mersed into a water-bath at 37 °C for thawing and rewarming. The fragments were washed with Krebs-Henseleit solution at 37 °C and the contractility examined. The contractility, i.e. percentages of contracting fragments 80 min after rewarming, was used as a criterion for viability. Contraction of the fragments was triggered by electric stimulation, using rectangle pulse, 12 V, 2 msec, in special chambers perfused with oxygenated Krebs-Henseleit solution and addition of 3 µg/l adrenaline.

Results and discussion. In the present experiments, we have observed the effects of changes in composition of the cryoprotective medium on contractility of heart auricle fragments following storage at −196 °C. Earlier studies from this laboratory have shown that variations in electrolyte composition of preservation solutions can account for the differences in contractility of auricle fragments occurring after short-term storage at temperatures between −15 °C and −50 °C[9,10]. Now we could observe that by increasing the potassium ion, magnesium ion and calcium ion concentrations in the preservation solution B at a constant level of DMSO, about 40% of frozen and thawed heart fragments could survive a storage in liquid nitrogen (table 2). With an additional increase of the glucose level in the preservation solution to 166 mM, the number of heart fragments able to contract after a storage of 24 h at −196 °C increased to 60% (table 2). The average differences were statistically significant and therefore show the cryoprotective effectiveness of the changed electrolyte and glucose composition.

Corresponding to our experience and to that of Sumida[5] we had chosen a relatively high concentration of DMSO. Under these conditions, considerable injuries by osmotic and toxic influences of the cryoprotectant should be taken into consideration, even with hypothermia[9,11]. Figure 2 shows a comparison of the cryoprotective effectiveness of preservation solution A, B and C. It is evident that K^+-, Mg^{++}- and Ca^{++}-rich preservation solutions act during the first stages of cryopreservation, whereas the degree of tissue damage occurring between −25 °C and −196 °C can be reduced by addition of glucose in preservation solution C. The level of ATP in ischemic heart tissue has been associated with contractility restoration[12,13]. The use of cardioplegic intracellular type solution under hypothermic conditions results in a reduction of metabolic requirements of heart tissue and in a better maintenance of energy-rich phosphates[14,15]. A comparison of our results in the various stages of storage shows the advantageous effect of depolarizing solution B. The improved contractility by increased glucose concentration in solution C suggests that glucose in connection with DMSO has a protective influence on the cell membrane and/or results in additional dehydration of heart tissue during freezing (smaller degree of damage at stages 2 and 3 in figure 2). Therefore the influence of glucose could be explained as an additional or competitive effect related to the cryoprotective effect of DMSO.

Our experiments show that, not only isolated heart muscle cells, but also heart auricle fragments of adult rats, can be frozen and thawed with a high degree of survival of contracting fragments. The composition of the basal medium in the cryoprotective medium used for cryoprotection plays an important role in the cryopreservation of biological materials.

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Increased blood pressure in the SHR is not related to a deficit in renomedullary PGE_2[1]

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Summary. Synthesis of prostaglandin E_2 by renal medulla from SHR and WKY rats was compared during early postnatal development. Although arterial blood pressure was significantly higher in SHR as early as 6 weeks of age, no difference in renal medullary prostaglandin synthesis was observed.

Current research on hypertension has been directed, in part, toward the hypothesis that deficiencies of renal vasodilators could play a role in the etiology of essential hypertension[2,3]. Attention has focused on prostaglandin E_2 (PGE_2) because this potent vasodilator[4,5] is synthesized in large amounts by the renal medulla[6]. Therefore, a natural hypothesis was that a deficiency in renal PGE_2-production and/or release contributed to the etiology of hypertension[7]. Indeed, several groups have reported decreased release of PGE_2 from kidneys of hypertensive rats[8-10].

Unfortunately, most animal models of hypertension require either surgical or pharmacological manipulation of the kidneys, making extrapolation of the data to the etiology of essential hypertension in man difficult. The development of the spontaneously hypertensive rat (SHR) made it possible to quantify renal vasodilators in a hypertensive rat model that did not require prior manipulation of the kidney.

If a deficiency of PGE_2 is involved in the etiology of hypertension, the deficiency should appear before the development of hypertension. Alternatively, if a deficit in

PGE_2 occurs as a result of the hypertension, no difference in renal PGE_2-metabolism should occur until after the development of hypertension. These investigations were designed to determine if a decrease in the ability to synthesize renomedullary PGE_2 occurred prior to the development of hypertension in the SHR.

Methods. Renomedullary PGE_2-production was determined in Okamoto SHRs and age matched Wistar Kyoto normotensive rats (WKYs) at 3–15 weeks of age. This age range allowed the measurement of PGE_2-production before, during and after the development of hypertension. SHRs and WKYs were raised in our colony or purchased from Laboratory Supply Co., Indianapolis, Indiana. Systolic blood pressure was measured indirectly by tail plesmography on animals 6 weeks of age and older.

Prostaglandin production was determined by an in vitro incubation technique. Rats were killed by a blow to the head and the kidneys rapidly removed and placed in cold saline. Medullary tissue was dissected from cortex, minced and held in cold saline (0.9% NaCl) until incubation. From 3 to 7 weeks of age, medullae from several rats were pooled to insure sufficient tissue for incubation. Tissue samples (80–200 mg) were weighed and placed in a beaker with Krebs-Henseleit medium (1.5 ml/100 mg tissue) and preincubated for 15 min at 37 °C in an atmosphere of 95%–5% O_2–CO_2 to remove PGE_2 generated during tissue preparation. After preincubation samples were moved to fresh medium and incubated 30 min under the same conditions. Tissue PGE_2-concentration was then determined by mass-fragmentography; PGE_2-concentration in the incubation medium was determined by radioimmunoassay. Both assays have been described in detail elsewhere[10].

Results. Blood pressure in SHRs was significantly greater than in WKYs as early as 6 weeks of age (figure 1). With 2 exceptions this was the case for all ages investigated. Tissue PGE_2 in SHRs and WKYs was lowest at 4 weeks of age, increased to 9 weeks and plateaued (figure 2). Similarly, PGE_2 released into the medium was low at 4 weeks, increased and plateaued (figure 2).

Discussion. In general, no difference in renal PGE_2-production between SHRs and WKYs was observed during development despite significant differences in blood pressure. A decrease in PGE_2-concentration in adult SHRs has been reported by others[11], suggesting that deficiencies of PGE_2 in the kidneys of SHRs occur after the increase in blood pressure.

Dunn[12] reported significantly higher prostaglandin synthetase activity in renal medullary microsomes from SHRs than from WKYs. Under ideal conditions of incubation the biosynthetic capacity of the SHR may exceed that of the WKY. However, PGE_2-concentration was the same in SHR and WKY renal medullae[12], suggesting that synthesis and degradation function concomitantly to maintain similar net PGE_2-concentrations in renal medullae of SHRs and WKYs. On the other hand, Pace-Asciak[13] reported decreased activity of prostaglandin 15-hydroxydehydrogenase (15-PGDH) in renal cortex of SHR. If medullary PGE_2 must pass through the cortex before reaching the systemic circulation, decreased activity of 15-PGDH could result in increased PGE_2 in renal cortex of SHR. Malik and McGiff[14] have attributed vasoconstrictor properties to PGE_2 in rat kidneys. Therefore, increased intrarenal PGE_2 resulting from a defect in cortical 15-PGDH could be involved in the maintenance or attainment of hypertension. The data presented in this paper indicate that differences in blood pressure exist between SHRs and WKYs at 6 weeks of age. No difference in renomedullary PGE_2-production was observed in the 2 groups. Therefore, no apparent decrease in the ability to synthesize PGE_2 occurs until after the development of hypertension, suggesting that abnormalities in renomedullary metabolism of PGE_2 are not involved in the etiology of hypertension in the SHR.

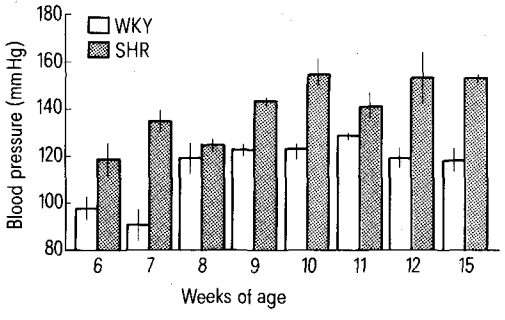

Fig. 1. Mean systolic blood pressure for SHR- and WKY-rats. Blood pressure of SHRs were significantly higher ($p < 0.05$) than WKYs at 6, 7, 9, 10, 12 and 15 weeks of age. Blood pressure measurements represent the mean±SE for at least 3 animals.

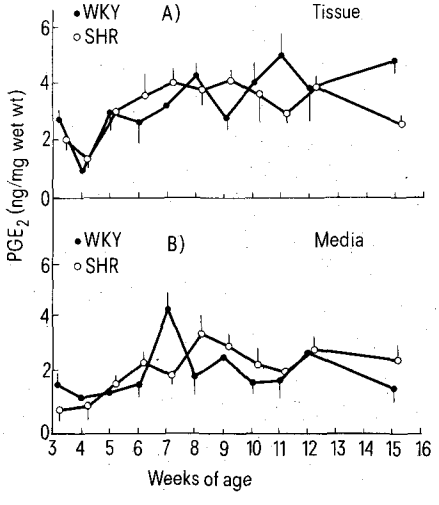

Fig. 2. Prostaglandin concentration plotted as a function of age for SHRs and WKYs in tissue (panel A) and medium (panel B). Each point represents the mean±SE of at least 4 determinations.

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