chromatography according to the method detailed in the preceding paper by Boctor[8].

Results and discussion. The data available on qualitative and quantitative estimation of free amino acids in mites are still very scarce. Rodriguez and Hampton[13] determined the essential amino acids in young adult *Tetranychus urticae* females. 18 protein amino acids and 3 nonprotein amino acids were detected. In the present investigation, analysis of whole body extract of adult mite by 2-dimensional paper chromatography revealed the presence of 12 amino acids and 2 amides. Asparagine, ornithine, histidine, aspartic acid, serine, lysine and glycine are the major components of the free amino acid pool, comprising 83.94% of the total amino acid content. As shown in the table, asparagine and glutamine generally predominate in insect haemolymph and certain tissues[1,8,9,14]. The second most concentrated amino acid in mite extract is histidine. In most insects, histidine was also found at a high level[1,8,9].

Our results also show that glutamine, glutamic acid and cystine occurred in relatively smaller amounts. The concentrations of alanine, threonine and citrulline were almost the same and were found in the smallest quantity. Proline was detected on the chromatogram but because of its low concentration, could not be measured quantitatively. The trace of proline in mite tissue contrasts with relatively high concentrations in insects, probably owing to its special role in insect flight metabolism[15–17], and the difference between muscle energetics of walking mites and flying insects. Tyrosine was not detected in mite tissues. In insect haemolymph, tyrosine occurred in substantial amounts owing to its role in insect cuticle sclerotization[18,19].

Free amino acids of female *E. orientalis* adults

Amino acids	µmoles/100 g tissues	Percent of total content
Glycine	1065.944	10.94
Alanine	10.551	0.11
Serine	850.699	8.73
Threonine	15.782	0.16
Aspartic acid	742.449	7.62
Glutamic acid	415.754	4.27
Glutamine	515.223	5.29
Lysine	740.406	7.60
Histidine	1395.144	14.32
Citrulline	18.836	0.19
Ornithine	1353.060	13.89
Cystine	587.500	6.03
Asparagine	2029.970	20.84
Proline	Trace	Trace
Tyrosine	–	–
Totals	9741.318	99.99

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Alterations of β-adrenoceptor-density and cAMP-synthesis in rat-erythrocytes after stress erythropoiesis[1]

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Summary. During the maturation of red blood cells from rats after stress erythropoiesis, adenyl cyclase activity and β-adrenoceptor density (pmoles/mg protein) decrease at distinctly different rates suggesting a different turnover of these membrane units.

Previous investigations[2,3] have shown that mature erythrocytes from rats contain very low adenyl cyclase activity which can be stimulated specifically by isoprenaline (Ipn). In contrast, by means of ligand binding, e.g. with (-) (^{3}H) dihydroalprenolol (DHAP), a relatively high density of β-adrenoceptors has been demonstrated in membrane preparations from mature red blood cells[4]. When stress erythropoiesis was induced in rats by treatment with acetylphenylhydrazide (APH), a pronounced increase of Ipn-stimulated adenyl cyclase activity (about 100-fold) and also an increase of density of β-adrenoceptor sites (about 4–6-fold) occurred. These increments were linearly correlated to the respective reticulocyte counts, the latter depending on the dose of APH used[4]. These results are in accordance with the hypothesis that in the cytoplasmic membrane, the enzyme adenyl cyclase and the β-adrenoceptor are different entities[5–7] which may have independent and different turnover rates during the maturation process of the red blood cell[8].

It seemed of interest, therefore, to investigate the increase and decrease of the Ipn-stimulated adenyl cyclase activity and the density of β-adrenoceptor sites during the time course of the reticulocyte crisis produced by treatment of the animals with a fixed dose of APH.

Methods and materials. Male Wistar rats weighing 150–200 g were injected with 40 mg/kg acetyl-phenylhydrazide

(APH) on 3 consecutive days. The animals were decapitated and exsanguinated at the intervals given in figure 2. Adenyl cyclase activity stimulated by (-) isoprenaline (Ipn) was measured in freeze-dried membranes as described previously[9]. Maximal velocity of the stimulated enzyme activity (V_{max}-values) were calculated from Lineweaver-Burk plots. Maximal stimulation of cAMP synthesis in intact red blood cells was measured in the presence of Ipn (10^{-5} M) and the potent phosphodiesterase inhibitor Ro 20-1724 (10^{-4} M)[4]. Density of β-adrenoceptor sites in membrane preparations was determined in binding studies using (-) (^{3}H) dihydroalprenolol (DHAP; sp. act. 33 Ci/mmole; NEN, Dreieich)[4]. Reticulocytes were counted as percent of total erythrocytes in blood smears stained with brillant cresyl blue. Cell concentrations were determined in a coulter counter. Protein was measured using bovine serum albumin as a reference standard[10]. The results depicted in figures 1 and 2 are mean values from 3 individual experiments. All biochemical determinations were performed in quadruplicate.

Results. As reported previously[4], during increase of reticulocytosis from about 2 to 45% (figure 1), the maximal velocity (V_{max}-values) of the Ipn stimulated adenyl cyclase activities in membrane preparations is enhanced about 50-fold without alteration of the K_a-values for Ipn (10^{-7} M). Also cAMP synthesis measured in intact red blood cells increases about 7-10-fold. Concomitantly the β-adrenoceptor density in membrane preparations calculated from Scatchard plots (B_{max}-values) increases from 0.2 to 0.74 pmoles/mg protein, i.e. less than 4-fold. Only minor changes of the dissociation constants for (-) (^{3}H) dihydroalprenolol (DHAP; $K_D = 4.17$-5.3 nM) are observed. Maximal values of all parameters determined are reached on the 7th day after the 1st injection of acetyl-phenylhydrazide (APH; c.f. figures 1 and 2). Thereafter, with decreasing reticulocyte counts, the activity of Ipn-stimulated cAMP synthesis, in membranes as well as in intact red blood cells, falls steeply to reach control values at reticulocyte counts of about 2% (i.e. on the 21st day after the 1st injection of APH; figure 1). In contrast, an obvious delay in the decrease of receptor density is observed (figure 1).

The time course of the changes of the parameters measured is depicted in figure 2. Again it becomes evident that, concomitantly with the rapid decreasing reticulocyte counts, Ipn-stimulated cAMP synthesis, in membrane prep-

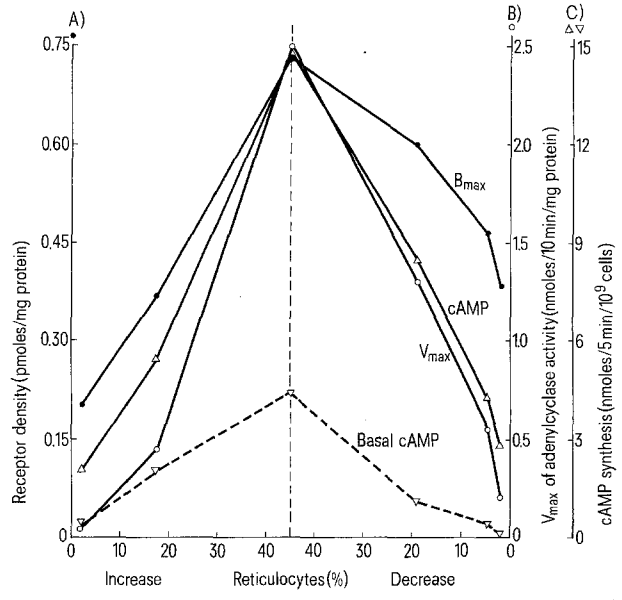

Fig. 1. Alterations of the β-adrenoceptor-adenyl cyclase-system in red blood cells from rats during stress erythropoiesis after treatment with 3×40 mg/kg acetylphenylhydrazide (APH). The reticulocyte counts (abscissa) are correlated with receptor density (B_{max}-values: A), isoprenaline (Ipn) stimulated adenyl cyclase activity in membranes (V_{max}-values: B) and in intact red blood cells (cAMP: C). The measurements were performed before and 4, 7, 10, 14 and 21 days after the 1st injection of APH. Basal cAMP (C): cAMP content of cell suspensions without addition of Ipn.

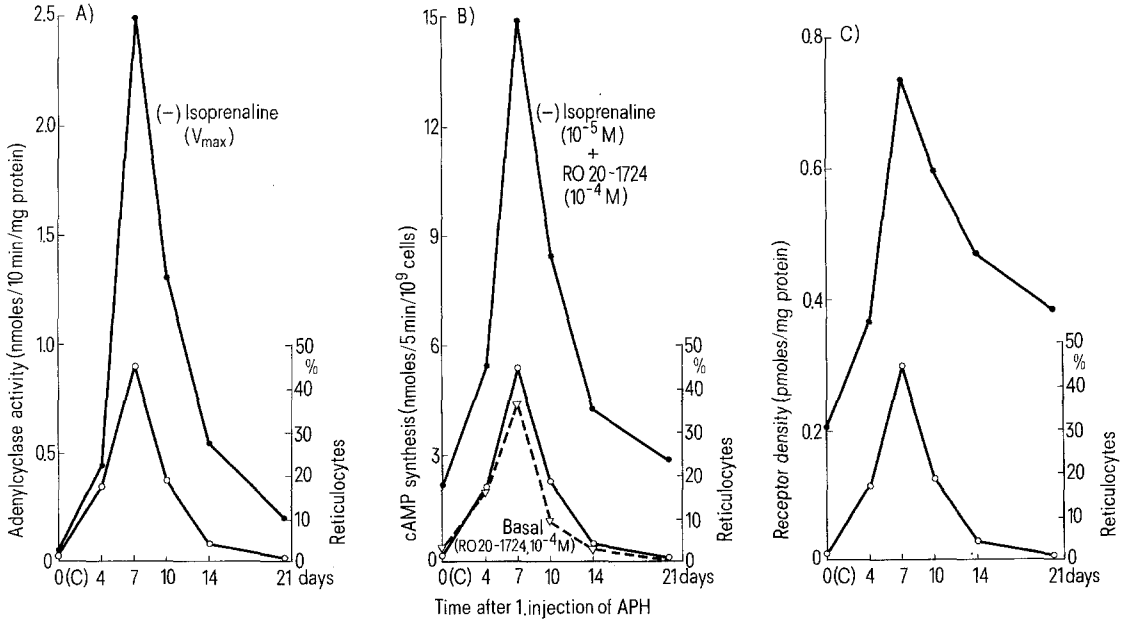

Fig. 2. Time course of alterations of the β-adrenoceptor-adenyl cyclase system and reticulocyte counts after treatment with 3×40 mg/kg APH. O---O: Reticulocyte counts. *A* Ipn-stimulated adenyl cyclase activity (V_{max}-values) in membranes. *B* Ipn-stimulated cAMP synthesis in intact red blood cells; ▽---▽: Basal values of cAMP without addition of Ion. *C* Density of β-adrenoceptors in membrane (B_{max}-values). O(C) = Control, i.e. without treatment with APH.

arations (A) as well as in intact cells (B), decrease with an approximate half-time of 2–4 days (evaluated from semi-log plots).
Again a significantly slower disappearance of β-adrenoceptor sites ($t_{1/2}$ approximately 8 days) is observed (C).
Discussion. From results presented previously, it has been concluded that, during the maturation process of the red blood cell from rats, the enzymatic activity of adenyl cyclase and the β-adrenoceptor are lost at the same rate: the fluoride activated enzyme activity and the isoprenaline (Ipn) stimulated enzyme activity decreased parallel with decreasing reticulocyte counts[2,3]. The results of ligand studies presented here reveal that this previous conclusion (drawn only from enzymatic experiments) was incorrect: cAMP synthesis and β-adrenoceptor density decrease at distinctly different rates[8,11]. These observations, however, are not in agreement with findings in growing rats[12] with decreasing reticulocyte counts; in this instance, a parallel decrease of adenyl cyclase activity and receptor density has been assumed. These results[12], however, were obtained in only 2 groups of animals of different age.
It must be taken into account, however, that the β-adrenoceptor unit is coupled to the enzymatic unit by a GTP-binding unit[13]. If this subunit of the receptor-effector system disappears more rapidly during the maturation process than the enzymatic unit, the whole system will be uncoupled. This would result in an apparently more pronounced decrease of adenyl cyclase activity. Furthermore, a relative lack of the activator of the coupling unit, i.e. GTP, could lead to such an uncoupling process. It is known that the GTP concentration in mature red cells is much lower than in immature cells[14].

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Studies on the metal-complex of acetyl salicylic acid (aspirin)

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Summary. The present communication deals with the isolation of acetyl salicylic acid (aspirin) complexes with Bi^{+3}, Zn^{+2} and UO_2^{+2}. The characterization of 1:2 complexes have been carried out with the help of conductometric, pH metric, elemental analysis and IR spectral studies. Spectrophotometric studies in case of UO_2^{+2} (the only colored complex) in range of 4.2 to 5.5 pH show absorption at 490 nm and complex obey Beers Law at the concentration range of 0.01 M to 0.1 M.

Very little work has been reported in the literature[1-5] on the complexes of metals and acetyl salicylic acid (aspirin) which is used for various pains of the human body. The complexes of metal ions like Pb^{+2}, Sn^{+2}, Al^{+3}, Cu^{+2}, In^{+3}, Ni^{+2} and Cd^{+2} have been reported. However, no work has been done on the complexes of Bi^{+3}, Zn^{+2} and UO_2^{+2}. The present communication deals with the isolation, characterization by elemental analyses and various studies in solutions with the help of spectrophotometer, conductometer, pH meter and IR spectral studies of the complexes. Spectrophotometric studies in the case of UO_2^{+2} (the only colored complex) in the range of 4.2 to 5.5 pH show absorption at 490 nm and complex obeys the Lambert and Beer Law at the concentration range of 0.01 M to 0.1 M. The pH metric, conductometric titration and Job's method of continuous variation observed 1:2 ratio (metal:ligand) in the complexes; this fact was confirmed by elemental analyses of the complexes.
Experimental. Acetyl salicylic acid has been isolated and crystallized as reported[6]. The nitrate of bismuth chloride of zinc and uranyl acetate used were of AnalaR grade. The standard solution of bismuth nitrate was prepared by dissolving it in HCl and making it upto mark with absolute alcohol in maintained pH up to 2. The solutions of zinc chloride and uranyl acetate were prepared by dissolving them in alcohol and conductivity water, respectively. All the conductometric titrations were performed by using Toshniwal conductivity bridge type CL01/02A and a dip type cell. pH metric studies were recorded with a Elico pH meter model L1-10 using hydrogen and calomel electrodes. Spectrophotometric studies were done on Bausch and Lomb's spectrophotometer model spectronic-20[7]. The IR-spectra of bismuth complex was taken in KBr while that of zinc and uranyl acetate complex were performed in nujol mull on a Perkins-Elmer model-621 spectrophotometer in the range of 4000 to 200 cm^{-1}.
The complexes of Bi^{+3} and Zn^{+2} were isolated as a crystals in alcoholic medium. The solutions of metal and ligand were mixed in the molar ratio of 1:2 (metal:ligand) stirred with magnetic stirrer. The solutions were then refluxed on a water bath; on cooling over night, defined pinkish crystals of Bi^{+3} and white crystals of Zn^{+2} complexes were separat-