Trace metals especially Fe^{+++} as dietary factors for leafhoppers[1]

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Summary. Some trace metals are required for development and reproduction, and therefore for continuous rearing of the aster leafhopper, *Macrosteles fascifrons,* on a holidic diet. Requirement for Fe^{+++} becomes apparent immediately in the 1st generation; for Cu^{++} and Zn^{++}, in the 3rd. The leafhopper has grown normally and continuously for 3 generations in the absence of manganese.

Continuous rearing of aphids was achieved with the incorporation of some metals in trace amounts into holidic diets. In general, at least 4 cations, i.e. Fe^{+++}, Zn^{++}, Cu^{++} and Mn^{++}, are regularly included in the aphid diets. The function of these ions was found to be mainly involved in reproduction[2]. The essentiality of some cations (Mn^{++}, Zn^{++}) for ovarial transmission of symbiotes through generations in *Blattella germanica* has been reported[3]. These 4 cations were needed for the maintenance of the intracellular symbiotes in feeding *Neomyzus circumflexus* on a holidic diet[2]. Therefore, the trace metals may be required directly for metabolic processes, with or without an indirect effect on perpetuation of the symbiotes, which are related to development and reproduction of many insects.

Leafhoppers constantly harbor different types of intracellular symbiotes, possibly vital in nutrition. There is a lack of report dealing with dietary effects of trace metals for leafhopper growth. Since artificial rearing of the aster leafhopper, *Macrosteles fascifrons,* has succeeded[4], studies on dietary effects of some trace metals on its growth and reproduction become feasible and are reported here.

The basal diet for this study was principally the same as the holidic diet (3% sucrose instead of 5%) listed elsewhere[4], i.e. the standard diet minus the factor being tested. Individual trace ions, viz., Fe^{+++}, Zn^{++}, Cu^{++} and Mn^{++}, were omitted. The control diets were both the standard diet complete with all trace metals and the trace metal-deficient diet containing none of these 4 cations. Feeding experiments were started with 2-day-old nymphs of *M. fascifrons* and continued through 3 generations, if possible. Development and reproduction on each diet were regarded as criteria for evaluating the efficacy of these inorganic nutrients.

In table 1, it is shown that the omission of iron affects development of the 1st generation immediately. Although the nymphs grew normally from 1st to 5th instar, they had difficulty in the imaginal ecdysis. Only a few insects developed to the adult stage and they had deformed wings. These adults survived for a short period without reproducing. Therefore, continuous rearing through generations is impossible in the absence of iron. The pea aphid, *Acyrthosiphon pisum,* was reported to show poor growth and reproduction in the 1st generation when fed a low level of iron; but its reproduction was enhanced by adding iron into the diet[5,6]. Similar results were reported in *Myzus persicae*[7]. How the iron influences the leafhopper growth remains obscure.

Omission of manganese did not show any deterrent effect on growth and development of the leafhoppers through 3 generations (table 1). The females deposited as many eggs as those on the complete diet (table 2). Variable results were reported in different aphid species[2,6,7]. However, a high level of this metal was required for reproduction in *A. pisum*[5]. The reported lack of effect of manganese for the leafhopper as well as for some aphids can probably be challenged because of the possibility of contamination from other constituents.

Both copper and zinc were required for development of the leafhopper to the adult stage and for reproduction in the 3rd generation, but they had no apparent effect on growth in the 1st and 2nd generation (table 1). Deficiencies of zinc somewhat decreased egg reproduction during the 1st and 2nd generations, whereas deletion of copper showed variable results in different generations. Reproduction was halted in the 3rd generation with lack of copper or zinc (table 2). Deficiencies of zinc slow down the growth of *M. persicae* in the 1st generation[7]. The mean adult weights of *A. pisum* fed 400 mg Zn/100 ml were significantly higher than those fed a lower level; but addition of zinc improved growth and reduced mortality only slightly[5,6]. These disparate results were possibly due to variations in nutrient balances, manner of preparation, of undetected contaminations. Copper did not increase rate of reproduction or gain in weight for *Schizaphis graminum*[8], but it is necessary for other aphid species[2,7].

Omission of all 4 metals from the basal diet for the leafhopper resulted in poor adult emergence in the 1st generation similar to that on the iron-deficient diet (table 1). Therefore, trace metals can be regarded as important dietary factors for continuous rearing with holidic diets. Since the requirements for some trace metals show up rather slowly, for example, effects of copper and zinc on the aster leafhopper became apparent only in the 3rd generation, we suggest that studies on mineral requirements

Table 1. Effect of trace metals on development of the aster leafhopper, *M. fascifrons*

	Survival* (%)			Nymphal period (days)			Adult emergence (%)		
Trace metals deleted	1st G	2nd G	3rd G	1st G	2nd G	3rd G	1st G	2nd G	3rd G
Fe	83.3	–		22	–		33.3 N 33.3 D	– –	
Zn	85.2	83.3	61.9	22	24	28	57.5 N 11.5 D	71.4 N	33.3 N
Cu	70.4	72.2	61.1	22	21	26	66.7 N	64.3 N	22.2 N 33.3 D
Mn	76.9	93.3	73.3	22	24	24	76.9	93.3 N	73.3 N
Fe, Zn, Cu, Mn	75.0	–		24	–		37.0 N 22.2 D	– –	
None**	80.0	81.3	76.2	22	24	24	75.0 N	75.0 N	76.2 N

* On 20th day; ** standard diet; -, no 2nd-generation nymphs obtained; G, generation; N, normal adults; D, deformed adults.

Table 2. Effect of trace metals on reproduction of the aster leafhopper, *M. fascifrons*

Trace metals deleted	Egg production* 1st generation	2nd generation	3rd generation
Fe	-		
Zn	+	+	-
Cu	+ +	+ + +	±
Mn	+ + +	+ + +	+ + +
Fe, Zn, Cu, Mn	±		
None	+ + +	+ + +	+ + +

* Observation on 10 females each diet; -, 0 eggs/female/day; ±, 0.1–0.9 eggs/female/day; +, 1.0–2.0 eggs/female/day; + +, 2.1–2.9 eggs/female/day; + + +, 3 or more eggs/female/day.

should be done through generations, rather than carried out in a short period of time. Carry-over by mothers and ionic contaminations in other components can not be overlooked when evaluating the nutritive values.

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A mathematical analysis of the disk-sphere transition of the human red cell

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Summary. By means of analytical calculations, an attempt is made to approximate the profile of the human red cell during the 'disk-sphere' transition induced by variation of the tonicity of the medium.

According to several authors[1,2] Cassini's oval is a plane curve of particular interest when one wishes to approximate the profile of any meridian section of the human red cell (HRC). It is well known that Cassini's oval is the locus of the points which satisfy the equation $(x^2+y^2+a^2)^2-4a^2x^2=m^4$, where a and m are 2 geometrical parameters. The purpose of this work is to attempt to find a law by which a and m may be related to a physical parameter T (tonicity) or, in other words, to supply the explicit expression of 2 functions of the following type:

$$\text{(I)} \qquad m=f(T); \qquad a=g(T).$$

Methods. We hypothesized that T is linked to $\mathring{x}$ (equatorial radius) and to $\mathring{y}$ (polar radius) by means of simple relations of polynomial type, i.e.

$$\text{(1)} \qquad \mathring{x}=\sum_{i=0}^{2} A_i T^i; \qquad \mathring{y}=\sum_{i=0}^{2} B_i T^i.$$

On the basis of the control values of $\mathring{x}$ and $\mathring{y}$, reported by Evans and Fung[3] for 3 T levels ($T_0=300$ mOsm/l; $T_1=217$ mOsm/l; $T_2=131$ mOsm/l), we were able to determine the coefficients A_i and B_i by means of the Hermite-Lagrange formula[4]. Given that:

$$\text{(I bis)} \qquad m^2=0.5\,(\mathring{x}^2+\mathring{y}^2); \qquad a^2=0.5\,(\mathring{x}^2-\mathring{y}^2),$$

we finally obtained the functions:

$$\text{(I ter)} \quad m=\left(\sum_{j=0}^{4}\alpha_j T^j\right)^{1/2}; \qquad a=\left(\sum_{j=0}^{4}\beta_j T^j\right)^{1/2}$$

where
$$\alpha_0=\frac{1}{2}(A_0^2+B_0^2) \qquad \beta_0=\frac{1}{2}(A_0^2-B_0^2)$$
$$\alpha_1=(A_0A_1+B_0B_1) \qquad \beta_1=(A_0A_1-B_0B_1)$$
$$\alpha_2=(A_0A_2+B_0B_2)+\frac{1}{2}(A_1^2+B_1^2) \qquad \beta_2=(A_0A_2-B_0B_2)+\frac{1}{2}(A_1^2-B_1^2)$$
$$\alpha_3=(A_1A_2+B_1B_2) \qquad \beta_3=(A_1A_2-B_1B_2)$$
$$\alpha_4=\frac{1}{2}(A_2^2+B_2^2) \qquad \beta_4=\frac{1}{2}(A_2^2-B_2^2)$$

By varying T in the experimentally significant range between 300 mOsm/l and 131 mOsm/l, we determined the corresponding configurations of Cassini's oval, and numerical values of the surface area and volume of the 'mathematical cell' produced by the rotation of the curves around the polar axis. We used the following formulae:

$$\text{volume} = 4\pi\int_0^{\mathring{x}} x f(x)\,dx,$$

$$\text{surface area} = 4\pi\int_0^{a} x\sqrt{1+[df(x)/dx]^2}\,dx + 4\pi\int_{f(a)}^{0} x\sqrt{1+[dh(y)/dy]^2}\,dy,$$

where f(x) is the equation of Cassini's oval explicated with respect to y, and h(y) is the inverse function in the interval $(a, \mathring{x})$. The inverse function and the particular limits of integration were chosen in order to eliminate any possible singularity of the surface area integral. The numerical calculation of the integrals was made using an IBM computer 370/158 (Fortran IV language) on the basis of the Gauss 10 points quadrate formula[5]. Profiles were obtained using the CALCOMP 925/1036 unit.

Results. The results we obtained are reported in tables 1 and 2.

Table 1. For explanation see text.

Stage	Tonicity (mOsm/l)	$\mathring{x}$ distance (μm)	$\mathring{y}$ distance (μm)	Surface area (μm²)	Volume (μm³)
* 1	300	3.910	0.404	135.318	106.887
* 2	250	3.873	0.604	132.744	106.790
3	230	3.831	0.844	130.286	108.001
* 4	217	3,795	1.050	128.698	109.897
* 5	170	3.610	2.118	128.486	128.571
6	140	3.446	3.065	138.853	153.364
* 7	135	3.415	3.243	141.785	158.700
8	133	3.403	3.316	143.067	160.944
9	132	3.396	3.353	143.733	162.090
*10	131	3.390	3.390	144.415	163.251
* 1	300	3.910	0.404	135.000	94.000
* 4	217	3.795	1.050	135.000	116.000
*10	131	3.390	3.390	145.000	164.000