amine is formed from para-tyramine by non-specific transmethylase[20]. DMPEA has been identified in the urine of both schizophrenic and normal subjects[21]. Therefore, these amines might be actively metabolized by MAO in vivo in mammalian tissues.

Many workers employ phenylethylamine as a type B-specific substrate. However, since phenylethylamine loses its specificity for the type B enzyme under various conditions[13,14], MPEA as well as benzylamine[13] is to be recommended as a more specific substrate for type B MAO.

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Levels of erythrocyte 2,3 DPG and ATP in heavy hashish smokers

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Summary. The variations in concentration of ATP and 2,3 DPG, and in lactic acid production as a function of time, were measured in the erythrocytes of heavy hashish smokers. Results indicate that the most remarkable reduction in concentration of the compounds examined occurred at 30 and 45 min after hashish smoking. The findings are discussed in connection with the possible influence of hashish components, especially Δ^9-THC, on the erythrocyte glycolytic pathway.

Hashish compounds are known to affect the metabolism of proteins, nucleic acids and lipids[1-3], and therefore probably influence carbohydrate metabolism. The connection between hunger – especially for sweet foods – and hashish smoking[4], led investigators to search for a hypoglycemic effect of the drug; none was observed[5,6]. On the contrary, Podolsky et al.[7] have detected hyperglycemic changes which were attributed to hashish constituents. Another interesting observation is that hashish compounds can inhibit glucose transport from the plasma into the erythrocytes[8]. Functional and structural changes induced by cannabinoids have also been described[3,9]. These observations prompted us to examine the possible variation of erythrocyte ATP and 2,3 DPG, and lactic acid production in chronic hashish users, under experimental conditions.

Materials and methods. 25 volunteers, heavy hashish smokers (aged 45–56), were examined in this study. These subjects had been using hashish for 20–30 years. Some of them had obvious signs of chronic cannabism. According to the information given by them they had every kind of hashish product (pure resin, marihuana etc.) that could possibly be found, always using a narghile pipe. Some of them reported smoking up to 100 g of crude hashish at a time. The hashish product used in this experiment was pure hashish resin extracted from the flowering tops of female cannabis plants. The hashish resin used was first analyzed by chromatographic methods and thus the concentration of the active constituents, especially Δ^9-THC, was accurately known (3,6%). Each of the hashish users was allowed to smoke 20 g of pure resin using a narghile pipe for a period not exceeding 10 min. In all cases blood samples were drawn from an antecubital vein prior to smoking and at 15, 30, 45 and 60 min following smoking, and transferred immediately into siliconized capillary tubes. After the whole blood leukocytes and platelets were separated, the erythrocyte residues were mixed with a 9:1 v/v 1.5% EDTA solution in 0.9% NaCl and centrifuged at 13,000 rpm for 30 min at 4 °C (Sorvall RC-2B). In the erythrocyte pellet ATP was estimated as described by Lamprecht and Trantschold[10]; 2,3 DPG was measured spectrophotometrically following the enzymatic method of Krimsky[11]. Finally lactic acid production was measured according to the method of Hohorst et al.[12]. The statistical analysis employed was Student's t-test.

Results. The values of erythrocyte 2,3 DPG, ATP and lactic acid production are presented in the table. It can be seen that the greatest alteration in 2,3 DPG and ATP as well as in lactic acid production are observed 30 and 45 min after smoking hashish. After 60 min the values tend to return to the level prior to smoking.

Discussion. The results obtained reflect the biochemical action of hashish components, especially Δ^9-THC, on erythrocyte glycolysis in heavy hashish smokers. The concentrations of ATP, 2,3 DPG and lactic acid production decreased in the erythrocytes of these subjects, with the greatest reduction being observed at 30 and 45 min after hashish smoking, when the highest concentration of the drug in the blood stream is noted[13]. This correlation indicates that the observed changes could be attributed to the direct and rapid effect of hashish constituents on erythro-

Erythrocyte 2,3 DPG and ATP concentrations and lactic acid production in heavy hashish smokers

Time (min)	2,3 DPG (mmole/mmole Hb)	p<	ATP (μmole/ml RBC)	p<	Lactic acid (μmole/ml RBC)	p<
0	1.17±0.06	-	1.71±0.10	-	1.54−0.09	-
15	1.10±0.04	0.0005	1.66±0.08	0.01	1.48−0.10	0.025
30	1.04±0.04	0.0005	1.54±0.07	0.0005	1.34−0.07	0.0005
45	0.93±0.02	0.0005	1.34±0.04	0.0005	1.25−0.05	0.0005
60	1.16±0.05	NS	1.62±0.06	0.0005	1.42−0.10	0.0005

Values are expressed as mean±SD.

cyte glycolysis. A possible explanation for this interesting finding is that of Schurr et al.[8], who proposed that for hashish smokers a temporary halt in glucose transport from the plasma to the erythrocytes occurs. This could explain the effective concentrations of the drugs in inhibiting glucose transport and correlates with the doses leading to physiological reactions. According to Schurr et al.[8] the decreased concentration of ATP, 2,3 DPG and lactic acid production observed in the present study could be explained by the low rate of efflux of glucose into erythrocytes and consequently a reduced amount of substrate for anaerobic glycolysis. However, the possibility that these findings are associated with the influence of hashish constituents on enzymes regulating the erythrocyte glycolytic rate cannot be excluded.

The reduced concentration of erythrocyte ATP is of great interest. It is well known that ATP is involved in the biosynthesis of phosphatidic acid intermediates in the production of phospholipids[14]. The reduced concentration of ATP supports our previous observations of a decreased phospholipid concentration in erythrocytes 30–60 min after hashish smoking[3]. Since it is clear that phospholipids play a functional and structural role in membrane integrity and are probably correlated with the changes observed after hashish smoking[3,9], it is evident that reduced ATP concentrations are incriminated in these changes.

The reduced concentration of 2,3 DPG after hashish smoking is another interesting finding in the present study. It is well established that this intermediate product of glycolysis is the main regulator of oxygen release from hemoglobin to the tissues, shifting the oxyhemoglobin dissociation curve (ODC) to the right[15]. Thus, the decreased level of 2,3 DPG after hashish smoking probably shifts the ODC to the left resulting in tissue hypoxia. This could explain the observation that after hashish smoking the peripheral blood flow is increased[16], since it is well documented that disturbances of tissue oxygen tension lead to adaptive changes in peripheral blood flow[17].

The results of this study clearly indicate a disturbance in the erythrocyte glycolytic pathway among heavy hashish smokers and warrants further investigation to identify the possible effect of hashish constituents on enzymes regulating the rate of erythrocyte glycolysis.

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Properties of a calcium-dependent apyrase in the saliva of the blood-feeding bug, *Rhodnius prolixus*

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Summary. The saliva of the blood-feeding bug *Rhodnius prolixus* contains at least 11 proteins, and has Ca^{2+}-dependent apyrase activity. The activity has a broad pH optimum between pH 7 and 9, and is inhibited by Mg^{2+}. Polyacrylamide gel electrophoresis and gel filtration suggest the possibility of at least 2 enzymes responsible for the activity.

Rhodnius prolixus is a triatomine hemipteran which feeds exclusively on blood. In the laboratory, it will gorge on a variety of inorganic solutions if they contain μmolar concentrations of ATP[2]. It is probable that in vivo this response to ATP enables the insect to detect an adequate supply of red blood cells[3]. Recently, the saliva of *R. prolixus* has been reported to have a potent ATPase activity[4]; one effect of this is to alter the apparent sensitivity of the bug to ATP in artificial diets. Here we report some properties of the enzyme or enzymes responsible.

Material and methods. Saliva was obtained from a laboratory stock of *R. prolixus.* Heads were pulled free of the body in 5th instar larvae, and the large, pink salivary glands were removed from the heads by pulling their ducts free of