# Increased Stool Mercury Excretion in the Rat: The Effect of Spironolactone[1] (36536)

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(Introduced by R. Klein)

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Selye has presented evidence that spironolactone pretreatment protects the rat against a variety of toxins including digitoxin (1), anesthetics (2), indomethacin (3), and mercury (4). Spironolactone pretreatment for 4 days protects rats both from renal damage and from death which would otherwise result from iv $HgCl_2$. If spironolactone treatment is initiated after iv $HgCl_2$ administration, no protective effect is demonstrable. The mechanism of spironalactone prophylaxis is unknown, although Seyle has suggested several possible explanations (4). The present experiments were undertaken in an effort to elucidate spironolactone's protective effect by tracing the fate of injected $^{203}HgCl_2$ in intact and adrenalectomized control animals and in intact and adrenalectomized animals pretreated with spironolactone.

*Materials and Methods. Experiment No. 1—Intact animals.* Twenty-seven male Sprague–Dawley rats with a mean body weight of 110 g were divided into two groups. Group 1 animals were maintained on standard laboratory chow and tap water and served as controls. Group 2 animals were maintained similarly and were given 10 mg spironolactone in 2 ml water twice daily by gavage throughout the experiment. On the fourth day both groups of animals received a single iv dose of 400 $\mu$g $HgCl_2$ in 0.5 ml isotonic saline which also contained 1.5 $\mu$Ci of $^{203}HgCl_2$. The amount of radioactivity administered was calculated from the weight difference of the administering syringe before and after injecting, and a similarly weighed sample was saved for standardization of gamma well counting. Nine Group 1 animals and eight Group 2 animals were placed in metabolic cages and were sacrificed with ether at the end of 24 hr. An additional three Group 2 animals (two in metabolic cages) and an additional four Group 1 animals (two in metabolic cages) were kept as long as 48 hr before being sacrificed. Radioactive mercury was measured in all collected stool and in measured aliquots of urine from all animals in metabolic cages. The intact gastrointestinal tract (pylorus to anus) with its contents was measured for radioactivity in four animals from each group sacrificed at 24 hr. Additionally, radioactive mercury was measured in hearts, brains, measured liver aliquots and both kidneys in animals from both groups as indicated in Table I.

*Experiment No. 2. Adrenalectomized animals.* Seven male Sprague–Dawley rats with a mean body weight of 110 g were adrenalectomized and then maintained on 1% sodium chloride solution and standard laboratory chow. Animals 1–3 served as controls, while animals 4–7 were given spironolactone, 10 mg in 2 ml of water by gavage twice daily beginning on the day of adrenalectomy. On the fourth experimental day all animals received a single iv dose of 200 $\mu$g $HgCl_2$ in 0.5 ml isotonic saline which also contained 1.5 $\mu$Ci of $^{203}HgCl_2$. Injected material was standardized as in Expt. No. 1. The animals were then placed in metabolic cages and sacrificed at the end of 24 hr. Excreta and tissues were measured as in Expt. No. 1 with one exception; GI tract contents were removed and measured separately.

*Results.* Animals in Expt. No. 1 behaved as Selye has described (4). All four control

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TABLE I. Intact Rats.

| | Percent total injected counts | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Liver (24 hr) | Kidneys (24 hr) | Urine (0–4 hr) | Urine (4–24 hr) | Urine (0–24 hr) | Stool (0–24 hr) | Heart (24 hr) | Brain (24 hr) |
| Group 1 (controls) | | | | | | | | |
| Mean | 31.41 | 20.97 | 3.90 | 2.65 | 6.55 | 4.55 | .245 | .087 |
| SEM | 3.81 | 0.88 | .56 | .60 | .94 | .69 | .029 | .0057 |
| No. | 7 | 9 | 11 | 11 | 11 | 11 | 4 | 4 |
| Group 2 (Spironolactone) | | | | | | | | |
| Mean | 27.01 | 13.14 | 0.77 | 5.39 | 6.16 | 24.44 | .126 | .066 |
| SEM | 4.22 | 1.15 | 0.249 | .27 | .67 | 2.76 | .011 | .0039 |
| No. | 7 | 8 | 10 | 10 | 10 | 10 | 4 | 4 |
| Significance of Differences | — | $<.001$ | $<.001$ | $<.001$ | — | $<.001$ | $<.05$ | $<.1$ |

animals not sacrificed at 24 hr died before 48 hr; while three simultaneously observed Group 2 animals were alive at the end of 48 hr. Kidneys from six control and six spironolactone-pretreated animals, dissected at between 24 and 48 hr, were examined without knowledge of their experimental group by two observers. Gross mottling, similar to that described by Selye (4), was found in all six control animals and in only one of six experimental animals.

The first experiment is summarized in Table I. The most striking difference between the two groups was in the stool $^{203}Hg$ excretion where animals pretreated with spironolactone excreted approximately five times as much $^{203}Hg$ in the stool as did controls. In this pretreated group the mean 24 hr stool excretion was 24.4% of the total injected mercury and represented the major excretory pathway. The very large increase in stool mercury in pretreated animals allowed them to excrete a total of approximately one-third the injected mercury in the first 24 hr, and this was approximately two and a half times as much as controls were able to excrete. Two group 2 rats kept in metabolic cages for 48 hr continued to show a high rate of stool mercury excretion during the second 24 hr, while two simultaneous controls died during that time period and put out only small amounts of stool mercury from 24 hr up to the time of death. Tissue levels of radioactivity measured at 24 hr were generally lower in pretreated animals and reflected the increased $^{203}Hg$ excretion. The rate of urine mercury excretion was higher in the first 4 hr than in the next 20 hr in the control rats, and probably indicated progressive renal damage. Intact GI tract measurements, not included in Table I, were similar in both groups and accounted for approximately 3% of the total injected radioactivity.

Because of the small number of animals involved in the second experiment, Table II contains individual rather than mean values. Tissue counts have not been included, since they were similar to those in the first experiment and appeared to reflect the amount of mercury excreted. Adrenalectomy did not influence the effect of spironolactone pretreatment on mercury excretion. The pretreated animals put out a much increased amount of mercury in the stool, and again stool represented the major excretory product. Stool and GI contents, when added individually and compared, showed an approximate fivefold difference between the two groups; this was similar to the ratio in the first experiment. Total excreted $^{203}Hg$ showed an approximate 2½-fold increase in pretreated adrenalectomized animals and this,

TABLE II. Adrenalectomized Rats.

| | Percent total injected radioactivity | | | | | | |
|---|---|---|---|---|---|---|---|
| | Controls | | | Spironolactone pretreated | | | |
| Animal No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Stool (0–24 hr) | 1.1 | 0.7 | 5.0 | 31.8 | 25.4 | 25.0 | 22.8 |
| GI Contents | 4.5 | 5.9 | 1.7 | 8.6 | 7.3 | 6.7 | 5.6 |
| Urine (0–4 hr) | .8 | .3 | .4 | .2 | .4 | .1 | .2 |
| Urine (4–24 hr) | 7.4 | 8.8 | 6.4 | 3.2 | 6.1 | 9.6 | 3.6 |
| Total excreted | 13.8 | 15.7 | 13.5 | 43.8 | 39.2 | 41.4 | 32.2 |

again, was comparable to figures in the first experiment with intact animals.

*Discussion.* The toxic effects of mercury in the rat are modified by pretreatment with spironolactone, and it appears that this protective effect results from increased stool mercury excretion. The adrenal gland is not necessary in order for spironolactone to exert its influence. The mechanism for increased stool mercury excretion is unknown, although there are a number of possibilities such as hepatic enzyme induction, increased active transport into the gut, or interruption of enterohepatic circulation. Selye has shown that spironolactone protects the rat against a number of other toxic agents, but it would be premature to suggest that the same mechanism is present for them, also. Further investigation is indicated to define spironolactone's influence on a variety of agents in the rat, and eventually, in the human.

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