inner side of the skin were read. The PCA titres were expressed as the reciprocal of the greatest dilution of serum giving a visible skin reaction. The magnitude of the reaction was expressed by the mean of the long and short diameter of the reaction site.

Histamine-induced airway constriction was studied using 4 guinea-pigs anesthetized with an i.p. injection of pentobarbital sodium (80 mg/kg). For i.v. injections a small polyvinyl chloride catheter was inserted through a skin window into a peripheral vein. Airway resistance was then measured by the overflow technique. The trachea was cannulated and the animal ventilated by means of a Starling miniature respiration pump at a rate of 60 strokes/min. The air overflow from the water valve passed through a pneumotachograph tube connected to a differential air pressure transducer. Changes in overflow were displayed on a recorder. The animal was challenged with various doses of histaminase and histamine. Inhibition of histamine induced bronchoconstriction was recorded as percent of the maximal overflow. Histaminase and histamine were injected within 30 sec.

Results and discussion. The enzyme purification is summarized in the table. With this method histaminase activity was purified 2500-fold from starting materials. The titre of passive cutaneous anaphylaxis was 32 or higher in group A (control) but in group B (3000 units of histaminase) the titre was 32 or lower in all animals. The titre was greatly decreased in group C (10,000 units) to 8 or lower but the effect of histaminase on PCA disappeared within 30 min (group D). The PCA reaction was decreased in size in the animals pretreated with histaminase. At the site injected i.d. with 32 times diluted antiserum the mean diameter of the reaction in group A was 23.5 mm, but in the group B the mean diameter was decreased to 5.5 mm (fig. 1).

I.v. injection of histamine resulted in a marked increase in airway resistance. The time course of airway resistance after i.v. infusions of histaminase and histamine is given in figure 2. 50,000 units of histaminase completely inhibits the histamine-induced bronchoconstriction, but its effect in vivo disappears within 30 min. I.v. infusion of pig kidney histaminase did not cause any allergic side-reaction in guinea-pigs.

Histaminase may be a useful tool in respiratory physiology to distinguish the action of histamine from that of other mediators.

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Effect of streptozotocin diabetes on adenosine-5′-triphosphate, oxygen consumption and steroidogenesis in testis mitochondria from rats

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Summary. The activity of the enzyme cleaving the side-chain of cholesterol (rate limiting step in steroidogenesis) was considerably reduced in experimentally induced diabetes. This result was accompained by both an increase in oxygen consumption and an increase in ATP synthesis. Insulin treatment prevented them.

It is well known that the diabetic state produces reproduction disturbances such as impaired fertility and reduced steroidogenic activity[2-5]. On the other hand it is likely that the respiratory chain and the cholesterol side-chain cleavage enzyme complex of testis mitochondria are linked in some manner, but it is not clear whether energy obtained from substrate oxidation can support both ATP production and mitochondrial steroidogenesis.

We have investigated this problem by studying the metabolism of testis mitochondria from diabetic rats and from diabetic rats treated with insulin.

Material and methods. Male Sprague-Dawley rats, weighing 250–300 g were used in all experiments. Diabetes was induced in 2 groups of animals by i.p. administration of streptozotocin (STZ) solution made in 0.1 M citrate buffer, pH 4.5 at a dose of 40 mg/kg b.wt. 2 days after administration of STZ, 1 group of the diabetic rats was given a protamine zinc insulin s.c. injection at the same time each morning for 28 days, in an amount (1–5 IU) that was adequate for normalizing the diabetic state. The rats were used 30 days after injection of STZ and were killed by decapitation without anesthesia. Testes were removed, decapsulated and homogenized in 250 mM sucrose solution pH 7.4 containing 20 mM KCl and 1 mM EDTA. Mitochondrial ATP synthesis and [4-^{14}C]-cholesterol conversion were determined simultaneously under the same experimental conditions. The mitochondrial preparations were obtained by the method of Dimino et al.[6] and mitochondrial ATP synthesis rate was assayed by measuring the disappearance of inorganic phosphate used for mitochondrial phosphorylation of ADP by trapping the synthesized ATP as glucose-6-phosphate[6,7]. Steroidogenic

Effect of diabetes and insulin treatment on: glycemia; body weight; testicular weight; oxygen utilization, ATP synthesis and cholesterol conversion by testis mitochondria

	Glycemia post prandial serum glucose	Body weight at autopsy (g)	Testes weight (g/100 b. wt)	Oxygen uptake (µl/mg protein × h)	ATP synthesis (µmoles/mg protein × 2 h)	% [4− ^{14}C]-cholesterol conversion/mg protein × 2 h
Control	105± 7	314±15	1.31±0.06	9.7±1.2	96.7± 7.7	6.01±1.7
Diabetic	358±23*	154±13*	0.75±0.07*	18.1±2.9*	156.7±22.6*	3.57±1.4*
D+insulin	115±10	252±14	1.25±0.03	10.9±0.1	124.9±22.2	6.99±0.6

*Significantly different from control rats. Statistical significance is given in the text.

activity of testis mitochondria was determined by measuring the percentage of [4-^{14}C]-cholesterol converted to [4-^{14}C]-pregnenolone and [4-^{14}C]-progesterone by the modified method of Robinson and Stevenson[8]. The total volume was 1.5 ml, which included 0.1 ml of 70 mM succinate used as the source of reducing equivalents. 2 mg mitochondrial protein and 20,000 cpm of [4-^{14}C]-cholesterol (50-60 mCi/mmole, New England Nuclear) were used in each assay. Oxygen consumption of testis mitochondria was determined by Warburg's manometric method, as has previously been described in detail[9]. The assays were initiated by the addition of 10 µl 1 M succinate. Protein was determined by the method of Lowry et al.[10], with bovine serum albumin used as standard. The significance of differences between means was tested by Student's test; p values < 0.05 were considered significant in the present study.

Results and discussion. Our findings are given in the table. Diabetes caused a body-weight loss of 51% ($p < 0.05$) and a testis weight loss of 44% ($p < 0.002$). Insulin treatment partially prevented these. Glycemia during diabetes was severely increased, and insulin normalized the diabetic state. Similar results were obtained by Paz and Homonnai[11]. Oxygen utilization was increased with succinate as substrate by 50% ($p < 0.002$) in the diabetic state, and insulin treatment completely prevented abnormal oxygen uptake. [4-^{14}C]-cholesterol conversion activity was severely inhibited ($p < 0.002$) and insulin treatment completely prevented it even to supranormal level. As this reaction is considered to be the rate-limiting step in steroidogenesis, this finding may be indicative of a reduction of testosterone production[12]. Coupled with this decrease in mitochondrial steroidogenesis there was an increase in the ATP synthesis rate by 39% ($p < 0.025$).

These observations suggest that the energy obtained from substrate oxidation can be diverted from ATP production to support steroidogenesis in testis mitochondria. In control animals the energy derived from succinate oxidation could be used for ATP synthesis, and this used to support other mitochondrial functions including reversed electron transport and the energy dependent transhydrogenase enzyme. If succinate supplies reducing equivalents for cholesterol conversion, it is probable that it would reduce NAD^+ by reversed electron transport, which then could reduce NAD^+P by the transhydrogenase enzyme[6,13]. However, in the diabetic state the reducing equivalents or energy that were potentially available to support mitochondrial steroidogenesis could now be used, at least in part, to enhance ATP synthesis. Similar observations, suggesting that energy obtained from succinate oxidation could be diverted from phosphorylation to support steroidogenesis, have been previously reported for ovarian mitochondria[14].

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Association between apolipoproteins A-I and A-II as evidenced by immunochemical approach[1]

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Summary. Apolipoprotein A-I isolated from human plasma high density lipoproteins were studied for its possible association with ^{125}I-labeled A-II. Using immunoprecipitation technique, we found that A-II, but not C-II and C-III, associated with A-I. The association was completed within 30 min and was temperature dependent.

Apoproteins A-I and A-II are the Major protein moieties of human plasma high density lipoproteins (HDL). The apoproteins are synthesized in the liver and intestine[2,3]. The mechanism involved in the assembly of apoA-I and apoA-II into HDL in vivo is still not clear. The molecular and solution properties of the apoproteins have recently been reviewed by Osborne and Brewer[4]. The proteins tend to self-associate and form aggregates in aqueous solution. The degree of self-association is dependent on the concentration[4] and on the temperature[5]. However, whether or not this apoprotein exists as an equilibrium mixture of monomers and dimers, dimers and tetramers, or monomers, dimers, tetramers and octamers is still controversial[4-9]. Besides self-association, these two apoproteins may associate with each other and form a hybrid. Using a cross-linking reagent, it has shown some degree of interaction between apoA-I and apoA-II[5]. Whether or not the association is temperature dependent is not known. In addition, the ability of apoA-I to associate with apoproteins C has not been reported. Alternatively, we now report the association between apoA-I and apoA-II using an immunochemical method. The association between apoA-I and ^{125}I-labeled apoC-II and apoC-III was also investigated. Understanding of the protein-protein interaction of these apoproteins may help us to elucidate the molecular orientation of the apoproteins in HDL as well as their metabolic pathway in vivo.

Materials and methods. ApoA-I and apoA-II were isolated by gel filtration on Sephadex G-150 in the presence of 5 M guanidine HCl, pH 8.0[10]. Homogeneity of apoproteins was determined by polyacrylamide gel electrophoresis and by amino acid analysis. Goat and rabbit anti-apoA-I antisera were obtained using the procedures described previously[10-13]. The antisera were then partially purified by