Arylesterase Isoenzymes in Rats Bred for Susceptibility and Resistance to the Hypertensive Effect of Salt[1] (36141)

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Three groups of investigators, Smirk and Hall (1), Dahl *et al.* (2), and Okamoto and Aoki (3), have developed strains of rats which are genetically hypertensive and which serve as experimental models for human hypertension. Genetic studies with all three models indicate that the blood pressure differences between hypertensive and normotensive strains are due to multiple genes, which largely act in an additive way (4–7). Blood pressure in mice is similarly inherited (8). A very important aspect of work with these models is to identify the specific biochemical functions controlled by the genes, which result in hypertension. Such genes, obviously may or may not be common to the three independently developed hypertensive strains. A recent report by Yamori and Okamoto (9) described a difference in the arylesterase isoenzyme patterns between the spontaneously hypertensive (SH) rats of Okamoto and Aoki and control Wistar rats. The sole purpose of the present investigation was to determine if a similar difference existed between the hypertensive and control strains developed by Dahl.

Methods. The rats used were developed by Dahl *et al.* (2) from Sprague-Dawley stock by selective breeding on the basis of the blood pressure response to salt. These strains are highly susceptible (S) or resistant (R) to the hypertensive effects of high salt intake. The blood pressure difference between S and R strains is pronounced and has been well characterized (2, 5, 10, 11). For comparison, Wistar and "spontaneously hypertensive" (SH) rats of Okamoto and Aoki were obtained commercially (Purina Laboratory Animals, Vincentown, NJ).

Tissue esterase isoenzymes were studied by electrophoresis on 5% acrylamide gel (E-C Apparatus Corp., Philadelphia, PA). Tissues (liver, small intestine, or kidney) were homogenized in a 0.9% NaCl (1 g/3 ml), and centrifuged for 20 min at 20,000*g*, 4°. The supernatant (5–10 μl) was used for electrophoresis with 0.3 *M* borate buffer (pH 8.0) in the buffer chambers and .03 *M* borate buffer (pH 8.5) used to prepare the gel. Following electrophoresis, gels were reacted (stained) at room temperature with 30 mg/100 ml of α-naphthyl butyrate and 100 mg/100 ml of fast blue RR salt (4-benzoylamino-2, 5-dimethoxyaniline) in 0.05 *M* Tris buffer (pH 7.1) for 2 hr. This is a standard procedure which allows visualization of nonspecific esterase bands which hydrolyze α-naphthyl butyrate (arylesterases) to α-naphthol which reacts with the fast blue dye to form a visible (brown) product.

Results. Small intestine. Figure 1 shows a survey gel of arylesterases from the small intestine of salt susceptible (S), salt resistant (R), spontaneously hypertensive (SH), and normal Wistar (W) rats. The isoenzymes of moderate mobility show the characteristic difference between SH and W reported by Yamori and Okamoto (9), *i.e.*, a band of slower mobility in SH than in Wistar. These are labeled MS and MC, respectively, as done in Ref (9). In the nomenclature of Ref. (9), M stands for moderate mobility, S for SH rats and C for control Wistar rats. The

[1] J. P. R. is supported by the U.S. Public Health Service (USPHS; HE-11293) and the American Heart Association (71-661). L. K. D. is supported primarily by the U.S. Atomic Energy Commission with partial support from the American Heart Association (70-772) and the USPHS (HE-13408).

[2] Recipient of a Research Career Development Award (USPHS; AM-10543).

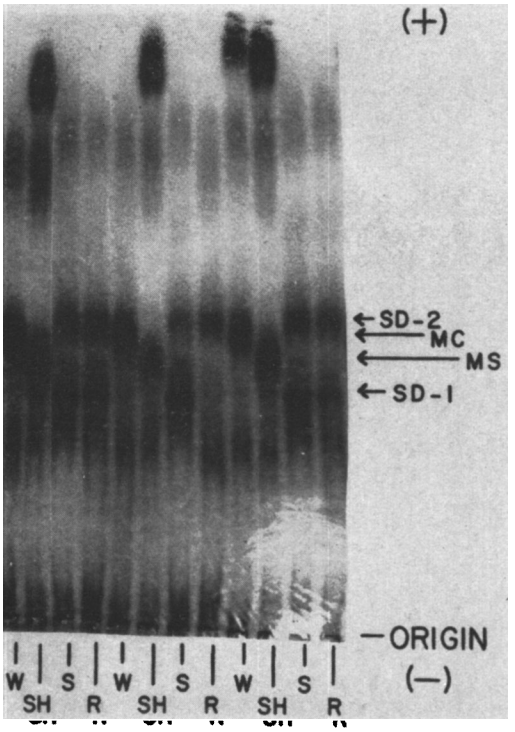

FIG. 1. Acrylamide gel electrophoresis of arylesterase isoenzymes from the small intestine of salt susceptible (S), salt resistant (R), spontaneously hypertensive (SH), and Wistar (W) rats: SD-1 and SD-2 refer to Sprague-Dawley isoenzymes 1 and 2, respectively; MS and MC are the designations previously used by Yamori and Okamoto (9) in designating isoenzymes of moderate mobility of the SH and Wistar (control) strains, respectively.

Sprague-Dawley S (salt susceptible) or R (salt resistant) rats were identical with respect to the major band of moderate mobility which is labeled SD-2 (SD = Sprague-Dawley) in Fig. 1. SD-2 appears in Fig. 1 to run slightly faster than the Wistar rat isoenzyme MC. Figure 2 better illustrates the small but consistent difference in mobility between SD-2 and MC isoenzymes. The minor band, SD-1, was inconsistently present in Sprague-Dawley rats and not different between S and R (Fig. 1 and 2).

The SD-2 band was not present in all Sprague-Dawley rats. Figure 3 shows a survey gel of S and R rats in which several animals essentially lack the isoenzyme. The frequency of rats lacking SD-2 was $10/26 = 38.5\%$ for R and $16/26 = 61.5\%$ for S.

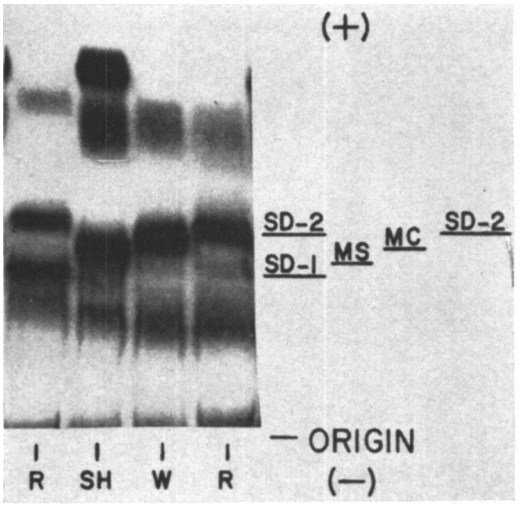

FIG. 2. Acrylamide gel electrophoresis of arylesterase isoenzymes from the small intestine: This gel demonstrates the small, but consistent, difference in mobility between SD-2 (Sprague-Dawley) and MC (Wistar) isoenzymes.

These frequencies were not statistically different ($p > .1$) by a chi-square test. The liver,

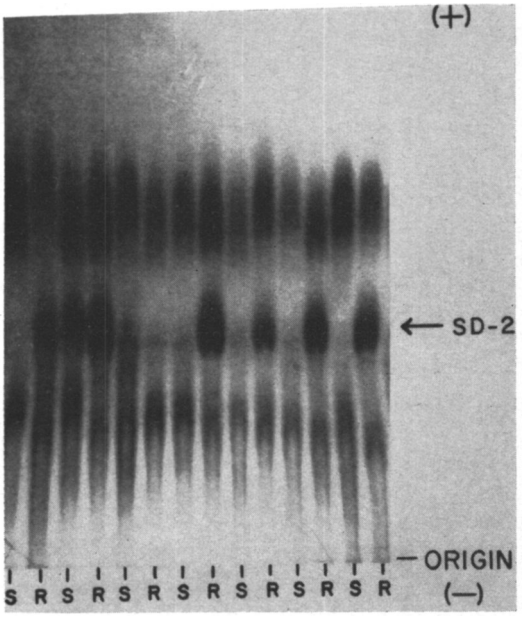

FIG. 3. Acrylamide gel electrophoresis of arylesterase isoenzymes from the small intestine of Sprague-Dawley (S and R) rats: A significant proportion of rats essentially lack the isoenzyme labeled SD-2.

as well as the small intestine, was studied in 20 rats. Whenever SD-2 was lacking in the small intestine, it was lacking in the liver pattern. Figures 1–3 incidentally demonstrate another difference between rats of Wistar origin (SH and Wistar controls) and rats of Sprague-Dawley origin (S and R). Wistar origin rats sometimes showed a strong band of high mobility in their intestinal patterns, seen as the fastest band in Fig. 1 and 2. This isoenzyme was completely absent in all rats of Sprague-Dawley origin which were tested.

Kidney medulla. The arylesterase isoenzyme pattern of the renal medulla was tested in 6 rats of each strain. The S, R, and Wistar rats were identical. They all showed two discrete bands which had the same mobility in all 3 strains. The SH rats showed the same difference in isoenzymes of the renal medulla as reported by Yamori and Okamoto (9). That is, the two medullary bands ran faster in SH than Wistar and therefore, were also faster than the S and R (Sprague-Dawley) rats which were identical to Wistar (Fig. 4).

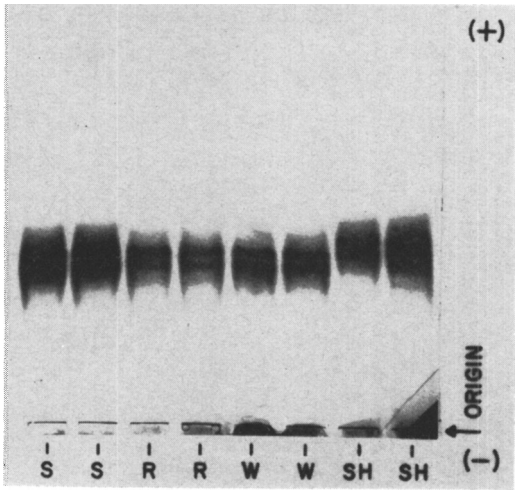

FIG. 4. Acrylamide gel electrophoresis of arylesterase isoenzymes from the renal medulla.

Discussion. The results showed: (a) that the arylesterase patterns of kidney, small intestine, and liver were similar in S and R strains; (b) that the isoenzymes described by Yamori and Aoki (9) in SH rats are, so far at least, unique to the SH strain of hypertensive rats.

The meaning of the esterase patterns in terms of the hypertension of SH rats is unclear at present. The arylesterase patterns in SH and W rats were shown to be controlled by a Mendelian gene inherited by codominance (9). If the SH patterns are subsequently shown to be correlated with an increment in blood pressure in F_2 and backcross populations obtained from SH and Wistar strains, then the gene controlling the SH esterase isoenzymes is probably one of several genes accounting for the blood pressure differences between SH and Wistar rats. Given the multigene character of the inheritance of blood pressure in rats (4–7), there is no *a priori* reason to expect different hypertensive strains to all carry exactly the same genes for hypertension. The existence of a specific gene in a specific hypertensive strain will depend largely on whether or not the gene in question existed in the original stock. The present data suggest that SH and S rats differ in at least one gene involved in blood pressure regulation. That is, SH rats carry an arylesterase gene presumed to be involved in hypertension but S rats do not carry this gene.

Summary. Tissue arylesterase isoenzyme patterns of salt susceptible (S), salt resistant (R), spontaneously hypertensive (SH), and Wistar (W) strains of rats were examined by acrylamide gel electrophoresis. The isoenzyme patterns in renal medulla and small intestine described by Yamori and Okamoto as characteristic of SH rats were not seen in the hypertensive, S strain of rats. This result suggest that SH and S rats differ by at least one gene which has tentatively been associated with hypertension. S and R strains were similar in tissue arylesterase isoenzyme patterns.

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Received Sept. 1, 1971. P.S.E.B.M., 1972, Vol. 139.