AN ENORMOUS NUMBER OF MICROBES populate external and internal surfaces of humans influencing a variety of aspects of host physiology such as providing nutrients and vitamins. The microbiome influences Phase I and Phase II drug metabolism (43). In recent years, a number of studies have pointed to links between the gut microbiota and pathophysiology of diabetes (8, 36), cancer (27), bowel disorders (9, 15), liver disease (46), immune conditions (33), and the metabolic syndrome (12). Increased understanding of these processes may unravel tremendous potential for therapeutics. Gut microbiota can influence a number of processes that affect the control of blood pressure.

Interesting studies linking gut microbiota with hypertension in the Dahl rat have been reported in this journal by Mell and colleagues (28). The cecal contents of salt-sensitive (S) rats were significantly different to salt-resistant (R) rats. To test further if differences in microbiota contribute to the extent of blood pressure regulation, microbial transplantation experiments were performed. The hypertension of S rats was exacerbated when the S rats were given R rat microbiota. Another recent publication (47) showed an altered gut microbiota in spontaneously hypertensive rats (SHR) compared with Wistar Kyoto (WKY) rat models and a small cohort of hypertensive patients. They claimed an association of gut dysbiosis with hypertension. I would like to draw to the attention of the readers a historical foundation, from more than 30 years ago, to such associations with hypertension. Observations in humans of the enterohepatic circulation of corticosterone (17) were taken further with studies in rats (18, 19, 21, 22, 24) where the experimental increase of blood pressure through the action of steroids was prevented by administration of antibiotics.

Clinical Evidence for Enterohepatic Circulation of Steroids

Intestinal metabolism was described in studies of humans with a rare disorder of synthesis of cortisol and sex steroids due to a genetic defect of steroid cytochrome 17-hydroxylase (CYP17), a condition defined by Biglieri et al. in 1966 (2). The hypertension in such patients is attributed to excess production of deoxycorticosterone, which causes renal sodium retention (see Fig. 1 for pathways of steroid synthesis). Plasma renin activity is suppressed and aldosterone production low. The urinary steroid metabolome of this condition was described in 1978 (17). Among the steroid metabolites of corticosterone were a high proportion of 21-deoxycorticosterone steroids. In a tracer experiment the 21-deoxy steroids retained the radioactive label of injected corticosterone (38). When steroid production in a CYP17-deficient patient was stimulated with adrenocorticotropic hormone (ACTH) or suppressed with dexamethasone, the changes in corticosterone metabolites with 21-hydroxyl groups preceded the changes of the 21-deoxycorticosterone products by 1 or 2 days (20). These observations mimicked changes in corticosterone metabolism of germ-free rats that did not produce the 21-deoxycorticosterone metabolites seen in conventional rats with normal gut microbiome (10). The micro-organism *Eubacterium lentum* was found to be capable of the 21-dehydroxylation (11), and this action was used in vitro to synthesize a number of 21-deoxy steroids (3, 4, 39).

Steroid Hypertension in the Rat

To address the possible relevance to hypertension of gut derived steroids and the enterohepatic circulation of metabolites, studies were performed in Sprague-Dawley rats made hypertensive with corticosterone or by administration of ACTH. The first results were presented at a Serono Symposium: Endocrinology of Hypertension, Padua, Italy, in October 1981 (18), the 164th meeting of the Society for Endocrinology (19), and at the Sixth International Congress on Hormonal Steroids, Jerusalem, Israel, in September 1982 (21). Hypertension was prevented by prior treatment of the rats with neomycin (22, 24); vancomycin had a weaker effect (24). Neomycin also slowed the development of hypertension in a spontaneously hypertensive rat of stroke-prone substrain (24). The gut bacteria were examined with conventional techniques at that time. Dilutions of fecal samples were spread on agar plates enriched to selectively support growth of organisms. Plates were incubated aerobically and anaerobically. The findings were insufficiently detailed to assign links with steroid metabolism and needed verification with extensive laborious investigations outside the scope of the laboratory. Culture-dependent methods for enumerating bacterial numbers are known to be biased and prone to errors. The studies were not taken further in the Honour laboratory. The effect of neomycin on blood pressure was repeated in the Florey Institute (Melbourne, Australia) with rats given ACTH and corticosterone (14) and in the rat model of one clip, one kidney (CSK or more usually 1K-1C) hypertension but not deoxycorticosterone-salt hypertension (26). Neomycin had no effect on the blood pressure or metabolic response to ACTH in sheep (45). The Florey group partially characterized a group of “hypertensinogenic” steroids (36).

Receptor Promiscuity

More is now known of the mechanisms for hypertension involving responses of steroid receptors (31). One extremely important feature has been acceptance that cortisol (and corti-
costerone), normally a glucocorticoid, can bind to the mineralocorticoid receptor, but under normal circumstances this is rendered inactive by oxidation of cortisol at carbon 11 to cortisone (11-dehydrocorticosterone), the 11B-hydroxysteroid dehydrogenase type 2 (HSD11B2) enzyme. A genetic defect of HSD11B2 in the kidney is associated with severe hypertension (42) from childhood (13, 23, 40) and is usually referred to as apparent mineralocorticoid excess syndrome because the increased sodium retention and high blood pressure are features of mineralocorticoid excess. HSD11B1 is a reductive enzyme familiar to clinicians for its ability to activate cortisone by conversion to cortisol. Liquorice was known for a long time to cause hypertension (7), and we now know that the active glycerrhetinic acid of liquorice is an inhibitor of HSD11B2 (41). Drugs are under development to inhibit the reductive HSD11B1 as targeted treatment for the metabolic syndrome (43).

Potential Clinical Studies

Morris et al. (29) characterized a number of steroids with similar actions to liquorice called glycerrhetinic acid-like factors (GALFs) and later suggested that corticosterone metabolites related to 11-hydroxyprogesterone (or 21-deoxycorticosterone) metabolites that are known to be potent GALFs (6) may contribute to the hypertension in Biglieri syndrome (30). Morris makes the proposition in the more recent paper that administration of antibiotics to patients with 17-hydroxylase, along with steroid metabolomic studies, might reveal the extent to which gut dysbiosis influences hypertension. These patients are very rare, and to be included in any studies with antibiotics the patients would first need to be taken off replacement steroid treatment. With time of treatment the hypothalamic-pituitary-adrenal axis will have been suppressed, and the adrenals would have atrophied, so normal adrenal function and recovery of a state of corticosterone excess might take some time during which blood pressure would increase. Neomycin, however, is not recommended for parenteral use in humans because of ototoxicity and nephrotoxicity; furthermore, there are now less toxic agents that can be used. Depletion of microbiota requires a combination of antibiotics such as rifaxim and metronidazole for weeks.

As an alternative, another group of patients worthy of investigation are patients with prostate cancer treated with abiraterone to suppress sex steroid production (1). This drug acts at CYP17 to inhibit steroid 17-20 lyase but also suppresses 17-hydroxylase, leading to low cortisol and androgens with...

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Fig. 1. Biosynthesis of adrenal steroids. Rats do not produce cortisol. Deoxycorticosterone excess causes hypertension. Progesterone can also be converted to 11-hydroxyprogesterone by the action of CYP11B2. Most steroids are excreted in urine after reduction of the A-ring and conjugation with glucuronic acid. Corticosterone and sex steroids are excreted in bile. Steroids in the intestine are subject to bacterial metabolism. Tetrahydrocorticosterone can lose the 21-hydroxyl group and 21-deoxysteroid metabolites, in effect metabolites of 11-hydroxyprogesterone, are reabsorbed from the intestine. StAR, steroidogenic acute regulatory protein; CYP11A1, side chain cleavage; HSD3B2, 3-beta hydroxysteroid dehydrogenase; CYP17A1, 17-hydroxylase; CYP21A2, 21-hydroxylase; CYP3A12, 11B-hydroxylase; CYP11B1, 11B-hydroxylase and aldosterone synthase; HSD11B, 11B-hydroxysteroid dehydrogenase; HSD17B, 17-hydroxysteroid dehydrogenase.
high corticosterone production. Some patients need hydrocortisone replacement, and some patients become hypertensive. The mechanism for increased blood pressure has not been investigated, but the production of corticosterone metabolites in the gut should be explored with detailed metabolomic studies.

**Gut Dysbiosis and Hypertension**

Differences in the gut microbiota have been demonstrated in Dahl rat models (28). The gut microbiomes of Dahl S were compared with R rats. Bacteroides (especially family S24-7) and Veillonellaceae counts were higher in S rats compared with R rats. Animals from both strains were maintained on high-salt diets, administered the antibiotics vancomycin and meropenem, and then transplanted with rat cecal contents. Systolic blood pressure was significantly elevated for the high-salt diets, administered the antibiotics vancomycin and with the R rats. Animals from both strains were maintained on and Veillonellaceae counts were higher in the S rats compared with R rats. Lower levels of Veillonellaceae and increased plasma acetate were features of the higher blood pressure in S rats given cecal contents from R rats.

The gut microbiome of SHR was compared with the microbiota of the WKY strain (47). Microbial richness, evenness, and diversity were all decreased in SHR with diminished Actinobacteria. The Firmicute-to-Bacteroidetes ratio (F/B) was higher in SHR. Yang and colleagues (47) also studied rats made hypertensive with angiotensin II infusion. This increased the F/B ratio. The microbiota was then changed with minocycline, a broad-spectrum tetracycline antibiotic frequently used in humans for treatment of acne vulgaris. Minocycline targets Actinobacteria and significantly reduced the F/B ratio due to an increased B and reduction in F counts similar to the differences between SHR and WKY. Neomycin is an aminoglycoside with activity against strains of staphylococci and enteric Gram-negative rods (Bacteroidetes). The gut of the human is dominated by B and F species with minor populations of Actinobacteria, Fusobacteria, and Cyanobacteria. Bacteroidetes include three large classes of Gram-negative, rod-shaped bacteria. The F genera have a Gram-positive cell wall and include Clostridia, which are anaerobic and Bacilli that are facultative aerobes. Steroid 21-dehydroxylation has been described for Clostridia species and Eggerthella (then Eubacterium), which is a genus of Actinobacteria (4, 11).

**Gut Microbiota and Hypertension**

Mechanisms for hypertension other than steroid metabolism may be influenced by gut dysbiosis as highlighted by Gustafsson recently (16). Yang and colleagues (47) also considered the impact of decreased acetate- and butyrate-producing bacteria with increased lactate-producing bacteria in SHR, because links with diabetes mellitus and cardiovascular dysfunction have been reported (35). The short chain fatty acids (SCFA), particularly butyrate, were not measured in the studies, but they are known to reduce inflammation, improve insulin sensitivity, and protect against diet-induced obesity and cardiovascular disease. Plasma acetate was higher in S rats given cecal contents of R rats (28). Propionate, another bacterial SCFA, has a number of actions through G protein-coupled receptors and has been shown to mediate olfactory chemosensors and modulate blood pressure via the kidney and release of renin (34). Gene knockout studies confirmed the vasodilation induced by propionate was differentially modulated by disruption of Olfr78 and Gpr41 gene expression.

**Conclusion**

A number of studies over the past 30 years and more have suggested a role for the gut microbiome in the development of hypertension in rats and man. The original work in 1981 was based on disrupting the enterohepatic circulation of steroids by administration of antibiotics to suppress intestinal bacteria. Rapid enumeration of bacteria was not available then but can now be achieved with a variety of molecular techniques as used separately by Mell et al. (28), Yang et al. (47) and Pluznick et al. (34). Changes in the microbiome can be demonstrated, but as yet the findings haven’t pinpointed the mechanism although salt was a common factor. The modern molecular approach to enumerating bacterial changes combined with targeted metabolite detection should lead to explanations of the mechanisms for gut dysbiosis on hypertension. Central effects of antibiotics and gut-derived metabolites can also not be discounted. The involvement of nuclear receptors will need to be clarified. Further experiments demonstrating effects of gut dysbiosis on hypertension are clearly necessary. The older and new data in rats and humans provide a rationale for further investigations since this concept may offer a new mechanism for treating hypertension with diet, probiotics, antibiotics, or fecal transplants as seen with other pathologies (5, 25, 32, 37).

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: J.W.H. conception and design of research; J.W.H. performed experiments; J.W.H. analyzed data; J.W.H. interpreted results of experiments; J.W.H. prepared figures; J.W.H. drafted manuscript; J.W.H. edited and revised manuscript; J.W.H. approved final version of manuscript.

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