of the bands, but the number of isoenzyme bands were the same in Chinese Spring and the Transfer for the respective tissues (Figure). The anodal peroxidase isoenzymes were found to be 10 in all, and have been numbered from Px-1^a (the slowest moving) to Px-10^a (the fastest moving). The isoenzyme Px-6^a was found to be specific to the coleoptile in both samples. In general the roots had the highest peroxidase activity of the isoenzymes, and the leaf tissue showed the lowest activity.

The cathodal side of the gel showed interesting differences in the number and intensities of isoenzyme bands. In total there were 8 peroxidase isoenzymes which were found to have moved towards the cathodal side. The isoenzymes are numbered from Px-1^c to Px-8^c, starting from the origin. The isoenzyme Px-6^c was found to be present in the root and coleoptile, but was absent from the leaf tissue of both Chinese Spring and the Transfer. The presence or absence of one or more isoenzyme bands from one of the tissue types from the seedling indicates that the isoenzymes give a good measure of changing gene function, and in the words of MARKERT[6], 'provide some insight into the regulation of gene function which leads to the synthesis of these various proteins at the right time and place and in the correct proportion to serve the needs of the organism'.

The most characteristic feature was the absence of isoenzyme Px-5^c from all the 3 tissues of Chinese Spring and its presence in the 3 tissues of Transfer. The isoenzyme Px-5^c showed the same degree of band intensities with all the hydrogen donors used. This additional peroxidase isoenzyme Px-5^c present in the tissue extracts of Transfer seedlings also showed poly-phenol oxidase activity when stained for poly-phenoloxidases. Since the conditions for growing the seedlings, preparation of the tissue extracts and gel electrophoresis were uniform for both the samples, the isoenzyme differences observed, between the parent variety Chinese Spring and Transfer with the *umbellulata* chromosome segment, would therefore be the result of the genetic information on *umbellulata* chromosome segment governing the synthesis of this additional peroxidase isoenzyme Px-5^c in all the 3 tissues studied.

At present it is very difficult to correlate the observations of BHATIA and SMITH[4] and the present findings, because of the fact that the additional protein bands observed by BHATIA and SMITH[4] in the leaf extracts of Transfer were anodally moving, whereas the additional peroxidase isoenzyme Px-5^c recorded during the present study was cathodal[7,8].

Résumé. Des essais par électrophorèse à gel avaient révélé dans les tissus de semis de 8 jours du Transfert (IC 13296) la présence d'une isoenzyme de péroxydase, Px-5^c, qui ne se trouve pas chez la variété parente, «Chinese Spring». Il est suggéré que cette isoenzyme basique de péroxydase supplémentaire est le produit de renseignements génétiques transmis par le segment de chromosome de l'*umbellulata*.

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Gene Frequencies of *Pl* Alleles for the Alkaline Phosphatase of Human Placenta in a Random Sample of the Population of Rome

From the results obtained by BOYER in 1961[1] the existence of a polymorphism for alkaline phosphatase of human placenta appeared clearly. Later on ROBSON and HARRIS[2,3] by means of electrophoretic analysis on starch gel at pH 6.0 and pH 8.6, demonstrated the existence of 6 common electrophoretic patterns of placental alkaline phosphatase (approximatively 2% of the placentae showed a different pattern). Their frequencies are in agreement with the hypothesis that they are determined by 3 common codominant alleles (Pl^s, Pl^f, Pl^i) of one autosomal locus *Pl*.

The enzyme is of foetal origin: it is present in the serum of almost all women by the 28th week of gestation and disappears by the 6th week after delivery.

The data of ROBSON and HARRIS, and more recently those of BECKMAN et al.[4,5], show wide interracial variations in the frequency of *Pl* alleles. The biological significance of this polymorphism is not clear: it may be relevant in the problems of maternal-foetal interactions.

In this communication we report the *Pl* gene frequencies observed in a random sample of 175 placentae from single births which occurred in the Roman population between June 1966 and July 1967.

The placental extracts were prepared according to BOYER[6]; the starch gel electrophoresis was carried out according to ROBSON and HARRIS[2], and the enzyme activity was developed according to BOYER[1].

In the Tables I and II are reported the data concerning 172 cases; each of the remaining 3 showed a different uncommon phenotype, a finding that is in agreement with the 2% frequency observed by ROBSON and HARRIS for rare phenotypes.

All of the 6 common phenotypes described by ROBSON and HARRIS were found and showed the frequency expected according to the HARDY-WEINBERG equilibrium

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Table I. Observed and expected frequencies of phenotypes for alkaline phosphatase of human placenta

Phenotypes	*S*	*F*	*I*	*FS*	*FI*	*SI*	Totals
Observed	81	12	2	55	9	13	172
Expected	76.887	11.256	0.982	58.838	6.651	17.382	171.99
χ^2	0.220	0.005	0.273	0.250	0.514	0.866	2.128

The 6 most common phenotypes have been found. Their frequencies are in good agreement with the expectation based on the HARDY-WEINBERG equilibrium.

Table II. Gene frequencies of the 3 placental alkaline phosphatase genes, Pl^s, Pl^f and Pl^i in the population of Rome

Alleles	Pl^s	Pl^f	Pl^i	Totals
Observed	230	88	26	344
Frequencies	0.669	0.256	0.076	1.001

The gene frequencies of the 3 most common *Pl* alleles in the Roman population are very similar to those observed in the English and Swedish populations.

($\chi^2_{3df} = 2.128$; $P > 0.5$). The gene frequency of the 3 common alleles (Table II) were quite similar to that found in some North-European populations[3, 4].

Riassunto. Sono state studiate le frequenze degli alleli al locus *Pl* per la fosfatasi alcalina della placenta umana in 175 parti singoli verificatisi nella popolazione di Roma. Sono stati identificati tutti e 6 i fenotipi più comuni descritti in precedenza; la loro frequenza corrisponde a quella attesa secondo la legge di HARDY-WEINBERG. Le frequenze geniche sono risultate sovrapponibili a quelle riportate per le popolazioni Inglese e Svedese.

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Increase of the Amount of DNA-Feulgen in Mammalian Tissue by Schiff Reagents at Less Acid pH

It has been shown by SWIFT[1] that a progressive increase of the intensity of Feulgen staining occurs as the pH of Schiff reagent increases from 0.8 to 3.6. ITIKAWA and OGURA[2] have prepared SO_2-treated Schiff reagent of various pH values – the higher pH values being obtained by the addition of alkali – and have demonstrated that optimum Feulgen staining is obtained at pH 3.0–4.3. DUTT[3] has also shown that when the pH of Schiff reagent, prepared with basic fuchsin, is increased by the addition of a dilute solution of sodium hydroxide, the staining potentiality of such a reagent is increased in hydrolysed mammalian and plant cell nuclei as compared with one in which the pH varies between 1.55 and 1.80. It has recently been shown[4] that different alkaline chemicals can also decrease the hydrogen ion concentration of Schiff reagent without inhibiting its capacity to serve as a potent reagent for histochemical demonstration and quantitation of DNA in tissue sections. CONN[5] stated that basic fuchsin, which is used in the preparation of Schiff reagent, contains 3 different dyes of the triphenylmethane series, viz. pararosaniline (magenta O), rosaniline (magenta I) and new fuchsin (magenta III, which differ from one another with respect to the absence or presence of 1 or 3 substituent methyl groups. In an effort to find out whether response to change of pH is exhibited by any one or all the constituents of basic fuchsin, the present experiments were undertaken.

Pararosaniline (C.I. No. 42500) and new fuchsin (C.I. No. 42520), both manufactured by National Aniline Division, New York, and rosaniline hydrochloride, manufactured by British Drug Houses, England, were individually used in the preparation of Schiff reagents according to DE TOMASI[6]. The pH values of these reagents were raised so as to make them less acid by the addition of a 0.2*M* aqueous solution of borax. The materials used in these experiments were kidney and liver of a male Indian water buffalo (*Bubalus bubalis* L.) which were fixed in 10% neutral formalin for 24 h and subsequently washed overnight in running tap water. Paraffin sections of these materials, 10 μ in thickness, were used throughout. Sections were hydrolysed in 1*N* HCl at 60 °C for 7 min and then stained for 50 min at 5 °C. The optimum time of hydrolysis was determined in a previous experiment. A couple of slides, one stained at the initial pH (control) and the other at less acid pH (experimental), were processed simultaneously. Staining of slides by the different stains was done separately. Following staining, sections were treated with the usual bleaching solution for 15 min with 3 changes of 5 min each. Afterwards they were dehydrated through grades of alcohol, cleared in dimethylaniline and then mounted in DPX for microspectrophotometric determination of the amount of DNA-Feulgen in nuclei that were located at random. However, care was taken to measure more or less the same number of nuclei from the periphery as well as the centre of the sections in both the control and the experimental material stained by the different reagents. The microspectrophotometer used in this investigation has already been described by the author[4]. The optics consisted of a Leitz × 54 fluorite oil immersion objective and a × 6 eye-piece. For measurement of DNA-Feulgen, the two-wavelength method was followed in which λ_1 was considered as 570 nm and λ_2 as 500 nm. The values in arbitrary units were calculated according to PATAU[7].

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