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Glomerular Hyperfiltration during the Onset of Diabetes Mellitus in Two Strains of Diabetic Mice (C57BL/6J *db/db* and C57BL/KsJ *db/db*)

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Summary. GFR estimated by the total clearence of ^{51}Cr-EDTA increases from 41 to 60 ml/min · m^2 at the onset and during the development of marked hyperglycaemia and obesity in female diabetic mice (*db/db*) of the C57BL/6J and the C57BL/KsJ strains (blood sugar rises from 160 to 400 mg/100 ml). GFR decreases slowly in older diabetic mice (> 120 days old) approaching the control values (42 ± 7 ml/min · m^2) and then decreases still further. The total clearance of ^{14}C-hippuric acid is unaltered in *db/db* mice and controls between 45 and 150 days of age (96 ± 20 ml/min · m^2). This suggests no alteration of RPF during the development of the diabetic syndrome. The glomerular hyperfiltration of diabetic mice lasts until they are 120 days old and shows no correlation with the different blood glucose levels typical for diabetic mice (*db/db*) of the two strains.

Key words: Diabetes mellitus, insulin, ^{51}Cr-EDTA total clearance, ^{14}C-hippuric acid total clearance.

Longterm diabetes mellitus in human beings is often associated with lesions of the blood capillaries in different organs. The specific kidney alterations are well known; however, the pathogenesis of this microangiopathy is still undefined [2, 26, 27]. Mogensen et al. [22] and other authors [1, 9, 20, 23] observed an elevation of the glomerular filtration rate and the filtration fraction as well as an increased kidney size in early juvenile diabetes mellitus in man. This glomerular hyperfiltration precedes the morphological alterations. The cause of the elevated filtration rate and the elevated filtration fraction is not clear and its possible role in the pathogenesis of the specific kidney disorders in diabetes mellitus is not established.

Investigations with diabetic mice may extend our understanding. Diabetic (*db/db*) mice of both the C57BL/6J (BL/6) and C57BL/KsJ (BL/Ks) strains have a diabetes like syndrome; its pathogenesis is different from that of juvenile diabetes in humans [6]. Nevertheless, Like et al. [19] found a high incidence of glomerular lesions in the kidneys of these mice, older than 150 days, similar to those found in longterm diabetes mellitus in humans. The kidney weight of these animals also increased [22].

The present investigations studied whether glomerular lesions found in the kidneys of older diabetic mice are also preceded by glomerular hyperfiltration.

Materials and Methods

Female diabetic mice (*db/db*) of the BL/Ks and BL/6 inbred strains and homozygous normal controls (+/+) from each strain were repeatedly investigated at different ages by the following total clearance methods. There was an interval of more than ten days between two repeated measurements in the same animal. Each age group was supplemented with a few untreated animals in order to recognize influences of the preceding treatment.

GFR Estimations. About 0.5 µCi ^{51}Cr-EDTA (New England Nuclear, Mass.) in 0.2 ml saline was injected in the tail vein. Blood (0.05 ml) for γ-counting, was collected by retrobulbar puncture 15, 25, 35 and 45 min after injection. The plasma volume in these samples was calculated by the packed cell volume, estimated 2 hours after ^{51}Cr-EDTA loading. The biological half-time and the distribution space of the injected compound and its total clearance were calculated from the semilogarithmic regression line of counts per ml serum versus time, according to Dost [10]:

ln counts/ml serum = a · t + b;
t = time in minutes after injection

$$\text{space} = \frac{\text{injected counts}}{e^b} \text{ [ml/mouse]}$$

$$\text{biological half-time} = \frac{\ln 2}{a} \text{ [min]}$$

clearance EDTA = a · space [ml/min · mouse]

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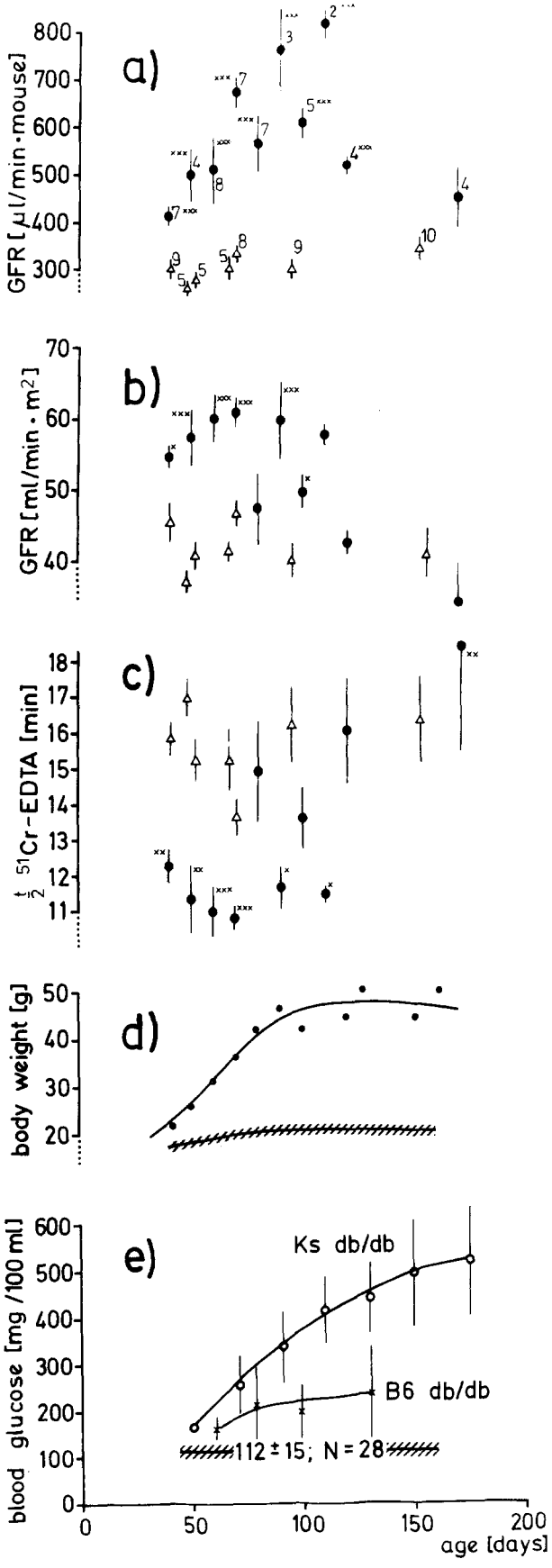

Estimations with coefficients of correlation $r < 0.98$ are rejected. Body surface area was calculated as follows: surface area = 10 · body weight (in grams) $^{2/3}$.

Total Clearance of Hippuric Acid. 1.2 µCi hippuric acid [glycine-1 ^{14}C] (3.89 mCi/mmole; ICN Pharmaceuticals, Jovine, California) plus 7 µg unlabelled hippuric acid and 3 µg gentamycin sulphate (preventing microbiological degradation of the compound during storage) dissolved in 0.25 ml saline (0.154 mol/l) was injected into the tail vein. Blood (0.05 ml) was collected by retrobulbar puncture, 5, 8 and 11 min after injection. After centrifugation the β-activity in 0.02 ml serum was estimated by liquid scintillation counting. The biological half-time and the distribution space of the injected ^{14}C-hippuric acid and its total clearance were calculated as previously described.

The glucose concentration in the blood which was collected about midday from animals fed *ad lib.* was estimated by a glucose-oxydase method (Sigma Chemical Comp., St. Louis).

Before and during the experimental procedures the mice were housed in groups of two to four animals per plastic cage, with wooden shavings, and fed a pelleted diet (Diet 96, Emory Morse Company, Guilford, CT; containing 19% total protein, 6% total fat; 0.66 ± 0.14% sodium), and received tap water *ad libitum.* Room temperature was 22 ± 2°C, light from 0700–1900 hours. The significance of differences between the means of the various groups was determined by means of analysis of variance and Duncan Test or by T-Test (31).

Results

Glomerular Filtration Rate (GFR)

GFR was estimated by the total clearance of ^{51}Cr-EDTA. This was performed 38 times in 25 female BL/6 diabetic animals, 13 times in 13 female diabetic BL/Ks and 45 times in 10 healthy female controls (+/+) from each of the BL/6 and BL/Ks strains. No differences in GFR, biological half-life of ^{51}-Cr-EDTA or the distribution space of this compound was obvious between strains in either the diabetic (*db/db*) or the control animals (+/+). Therefore the results for each strain were combined and are shown in Figure 1. The development of the obesity-diabetes syndrome is demonstrated by the increase of the blood glucose concentration and the body weight with age (Fig. 1e and 1d). Diabetic females older than 40 days differ significantly ($p < 0.05$) in body

Fig. 1. Glomerular filtration rate (GFR) estimated by the total clearance of ^{51}CR-EDTA in correlation with the age in female diabetic (*db/db*) mice (•) and in controls (+/+) (△) of the C57BL/6J and the C57BL/KsJ strains.
Mean and SEM, numbers near the mean indicate number of animals per group; stars indicate a significant difference from controls (× = $p < 0.05$; ×× = $p < 0.01$; ××× = $p < 0.001$). **a** total clearance of ^{51}Cr-EDTA per mouse; **b** total clearance of ^{51}Cr-EDTA calculated on an equal body surface area; **c** biological half-life of ^{51}Cr-EDTA; **d** body weight; **e** blood glucose concentration in C57BL/6J *db/db* (×) or C57BL/KsJ *db/db* (○) mice and controls (hatched area)

weight and blood glucose concentration when compared with the controls. In the BL/Ks *db/db* animals with the severe diabetes syndrome blood glucose concentrations higher than 500 mg/100 ml were reached by 150 days of age. In contrast, the BL/6 *db/db* females at the age of about 100 days and more, showed only a mild hyperglycaemia. On average this reached 200 mg/100 ml. However, both had similar body weights and similar alterations of the glomerular filtration rate. GFR was significantly ($p < 0.01$) higher in the *db/db* mice than in the controls before pronounced hyperglycaemia and severe adiposity were apparent (around 40 days of age) (Fig. 1a). GFR further increased until 100 days of age when the obese animals reached a plateau in respect to increase in body weight. This elevation correlated with the increased body weight. When the GFR was calculated on an equal body surface area, the *db/db* animals also showed an increased GFR ($p < 0.05$) at the onset of hyperglycaemia which did not increase further during the development of the diabetes-like syndrome (Fig. 1b). This raised GFR contributes to a quicker ($p < 0.01$) wash-out effect of the extracellular water in the obese animals, as demonstrated by the lower ^{51}Cr-EDTA-half-time (Fig. 1c). However, this increase in the GFR and the decrease of the EDTA-half-time is not seen in older animals. GFR decreases slowly in obese animals, older than 120 days, and approaches the levels of the controls while the EDTA-half-time increases. In a few 170 day old animals belonging to the BL/Ks strain the GFR may be less than in the controls (Fig. 1b).

The increased body weight of the diabetic animals also changes the distribution space of the ^{51}Cr-EDTA. The increase in the ^{51}Cr-EDTA distribution space and the body weight have no linear proportionality in the obese animals. In a lean animal of 20.0 g body weight the relative space amounts to about 35%. However, in an obese animal of 50.0 g body weight the relative space decreases to around 26% of the body weight. The correlation of body weight (w) with distribution space (S) follows the equation $S = 72.1\ w^{-0.26}\%$ as calculated from space determination in 59 animals with body weights ranging from 16 to 55 g.

The total clearance of ^{14}C-hippuric acid was estimated 22 times in 15 female BL/6 diabetic animals, 15 times in 13 female BL/Ks diabetic animals and 19 times in 8 healthy controls (+/+) of each strain. No difference in biological half-life, the distribution space or the total clearance of this compound was seen between the two strains in either the diabetic or the control animals. Therefore the results for each strain were combined and are shown in Figure 2. In

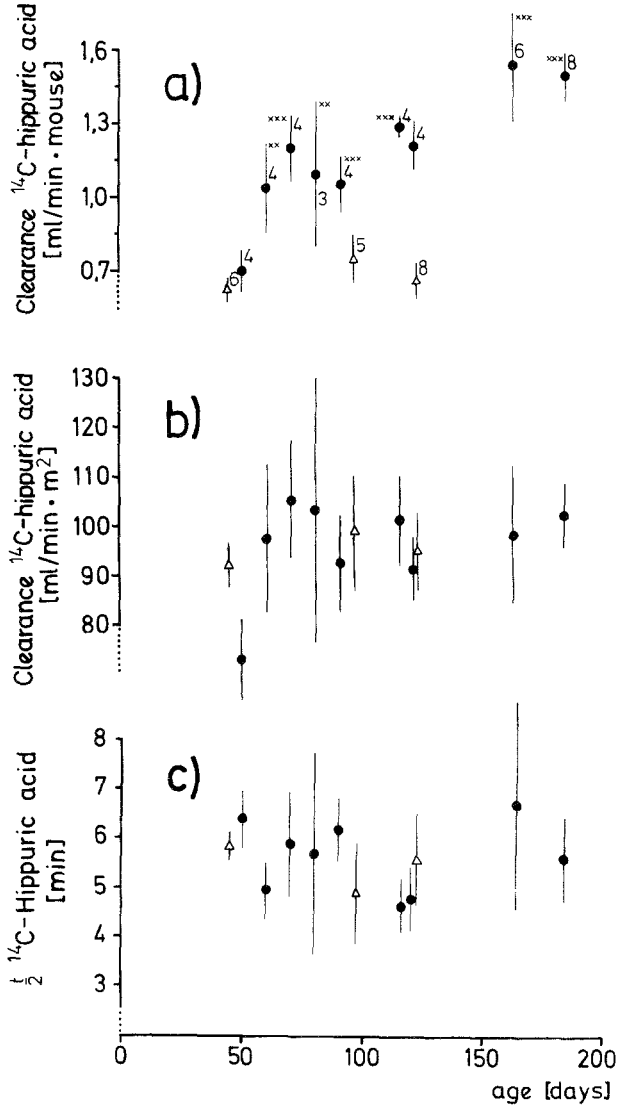

Fig. 2. The total clearance of ^{14}C-hippuric acid in correlation with age in female diabetic (*db/db*) mice (•) and in controls (+/+) (△) of the C57BL/6J and C57BL/KsJ strains. Mean and SEM, numbers near the mean indicate number of animals per group; stars indicate a significant difference from controls ($\times = p < 0.05$; $\times\times = p < 0.01$; $\times\times\times = p < 0.001$). **a** total clearance of ^{14}C-hippuric acid per mouse; **b** total clearance of ^{14}C-hippuric acid calculated on an equal body surface area; **c** biological half-life of ^{14}C-hippuric acid

the young *db/db* females (45–55 days old) hippuric acid clearance per mouse was similar to the controls, but it increased in older *db/db* females (Fig. 2a). This increase corresponds with the increase of body weight. No differences between *db/db* mice and their controls could be detected by the calculation of the total clearance of hippuric acid on an equal body surface area (Fig. 2b). This finding agrees with the unaltered half-time of the hippuric acid (Fig. 2c). No differences could be detected between *db/db* animals

and the controls at any age. The combined biological half-time for clearance of the ^{14}C-hippuric acid was 5.6 ± 1.6 min for all animals. The hippuric acid distribution space corresponds to 22.9 ± 2.2% of the body weight in both the lean and the obese animals.

Discussion

Only a few papers evaluate kidney function in mice by the classical clearance methods. Estimations of the GFR by renal or total clearance of ^{51}Cr-EDTA have been used successfully in human beings and animals [11, 12, 24, 25, 28]. The GFR values, estimated here in healthy mice (0.30 ml/min · mouse), are in accordance with the results of Katz et al. [17] who used the renal clearance of inulin to measure GFR in mice. This suggets that the estimation of GFR by the total clearance of ^{51}Cr-EDTA is suitable for mice.

It is well known that a correct estimation of the renal plasma flow (RPF) by a single-shot-technique is impossible. The total clearance of different hippuric acid compounds has been found by different investigators to be smaller than its renal clearance in different species [3, 4, 24, 25]. Our total clearance of ^{14}C-hippuric acid are also smaller than those of Katz et al. [17], estimated by renal clearance of ^{125}I-p-amino-hippuric acid in a few mice. Despite the deviation we are of the same opinion as other authors [3, 4, 24, 25] that the total clearance of ^{14}C-hippuric acid in mice also reflects alterations of RPF but does not give any information about its real values.

Glomerular hyperfiltration occurring during the onset of human juvenile diabetes has been shown to precede the specific diabetic angiopathy [1, 9, 18, 20, 21, 23]. The studies presented here show that glomerular hyperfiltration occurs at the onset of the diabetes-like syndrome and precedes glomerular microangiopathy in BL/6 *db/db* and BL/Ks *db/db* mice. We found increased GFR but no increase of the ^{14}C-hippuric acid clearance at the beginning of the hyperglycaemia. During this early phase of hyperfiltration the basal membrane and the mesangium of the glomerula are not different from those of the controls, as described in humans by Osterby *et al.* [23]. Like [19] did not find morphological differences in these structures between BL/Ks *db/db* mice and controls until 120 days of age. It is not known if the glomerular hyperfiltration in mice is associated with enlargement of the tubular tuft, caused by capillary distension and cellular hypertrophy, as described in human beings [18, 23]. In addition to similarities between mice and humans, with respect to hyperfiltration occurring at the onset of hyperglycaemia, there may be differences between these species in the early phase of the diabetic nephropathy. In humans glomerular hyperfiltration has some correlation with the long term elevation of blood glucose level [21]. In mice the GFR is markedly increased at the onset of hyperglycaemia or obesity and is not connected with the well known [7, 13, 15, 29, 30] differences in severity of hyperglycaemia characteristic of each strain of diabetic mice. This finding raises the question whether hyperfiltration in the *db/db* mice could be caused by other metabolic or endocrine alterations occurring before the onset of severe hyperglycaemia or is induced earlier by mild hyperglycaemia. *db/db* mice, 40–60 days of age show hyperfiltration at blood glucose concentrations of about 159 ± 25 mg/100 ml in the BL/6 strain or 167 ± 40 in the BL/Ks strain. This mild hyperglycaemia did not arise earlier than 10–25 days before and was not accompanied by glycosuria. Coleman and Hummel [6] found hyperinsulinaemia associated with normal blood glucose values in these mice as early as ten days of age and this increases progressively until it reaches a peak at the age of about 3 months in BL/Ks *db/db* mice and then gradually declines to near normal values. Desjardin [8] found decreased growth hormone content in young *db/db* mice, which increased in 12 and 13 weeks old animals. The diabetic syndrome in mice is also associated with signs of hypercorticism [7, 14]. Neither growth hormone, insulin, nor corticosterone are able to provoke an isolated increase of the GFR when injected in animals [16], suggesting that hyperfiltration in the early diabetes-like syndrome may have a complex aetiology.

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