In 2007 he and colleagues published in the NEJM how left ventricular apex pacing cured a child [3], and this was confirmed five years later in a large clinical trial. A perfect, convincing example of so-called translational medicine, directly from the animal experiment to the bedside.

Without Prinzen's dog studies, this life-saving success wouldn't have been possible. Some years later, when I was still Scientific Director of CARIM - the Cardiovascular Institute of Maastricht University, the animal protectionist had won the battle: Frits Prinzen was forced to stop his experiments in dogs, and I couldn't avert it. His comment: "Experiments in dogs are a sensitive topic in the general public, related to the strokability of these animals. However, there was a considerable literature and experience from the own laboratory that effects on ventricular pacing were significantly different when testing in other large animals like pigs and goats. Therefore, doing these studies in dogs was the only way to reach the goal of a better treatment for pacemaker patients." And: " The question arises whether it is ethically acceptable to take a dog's life to safe a human life". I would say: Yes, it is. But not everyone shares this opinion. In the Netherlands, for instance, there is a political move to ban all animal experiments by 2025.

So, let us open the discussion on animal experiments in hypertension by a number of articles on the issue in this and a further issue of "Hypertension News". You, the readers, are welcome to send us your comments via any media, and we will try to create a dedicated, lively forum in our journal.

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LEARNING THE ROPES (2)

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Role of animal experiments in hypertension research

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From the early times of hypertension research animal models have been instrumental to acquire novel insights in the regulatory principles defining blood pressure. Moreover they have been essential for the discovery of therapeutic targets and the development of corresponding drugs as particularly exemplified in the renin-angiotensin system. Recent technological revolutions in the detailed analysis and in the targeted alteration of genomes will resume and even accelerate this process in the future. In conclusion, animal experiments have been essential and will remain irreplaceable in hypertension research. Animal experiments have been essential for hypertension research from its beginnings. Already 1898, Tigerstedt and Bergmann injected rabbit kidney extracts into recipient rabbits to discover a hypertensinogenic substance, which they called renin [1] (Figure 1). Nearly 40 years later Harry Goldblatt clipped the kidney of a dog, thereby released renin and induced hypertension in the animal [2] (Figure 2).

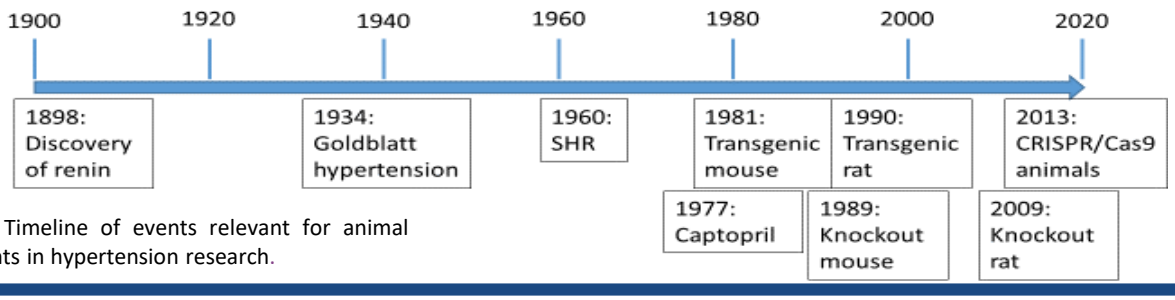

Figure 1: Timeline of events relevant for animal experiments in hypertension research.

This created the classical renovascular model of hypertension (Table 1). It was later mainly applied in the rat which became the most frequently used animal species in hypertension research. In the 1950s and 60s several laboratories intended to generate rat models of human essential hypertension by selecting and inbreeding rats with the highest blood pressures in outbred strains (Table 1). This way the spontaneously hypertensive rats (SHR) were established in Japan, the Dahl salt sensitive rats in the US and several other models all over the world [3]. These experimental and genetic rat models of hypertension helped to discover mechanisms which regulate blood pressure and elicit the hypertensive damage in target organs such as heart, vessels, and kidney. In particular however, they were instrumental in the development of novel antihypertensive drugs. The first inhibitor of the angiotensin-converting enzyme (ACE), captopril, and losartan, the first blocker of the AT1 receptor for angiotensin (Ang) II, were tested in Goldblatt-hypertensive rats [4].

The genetic rat models of hypertension were also used to reveal novel genes involved in the pathogenesis of hypertension by quantitative trait locus (QTL) analysis exploiting polymorphisms between the hypertensive strain and a normotensive control strain. One of the genes discovered by this method codes for ACE2, which turned out to be less expressed in SHR compared to control rats [5]. ACE2 is a major regulator of the renin-angiotensin system since it metabolizes Ang II to Ang-(1-7) and thereby converts a vasoconstrictive peptide into a protective one. We could confirm the importance of this gene for the pathogenesis of hypertension in SHR by reconstituting its expression in vessels of these rats and thereby reducing their blood pressure [6].

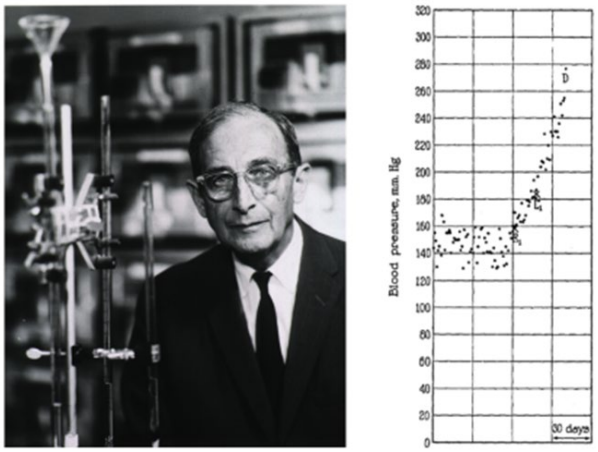

Figure 2: Harry Goldblatt with his blood pressure measuring device (left) and blood pressure response of a dog after clipping of one renal artery (right) [2].

Such experiments became possible in the early 1980s when a new era in animal research begun allowing permanent changes in the genome of animals (Figure 1). First only "transgenes" could be added to the genome by random integration of DNA-constructs but later also targeted alteration of genes was achieved using homologous recombination in embryonic stem (ES) cells, which was mainly used to ablate genes ("knockout technology"). Unfortunately, these techniques were first available only in the mouse, a species which had previously been avoided by hypertension researchers owing to its small size. At least only with a short delay, transgenic technology was also established for the rat in 1990 [7]. Interestingly, the first transgenic rat (TGR(mREN2)27) overexpressed the mouse renin gene Ren-2 and became another classical model of hypertension (Table 1). Other transgenic rats confirmed the importance of the brain and in particular the central Ang II generation in blood pressure control [8].

Table 1: Classical Hypertension Models

Name	Induced by
Goldblatt hypertension	Clipping of one kidney artery
DOCA-salt hypertension	DOCA (mineralocorticoid) pellet implantation, high salt diet
L-NAME hypertension	L-NAME (NO synthase inhibitor) infusion
Ang II hypertension	Angiotensin II infusion
Spontaneously hypertensive rat (SHR)	Selective breeding, polygenic
Milan hypertensive rat	Selective breeding, polygenic
Lyon hypertensive rat	Selective breeding, polygenic
Dahl salt-sensitive (DSS) rat	Selective breeding, polygenic, high salt diet
SABRA hypertensive rat	Selective breeding, polygenic, high salt diet
ISIAH rats	Selective breeding, polygenic, immobilization stress
TGR(mREN2)27 rat	Transgenic expression of the mouse Renin-2 gene
eNOS-knockout mouse	Genetic deletion of endothelial NO synthase

However, knockout technology remained restricted to the mouse for nearly 20 years and forced hypertension researchers to accommodate to the mouse as model species to exploit this powerful method for functional genomics. Cardiovascular phenotyping methods were down-scaled to the mouse, but still remain less reliable as the ones in the rat. For example the state-of-the-art method to measure blood pressure in awake, freely moving animals, the implantation of telemetry transmitters, inevitably alters blood pressure regulation in mice, but not in rats, by blocking blood vessels.

Nevertheless, knockout mouse models were instrumental to discover unpredicted players in blood pressure regulation such as the immune system, by the discovery that Rag2-knockout mice lacking T-cells do not respond to Ang II infusion with increased blood pressure [9]. Surprisingly, mice lacking AT1 specifically in vessels showed that this mechanism was even more important for the hypertensive effect of Ang II than the expected vasoconstriction [10].

Between 2008 and 2013, four new technologies were developed one after the other which finally also allowed targeted genetic alterations in the rat (Figure 1). First rat ES cells and classical knockout technology became available and shortly thereafter zincfinger- and TALE-nucleases were developed. These are restriction enzymes which are guided by specific DNA-binding protein domains to selected sites in the genome. After cutting of the DNA double strand at this site, cellular repair processes religate the DNA, but include small deletions or insertions and thereby often frame-shift mutations in genes. Moreover by offering a repair template, predesigned mutations can be included at the target site. Of note, these powerful techniques were first developed in rats, since there was an urgent need to generate knockout animals in this species mainly driven by cardiovascular researchers. But finally all these techniques were superseded by CRISPR/Cas9 technology, in which the DNase Cas9 is targeted to a specific site in the genome by a homologous guide RNA. When Cas9 cuts, the same options for gene destruction or alteration are available as for zincfinger- and TALE-nucleases. This technique is available in all animal species including the rat and may allow at least a partial renaissance of this species in modern hypertension research.
In parallel to this revolution in transgenic technology, rapid progress in DNA-sequencing methodology allowed the elucidation of whole genomes. By comparing thousands of genomes of hypertensive and normotensive individuals in genome-wide association studies (GWAS) several hundred genes were found to be linked to increased blood pressure. These genes may code for novel targets for antihypertensive therapy. The most straight-forward way to raise this treasure will be to mimic the hypertensive gene variants in suitable animal models using modern transgenic technology and reveal their impact on blood pressure regulation. In the ideal case the same animal models could also be used for the development of drugs interfering with the novel target. This would resume the use of animal models in hypertension research as it started more than a century ago.

In conclusion, hypertension research will stay dependent on experiments in whole mammalian organisms, since cell cultures or organoids can never recapitulate the complex interaction of cardiovascular organs such as heart, vessels and kidney, with different arms of the nervous system, the immune system, and numerous hormones, which set the blood pressure level and thereby determine hypertension in animals including humans.

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