the average amount of apoprotein synthesized in excess of the amount catabolized over the 6-day period can be calculated from the plasma levels, assuming a plasma volume of 3.8 l[14]. It thus appears that there was a total body increment of 88 mg/day of apoA-II, 4 mg/day of apoC-II, and 23 mg/day of apoC-III as calculated from the table.

The finding that apoprotein levels return to levels near the baseline before the plasma cholesterol achieves its pre-exchange level is significant, as it suggests that plasmapheresis can be repeated at intervals that would permit successive decrements in the plasma cholesterol and a decrease in total body cholesterol while permitting recovery of plasma apoA-II, apoC-II, and apoC-III levels. This observation requires further documentation both in additional patients and also for the other apoproteins. We are currently engaged in work that would permit this to be done.

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Human plasma dopamine-β-hydroxylase: oxygen and thermal stability[1]

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Summary. There are wide individual variations in the thermal stability of human plasma dopamine-β-hydroxylase (DBH). Thermal stability variations have proven of value in biochemical genetic studies of many enzymes. The development of DBH thermolability depends on the exposure of plasma to oxygen. This observation may help to elucidate the biochemical basis of the genetic regulation of DBH.

Dopamine-β-hydroxylase (E.C. 1.14.17.1, DBH) catalyzes the conversion of dopamine to the neurotransmitter norepinephrine, is localized to catecholamine containing vesicles in sympathetic nerves and the adrenal medulla, and is found circulating in blood[2]. Plasma DBH has often been measured because of the possibility that it might reflect the status or function of the sympathetic nervous system[2]. However, it is now known that most of the variance in basal human plasma DBH enzymatic activity and immunoreactive protein is due to the effects of a single genetic locus, *DBH*[3-9]. Recent studies have shown that, in addition to variation in basal enzyme activity, there are wide individual variations in the thermal stability of human plasma DBH[7,10]. Variations in thermal stability reflect variations in protein structure and have been helpful in the study of genetic polymorphisms of a variety of enzymes[6,11-13].

Approximately 10% of a randomly selected population has thermolabile plasma DBH. The trait of thermolability is a characteristic of the DBH molecule itself, and has a significant familial aggregation[10]. Family studies indicate that although this characteristic is probably inherited, it does not segregate with the alleles at the locus *DBH* that control basal enzyme activity[10,13,14].

Thermolability of DBH cannot be demonstrated with freshly obtained plasma, but rather it is 'developed' after plasma is incubated at 37 °C for 18-24 h in vitro. This step must then be followed by thermal inactivation at 55 °C for 20 min, a procedure required for the 'expression' of thermolability[10].

Elucidation of the biochemical mechanism responsible for the development of the characteristic of thermolability would contribute significantly to our understanding of individual biochemical differences in human DBH and might prove useful in studies of the structural gene of this important catecholamine biosynthetic enzyme. We have studied the possibility that exposure to oxygen during the development of thermolability might be the critical element in uncovering individual variations in the thermal stability of human plasma DBH.

Heparinized blood samples were obtained from selected laboratory personnel known to have either thermolabile or thermostable DBH and from 99 consecutive, randomly selected blood donors at the Mayo Clinic Blood Bank. Preincubation for the development of the characteristic of thermolability was performed by incubating 200 µl of each sample at 37 °C for 18 h in stoppered 2 ml vacutainer tubes. The gas in the vacutainer was changed by evacuating the tube for 20 sec under a vacuum of 20 inches of mercury, followed by introducing nitrogen, oxygen or air into the tube through a 25 gauge needle for 20 sec with 10 psi of pressure. The procedure for changing the gas in the tube was then repeated a 2nd time. After the preincubation step, plasma was diluted with water 1:50 (vol:vol) and 200 µl aliquots were placed in reaction tubes. The thermal inactivation step necessary to bring about the expression of thermolability was performed by incubation of the diluted plasma at 55 °C for 20 min. Control samples were kept on ice at 4 °C during the thermal inactivation step. DBH

enzymatic activity was measured by the method of Molinoff et al. as previously described[10,15]. 1 unit of enzyme activity represented the formation of 1 nmole of β-phenyl-β-ethanolamine per h. The ratio of enzyme activity in a sample heated at 55 °C to the activity in the control sample, a so-called H/C ratio (heated divided by control), served as an index of thermal stability[10,13].

To test the hypothesis that oxygen plays a role in the development of DBH thermolability, plasma samples from 2 subjects known to have thermostable and 2 subjects known to have thermolabile DBH were incubated at 37 °C for varying periods of time from 0 to 18 h in the presence of air, nitrogen or 100% oxygen. No significant change in the basal DBH activity occurred in any sample as a result of this treatment. However, thermal stability after incubation at 55 °C as measured by H/C ratios decreased dramatically in the thermolabile samples following incubation with air or oxygen (figure 1). These same samples showed no decrease in thermal stability after incubation in the presence of nitrogen (figure 1). At all time periods tested, preincubation with oxygen resulted in greater thermolability than did incubation in the presence of air. Plasma samples with thermostable DBH did not become labile when incubated with air, oxygen, or nitrogen (figure 1).

In a separate experiment, after preincubation at 37 °C in the presence of air it was shown that there was no difference in the expression of the trait of thermolability for sample A (figure 1) when the 55 °C thermal inactivation step was performed under an atmosphere of either nitrogen or air. This result indicated that the presence of oxygen was not required for the expression of DBH thermolability even though it was required during the development step.

DBH enzymatic activity is usually measured in the presence of catalase because the enzyme can be inactivated by peroxides formed from oxygen during the assay procedure[2]. An experiment was performed to determine whether catalase might alter the development or expression of DBH thermolability. Plasma from a subject with thermolabile DBH was incubated at 37 °C in the presence of air for 18 h with and without the addition of 6500 units per ml of beef liver catalase (Boehringer-Mannheim). The DBH in the sample incubated in the absence of catalase was thermolabile with an H/C ratio of 0.437 ± 0.046 (mean $\pm$ SEM, $n = 3$). The H/C ratio was not significantly changed at 0.388 ± 0.037 following incubation in the presence of catalase. These results made it much less likely that peroxide formation played a significant role in the development of DBH thermolability in samples exposed to air or oxygen.

One reason for interest in DBH thermolability would be its possible use for the detection of genetic polymorphisms. Exposure of plasma samples to oxygen might result in the detection of a subgroup of subjects with thermolabile plasma DBH. Therefore, fresh plasma samples from 99 randomly selected subjects were pretreated for 18 h at 37 °C in the presence of oxygen prior to incubation at 55 °C. The average basal enzyme activity in these samples was 698 ± 39 units/ml (mean $\pm$ SEM), very similar to values reported previously for randomly selected population samples[2,3]. The frequency distribution of H/C ratios for this population sample is shown in figure 2. Most subjects had stable DBH (H/C ratios > 0.8) while 9 of the 99 subjects had thermolabile enzyme with H/C ratios of less than 0.8. This frequency distribution is not strikingly different from that reported previously for plasma samples exposed to air rather than oxygen during the 37 °C pretreatment step[10].

Plasma DBH enzymatic activity and immunoreactive protein have been measured as potential markers for the function and/or status of the sympathetic nervous system in man[2]. Unfortunately, large individual variations in enzyme activity and immunoreactive protein due to the effects of inheritance have complicated interpretation of the results of these experiments[3-9]. Thermal stability has been a useful technique for detecting genetic polymorphisms of a variety of enzymes[10-13]. There are wide individual variations in the

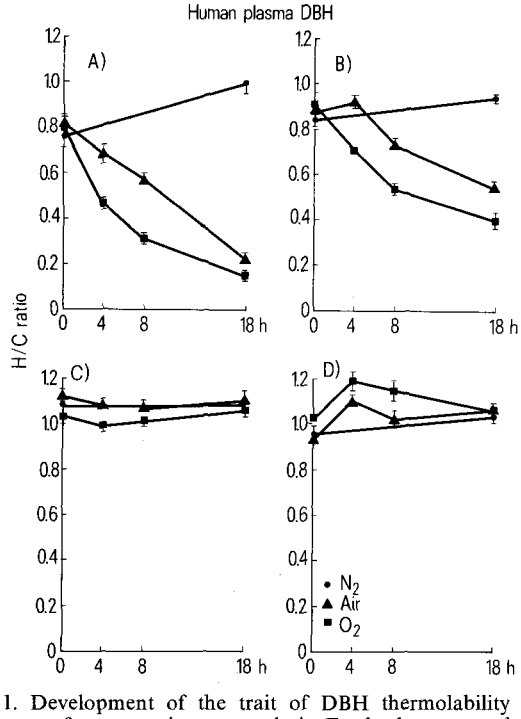

Fig. 1. Development of the trait of DBH thermolability in the presence of oxygen, nitrogen, and air. Fresh plasma samples from subjects with thermolabile DBH (A and B) and thermostable DBH (C and D) were pretreated at 37 °C in the presence of nitrogen (●——●), air (▲——▲), and oxygen (■——■) prior to determination of thermal stability by treatment at 55 °C. Each value represents the mean $\pm$ SEM of 3 determinations of both the control and heated samples. Basal enzyme activities before treatments of samples A, B, C, and D were $159 \pm 2{,}4$, 381 ± 9, 988 ± 37 and 416 ± 15 units/ml (mean $\pm$ SEM), respectively. Basal values did not change significantly during the 37 °C pretreatment. (See text for details.)

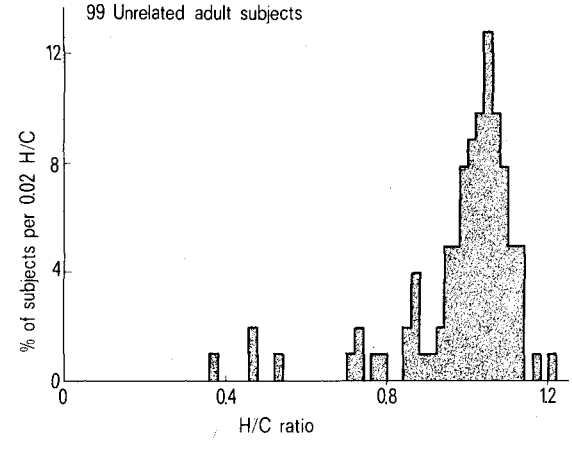

Fig. 2. Frequency distribution histogram of human plasma DBH thermal stability after pretreatment with oxygen. Fresh plasma samples from 99 randomly selected adult subjects were pretreated by incubation at 37 °C for 18 h in the presence of oxygen prior to thermal inactivation at 55 °C for 20 min. H/C ratios were used as a measure of thermal stability.

thermal stability of human plasma DBH[10]. It is important that the biochemical basis of these variations in thermal stability be elucidated. We have shown that the development of plasma DBH thermolability is dependent on the presence of molecular oxygen. This observation may serve as a basis for future studies of individual variations in the biochemical properties of the DBH molecule and may be applied to studies of the structural gene for DBH in man.

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Effects of various inhibitors and 2,4-dinitrophenol on adenosine triphosphatase from Malpighian tubules of *Locusta migratoria* L.

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Summary. Both Mg^{2+}-ATPase and HCO_3^--stimulated ATPase activity were inhibited by sodium azide and to a lesser extent ethacrynic acid and amiloride. 1 mM DNP stimulated Mg^{2+}-ATPase activity by 22% and HCO_3^--stimulated ATPase activity by 7%.

Since Kasbekar and Durbin[1] and Sachs et al.[2] demonstrated the presence of a Mg^{2+}-dependent HCO_3^--stimulated ATPase in frog gastric mucosa, numerous other researchers have reported similar anion-sensitive ATPase activity in homogenates of various epithelia from a variety of different species. These include mammalian pancreas[3-5], submandibular gland[6,7], renal proximal tubule[8,9], K^+-transporting midgut of *Hyalophora cecropia*[10], rectum of *Schistocerca gregaria*[11] and the Malpighian tubules of *Locusta migratoria*[12]. Such anion-sensitive ATPase has been implicated in a variety of ion transport systems. For example, H^+/HCO_3^- transport[3-5], Na^+/H^+ exchange[8,9] and Cl^- transport[11]. Since ion transport is considered to be the 'driving force' in fluid secretion by insect Malpighian tubules, the nature of the HCO_3^--stimulated ATPase reported in this tissue[12] warrants further investigation. The present study has been carried out to determine the effects of 2,4-dinitrophenol (DNP), ethacrynic acid, amiloride and sodium azide on the Mg^{2+}-dependent ATPase of *Locusta* Malpighian tubules as a basis for future physiological studies and to permit comparison between this anion-sensitive enzyme and ATPases from other sources.

Materials and methods. Mature adult locusts, *Locusta migratoria* L., were used and these were taken from a population maintained under crowded conditions at 28 ± 0.5 °C and 60% relative humidity.

The methods of preparation of the membrane microsomal homogenate, of enzyme assay and of protein determination were essentially as described previously[12]. In determining enzyme activity, 3 incubation media, having the following composition, were used: 1. 2 mM magnesium chloride; 2. 2 mM magnesium chloride and 20 mM sodium chloride; 3. 2 mM magnesium chloride and 20 mM sodium bicarbonate, unless otherwise stated. Each medium contained 3 mM ATP (sodium salt) final concentration and 20 mM imidazole/HCl, pH 7.5. All tubes were thermoequilibrated for 15 min before starting the reaction by the addition of 0.5 cm^3 microsomal suspension. Incubations were carried out at 30 ± 0.1 °C. All solutions were made up in glass-distilled, deionized water. All inorganic salts were AnalaR grade or the best commercially available. Imidazole and ATP (di-sodium salt) were obtained from Sigma Chemical Co., sodium azide and 2,4-dinitrophenol from BDH Chemicals Ltd, Ethacrynic acid and amiloride were a gift from Merck, Sharp & Dohme Ltd.

Results. The inclusion of 1 mM sodium azide in the reaction media resulted in almost complete inhibition of Mg^{2+}-dependent ATPase ($\pm$ 20 mM NaCl) and Mg^{2+}-dependent HCO_3^--stimulated ATPase activity; Mg^{2+}-dependent ATPase activity being reduced from 315.6 ± 21.4 to 2.0 ± 0.3 nmoles Pi liberated/mg protein/min ($n = 3$) and from 322.0 ± 20.0 to 2.1 ± 0.4 nmoles Pi liberated/mg protein/min (+20 mM NaCl; $n = 3$) whilst the Mg^{2+}-dependent HCO_3^--stimulated ATPase activity was reduced from 526.3 ± 37.9 to 4.2 ± 0.3 nmoles Pi liberated/mg protein/min ($n = 3$). In contrast, amiloride had no effect on Mg^{2+}-dependent ATPase ($\pm$ 20 mM NaCl) over the concentration range 5×10^{-5} M to 5×10^{-8} M. There was, however, some slight inhibition of activity at the highest concentration of amiloride used (5×10^{-4} M); the inhibition of Mg^{2+}-