since uptake of exogenous polyamine is limited in these parasites[7]. Exogenous supply of these biogenic amines has not altered the course of *Trypanosoma brucei* infection in mice[2]. *In vitro* studies revealed that exogenous arginine and ornithine stimulate polyamine synthesis in trypanosomes[7]. The increased parasitaemia levels by the administration of exogenous arginine and ornithine observed in the present study may be attributed to increased production of polyamines. The arrest of trypanosome multiplication by the administration of DFMO along with ornithine supports this theory as DFMO is a specific suicide irreversible inhibitor of ODC.

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Catecholamines are present in hen's egg yolk in fairly stable form: elevated adrenaline indicates stress

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Levels of the catecholamines adrenaline, noradrenaline and dopamine in egg yolk did not change significantly on incubation of yolk for 30 min at 41°C. Enzymes that metabolize these amines may be absent or, if present, they may be in inactive state. Levels of these amines declined on exposure to heat, although considerable amounts still remained in the yolk of boiled eggs. Handling of the birds, increased density of birds in cages, and social stress induced marked elevation of adrenaline in egg yolk. The findings may be of importance to the poultry industry.

ADRENALINE and noradrenaline are potent stimuli for increased metabolic rate (thermogenesis), lipolysis, hyperglycaemia, and anxiety and fear in human beings[1–5]. They have also been implicated in modulation of cardiovascular[5,6], reproductive[7–10], platelet-aggregation[11] and several other functions[5,12]. Dopamine is well known for its role in the brain, but also acts in the kidney, inhibiting aldosterone[13–15]. In birds, catecholamines have been implicated in stress[16,17] but the elevation of catecholamines has usually been assumed, through fall in skin temperature following vasoconstriction, and increase in heart rate, blood pressure and blood glucose[18]. Handling of birds, increase in density of birds in cages, and social changes induce stress in birds[19,20]. Here we show the presence of catecholamines in yolk of hen's eggs and that these amines are quite stable in yolk on incubation or heating, or boiling of eggs. These results may be important in regard to consumer interest. We also show that there is marked elevation of adrenaline in yolk under stressful conditions. The catecholamine synthesis blocker α-methyl-*p*-tyrosine caused a dose-dependent reduction in levels of all three catecholamines.

The catecholamines were determined in 0.5 g of thoroughly mixed yolk, as for ovarian follicles[21]. Recovery of catecholamines added to acid butanol matched recovery of standard catecholamine added to yolk extract (Table 1). So acid butanol was used as extraction medium for determination of catecholamine in yolk. Table 2 gives catecholamine content of egg yolk after incubation for 30 min at 41°C and heating of thoroughly mixed yolk, in each case from the same eggs, for 5 or 10 min, in test-tubes of the same size ($60 \times 15 \times 0.5$ mm) placed in boiling water, and of yolk of boiled eggs. Incubation of yolk for 30 min at 41°C did not result in significant drop in levels of the catecholamines. Considering their very short half-life in the circulation, it is likely that the metabolizing enzymes for these catecholamines may be absent in yolk or, if present, may be in an inactive state. While there was a significant drop in levels after heating of yolk or boiling of eggs, considerable quantities were still present.

The effect of the catecholamine synthesis blocker α-methyl-*p*-tyrosine was also studied. Control birds were injected vehicle alone. Table 3 shows that the administration of vehicle alone caused significant increase in adrenaline content of egg yolk, indicating that the handling of birds for injection caused stress to the birds and an increase in yolk adrenaline. The catecholamine synthesis blocker caused a dose-dependent reduction in yolk catecholamine.

The yolk catecholamines were also determined six days after two birds were put together in the same standard-size individual-bird cage and again after separating them and putting them in separate cages. The increase in cage density and the social change, both considered to act as stressors[19,20], caused significant

Table 1. Test of recovery of egg yolk catecholamine using acid butanol.

	Catecholamine added (ng/2 ml)	Dopamine recovered		Noradrenaline recovered		Adrenaline recovered	
		Amount (ng)	(%)	Amount (ng)	(%)	Amount (ng)	(%)
Acid butanol alone	—	—	—	—	—	—	—
Acid butanol + catecholamine	250	162 ± 7	64.8	163 ± 10	65.2	165 ± 10	66.0
Acid butanol + catecholamine	500	325 ± 6	65.0	324 ± 10	65.8	326 ± 14	65.2
Yolk extract alone	—	2233 ± 32	—	1827 ± 35	—	734 ± 21	—
Yolk extract + catecholamine	250	2395 ± 36	64.8	1991 ± 30	65.6	898 ± 28	65.6
Yolk extract + catecholamine	500	2557 ± 36	64.8	2169 ± 45	68.4	1061 ± 23	65.4

Each value is mean ± SE of six determinations.

Table 2. Effect of heating of yolk and boiling of eggs on yolk catecholamine content.

Treatment	Catecholamine (ng/g) in yolk		
	Adrenaline	Noradrenaline	Dopamine
Control	377 ± 15[a]	986 ± 14[a]	1097 ± 26[a]
Incubation of yolk at 41°C for 30 min	359 ± 14[a] (4.77)*	976 ± 14[a] (1.01)	1094 ± 20[ab] (0.27)
Heating of yolk (0.5 g) in text-tube kept in boiling water for 5 min	263 ± 14[c] (30.24)	885 ± 22[b] (10.24)	1002 ± 28[cd] (8.66)
Heating of yolk (0.5 g) in test-tube kept in boiling water for 10 min	225 ± 10[d] (40.32)	827 ± 19[c] (16.13)	970 ± 15[d] (11.58)
Boiling of eggs	302 ± 7[b] (19.89)	910 ± 10[b] (7.70)	1037 ± 18[bc] (5.47)

Each value is mean ± SE of six determinations.
*Values in parenthesis are per cent decrease with respect to control in each case.
Values with different superscripts significantly different ($P < 0.05$).

Table 3. Effect of handling of birds and injection of α-methyl-*p*-tyrosine on yolk catecholamine content.

Treatment		Catecholamine (ng/g) in egg yolk		
		Adrenaline	Noradrenaline	Dopamine
Control (vehicle IP)	Pre-treatment	354 ± 10[b]	943 ± 26[ab]	1156 ± 15[a]
	Post-treatment*	469 ± 17[a] (↑32.49)†	1011 ± 32[a] (↑7.29)	1176 ± 20[a] (↑1.73)
α-Methyl-*p*-tyrosine (25 mg/kg each time IP)	Post-treatment	400 ± 25[b] (↑12.99)	869 ± 31[b] (↓7.85)	1006 ± 35[b] (↓4.33)
α-Methyl-*p*-tyrosine (50 mg/kg each time IP)	Post-treatment	283 ± 17[c] (↓20.06)	736 ± 34[c] (↓21.95)	837 ± 30[c] (↓28.00)

Each value is mean ± SE of six determinations.
*Treatment consisted of two injections at 10.30 a.m. and 4.30 p.m. each day for six days.
†Values in parenthesis are per cent increase (↑) or decrease (↓) with respect to control in each case.
Values with different superscripts significantly different ($P < 0.05$).

Table 4. Effect of density of birds in cages on yolk catecholamine content.

Condition	Catecholamine (ng/g) in egg yolk		
	Adrenaline	Noradrenaline	Dopamine
Single hen in each cage	370 ± 15[a]	991 ± 24[a]	1113 ± 31[a]
Two hens in a cage (6 days)	507 ± 16[b] (37.03)*	1067 ± 34[a] (7.67)	1147 ± 43[a] (3.06)
Hens back to separate cages	369 ± 21[a]	998 ± 28[a]	1105 ± 36[a]

Each value is mean ± SE of six determinations.
*Values in parenthesis are per cent increase.
†Values with different superscripts are significantly different ($P < 0.05$).

increase in adrenaline content of egg yolk of stressed birds, but not in noradrenaline and dopamine (Table 4). The adrenaline content returned to normal level a week after removal of stress.

The results show that the catecholamines adrenaline, noradrenaline and dopamine are fairly stable in yolk of hen's egg and any stress to the birds causes an increase in adrenaline content of yolk.

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Presence of phosphoribosylpyrophosphate synthetase in chloroplasts from fronds of *Bryopsis*

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The subcellular localization of phosphoribosylpyrophosphate (PRPP) synthetase activity in the fronds of a green alga, *Bryopsis* sp. was examined. The subcellular components of *Bryopsis* cells were squeezed out from the algar fronds without any requirement for homogenization, and intact organelles were easily obtained. Most of the activity of PRPP synthetase was distributed in the cytosol and chloroplasts.

5-PHOSPHORIBOSYL-1-PYROPHOSPHATE (PRPP) is a donor of phosphoribosyl groups for the synthesis of nucleotides. PRPP synthetase [ATP: D-ribose-5-phosphate pyrophosphotransferase, EC 2.7.6.1] from higher plants has been characterized[1,2]. Preliminary studies on the subcellular distribution of PRPP synthetase in spinach indicated that the enzyme is located predominantly in the cytosol[1]. However, our recent studies on purine metabolism in intact chloroplasts purified from spinach leaves suggest that a salvage pathway for adenine operates in the chloroplasts (H. Ashihara, unpublished observation). Salvage of adenine seems to be performed by adenine phosphoribosyltransferase which is present in the chloroplasts[3]. PRPP, required for the reaction catalysed by the enzyme, may be transported only with difficulty from the cytosol to the interior of the chloroplasts. It can, therefore, be presumed that machinery for the synthesis of PRPP is present in chloroplasts. In the present study, the localization of PRPP synthetase in cellular extracts of algal fronds of *Bryopsis* sp. was examined. This material was chosen because of the ease of preparation of intact organelles[4].

Bryopsis sp., collected at Futtu in Chiba Prefecture, Japan, was used as plant material. This green alga appears to be an intraspecific variant of *Bryopsis plumosa* (Hudson) C. Agardh from its morphological characteristics[4]. The cellular organelles and cytosol from fronds of *Bryopsis* sp. were extracted by the methods of Misonou *et al.*[4] with a slight modification. The algal fronds (ca. 40 g fresh wt) were cut with scissors and the components of cells were squeezed into 40 ml of ice-cold extraction medium (50 mM Tris-HCl buffer, pH 8.5, 0.5 M sorbitol, 10 mM sodium EDTA) through two layers of nylon cloth (pores, 100 μm in diameter). The suspension was filtered through a layer of Miracloth (Calbiochem, La Jolla, CA, USA), and the filtrate was centrifuged at 1500 g for 5 min. The supernatant fraction was further centrifuged at 10,000 g for 20 min. Chloroplasts were pelleted by the first centrifugation, and mitochondria and nuclei by the second one. The pellets were washed once with washing medium (25 mM Tris-HCl buffer, pH 7.6, 0.25 M sorbitol, 5 mM sodium EDTA). In order to check the intactness of the chloroplasts, the chloroplast fraction was examined by gradient centrifugation in different concentrations of Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden)[3,5]. A single band of chloroplasts was always observed. Purity of chloroplasts was checked by light microscopy. The preparation was not contaminated by nuclei or mitochondria.

For the preparation of the enzyme, pellets of particulate fractions were dissolved in 50 mM HEPES–NaOH buffer (pH 7.6), and homogenized with a Polytron homogenizer, Type PT 10–35 (Kinematica, Littau, Switzerland), operated at maximum speed for 15 sec at 2°C. In the case of separation of stroma from chloroplast membranes, the homogenate was further centrifuged at 10,000 g for 20 min at 2°C.

The activity of PRPP synthetase was determined as described earlier[1]. The reaction mixture contained the following components in a final volume of 2.0 ml. Twenty-five mM HEPES–NaOH buffer (pH 7.6), 75 μM ribose 5-phosphate, 300 μM ATP, 7.5 mM $MgCl_2$, 50 μM [7–^{14}C]orotate (specific activity, 37 kBq