitive to prolonged freezing (5–6 months): hemagglutinating activity was only found in the component eluted with tyroglobulin.

The result so far obtained show that 2 protein subunits in the *H. polii* coelomic fluid could form hemagglutinin molecules heterogeneous in size, the largest being sensitive to freezing. As suggested in a previous paper[6], the hemolysins differ from the hemagglutinins in that they are smaller, more sensitive to temperature variations and can be distinguished by immunoelectrophoresis. The hemolysins could also contain a 68,000 dalton protein chain; however, further work is needed to clarify whether or not affinity exists between hemolysin and hemagglutinin subunits.

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2 Ryoyama, K., Biol. Bull. *146* (1974) 404.
3 Finstad, C.L., Litman, G.W., Finstad, J., and Good, R.A., J. Immun. *108* (1972) 1704.
4 Parrinello, N., Canicatti, C., and Rindone, D., Boll. Zool. *43* (1976) 259.
5 Ryoyama, K., Biochim. biophys. Acta *320* (1973) 157.
6 Parrinello, N., Rindone, D., and Canicatti, C., Devl comp. Immun. *3* (1979) 45.
7 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
8 Andrews, P., Biochem. J. *96* (1965) 595.
9 Davis, B.J., Ann. N.Y. Acad. Sci. *121* (1964) 404.
10 Shapiro, A.L., Vinuela, A.L., and Maizel, J.Y., Biochem. biophys. Res. Commun. *28* (1967) 815.
11 Weber, K., and Osborne, M., J. biol. Chem. *244* (1969) 4406.
12 Gavery, S.S., Cremer, N.E., and Sussdorf, D.H., Methods in immunology. W.A. Benjamin, Inc., London 1967.
13 Ouchterlony, O., and Nilsson, L.A., in: Handbook of experimental immunology, chapter 19, p.39. Ed. D.W. Weir. Blackwell, Oxford 1973.
14 Scheidegger, J.J., Int. Archs Allergy *7* (1955) 103.

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Beta-endorphin immunoreactivity in the plasma of patients with the Prader-Labhart-Willi syndrome and their normal siblings

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Summary. No significant difference was found in the range or mean values of ir-β-endorphin in the plasma of 6 patients with the Prader-Labhart-Willi syndrome compared to 7 of their normal siblings. The hypothesis that some of the symptoms of the P-L-W syndrome are due to excessive opioid activity is not supported by measurement of peripheral levels of ir-β-endorphin.

Excessive levels of immunoreactive beta-endorphin (ir-β-ep) occur in the pituitary of genetically obese rodents[2] such as *ob/ob* and *fa/fa* as well as in the plasma of obese hirsute women[3]. These forms of obesity involve overeating, reductions in energy-expending activities and a hypogonadal condition. The Prader-Labhart-Willi syndrome presents a similar set of symptoms including hyperphagia, hypogonadalism, hypotonia and obesity. The parents of Prader-Labhart-Willi children report that they seem insensitive to pain. Moreover, naloxone (an opioid antagonist) reduced the hyperphagia of 2 male patients with Prader-Labhart-Willi syndrome as reported by Kyriakides and colleagues[4]. These indications all seem compatible with the hypothesis of excessive opioid activity. Therefore we measured the quantity of ir-β-ep in the plasma of 6 patients with the Prader-Labhart-Willi syndrome and 7 of their normal siblings.

The subjects with the syndrome ranged in age between 12 and 28 years. Four of them were females and 2 were males. All the subjects were obese with a history of intense

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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2 Margules, D.L., Moisset, B., Lewis, M.J., Shibuya, H., and Pert, C.B., Science *202* (1978) 988.
3 Givens, J.R., Wiedemann, E., Andersen, R.N., and Kitabahi, A.E., J. clin. Endocr. Metab. *50* (1980) 975.
4 Kyriakides, M., Silverstone, T., Jeffcoate, W., and Laurence, B., Lancet *1* (1980) 876.
5 Inturrisi, C.E., Alexopoulos, G., Lipman, R., Foley, K., and Rossier, J., Ann N.Y. Acad. Sci. (1983) in press.
6 Ghazorossian, V.E., Dent, R.R., Otsu, K., Ross, M., Cox, B., and Goldstein, A., Analyt. Biochem. *102* (1980) 80.
7 Wardlaw, S.L., and Frantz, A.G., J. clin. Endocr. Metab. *48* (1979) 176.
8 Dent, R.R.M., Guilleminault, C., Albert, L.H., Posner, B.I., Cox, B.M., and Goldstein, A., J. clin. Endocr. Metab. *52* (1981) 942.
9 Zipf, W.B., O'Dorisio, T.M., Cataland, S., and Sotos, V., J. clin. Endocr. Metab. *6* (1981) 1264.
10 El-Salhy, M., and Grimelius, L., Regul. Pept. *2* (1981) 97.

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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