



# Trails of Discovery

## *A cornerstone of cardiovascular therapy: the thiazide diuretics*

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In this era of molecular biology and the apparently novel subdiscipline of translational medicine, it is perhaps salutary to review briefly the background to the discovery of the thiazide diuretics, which, even 50 years after the initial research leading to their discovery, play a pivotal role in cardiovascular therapy.<sup>1,2</sup> As so often in novel drug discovery, several seemingly unconnected scientific observations coincided to bring about the discovery of the thiazides. The first-generation diuretics were salts of mercury used primarily as topical antiseptics, especially in the management of syphilis, usually in the form of mercurous chloride (calomel). Toward the end of the 18th century, it was observed by astute clinicians that fluid retention secondary to cardiac failure ("cardiac dropsy") responded to calomel. However, it was the careful observations of Jendrassik in 1886 that showed that calomel could promote a diuresis of up to 7 or 8 liters per day, but only if marked fluid retention was already present. Calomel did not promote diuresis in normal subjects.<sup>3</sup> From that time, numerous attempts were made to discover mercurial diuretics that were active orally rather than parenterally. Prolonged administration of mercurial diuretics required

daily intramuscular injections and there was the serious risk of mercury poisoning.<sup>4</sup> The alternative orally active diuretic for treating congestive heart failure was theophylline (0.25 g four times daily). Its efficacy depended upon the degree of fluid retention, and tachyphylaxis was observed on long-term treatment. Clearly, there was a need for more effective and efficient diuretics, but there was little scientific evidence at the end of the 1920s to indicate which direction research should take.

### EARLY OBSERVATIONS

The events leading to the discovery of the thiazides cover a 30-year period, from 1930 to 1960 (see *Figure 1 page 176*, flowchart), during which important academic and industrial research played a complementary role. There were two pivotal, but seemingly unrelated, scientific discoveries in the early 1930s. Firstly, the demonstration by Gerhard Domagk, working in the laboratories of the IG Farbenindustrie, made the discovery that was to earn him the Nobel Prize in Medicine for 1939, that the red dye Prontosil (sulfamido-chrysoidine, or 4-[(2,4-diaminophenyl)azo]benzenesulfonamide), synthesized in 1932, had antibacterial activity when administered to streptococcal-infected mice. Surprisingly it was inactive in *in vitro* antibacterial studies.<sup>5</sup> Shortly afterwards it was shown that the active metabolite of Prontosil was sulfanilamide by the French group working in Bovet's labo-

ratory in France.<sup>6</sup> Sulfanilamide was found to have potent antibacterial properties in experimental infections in mice.<sup>7</sup> Secondly, Meldrum and Roughton, described the existence of the enzyme carbonic anhydrase in red blood cells and subsequently in the kidney, gastric mucosa, and central nervous system.<sup>8</sup>

In 1937, Southworth published a paper, in which he showed that administration of the first antibacterial sulfanilamide caused a fall in CO<sub>2</sub> combining power of the plasma in 15 patients receiving treatment for infection.<sup>9</sup> The following year, Strauss and Southworth reported on urinary changes due to sulfanilamide treatment in 3 healthy subjects.<sup>10</sup> These were observed over 12 days and given 3 to 5 g of sulfanilamide between days 3 and 6. This paper is perhaps impressive for several reasons:

- The 24-hour urinary volume, pH, and electrolyte changes were recorded over the entire 12-day period.
- Blood CO<sub>2</sub> combining power was measured daily.
- Plasma levels of sulfanilamide were measured, and a relationship demonstrated between drug dose and the increase in urine volume and electrolyte excretion (*Figure 2, page 177*).<sup>10</sup>
- All 3 volunteers developed raised body temperature of between 102 and 103° F, yet the paper does not speculate on the basis of these unwanted effects or that one subject had to stop dosing because of significant side effects.

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Two years later, Mann and Keilin, working in the Molteno Institute, University of Cambridge, published a paper demonstrating that sulfanilamide was a potent inhibitor of carbonic anhydrase.<sup>11</sup> They reported the structure-activity relationships of 16 analogs of sulfanilamide and showed that the amino group of sulfanilamide, which is essential for the antibacterial property, is not responsible for the inhibition

of carbonic anhydrase. Furthermore, they demonstrated that sulfonamides, which did inhibit carbonic anhydrase, were highly specific and did not affect other zinc-containing enzymes. Interestingly, the compounds used in this study were supplied by three separate pharmaceutical companies, but it is not clear if the scientists in these companies followed up the clinical implications of these observations.

In 1941, Davenport identified carbonic anhydrase in the kidney.<sup>12</sup> In 1942, Hober reported on the effects of eight sulfonamide analogs on changes in urinary pH in the perfused frog kidney.<sup>13</sup> He concluded that the pH changes due to the sulfonamide analogs could be due to inhibition of carbonic anhydrase, which was involved in the reabsorption of bicarbonate in the renal tubule.

Academic studies	Drug research studies
<p><b>1933</b> Meldrum and Roughton describe distribution and properties of carbonic anhydrase (CA)<sup>8</sup></p>	<p><b>1929-35</b> Gerhard Domagk, of IG Farbenindustrie, Germany, shows that Prontosil (sulfamido-chrysoidine), a prototype compound leading to the development of sulfonamide antibacterials, given orally, cures streptococcal-infected mice, but is inactive in vitro.<sup>5</sup> Awarded Nobel Prize in Medicine in 1939</p>
<p><b>1937</b> Southworth observes acidosis in Prontosil-treated patients<sup>9</sup></p>	<p><b>1935</b> Bove's group identifies sulfanilamide as the active metabolite of Prontosil<sup>6</sup></p>
<p><b>1938</b> Strauss and Southworth show sulfanilamide causes diuresis in volunteers<sup>10</sup></p>	
<p><b>1940</b> Mann and Keilin purify CA and suggest that nonantibacterial sulfanilamide analogs inhibit CA<sup>11</sup></p>	
<p><b>1941</b> Davenport and Wilhelmi identify CA in renal cortex of several species. Propose sulfanilamide causes diuresis by CA inhibition<sup>12</sup></p>	<p><b>1943-50</b> Sprague and Beyer, of Sharp &amp; Dohme, seek inhibitors of renal excretion of penicillin. Discover probenecid (Benemid), a sulfanilamide analog<sup>14</sup></p>
<p><b>1949</b> Schwartz shows that sulfanilamide improves heart failure by ↑NA, H<sub>2</sub>O loss<sup>16</sup></p>	<p><b>1950</b> Roblin and Clapp, of American Cyanamid, discover, based on a communication from Schwartz, acetazolamide, an orally active CA inhibitor<sup>18</sup></p>
<p><b>1950</b> Freis initiates clinical research in hypertension<sup>22</sup></p>	<p><b>1950-52</b> Beyer et al initiate diuretic research on sulfanilamide-induced CA inhibition and improved organomercurials. Clinical target is heart failure and hypertension<sup>15</sup></p>
<p><b>1957</b> Freis shows that chlorothiazide potentiates response of other antihypertensive agents<sup>23</sup></p>	<p><b>1955-57</b> Novel diuretic action of chlorothiazide discovered<sup>21</sup></p>
<p><b>1967</b> Freis et al show reduction in morbidity by long-term blood pressure control<sup>33</sup></p>	<p><b>1957-62</b> Numerous thiazide analogs developed</p>
<p><b>1971</b> Freis awarded Lasker Prize<sup>33</sup></p>	<p><b>1975</b> Beyer, of Merck, Sharp &amp; Dohme, awarded the Lasker Prize for the discovery of the thiazides<sup>15</sup></p>

**Figure 1.** Pathways to the discovery of thiazides. In the 1920s, research was focused primarily on finding potent orally active organomercurial analogs as diuretics, but their therapeutic margin was narrow.



In 1943, Karl Beyer set up a renal research program in the Sharp & Dohme pharmaceutical company in the United States. The initial purpose of their research was to examine the renal excretion of sulfonamide compounds, because crystalluria, due to sulfonamide treatment, was a common clinical problem. This necessitated studying renal transport mechanisms.<sup>14</sup> At that time, the company was working on analogs of sulfonamides for antibacterial purposes and also on the large-scale production of penicillin G.

The latter is rapidly excreted by the kidney, so studies were performed to find a chemical means of inhibiting its rapid elimination. In 1950, Beyer postulated that two renal enzyme systems were involved in ion transport. These were carbonic anhydrase and an unidentified sulfhydryl-containing enzyme that was possibly inhibited by mercurial diuretics. Initial research success came with the discovery of probenecid (Benemid), a benzoic acid derivative, which markedly inhibited the renal excretion of penicillin G.<sup>14</sup>

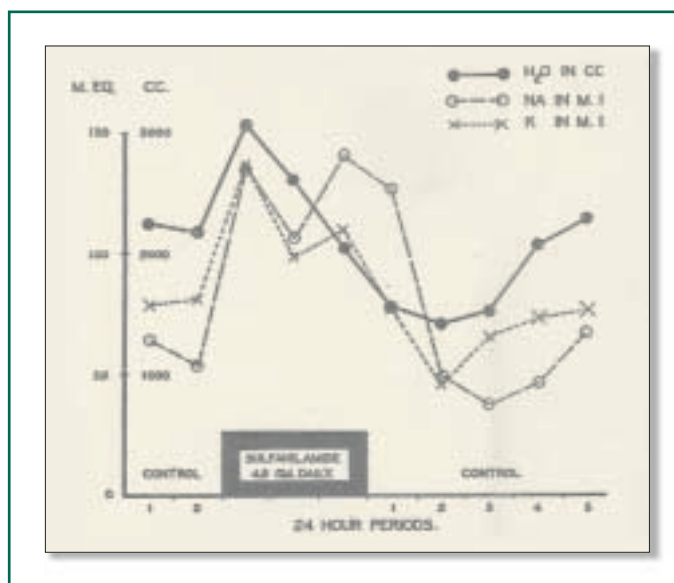
### STUDIES LEADING TO THE DISCOVERY OF THIAZIDES

Beyer and his chemical colleagues, Novello and Sprague, set up a research program seeking compounds that would inhibit the reabsorption of the cation sodium, resulting in a natriuretic effect. The therapeutic targets were edematous states and essential hypertension. They selected two separate chemical approaches. The priority program was based on analogs of sulfonamides, which were known to inhibit carbonic anhydrase. The secondary target was to discover nonmercurial analogs, which they believed acted by inhibiting sulfhydryl-catalyzed dehydrogenase enzymes in the kidney. Based on their previous experience seeking inhibitors of penicillin excretion by the kidney, they planned to

seek compounds that would inhibit carbonic anhydrase in the proximal renal tubule, would be poorly reabsorbed in the distal portion of the tubule and if possible should not act extracellularly, in order to minimize nonspecific systemic toxicity.<sup>15</sup>

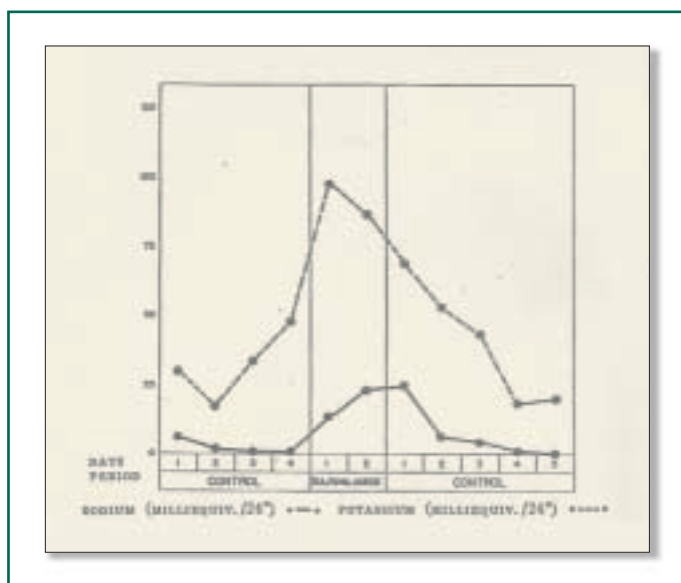
While this initial work was in progress in Sharp & Dohme (Sharp & Dohme merged with Merck in 1953 to become Merck, Sharp & Dohme: MSD), the industrial scientists Roblin and Clapp, working in the American Cyanamid Company, were also seeking novel orally active diuretics based on inhibition of carbonic anhydrase. Their research interest was triggered by the clinical studies of William Schwartz, working as a Research Fellow in the Peter Bent Brigham Hospital, Boston. He had studied the effects of sulfanilamide (4 to 6 g daily) on urinary function in 3 patients with severe congestive heart failure.<sup>16</sup> In his paper, he indicates that his study was prompted by the report by Pitts and Alexander, published in 1945, showing that sulfanilamide administered to dogs increased urinary

pH and reduced titratable acidity, probably by inhibiting renal tubular carbonic anhydrase.<sup>17</sup> Examples of the increase in urinary sodium excretion due to sulfanilamide administration are illustrated in a human volunteer (*Figure 2*) and a patient with congestive heart failure (*Figure 3, page 178*). The American Cyanamid scientists prepared analogs of sulfanilamide, seeking potent orally active inhibitors of carbonic anhydrase, based initially on suggestions made by Schwartz, which they acknowledge in their paper published in 1950.<sup>18</sup> One of their analogs proved to be 330 times more potent at inhibiting carbonic anhydrase than sulfanilamide. This compound, subsequently called acetazolamide, was evaluated clinically. Friedberg reported on its effects in 26 patients with heart failure, of which 18 responded with a marked weight loss, diuresis, and improvement in symptoms.<sup>19</sup> However, chronic administration of acetazolamide resulted in a loss of efficacy. Subsequently, a use was found for it in selected cases of epilepsy and glaucoma.



**Figure 2.** Urinary output of water, sodium, and potassium in a volunteer given sulfanilamide (0.1 g/kg/day).

Adapted from reference 10: Strauss NB, Southworth H. Urinary changes as a result of sulfanilamide administration. *Bull Johns Hopkins Hosp.* 1938;63:41-45. Copyright © 1938, The Johns Hopkins University.



**Figure 3.** Effect of sulfanilamide (4 to 6 g daily) on urinary sodium and potassium in a patient with severe heart failure.

Adapted from reference 16: Schwartz WB. The effect of sulfanilamide on salt and water excretion in congestive heart failure. *N Engl J Med.* 1949;240:173-177. Copyright © 1949, Massachusetts Medical Society.

In the meantime, the research at MSD progressed rapidly. In contrast to the American Cyanamid approach, which determined the potency of chemical analogs in an *in vitro* system for detecting inhibition of carbonic anhydrase, Beyer and his colleagues in MSD used the conscious dog in order to determine the effects of compounds on sodium and chloride urinary excretion. In a short time, a lead compound, CBS (*p*-carboxybenzene-sulfonamide), was identified. It was chloruretic, secreted by the renal tubules and rapidly excreted. These findings supported the validity of Beyer's hypothesis. Numerous analogs were evaluated and it became clear that the determinants of diuretic efficacy were not only dependent on the potency of the carbonic anhydrase inhibitors, but also on physicochemical properties of lipophilicity, protein binding, and the handling by the renal tubule.

The chemical synthetic program included the synthesis of cyclized analogs, one of which became chlorothiazide (Figure 4).<sup>20</sup> Its profile in the canine

experiments showed a marked (35-fold) increase in sodium and chloride excretion with a lesser (3.8-fold increase) in potassium and bicarbonate excretion. The compound was active orally in the dog, with maximal activity 30 minutes after administration. It soon emerged that the pharmacodynamic profile of chlorothiazide was markedly different to that of acetazolamide and had features similar to organomercurials.<sup>21</sup>

### SUBSEQUENT DEVELOPMENTS

#### Early clinical findings

Since chlorothiazide was active orally and had excellent tolerability, it was rapidly evaluated in both essential hypertension and congestive heart failure. It is salutatory to recall that in the early 1950s, the only agents available to treat hypertension were ganglion-blocking drugs and veratrum alkaloids, derived from natural products. Edward Freis, in his 1971 Albert Lasker Award paper,<sup>22</sup> subsequently describes

the evolution of the treatment of essential hypertension between 1946 and 1966. He was among the first investigators to describe the effects of chlorothiazide (1.5 g daily) in reducing blood pressure in patients with moderate-to-severe hypertension. His preliminary conclusion after studying 88 patients and 15 normotensives, none of which received a placebo, was:

- That chlorothiazide is an effective and well-tolerated antihypertensive agent that appears to be specific for the hypertensive state.
- Its mode of action appears to differ from previous antihypertensive agents. It produces significant reduction of blood pressure when used alone and additional reduction when combined with other antihypertensive agents, or when given to sympathectomized patients.
- It does not reduce blood pressure in normotensive subjects.<sup>23</sup>

Other investigators soon confirmed these observations in hypertensive patients<sup>24</sup> as well as showing efficacy in patients with congestive heart failure.<sup>25</sup>

### CHLOROTHIAZIDE ANALOGS

Beyer coined the term "saluretic" to distinguish chlorothiazide from previous agents whose characteristics were to cause a marked increase in urine volume, *ie*, diuretics.<sup>15</sup> The publication of trials of chlorothiazide led to an explosion of medicinal chemistry activity in other pharmaceutical companies. The objective of the research was to identify compounds that would be an improvement on chlorothiazide in terms of potency, specificity, systemic bioavailability, duration of action, and patentability. Chlorothiazide was between 10% and 20% orally bioavailable and was rapidly excreted unchanged in the urine with a plasma half-life of 1 to 2 hours, but a duration of action of 6 to 12 hours. The search for improved saluretics was to an extent con-



founded by the lack of correlation between in vitro tests for inhibition of carbonic anhydrase and the saluretic activity observed in in vivo studies. Detailed analysis of the structure-activity requirements for carbonic anhydrase inhibition was widely available in the literature,<sup>26</sup> but was of little help in making improved saluretics. In assessing the appropriate tests to detect saluresis, Beyer emphasized that the conscious dog model would identify compounds that would be overlooked by animal disease models. He wrote,

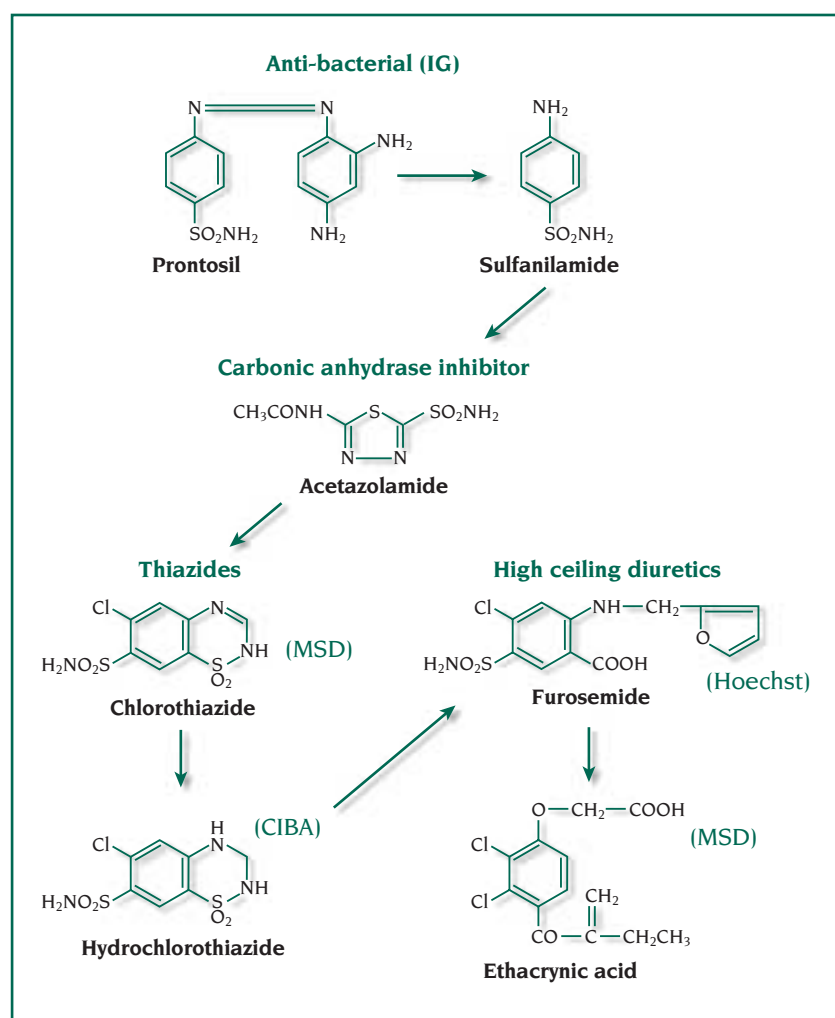
had one employed a model of cardiac decompensation or renal insufficiency adequate to induce fluid retention, the technical difficulty of maintaining a uniform colony

of such animals adequate to control and quantify a drug effect on fluid retention might be defeating... Had such other measures been employed as first-line procedures in dogs, both chlorothiazide and ethacrynic acid [see below] may have been missed if they had been administered as a single daily dose, because of their short duration of action.<sup>27</sup>

Within 18 months, the group in the CIBA laboratories in the USA published data showing that hydrochlorothiazide was possibly an improvement on chlorothiazide because it had more than 60% systemic bioavailability, a plasma half-life of 5 to 6 hours and was 10 times more potent. Surprisingly, it underwent enterohepatic recirculation, which may explain its apparent

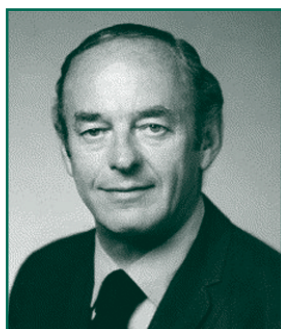
greater potency in human studies.<sup>28</sup> These pharmacokinetic characteristics were brought about by the seemingly trivial substitution of two hydrogens in the heterocyclic portion of the ring system (*Figure 4*). Subsequently, about 12 thiazide analogs, differing in potency and kinetic properties, were marketed. They all had about the same maximal saluretic effect.

During the late 1950s, chemists in the Hoechst Company, Germany, initiated a research program to find saluretic agents that could replace their organomercurial compound, mersalyl, which had been used for the previous 30 years before being displaced by the thiazides. The Research Group abandoned their studies to find improved carbonic anhydrase inhibitors and instead set out to find compounds with a higher maximal saluretic efficacy in comparison with thiazides. Muschawek has described the lengthy chemistry program, which was also based on sulfonamide analogs, specifically sulfamoyl benzoic acids, which they reacted with various amines. This finally led to the identification of furosemide (Lasix), the first compound with a much higher saluretic and diuretic effect.<sup>29</sup> More than 40 years after its discovery, furosemide and other "high-ceiling" diuretics remain the cornerstone of the treatment of congestive heart failure and pulmonary edema, as well as contributing to managing essential hypertension. They differ from the thiazides in primarily inhibiting ion exchange in the ascending loop of Henle and also in the distal tubule. They do not inhibit carbonic anhydrase. Beyer's Group at MSD discovered ethacrynic acid, another high-ceiling diuretic, as part of their program to discover non-mercurial compounds, which were as effective as the organomercurials. Their compound also originated from the sulfonamide analog synthetic program, but the Hoechst Group had priority of discovery with this new class of diuretic.



**Figure 4.** Evolution of chemical structures leading to three classes of oral diuretics.

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**Figure 5.** Edward D. Freis (1912-2005). All rights reserved.

Servier's indapamide is also to be cited here. Indapamide's action relies on a thiazide-type renal diuretic effect—although it lacks the thiazide ring of the true thiazides—as well as on specific vasodilator properties that contribute to its antihypertensive efficacy.<sup>30</sup>

**DISCUSSION**

The discovery of the thiazides provides illuminating examples of the complexity of the relationships between pathophysiology, pharmacology, and clinical science in discovering new medicines. For example, the discovery of the thiazides occurred at a time when the outstanding clinical scientist Professor Edward Freis (Georgetown University, Washington) (Figure 5) was already trying to find medical treatments for essential hypertension. The pivotal role of salt and water balance in determining hemodynamic and blood pressure status were recognized, as for example with the adoption of the Kempner salt-free diet, which, while effective, was impracticable.<sup>31</sup> Freis designed the classic first controlled trial in essential hypertension to demonstrate that reducing elevated blood pressure reduced morbidity and mortality in such patients.<sup>32</sup> This trial had several features that were unique at that time, including:

- A trial design requiring double blindness and placebo control.
- The independent assessment of clinical end points by a committee.

- The creation of a data safety monitoring board. As Barry Materson recently said of Freis,

he designed this study at a time when the conventional wisdom was that it was nonsense and perhaps even unethical to treat patients with hypertension. In essence, he made one of the most important contributions of the century to medical practice.<sup>33</sup>

- The treatment arm of the trial comprised a combination capsule containing a thiazide diuretic (Esidrex), reserpine (Serpasil), and hydralazine (Apresoline) given in a fixed combination 3 times a day (SER-AP-ES).

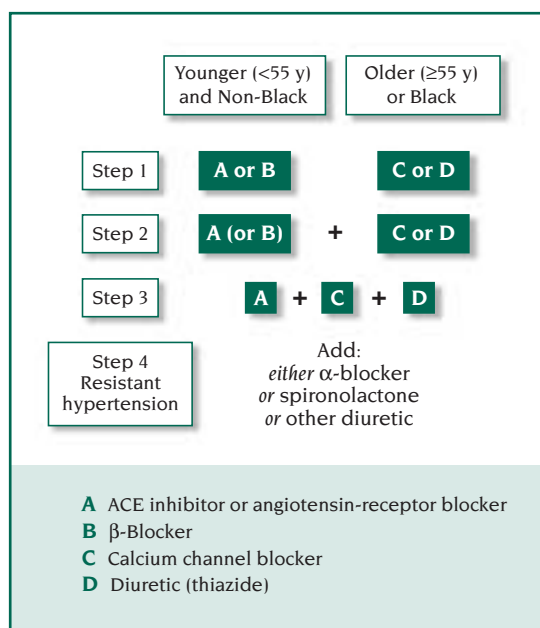
A subset of the total trial of 523 male patients comprised patients with a diastolic pressure between 115 and 129 mm Hg (73 on active drug, 70 on placebo). Four patients died in the control group of cardiovascular complications, but none in the treated group, while 17 in the control group developed severe nonfatal complications, while only 6 treated patients had these problems.<sup>34</sup> The success of the Veterans Administration trial led to the creation of the National High Blood Pressure Education Program and the award of the Lasker Prize to Freis in 1971.<sup>22</sup>

Sadly, Professor Freis died on February 1, 2005, aged 92 years. The Author met him at a Gordon Conference and asked him how he managed drug-resistant essential hypertension, to which he replied “I don't seem to encounter this very much, I just give them one of those little blue capsules 3 times a day!”

However, it has taken many years for it to be recognized that combination therapy is the best approach to the effective control of raised blood pressure. This has led to the approach exemplified by the modified Cambridge AB/CD rule (Figure 6).<sup>35</sup> There have been intensive studies attempting to relate the blood pressure response to different classes of antihypertensive agents to particular genetic polymorphisms, but the present opinion is that single gene effects on antihypertensive drug responses are small.<sup>36</sup> Despite intensive investigation over the last 30 years, there is no final agreement on the mode of action of the thiazides in lowering blood pressure, though their effects in improving the treatment of congestive heart failure are closely related to the reduction in salt and fluid retention. Thiazides

**Figure 6.** British Hypertension Society (BHS) recommendations for lowering blood pressure. Sequential therapeutic regimens recommended for achieving target blood pressure of <140/85 mm Hg, according to age and ethnic group.

Adapted from reference 35: Schwartz GL, Turner ST. Pharmacogenetics of antihypertensive drug responses. Am J Pharmacogenomics. 2004;4:151-160. Copyright © 2004 Adis Data Information BV.





lower the blood pressure during long-term treatment by reducing the peripheral vascular resistance, but how this is achieved is the source of continuing controversy.<sup>37-39</sup>

A second aspect of the complex relationships described above is how research intended to solve one problem—in this instance prolonging the action of penicillin by blocking its renal excretion—led to a research program designed to treat edema and hypertension. However, the feasibility of such research only became possible by the prior serendipitous observations of the effects of sulfanilamide on water and electrolyte excretion in humans.<sup>16</sup> Without the sulfanilamide template, medicinal chemists would have struggled to devise an appropriate chemical synthetic program. The final breakthrough relied on the combination of choosing physiologically based *in vivo* test systems (the conscious dog) and identifying the difference in pharmacodynamic profile between carbonic anhydrase inhibitors and the urine electrolyte excretory profile of chlorothiazide. In his Lasker Medal lecture,<sup>15</sup> Beyer attributed the success of his group to the following elements:

- (i) The physiological basis for the work and its relevance of the clinical situation seemed sound;
- (ii) our methodology seemed to be appropriate, adequate and accurate; and
- (iii) there was a need for many people to work together.

The evidence historically is that these criteria were fulfilled. It attests the reality of what we have fancied to call “designed discovery”—a process whereby clinical need and understanding biological concepts and analogies and chemical ingenuity and perseverance brought together in a stimulating environment can yield a specific result that has been empirically conceived.

This description of designed drug discovery written more than 30 years ago would seem to suggest that the current vogue for creating a subspeciality of “bench-to-bed translational research” was born at least 50 years ago.

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