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].

1 H. SWIFT, *The Nucleic Acids* (Academic Press Inc., New York 1955), vol. 2.
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3 M. K. DUTT, J. Histochem. Cytochem. *11*, 390 (1963).
4 M. K. DUTT, Nucleus, Calcutta *10*, 168 (1967).
5 H. J. CONN, *Biological Stains*, 7th edn (The Williams and Wilkins Co., Baltimore 1961).
6 J. A. DE TOMASI, Stain Technol. *11*, 137 (1936).
7 K. PATAU, Chromosoma *5*, 341 (1952).

Data showing mean nuclear diameters and their mean DNA content in kidney and liver nuclei stained by Schiff reagents at different pH

Dye	Treatment	No. of nuclei	Material	Mean nuclear diameter (in μ)	DNA content with S.E.	Difference between means	t-value	P
Pararosaniline	Control (pH 2.3)	23	kidney	6.48 ± 0.21	15.2 ± 2.23	12.5	6.80	< 0.001
	Experimental (pH 4.0)	23	kidney	6.48 ± 0.21	27.7 ± 2.66			
Rosaniline	Control (pH 2.7)	39	liver	6.32 ± 0.59	21.2 ± 1.22	17.0	5.75	< 0.001
	Experimental (pH 3.5)	39	liver	6.32 ± 0.49	38.2 ± 2.63			
New fuchsin	Control (pH 2.9)	35	liver	5.77 ± 0.50	15.4 ± 1.12	13.3	6.14	< 0.001
	Experimental (pH 3.7)	35	liver	6.00 ± 0.46	28.7 ± 1.82			

The results are presented in the Table. It is quite evident from these results that considerably higher DNA values are obtained in the experimental materials stained by the different dyes at less acid pH as compared with those of the controls stained at low pH. This shows that all the components of basic fuchsin, viz. pararosaniline, rosaniline and new fuchsin, in their leuco state are reactive to change of pH, thereby increasing the staining potentiality of cell nuclei, as noted by SWIFT[1], ITIKAWA and OGURA[2], and DUTT[3]. The most plausible explanation of this increase in the quantity of DNA-Feulgen may be that a much larger number of aldehyde molecules take part in the reaction at a less acid pH than is possible at a very low pH, as suggested by the author[3,4,8].

Zusammenfassung. Die Färbbarkeit der DNS mit dem Feulgenreagens nimmt mit steigendem pH des Reagens zu. Es wird nachgewiesen, dass die verstärkte Reaktion alle 3 Bestandteile des gebräuchlichen Fuchsins betrifft.

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Microorganisms and Ion Absorption by Roots

Experiments on absorption of K and several other ions by diverse tissues of many plant species have revealed a dual pattern thought to reflect the operations of 2 mechanisms of ion transport[1]. At concentrations below 1 mM, Michaelis-Menten kinetics describe the relation between the rate of absorption of K and its external concentration. The maximum is closely approached at 0.2 mM K, and the rate of absorption does not rise much between 0.2 and 1 mM – an indication that the transport mechanism operating (mechanism 1) is nearly saturated at these concentrations.

At higher concentrations of K (1–50 mM), the rate of its absorption rises to values far in excess of the plateau referred to – evidence that at these concentrations a second mechanism of transport comes into play. This mechanism 2 differs by several clear-cut criteria from mechanism 1[1].

Recognition of this dual pattern of ion transport has come from short-term experiments with excised tissues, most of them with excised roots. Unless aseptic technique is used, roots of seedlings as normally grown in the laboratory carry a population of microorganisms, as of course do roots in soil. The effect of this microflora on the results of experiments on ion transport by roots is usually considered negligible, for several reasons. (1) The mass of the microorganisms is extremely small compared with that of the tissue with which they are associated. (2) Only ions non-exchangeably retained are measured as having been absorbed by the tissue[2]. This value should not include the bulk of the ions absorbed by bacteria, since these ions are readily lost by exchange. (3) The kinetics of ion transport are remarkably consistent, whether examined in experiments with fibrous root tissue, storage tissue, or leaf tissue

[1] E. EPSTEIN, Nature *212*, 1324 (1966).
[2] E. EPSTEIN, W. E. SCHMID and D. W. RAINS, Pl. Cell Physiol. Tokyo *4*, 79 (1963).