ruvate, glycerol can serve as a source of the pyruvate-derived carbon atoms of pyridoxol. Since the final step on this route from glycerol to pyruvate, the dephosphorylation of phosphoenolpyruvate, is essentially irreversible and since reconversion of pyruvate into triose phosphate by alternative pathways is, in any case, unlikely to be important under the chosen experimental conditions, pyruvate cannot replace glycerol as a complete source of the carbon skeleton of pyridoxol. It has yet to be established whether phosphoserine, whose implication in pyridoxol biosynthesis has been inferred on the basis of growth studies with blocked mutants (*13*), enters by way of pyruvate, by way of triose phosphate, or directly.

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Mercury Poisoning: Prevention by Spironolactone

Abstract. *The renal tubular necrosis and calcification as well as the mortality induced by mercuric chloride in the rat are readily prevented by prior treatment with well-tolerated amounts of spironolactone.*

Spironolactone can protect the rat against intoxication with digitoxin (*1*), various anesthetics (*2*), dimethylbenzanthracene (*3*), indomethacin (*4*), and numerous other agents (*5*). Such protective effect is shared by many other "catatoxic steroids" which are known to act through the induction of hepatic drug-metabolizing enzymes; hence the question arose whether spironolactone could also protect against the poisoning with a toxic heavy metal salt, which could not be disposed of by enzymatic degradation.

Female Sprague-Dawley rats (30) with a mean body weight of 100 g (range 90 to 110 g) were divided into two equal groups. Group 1 was not treated; group 2 received 10 mg of spironolactone (Searle) in 1 ml of water by stomach tube, twice daily, beginning on the first day and continuing until termination of the experiment. On the fourth day the rats of both groups were given a single dose consisting of 400 μg of $HgCl_2$ in 1 ml of water intravenously.

The control group began to show signs of prostration within 48 hours, and all of them died within 3 days after the injection of $HgCl_2$. By contrast, the animals that were treated with spironolactone before being given the $HgCl_2$ remained in excellent condition and were killed with chloroform when mortality reached 100 percent in the controls.

At autopsy the kidneys of each of the control rats showed heavy cortical calcification with severe perirenal edema. In the spironolactone-treated animals the kidneys and their surroundings were normal. Upon histologic examination of alcohol-formol fixed specimens stained with the von Kóssa or the celestin blue techniques, calcification and necrosis were found to be limited to the proximal convoluted tubules. The rest of the renal tissue, particularly the medulla and the vascular system, remained unaffected. The histologic appearance of the kidneys of the spironolactone-treated animals was essentially normal; only in two rats did we find two to three calcified tubules per cross section (Fig. 1).

These findings are reminiscent of earlier observations on the protection by testosterone (*6*) and cortisol (*7*) against $HgCl_2$-induced renal damage in the mouse and rat; but spironolactone, though devoid of classic hormone actions, is much more potent in this respect.

It would be premature to speculate about the mechanisms through which steroids exert their prophylactic effect. Spironolactone, which possesses a thioacetate group, may be particularly efficacious in introducing sulfur into the organism for the detoxication of a heavy metal such as mercury. The less potent steroids might promote the synthesis of SH-containing compounds, enhance the elimination of mercury, protect the kidney directly through a renotrophic effect (for example, testosterone), or reduce the mercuric salt to the less toxic mercurous form. The latter possibility is suggested by the observation that ascorbic acid apparently protects

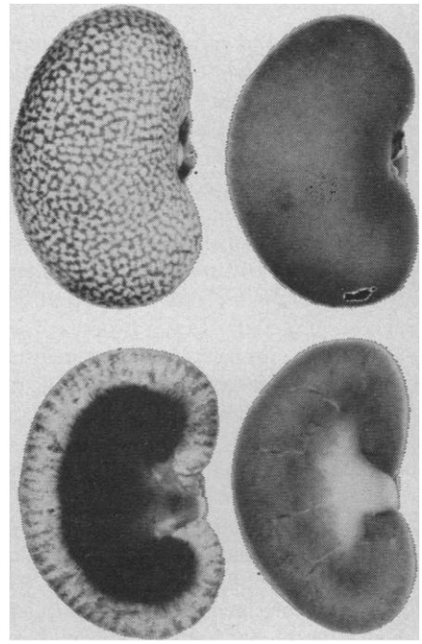

Fig. 1. Protection of the kidney by spironolactone against mercury-induced damage. Fresh specimens. (Left) Heavy calcification of the renal cortex in a rat of group 1 treated with $HgCl_2$. (Right) Complete prevention of calcification by prior treatment with spironolactone. (Top: external surface; bottom: cut surface.)

against $HgCl_2$ through this mechanism (*8*).

Ancillary experiments (*9*) have shown that, under otherwise similar conditions, equivalent or even greater amounts of sulfur offer no protection against this acute intoxication with mercury, if administered in the form of glutathione, cystine, cysteine, penicillamine, or thioacetamide. Sodium thioacetate and dimercaprol (BAL) can prevent the nephrocalcinosis, but only at nearly lethal dosages. On the other hand, two additional thioacetylated steroids, emdabol and spiroxasone, are approximately as potent as spironolactone in inhibiting this form of renal damage without inducing manifestations of toxicity. None of these steroids was of prophylactic value when administered after the intravenous injection of $HgCl_2$.

In any event, spironolactone, a virtually nontoxic and hormonally inactive steroid which increases resistance against many organic drugs, also offers excellent protection against an otherwise lethal, acute intoxication with mercuric chloride.

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Brain Weight Increases Resulting from Environmental Enrichment: A Directional Dominance in Mice

Abstract. *A genetic analysis of brain weights of 544 mice reared in either enriched or standard laboratory environments indicated significant directional dominance in the percentage of increase in brain weight as a result of enrichment.*

Rats reared in enriched environments from weaning to 105 days old have shown approximately 4 percent increase in cortical weight and 1 percent increase in whole brain weight over control animals reared in isolation (*1*). When different genetic lines were used in these studies, and in studies with mice (*2*), substantial genetic differences in brain weights also occurred, usually exceeding those produced by enrichment. Similarly, genetic analyses of inbred strains of mice (*3*) indicate a high heritability of brain size. Unfortunately these latter studies have been limited to animals reared in the relative restriction of laboratory cages, and studies involving enrichment have not been designed to permit quantitative estimates of genetic factors. Although we are aware of the considerable influences of both genetic makeup and enrichment on brain size, we have little information on how these factors interact.

I have carried out a genetic analysis of brain and body weights of house mice (*Mus musculus*) from a large number of genotypes reared in standard laboratory cages or in enriched environments. This report focuses on the effects of directional dominance and enrichment on whole brain weight (see *4*).

A total of 144 inbred mice from six strains (A/J, BALB/cJ, RF/J, C3H/HeJ, C57BL/10J, DBA/1J), 240 F_1 mice from the 30 possible crosses of the six strains, and 160 F_2 mice from ten four-way crosses derived from the F_1 strains were used. The four-way crosses were selected to represent parental lines in approximately equal proportions. At birth, half the litters in each genotype were assigned to be reared in the enriched cages, the remaining to be reared in standard cages. The standard laboratory cage was constructed of semi-transparent plastic 14 by 20 by 9 cm. The enriched cages were 55 by 25 by 15 cm high and contained a variety of small objects for climbing and exploring. These objects and their location in the cage remained constant and were identical in all 35 enriched cages used (Fig. 1). Animals were undisturbed except for weaning at 3 weeks and brief behavioral testing at 6 weeks. Between the 6th and 7th week they were killed and the body and brain weights were measured. With the exception of the anterior portion of the ophthalmic division of the trigeminal nerves, the entire brain extending to the 12th cranial nerve was removed and weighed within 5 minutes after the animal was killed.

Table 1. Brain and body weights of C3H × BALB hybrid mice reared in enriched, standard, and control cages.

Cage	Food hopper	Cage area	Weight (g)	
			Body	Brain
Enriched	Large	Large	19.1*	.469
Control 1	Large	Large	20.0*	.450*
Control 2	Large	Small	20.8*	.445*
Standard	Small	Small	15.3	.432*

* Not significant.

The mean brain weights were calculated for the combined inbreds, F_1 hybrids, and the F_2 four-way crosses reared in each environment (Fig. 2). In a design in which crosses from a number of lines were used, the average coefficient of inbreeding in both F_1 and F_2 progeny reverts to that of the base population (*5*). Therefore, mean scores for F_1 and four-way crosses should not be significantly different; however, any difference between inbreds and hybrids indicates directional dominance (or inbreeding depression). The brain weights of F_1 and F_2 animals in comparable environments were not significantly different from each other, but such animals had significantly larger brains than comparably reared inbred animals. Furthermore, among both hybrid groups enrichment led to a significant increase in weights of whole brain, whereas within the inbred parent strains, enriched and restricted animals were not significantly different. These data indicate that strong directional dominance is involved in increases in brain weight as a result of enrichment.

Contrary to the earlier studies with rats, where the relatively inactive restricted animals were generally heavier than their enriched counterparts, in this experiment weight increased (average, 21 percent) among enriched mice. Furthermore, since moderate genetic and environmental correlations existed between brain and body weights, and hybrids were generally heavier than inbred animals, it is important to demonstrate that the effects shown in Fig. 2 were not artifacts of differences in body weight. Two lines of evidence suggest that this was not the case. First, although some of the overall difference