fraction is the sex difference in the reduction of this fraction with the transition from internal synchronization to desynchronization (the reduction is nearly independent of the direction of desynchronization); on the average, this reduction is nearly 3 times greater in females than in males. As a consequence, the sex difference in the sleep fractions in internally desynchronized rhythms nearly disappears. In the total sample and in the 2 sub-samples separated according to the direction of desynchronization, the sleep fraction is still larger in females than in males, but no longer to a significant extent. Unfortunately, therefore, it cannot be decided whether or not a relevant sex difference exists in the sleep fraction of internally desynchronized rhythms, although there is a trend in the same direction as with internally synchronized rhythms. It can be stated, however, with certainty that freerunning female rhythms react, with respect to the sleep fraction, more strongly to the occurrence of rhythm disorders than do male rhythms, independent of the direction of the rhythm disorder. This sex difference in the change of the sleep fraction may reflect a higher sensitiveness of females than of males to rhythm disorders, in general.

As mentioned above, all results concerning internally synchronized rhythms obtained in the present study are in agreement with results of a previous study including another sample of subjects, with rhythms that remained internally synchronized in the long run[7]. The present results concerning sleep fractions are also in agreement with results of another previous study dealing with seasonality[8]. That study was based on a much larger but non-homogeneous sample of subjects, with internally synchronized and desynchronized rhythms; it described a seasonal variation in the sleep fraction (smaller fractions in spring and larger fractions in autumn) but always a larger sleep fraction in females than in males. Results concerning sex differences in freerunning human rhythms, unfortunately, are not known from other groups of authors. Corresponding results from animal experiments are also rare. Aschoff[1] reported a slight but not significant sex difference in the period of chaffinches (*Fringilla coelebs* L.): the freerunning period was, in different intensities of constant illumination, slightly longer in females than in males. The only other species where sex differences in freerunning rhythms have been reported, seems to be the hamster (*Mesocricetus auratus* L.); Davis et al.[2] found slightly shorter periods and slightly more sleep in females than in males; hence, the direction of the sex differences in these mammals were the same as in man; unfortunately, however, these animal results were not significant statistically, at an acceptable level.

1 Aschoff, J., Circadian activity rhythms in chaffinches (Fringilla coelebs) under constant conditions. Jap. J. Physiol. *16* (1966) 363–370.
2 Davis, F.C., Darrow, J.M., and Menaker, M., Sex differences in the circadian control of hamster wheel-running activity. Am. J. Physiol. *244* (1983) R93–R105.
3 Wever, R.A., The circadian multi-oscillator system of man. Int. J. Chronobiol. *3* (1975) 19–55.
4 Wever, R.A., The Circadian System of Man. Springer, New York/Heidelberg/Berlin 1979.
5 Wever, R.A., On varying work-sleep schedules: the biological rhythm perspective, in: Biological Rhythms, Sleep and Shift Work, pp. 35–60. Eds L.C. Johnson, D.I. Tepas, W.P. Colquhoun and M.J. Colligan. Spectrum Publ., New York 1981.
6 Wever, R.A., Behavioral aspects of circadian rhythmicity, in: Rhythmic Aspects of Behavior, pp. 105–171. Eds F.M. Brown and R.C. Graeber. L. Erlbaum Ass. Inc., Hillsdale N.J. 1982.
7 Wever, R.A., Properties of human sleep-wake cycles: parameters of internally synchronized freerunning rhythms. Sleep *7* (1984) 27–51.
8 Wirz-Justice, A., Wever, R., and Aschoff, J., Seasonality in freerunning circadian rhythms in man. Naturwissenschaften *71* (1984) 316-319.

0014-4754/84/111226-09$1.50 + 0.20/0

Immune-mediated glomerulonephritis induced by mercuric chloride in mice[1]

S. Enestrom and P. Hultman

Department of Pathology I, Linköping University, S-58185 Linköping (Sweden), 23 January 1984

Summary. The BALB/c mouse developed mesangial deposits of immune constituents and light microscopical changes characteristic of immune complex glomerulonephritis after 8 weeks' treatment with mercuric chloride given by s.c. injection. There were no signs of linear of granular immune deposits along the glomerular capillary basement membrane after 2 or 8 weeks. The antigen could not be identified. No antibodies to nuclear or renal structures were found. Using a histochemical method (silver amplification) mercury was detected by light and electron microscopy in tubular and glomerular structures. Mercury was present in secondary lysosomes of the mesangial cells after eight weeks of mercury poisoning.
Key words. Mercuric chloride; BALB/c mice; immunofluorescence; silver amplification.

Introduction

The kidney is the main target organ after exposure to inorganic mercuric salts[2], which are generally believed to damage the proximal tubular epithelial cells in both man[25,27] and rodents[16,21]. Brown Norway (BN) rats given mercurial substances orally, parenterally, or by inhalation develop a biphasic autoimmune disease[4,11,12] which is often self-limiting if the animal survives the initial phase[3,10]. Antibodies to the glomerular basement mem-

Table 1.

Group	Number of animals	Sex	Treatment	Individual dose injected (mg/100 g b.wt)	Number of injections[a]	Duration of experiments (weeks)	Time between last injection and killing (days)	Age when killed (weeks)
A	14	♂+♀	None	–	–	–	–	7
B	2	♂	0.9% NaCl[b]	–	6	2	3	10
C	8	♂+♀	0.5‰ $HgCl_2$[c]	0.44	5	2	3	9
D	14	♂+♀	None	–	–	–	–	16
E	8[d]	♂+♀	0.5‰ $HgCl_2$[c]	0.24	17	8	3	17

[a] All injections were given at 3-day intervals; [b] 0.1 ml of a sterile 0.9% NaCl solution was injected s.c; [c] The solution was given s.c. and the volume adjusted to b.wt to give the specified dose; [d] The group initially consisted of 15 animals (see results).

brane followed by immune complexes have been identified in the kidneys and serum[3, 10, 23]. The susceptibility of the rat to this nephropathy is strongly dependent on the animal's genetic constitution, and especially on genes linked to the major histocompatibility complex[8, 9, 24]. A biphasic disease similar to the one observed in the BN rat has been induced in rabbit[22]. Other rat strains have proved resistant or have developed an immune complex glomerulonephritis, sometimes with detectable antinuclear antibodies[9, 11, 15, 29].

The present investigation was carried out to see whether an immune-mediated glomerulonephritis can be induced in the BALB/c mouse by administration of mercuric chloride. A new animal model for studying the pathogenetic mechanisms of glomerulonephritis was thus established.

Material and methods

Animals. BALB/c mice of both sexes and weighing 18–30 g were used. They were classified according to the treatment given (table 1).

Experimental procedure. The animals were given subcutaneous (s.c.) injections of mercuric-II-chloride or of a sterile 0.9% saline solution. Some animals were not treated, and served as age-matched controls.

Mercuric-II-chloride (analytical standard, E. Merck, West Germany) was dissolved in redistilled water to give a solution of 0.5 g/l. This solution was injected s.c. in accordance with one of two dose schedules. Group C (table 1) received five s.c. injections of the solution at three-day intervals. The volume was adjusted to give an individual dose of 0.44 mg $HgCl_2$/100 g b.wt. Group E was given 17 s.c. injections of mercuric chloride at 3-day intervals, the volume being adjusted to give an individual dose of 0.24 mg $HgCl_2$/100 g b.wt. Group B received 6 injections of 0.1 ml of sterile saline at 3-day intervals. Groups A and D were given no injections. All treated animals were killed three days after the last injection.

Immunofluorescence method. Immunofluorescence was performed on 4-µm-thick, air-dried, ethanol-fixed cryostat sections from snap-frozen kidney blocks. The sections were washed in phosphate-buffered saline (PBS, pH 7.6) for 15 min before and after fixation. Using a direct method the sections were incubated for 45 min at room temperature in a moist chamber with FITC-conjugated specific goat anti-mouse antibodies to a) Fc-fragments of IgG and IgM, b) complement factor C3c (C3), and c) mouse albumin. The antiserum to albumin was diluted at 1:40 in PBS, the other antisera as described below. The sections were washed for 20 min in PBS and mounted in 20% glycerol-PBS.

Semi-quantitative determinations of the glomerular amounts of IgG, IgM, and C3 were made for every animal. The endpoints of positive immunofluorescence were determined using series of dilutions of each antiserum from 1:20 to 1:1 280 in PBS (pH 7.6). 20 glomeruli were evaluated for each animal, and the endpoint of the titer was set as the highest dilution with which at least 90% of the glomeruli showed specific positive fluorescence. The distribution and pattern of positive fluorescence were also determined. The sections were coded and the evaluation made without knowledge of the treatment given or other results.

For detection of IgA an indirect method was used. The air-dried, ethanol-fixed sections were washed in PBS. They were then incubated in specific goat anti-mouse IgA, diluted 1:40 with PBS, and then in specific rabbit anti-goat IgG, diluted 1:40 with PBS. They were next washed for 20 min in PBS, and were finally mounted in glycerol-PBS as described above. All incubations were made at the same time to minimize variations in staining resulting from external factors. The antisera were purchased from Nordic Immunological lab., Tilburg, The Netherlands.

Serological examinations. Serum was prepared at the time of killing, frozen, and kept at −70 °C until required. Before analysis, sera were heated to 56 °C for 30 min. After dilution 1:10 in PBS, sera from the different groups were incubated on cryostat sections of normal rat liver and normal and mercuric chloride treated kidney (group E) for 30 min. The sections were then rinsed in PBS and stained with specific fluoresceinated anti-mouse IgG (Fc-fragments) diluted 1:40 in PBS (pH 7.6).

Preparation for light microscopy. Tangentially cut kidney pieces were immediately prefixed in Stieve's fluid (saturated aqueous mercuric chloride, 76 ml; 40% formalin, 20 ml; and glacial acetic acid, 4 ml) for 2 h and postfixed in 4% PBS-buffered formalin for 24 h. The tissue was then dehydrated, cleared, and embedded in Paraplast®. 1-µm-thick sections were cut with glass knives[13] and stained with periodic acid-Schiff's reagent (PAS) and periodic acid silver-methenamine (PASM).

Preparation for electron microscopy. Small cubes of kidney tissue were immediately immersed in 2.5% glutaraldehyde in Sörensen's phosphate buffer solution, post-fixed in OsO_4, washed, dehydrated, and embedded in Epon. Ultrathin sections were then cut and stained with lead citrate and uranyl acetate.

The silver amplification method (SA-method). Silver amplification was performed on glutaraldehyde-fixed tissue as described by Danscher[7] using semithin sections or chopped slices. Briefly, the sections or slices were exposed to a solution of citrate buffer, hydroquinone, silver lactate, and gum arabic (gum arabic acts as a colloid to give an even suspension of molecules and to prevent autocatalytic reactions). During development complexes of mercuric sulphide and mercuric sulphide formed through metabolism of mercury act catalytically on the reduction of silver ions to metallic silver[6]. As the newly formed silver has a similar catalytic effect, steadily expanding shells of silver are created round mercury present in the tissue. The semithin sections were re-embedded in Epon after thorough washing, and ultrathin sections were cut. The chopped tissue was embedded in Epon; semithin sections were prepared for light microscopy and ultrathin for conventional electron microscopy. The ultrathin sections were poststained with uranyl acetate and lead citrate.

Statistical evaluation. The difference in fluorescence intensity between mercury-treated animals and control were analysed using the exact randomization test[26] and Student's two-tailed t-test.

Results

General survey. Of the 53 mice used seven died. All deaths occurred among the mercuric chloride-treated animals in group E, which initially consisted of 15 mice. One mouse died on each of days 13, 14, 18, 24, and 30, and 2 mice on day 11. No evaluation of the spontaneous deaths was made, because of the rapid post-mortem changes which make the interpretation of tissue changes difficult.

Immunopathological findings. A semi-quantitative evaluation of the glomerular deposits of IgG, IgM, and C3 was performed (table 2).

In groups A and D (controls given no injections) only small amounts of IgG, and C3 were found in the mesangial zones of the glomeruli in a coarse, granular fluorescent pattern. Only occasionally was weak fluorescence seen in the capillary walls or other renal structures. In controls given saline (group B), the intensity and character of the fluorescence did not differ from that found in age-matched animals given no injections (not shown). Animals given larger doses of mercury for two weeks (group C) showed no significant increase in mesangial immunoglobulins compared to age-matched controls (group A) as evaluated by the exact randomization test and the t-test. A significant difference ($p < 0.05$) in the amount of C3 between these groups emerged with the t-test, but not with the exact randomization test. We conclude that no certain difference existed between the experimental and control groups with regard to C3. When mercuric chloride was given in small doses for eight weeks (group E) great increase of the amount of IgG occurred in the mesangial zones of the glomeruli. A significant increase in IgM and C3 was also seen in this group compared to age-matched controls (group D) as evaluated by the exact randomization test and the t-test. The mesangial staining in both control and experimental mice was diffuse and global, although slight variations were seen both within and between glomuleri (figs 1–3). Slight staining was occasionally seen along short segments of the glomerular capillary wall. The immunereactive C3 was strictly localized to the same sites as IgG in the consecutive sections. The staining for IgA was weak, and only scattered mesangial deposits were seen in the glomeruli of both control and mercury-treated animals (not shown). Staining for mouse albumin was as a rule negative in all renal structures, and especially in the glomeruli. However, in the animals given mercury for eight weeks (group E) there was intense staining of tubular casts.

Serological tests. No staining was observed in normal rat liver or kidney sections from normal and mercuric-chloride-treated animals, after preincubation with sera from mercuric-chloride-treated mice.

Morphology. Mercuric chloride given for two weeks in high dosage (group C) resulted in slight changes in the glomerular mesangial cells resembling early mesangiolysis. Fewer than 1% of the glomeruli showed atrophic lesions. Neither cellular proliferation nor inflammatory cells were found. The proximal tubules showed focal degeneration of epithelial cells. The tubular basement membrane was wrinkled, but did not show duplications.

Table 2. Semi-quantitative determination of the glomerular amounts of IgG, IgM, and C3 based on endpoint immunofluorescence for serial dilutions of antisera. The number of animals showing positive immunofluorescence is given for each dilution

Animal groups		Dilutions of antisera: Neg	1:20	1:40	1:80	1:160	1:320	1:640	Significance*
Group E ($HgCl_2$ for 8 weeks n = 8)	IgG						3	5	$p < 0.001$
	IgM				3	3	2		$p < 0.001$
	C3			4	3	1			$p < 0.001$
Group D (Controls n = 14)	IgG	2	7	5					
	IgM			3	11				
	C3	6	4	4					
Group C ($HgCl_2$ for 2 weeks n = 8)	IgG	2	3	3					NS
	IgM			3	5				NS
	C3	1	6	1					$p < 0.05$**
Group A (Controls n = 14)	IgG	7	7						
	IgM			6	8				
	C3	7	7						

* Student's two-tailed t-test between groups E and D and between groups C and A, respectively; ** Not significant when tested by the exact randomization test.

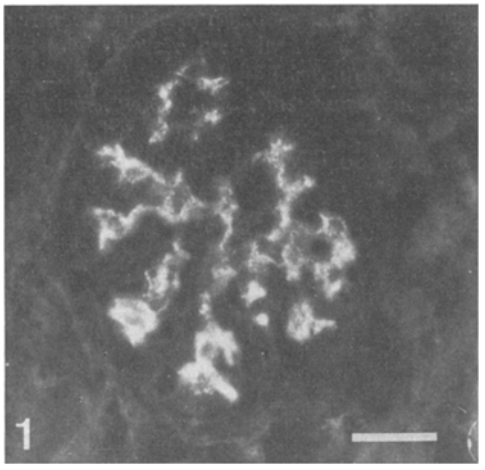

Figure 1. Cryostate sections of kidney from a mouse in group E after eight weeks' mercuric chloride treatment, stained with fluoresceinated antibodies to the Fc-fragments of IgG diluted 1:640. A predominantly mesangial, bright fluorescence is seen. Bar = 20 μm.

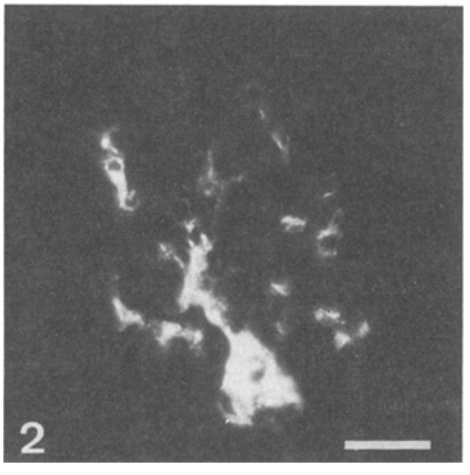

Figure 2. Cryostate section of kidney from a mouse in group E after eight weeks' mercuric chloride treatment. Staining by fluoresceinated antibodies to mouse IgM diluted 1:320. Coarse, granular staining with segmental accentuation is seen. Bar = 20 μm.

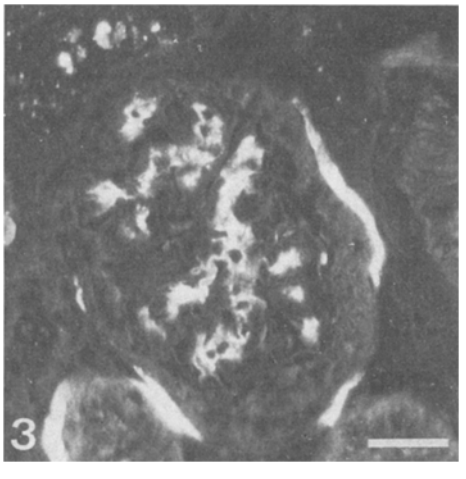

Figure 3. Cryostate section of kidney from a mouse in group E after eight weeks' mercuric chloride treatment. Staining with fluoresceinated antibodies to mouse C3 diluted 1:160 discloses a granular mesangial pattern. Part of Bowman's capsule is also outlined by fluorescence. Bar = 30 μm.

There was no inflammation in the interstitial tissue. When animals were given low doses of mercuric chloride for eight weeks (group E) all glomeruli showed slight endocapillary cell proliferation, moderate endothelial swelling, epithelial hypertrophy, and mesangial hyalinosis. However, 5–30% of glomeruli showed marked signs of retraction of the capillary tuft and wrinkling of Bowman's capsule (fig. 4). Age-matched controls (groups A and D) showed no such glomerular changes. Active focal epithelial cell proliferation occurred in the tubules in the group-E animals, and a slight inflammatory reaction was seen in the interstitial tissue.

In group C electron microscopy of the glomeruli revealed swollen endothelial and epithelial glomerular cells; mesangial deposits were rarely seen, and the centrolobular areas were slender. Group E showed electron-dense mesangial deposits and an increase of the dense mesangial matrix. Furthermore the endothelial cells had microvillous configuration with slender, tortuous, and arborized cytoplasmic extensions partly occluding the capillary lumina (fig. 5), and signs of degen-

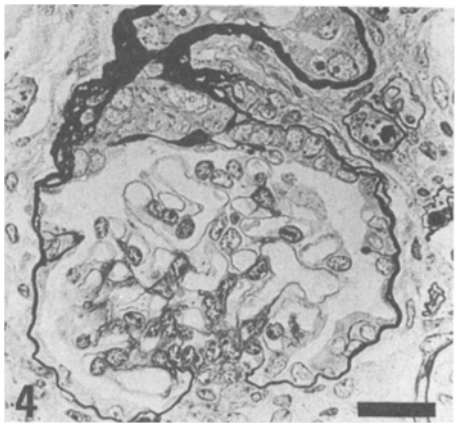

Figure 4. Glomerulus and proximal convoluted tubule from a mouse from group E. The capillary tuft shows low-grade cellular proliferation and wide capillary lumina, and is slightly retracted at the vascular pole. Bowman's capsule and the tubular basement membranes are wrinkled. Note the duplications of the membrane encircling small clusters of tubular epithelial cells, indicating grave damage. PASM. Bar = 20 μm.

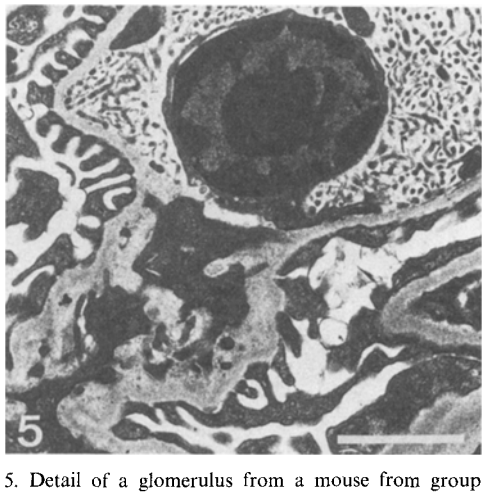

Figure 5. Detail of a glomerulus from a mouse from group E. The mesangial matrix is increased and dense. Note the extensive microvillar configuration of the endothelial cells and the heterochromatin-rich nucleus, suggesting pyknotic degeneration. Bar = 1 μm.

eration with dark nuclei. No deposits were found in the subendothelial or subepithelial spaces.
Dense proteinous casts were found in all animals given mercuric chloride, but in mice treated for eight weeks these were focally distributed.

Detection of mercury by the silver amplification method. Light microscopy disclosed dark precipitates in the proximal tubular epithelial cells, the centrolobular zones, and capillary walls of the glomeruli of groups C and E (fig. 6). The density of precipitates varied greatly between different glomeruli and between groups, but was generally higher in mice given larger individual doses (group C). By means of electron microscopy the silver amplified mercuric sulphide was found in the different laminae of the glomerular basement membranes, suggesting that mercury had traversed this barrier. Ultrastructural precipitates were present also in proximal tubular epithelial cells and in cellular debris within these tubules. Moreover, the precipitates were seen in secondary lysosomes of both mesangial (fig. 7) and proximal tubular epithelial cells. Controls showed a weak unspecific background staining.

Discussion

The results indicate deposition of immune complex in glomeruli of mice treated with mercury over a long period. Firstly, immunofluorescence revealed greatly increased deposition of IgG in the mesangium together with some increase in IgM and C3. Secondly, electron microscopy showed electron-dense bodies in the mesangium, probably the ultrastructural counterpart of the deposits shown on immunofluorescence. Thirdly, owing to the absence of inflammation and the constantly negative staining for mouse albumin in glomerular structures, nonspecific trapping of immune constituents is unlikely. Owing to the difference in molecular weights between albumin (mol.wt circa 70,000) and IgG and IgM (mol.wt circa 160,000 and 900,000, respectively), trapping of immunoglobulins in glomerular structures cannot strictly be excluded even if no albumin is found. However, animals given smaller doses of mercury leading to only subtle tubular damage with no casts and with preserved total glomerular filtration rate still show increased deposition of immunereactants in the mesangium (unpublished observations). Taken together, these facts constitute powerful evidence against nonspecific trapping of immunereactants owing to mechanical inhibition of glomerular filtration.
The immunofluorescent staining of the glomerular capillary wall for immunoglobulins reported to occur in mercury-treated BN rat[10,23] and in rabbit[22] never appeared in our animals. In particular, there was no linear capillary wall immunofluorescent staining. The localization of immune complex mainly to the mesangium

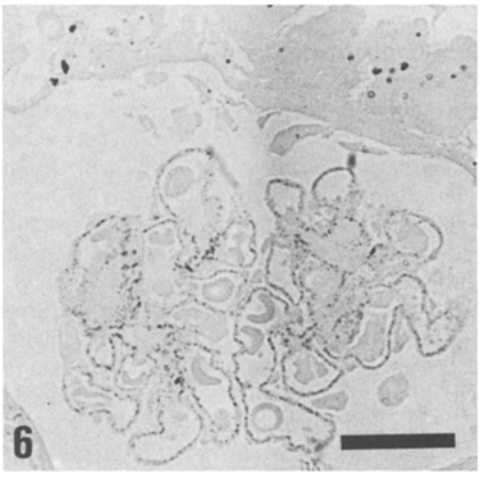

Figure 6. Glomerulus from a mouse in group E after silver amplification of the tissue. Granules can be seen outlining the capillary walls and in the centrolobular zones. Phase contrast. Bar = 20 µm.

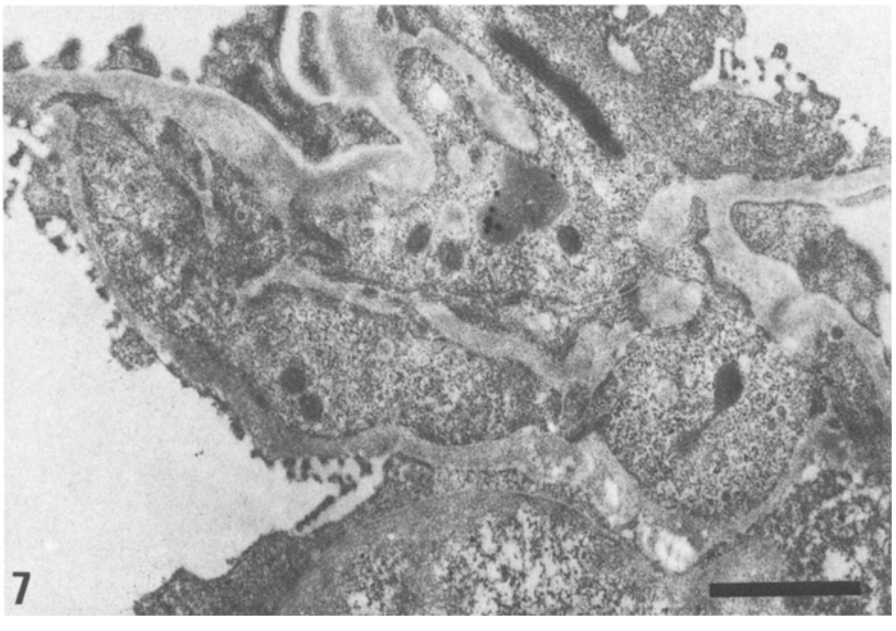

Figure 7. Part of a glomerulus from a mouse in group E. After silver amplification granules are seen to be clustered in secondary lysosomes of the mesangial cells. Bar = 1 µm.

after chronic mercury poisoning conforms with the findings in Wistar[20], Augustus and PVG/c rats[11]. The autoimmune reactions observed in rat and rabbit after exposure to mercuric chloride have been explained by immunoregulatory dysfunction with polyclonal B-cell activation[17]. We found no antinuclear antibodies in BALB/c mice after eight weeks of mercury treatment to indicate this kind of activation. However, such antibodies have not been found in all rat strains that develop immune complex glomerulopathy[11,15,29]. Thus, other, still unknown antigen-antibody systems may be involved both in rat and mouse.

The action of mercuric chloride need not be mediated via immune dysregulation, because theoretically mercury can act either directly or by a hapten mechanism on autologous structures, rendering these antigenic[15]. It is also possible that mercury present in the mesangial cells, as demonstrated by silver amplification, might impair the removal of immune complex from the glomerular centrolobular zone. Our observations on BALB/c show that this mouse develops endocapillary glomerulonephritis after chronic, low-dose inorganic mercury poisoning. The diseased glomerulus, however, shows a complex morphology, with a combination of injury and cell proliferation, which indicates that the glomerulopathy is not necessarily caused by immune-mediated reactions alone. Mercury seems to produce a cytotoxic effect on the endothelial cells, changing the normally smooth cellular surface to a microvillous configuration of the plasmalemma. Membrane configuration of this type appears to be an unspecific response to injury, and in fact has been observed in the plasmalemmal membrane of macrophages after exposure to reactive oxygen metabolites[28]. Mercury obviously attacks both endothelial and mesangial cells, leading to an atrophy of some glomeruli that resembles ischaemic lesions. Secondary ischaemia could be involved in the development of atrophic glomerular lesions. The fact that no occlusive lesions were seen in the vessels is evidence against this.

The silver amplification method has proved to be a sensitive means of demonstrating mercury in renal structures, especially when applied ultrastructurally. Because histochemical methods have been challenged on the basis of specificity[5], we have tried to confirm the value of this method by use of analytical electron microscopy[18]. The demonstration of mercury in glomerular structures of mercuric-chloride-treated mice is in contradiction to results obtained in rabbit by autoradiography[22]. This difference might be explained by the high sensitivity of silver amplification, which needs only three to six atoms of mercury to create a granule[19]. We believe that the silver amplification method will prove valuable in studies on the relation between renal lesions and mercury.

Further work is needed to show whether, a) prolonged exposure to mercuric chloride and/or the addition of immunostimulants will transform the disease into a more histologically active type, and b) the susceptibility to this mercury-induced glomerulopathy in mouse is genetically determined; we have found that inbred mouse strains with different H-2-haplotypes differ in susceptibility to this disease[14]. A third aim will be to determine the responsible immunological mechanism and antigen(s) and to establish a possible relationship to mercury accumulation in glomeruli.

1 We are deeply indebted to Dr. Gorm Danscher, Institute of Anatomy, B., University of Aarhus, Denmark, for valuable discussions and technical advice. This work was supported by the Swedish Medical Research Council, project no 6536.

2 Berlin, M., Mercury, in: Handbook on the Toxicology of Metals, pp. 503–539. Eds L. Friberg, G.F. Nordberg and V.B. Vouk, Elsevier/North-Holland Biomedical Press, Amsterdam/New York/Oxford 1979.

3 Bellon, B., Capron, M., Druet, E., Verroust, P., Vial, M-C., Sapin, C., Girard, J.F., Foidart, M.M., Mahieu, P., and Druet, P., Mercuric chloride induced autoimmune disease in Brown-Norway rats: sequential search for anti-basement membrane antibodies and circulating immune complexes. Eur. J. clin. Invest. *12* (1982) 127–133.

4 Bernaudin, J.F., Druet, E., Druet, P., and Masse, R., Inhalation or ingestion of organic or inorganic mercurials produces autoimmune disease in rats. Clin. Immun. Immunopath. *20* (1981) 129–135.

5 Clarkson, T.W., The pharmacology of mercury compounds. A. Rev. Pharmac. *12* (1972) 375–406.

6 Danscher, G., and Schröder, H.D., Histochemical demonstration of mercury induced changes in rat neurons. Histochemistry *60* (1979) 1–7.

7 Danscher, G., Histochemical demonstration of heavy metals. Histochemistry *71* (1981) 1–16.

8 Druet, E., Sapin, C., Günther, E., Feingold, N., and Druet, P., Mercuric chloride-induced anti-glomerular basement membrane antibodies in the rat. Genetic control. Eur. J. Immun. *7* (1977) 348–351.

9 Druet, E., Sapin, C., Fournie, G., Mandet, C., Günther, E., and Druet, P., Genetic control of susceptibility to mercury-induced nephritis in various strains. Clin. Immun. Immunopath. *25* (1982) 203–212.

10 Druet, P., Druet, E., Potdevin, F., and Sapin, C., Immune type glomerulonephritis induced by $HgCl_2$ in the Brown Norway rat. Ann. Immun. (Inst. Pasteur) *129 C* (1978) 777–792.

11 Druet, P., Ayed, K., Bariety, J., Bernaudin, J.F., Druet, E., Girard, J.F., Hinglais, N., and Sapin, C., Experimental immune glomerulonephritis induced in the rat by mercuric chloride, in: Advances in Nephrology, pp. 321–342. Eds J. Hamburger et al. Year Book Medical Publisher 15, London 1979.

12 Druet, P., Teychenne, P., Mandet, C., Bascou, C., and Druet, E., Immune-type glomerulonephritis induced in the Brown-Norway rat with mercury-containing pharmaceutical products. Nephron *28* (1981) 145–148.

13 Enestrom, S., Avantages des coupes semi-fines des biopsies rénales incluses en paraffine et réalisées par coteaux en verre au large tranchant. Archs. Anat. Cytol. Path. *29* (1981) 317–319.

14 Enestrom, S., and Hultman, P., Mercury induced glumerulonephritis in mice. Abstract, 6th World Congress of Academic and Environmental Pathology, Miami Beach, Florida, September 2–7, 1984.

15 Fournié, G.J., Lulé, J., Gayral-Taminh, M., Mignon-Conté, M., Conté, J.J., Bellon, B., Druet, E., Hirsch, F., Sapin, C., Druet, P., Izui, S., and Lambert, P.H., Immunological pathogenesis of experimental glomerulonephritis induced by some infectious and toxic agents. Contr. Microbiol. Immun. *7* (1983) 43–51.

16 Hinglais, N., Druet, P., Grossetete, J., Sapin, C., and Bariéty, J., Ultrastructural study of nephritis induced in Brown-Norway rats by mercuric chloride. Lab. Invest. *41* (1979) 150–159.

17 Hirsch, J., Couderc, J., Sapin, C., Fournié, G., and Druet, P., Polyclonal effect of $HgCl_2$ in the rat, its possible role in an eyperimental autoimmune disease. Eur. J. Immun. *12* (1982) 620–625.

18 Hultman, P., and Enestrom, S., Experimental Hg-nephropathy: correlation between histochemistry and analytical EM. J. Ultrastruct. Res. *85* (1983) 118–119.

19 Lelental, M., and Gysling, H.J., Tellurium Physical Development (TPD): A dry non-silver amplification process. J. Photogr. Sci. *28* (1980) 209–215.

20 Makker, S.P., and Aikawa, M., Mesangial glomerulonephropathy with deposition of IgG, IgM and C3 induced by mercuric chloride: A new model. Lab. Invest. *41* (1979) 45–50.
21 Rodin, A.E., and Crowson, C.N., Mercury nephrotoxicity in the rat 1. Factors influencing the localization of the tubular lesions. Am. J. Path. *41* (1962) 297–307.
22 Romano-Franco, A.A., Turiello, M., Albini, B., Ossi, E., Milgrom, F., and Giuseppe, A.A., Anti-basement membrane antibodies and antigen-antibody complexes in rabbits injected with mercuric chloride. Clin. Immun. Immunopath. *9* (1978) 464–481.
23 Sapin, C., Druet, E., and Druet, P., Induction of anti-glomerular basement membrane antibodies in the Brown-Norway rat by mercuric chloride. Clin. exp. Immun. *28* (1977) 173–179.
24 Sapin, C., Mandet, C., Druet, G., Günther, G., and Druet, P., Immune complex type disease induced by HgCl: genetic control of susceptibility. Transpl. Proc. *13* (1981) 1404–1406.
25 Schreiner, G.A., and Maher, J.F., Toxic Nephropathy. Am. J. Med. *38* (1965) 404–449.
26 Siegel, S., The Randomization test for two independent samples, in: Non-parametric statistics for the behavioral sciences, pp. 152–156. Ed. S. Siegel. McGraw-Hill, New York 1956.
27 Stejskal, J., Acute renal insufficiency in intoxication with mercury compounds III. Pathological findings. Acta med. scand. *177* (1965) 75–84.
28 Thaw, H.H., Forslid, J., Hamberg, H., and Hed, J., The effect of reactive oxygen metabolites (ROM) on the attachment and indigestion phase of C3b- and IgG-mediated phagocytosis by macrophages. Acta path. microbiol. immun. scand. *92* (1984) 1–8.
29 Weening, J.J., Fleuren, G.J., and Hoedemaeker, Ph.J., Demonstration of Antinuclear antibodies in mercuric-chloride-induced glomerulopathy in the rat. Lab. Invest. *39* (1978) 405–411.

0014-4754/84/111235-07$1.50 + 0.20/0

Effects of pre- and post-irradiation glucan treatment on pluripotent stem cells, granulocyte, macrophage and erythroid progenitor cells, and hemopoietic stromal cells

M.L. Patchen, T.J. MacVittie and L.M. Wathen

Experimental Hematology Department, Armed Forces Radiobiology Research Institute, Bethesda (Maryland 20814, USA), 12 April 1983

Summary. Glucan, a beta-1,3 polyglucose, was administered to mice either 1 h before or 1 h after a 650 rad exposure to cobalt-60 radiation. Compared to radiation controls, glucan-treated mice consistantly exhibited a more rapid recovery of pluripotent stem cells and committed granulocyte, macrophage, and erythroid progenitor cells. This may partially explain the mechanism by which glucan also enhances survival in otherwise lethally irradiated mice.
Key words. Mice; glucan treatment; Co^{60}-irradiation; stem cells, pluripotent; granulocytes; macrophages; erythroid progenitor cells; hemopoietic stomal cells; hemopoiesis.

Introduction

Glucan, a β-1,3 polyglucose isolated from the inner cell wall of the yeast *Saccharomyces cerevisiae*[1,2] is a potent stimulator of the reticuloendothelial system[3,4] and a dose-dependent modulator of the cellular[5,6] and humoral[7] immune systems and hemopoietic system[8–12]. Specifically, in terms of effects on hemopoiesis, the administration of glucan to normal mice results in an overall increase in the production of pluripotent stem cells and also granulocyte-macrophage, pure macrophage, and erythroid progenitor cells. The enhanced granulocyte and macrophage genesis observed in glucan-treated animals has been associated with glucan's ability to induced the production and/or release of granulocyte-macrophage colony-stimulating activity (CSA)[8,13]. At this time, the mechanisms of glucan's enhancement of other aspects of hemopoiesis are not fully understood.

Because of glucan's profound stimulatory effects on hemopoiesis at the stem cell and progenitor cell levels, its use as a therapeutic agent in cases of hemopoietic depletion induced by drugs and/or radiation has been suggested[10–12]. Recently, it has been reported that glucan induced increased numbers of endogenous pluripotent spleen colony-forming units (E-CFU)[14] and also increased numbers of peripheral blood granulocytes[15] when administered either before or after hemopoietically damaging doses (550–650 rads) of gamma radiation. The purpose of this study was to examine in detail the effects of pre- and post-irradiation glucan treatment on the recovery of hemopoietic stromal cells (HSC), transplantable pluripotent hemopoietic stem cells (CFU-s), granulocyte-macrophage progenitor cells (GM-CFC), macrophage progenitor cells (M-CFC), and erythroid burst and colony-forming cells (BFU-e, CFU-e).

Materials and methods

10- to 12-week-old female $B6D2F_1$ mice were used in all experiments. All mice were quarantined and acclimated to laboratory conditions for 2 weeks before experimentation. Particulate, endotoxin-free glucan was obtained from Accurate Chemical and Scientific Corporation (Westbury, N.Y.) and was prepared according to DiLuzio's modification[2] of Hassid's original procedure[1]. Glucan was diluted in sterile 5% dextrose, and 1.5 mg (approximately 75 mg/kg) was intravenously injected into experimental mice either 1 h before or 1 h after exposure to 650 rads of total-body cobalt-60 radiation.