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

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of terrestrial or aquatic higher plants[1,3,4]. Both the kind and degree of bacterial contamination must vary greatly for these diverse plant materials, and the consistency of the results therefore argues against any great effect of bacteria on the findings obtained.

In recent experiments, the role of associated microorganisms in phosphate absorption by plants has been examined and attention has been drawn to differences obtained with sterile and non-sterile roots[5,6]. I have therefore examined the kinetic pattern of absorption of K in sterile and non-sterile roots.

Five-gram samples of seeds of barley, *Hordeum vulgare* var. Arivat, were shaken in 80 ml 1.8% sodium hypochlorite (30% 'Purex' by volume) for 30 min. Each sample was then rinsed twice with 100 ml sterile water each time. The seeds were transferred to dry, sterile Petri dishes. A few seeds at a time were then transferred to sterile Petri dishes containing 1.06% agar and incubated for 3 days as a preliminary test for sterility. Seedlings from plates showing no evidence of contamination were transferred onto a cheesecloth supported by a stainless steel screen suspended in a 4 l beaker at such a height that the surface of a 3 l, 0.2 m*M* $CaSO_4$ solution was about 1 cm below the screen. This assembly, with a Teflon-coated magnetic stirring bar on the bottom and heavy aluminum foil over the top of the beaker, had previously been autoclaved. Dacron batting intervened between the rim of the beaker and the aluminum foil, which was crimped around the top of the beaker.

Each beaker, with about 30 seedlings on its screen, was placed on a magnetic stirring apparatus in a dark chamber maintained at 22 °C. Seedlings were then grown for 4 more days, the solution being agitated and aerated by means of the rotating stirrer bar.

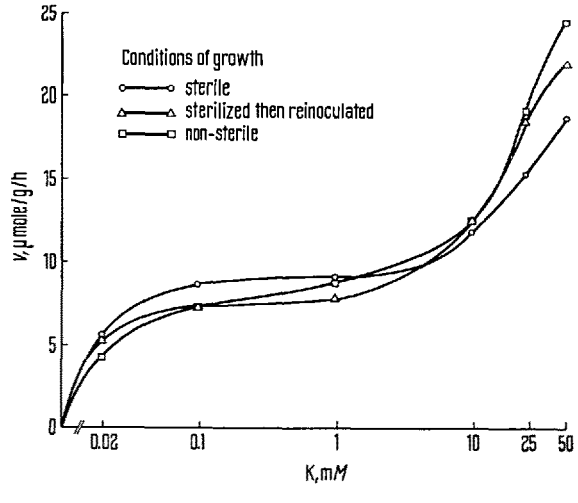
Thirty hours after transfer of the seedlings into this assembly the roots extended about 5 cm into the $CaSO_4$ solution. At this time, two 1 ml samples of the solution were withdrawn under sterile conditions and pipetted into 10 ml nutrient broth. These cultures were incubated at 30 °C for the remainder of the growth period of the seedlings and only seedlings from assemblies showing no contamination by this test were used for the sterile runs of the absorption experiments.

There were 2 control runs. One utilized roots from seedlings grown under non-sterile conditions in the usual manner[7]. Seeds for the other control treatment were sterilized as described above, plated on agar, and transferred to growth assemblies which were deliberately inoculated by dropping non-sterilized seeds into the beakers. This run was included to test the effect, if any, of the sterilization procedure itself, apart from that of subsequent growth under sterile conditions.

On the day of the experiment, the roots were excised into sterile water and absorption experiments performed as described before[2], except for the following 3 differences. (1) All solutions were sterilized by autoclaving. (2) Calcium was present as 0.5 m*M* $CaSO_4$ instead of $CaCl_2$. (3) Potassium was labeled with ^{86}Rb – a valid procedure for short-term absorption experiments with tissues of higher plants[3,8,9].

The results of many experiments were very similar. Those of 3 experiments done in succession were averaged and are presented in the Figure. At low concentrations, sterile roots absorbed K at a somewhat higher rate than did non-sterile roots. The reverse was true at high concentrations. Differences were not large; only at the top 2 concentrations was the difference between the sterile and the non-sterile treatments significant. There was no significant difference between the 2 non-sterile runs. The roots of all 3 treatments showed the characteristic dual pattern of transport outlined at the beginning of this report.

For absorption of K, therefore, our findings show only small differences between sterile and non-sterile roots. Similar results are likely for ions of other elements which are not incorporated into stable organic metabolites to any large extent, such as Na and Cl. The present conclusions in no way contradict those concerning P mentioned above since the rapidly dividing bacterial cells would preempt the extremely limited supply of phosphate present in those experiments[5,6]. This, coupled with the rapid incorporation of ^{32}P into nucleic acids of bacterial cells, causes large differences in patterns of labeling when sterile and non-sterile seedlings are compared[10]. The absence of large differences in our experiments with K shows that the results represent K absorption by the plant tissue[11].



Rate, *v*, of absorption of K by excised roots of barley as a function of the concentration of KCl, plotted logarithmically.

Résumé. Les comparaisons des taux d'absorption de K par des racines d'orge stériles et non-stériles, *Hordeum vulgare*, dans l'ordre de concentration de 0,02–50 m*M*, ont montré que le double mode de transport d'ion décrit auparavant pour des racines non-stériles, s'applique également aux racines stériles. La microflore de la racine n'a influencé que légèrement les taux d'absorption de K.

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