Further Observations on Development of a Colony of Spontaneously Hypertensive Rabbits.* (22443)

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In a previous paper we described the distribution of blood pressure values and pressure characteristics of rabbits with naturally occurring hypertension(1). We concluded that there is a hereditary factor in this spontaneous hypertension and that pressure characteristics namely, lability and elevation with age, simulate those of animals in normal rabbit population. These observations were made on animals raised between Sept., 1951, and June, 1954, and included 12 families, with 2 to 4 generations each, of various strains of rabbits. We continued to interbreed these families, especially along 3 main breeding lines, *viz.*, pure New Zealand, pure Dutch and a cross between New Zealand and Dutch. From June, 1954, to June, 1955, 131 rabbits were raised to 6 months and 116 to 8 months, representing 2 additional generations. They will subsequently be referred to as "new" population. Eighty-nine and 66 rabbits which were raised to 6 and 8 months, respectively, in the 3 preceding years were described in our first paper and will be referred to as "old" population. This paper presents the distribution of blood pressures among recent members of the colony, *i.e.*, the "new" population, and compares it to that of their progenitors, *i.e.*, the "old" population. In addition, further findings on blood pressure characteristics are described for rabbits selected from the colony and for closer study.

Methods. Rabbits have a natural life span of 3 to 7 years and rapid growth the first 2 months of life; they may be considered adults at 4 to 6 months. When rabbits are 3 to 4 months of age, we begin taking routine pressures at least twice a month up to 8 months of age. Unless animals are used as breeders or for research purposes, they are sacrificed at 10 to 12 months because of insufficient housing facilities. Other animals die of intercurrent diseases at various ages. Autopsies are performed whenever possible, and the findings are reported below. Routine auscultatory pressures are taken from the abdominal aorta using a sphygmomanometer cuff wrapped around the abdomen. This method was described in detail by McGregor(2). The animal is placed on the board, and 4 systolic and diastolic readings are made within 2 minutes. Another set of 4 readings is taken 5 minutes later. Four successive readings seldom vary more than 5 to 10 mm Hg. The average for each set of 4 readings is recorded along with heart rate, body weight, and condition of the animal. Averages of such sets of readings taken 5 minutes apart are often the same but may vary 10 to 15 mm Hg in either direction. The same results were reported by McGregor(2). When animals were brought into the laboratory, blood pressures were taken by the cuff method and, in addition, by direct femoral artery puncture with a Statham pressure transducer; the latter procedure and its correlation with cuff systolic pressures has been described earlier (1).

Results and Discussion. We selected from routine pressure readings the highest systolic pressure recorded during each of three 2 month periods. Table I presents the mean value of this highest systolic pressure of each indicated age group for both "new" and "old" populations. In addition, Table I shows the number, and per cent, of animals with systolic pressures above, and below, 150 mm Hg. The latter is the systolic value we consider to be the upper limit of normal(1). Fig. 1 shows complete frequency distribution of highest systolic pressure at 3-4 and 7-8 months of age in the "old" and "new" populations.

Table I and Fig. 1 point out that as the 2

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TABLE I. Distribution of Highest Systolic Blood Pressure† Obtained by Abdominal Cuff Method in a Spontaneously Hypertensive Rabbit Colony.

	"New" population					
Age (mo)	3–4		5–6		7–8	
Total No.*	125		131		116	
Systolic pressure (mm Hg)	111–150	151–200	111–150	151–200	111–150	151–200
No. rabbits	61	64	55	76	34	82
% total No.	49	51	42	58	29	71
Mean ± S.D.	151 ± 17		155 ± 15		162 ± 18	
	"Old" population					
Age (mo)	3–4		5–6		7–8	
Total No.*	84		89		66	
Systolic pressure (mm Hg)	111–150	151–200	111–150	151–200	111–150	151–200
No. rabbits	57	27	35	54	18	48
% total No.	68	32	39	61	27	73
Mean ± S.D.	144 ± 15		157 ± 16		158 ± 16	

* At 3-4 mo of age, some rabbits are too small to use for abdominal cuff determinations which accounts for fewer animals in this age group than at 5-6 mo. The reason less animals were available at 7-8 mo is that some died from intercurrent diseases.

Calculation of Fisher's "t" values showed that the means at 4 and 8 mo for both "new" and "old" populations are significantly different (p >.001) and means at 6 and 8 mo for "new" population are significantly different (p >.01). The difference in 4 mo means between the "old" and "new" populations is significantly different (p >.001).

† This refers to the set of 4 successive readings having highest average systolic pressure.

populations grew older their systolic pressures increased, and the "new" population had significantly higher pressures at 3-4 months of age, than their progenitors. Although mean systolic pressure at 8 months was somewhat higher in the "new" population, the difference between the two was not significant.

The "old" population did not show a pressure difference between the two sexes whereas the latest offspring did. The data in Table I for "new" population has been separated according to sex, and the results appear in

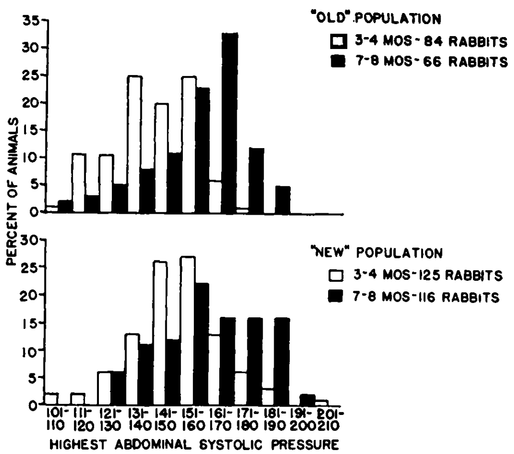

FIG. 1. Distribution of highest systolic pressure at 4 and 8 months in "old" and "new" populations.

TABLE II. Mean Values and Distribution of Highest Systolic Pressure* in "New" Male and Female Members of a Spontaneously Hypertensive Rabbit Colony.

	♂			♀		
No. of animals	62	68	63	63	63	53
Age in mo	3–4	5–6	7–8	3–4	5–6	7–8
Mean systolic pressure (mm Hg)	156	162	169	149	149	152
% animals above 150 mm Hg systolic	60	75	86	43	40	53

* This refers to set of 4 successive readings having highest avg systolic pressure.

Table II. Males had mean values for systolic pressure significantly higher than females, in all 3 age groups. Moreover, mean systolic pressure of "new" males is higher than in either male or female progenitors, whereas the mean systolic pressure of "new" females is lower than that of their progenitors. Other workers studying the effect of age on blood pressure in fowls found that females had lower pressures than males at both 10 and 50 months of age(3). On the other hand, no difference was found between sexes in Wistar rats known to develop high blood pressure with advancing age(4). In humans rate of rise in pressure with advancing age differs in male and female(5).

Diastolic pressure can be obtained with an

TABLE III. Femoral Artery Pressures in Spontaneously Hypertensive* and Normotensive Stock Rabbits.

	No. rabbits	No. determinations	Systolic Range	Systolic Avg	Diastolic Range	Diastolic Avg
Spontaneously hypertensives	25	35	131–180	151	78–124	97
Normals	10	11	121–136	128	83– 92	87

* From both "old" and "new" populations.

accuracy of ± 10 mm Hg, at best, by the abdominal cuff method. For this reason direct femoral artery punctures were made on a group of spontaneously hypertensive rabbits using normotensive stock animals as controls. Table III presents findings with accompanying systolic pressures. Spontaneously hypertensive rabbits had average diastolic elevation of 10 mm Hg above that of normals. Individual values indicated that small rabbits (Dutch breed), known to mature sooner than New Zealands, had higher diastolic pressures than the others.

One of the pressure characteristics in spontaneously hypertensive rabbits is marked lability, *i.e.*, a noticeable pressure variation from month to month. We brought animals into the laboratory to study this factor more closely. For a period of several months, daily or twice weekly, auscultatory pressure readings were taken in the usual manner. The pressure value recorded each time was the average of 4 successive readings which agreed within 5 to 10 mm Hg of each other. Despite the fact that an animal was tied down on a board in the unanesthetized state, the day to day fluctuation in average systolic pressure was only 10 to 15 mm Hg with an occasional fluctuation exceeding 25 mm Hg. This was true if pressures were taken at the same time and under the same conditions each day. However, during several weeks the average systolic pressure might gradually shift 20 to 30 mm Hg and stabilize at some new level. It is the extreme values of such pressure shifts which one may obtain by chance when only occasional pressures are taken, *e.g.*, twice a month, and which accounts for noticeable lability of pressure. Normotensive rabbits, under the same conditions, show the same magnitude and type of blood pressure fluctuations. McGregor found that daily readings obtained over a 21 day period had extreme fluctuations of 20 mm Hg(2).

Direct arterial measurement is the only accurate means of obtaining moment to moment pressure variations. Fig. 2 shows representative intraarterial tracings from the carotid arteries of a normotensive stock rabbit and two spontaneously hypertensive rabbits taken while they were supine and unanesthetized. Spontaneously hypertensive rabbits showed 2 characteristic fluctuations of pressure—small pressure fluctuations of about 1 second duration and relatively large fluctuations, 15 mm Hg of 4 to 7 seconds duration. The duration of small fluctuations corresponds to length of a single respiratory cycle, the latter being 1 to 2 seconds in rabbits. The 4 to 7 second duration waves resemble Traube-Hering waves of rabbits both in duration and magnitude(6). The normal animal's record in Fig. 2 shows shallow waves 3 to 4 seconds in duration; the 1 second duration waves are not present. Research on Traube-Hering waves has been carried out in partially curarized or anesthetized open-chest preparations. Such studies led to the conclusion that CO_2 concentration in vasomotor and respiratory centers affects duration of Traube-Hering waves while peripheral vascular tone determines their magnitude(6,7). The records in Fig. 2 indicate that Traube-Hering waves are present under normal physiologic conditions in rabbits. Since spontaneously hypertensive rabbits probably have increased vascular tone, this would account for their having greater wave magnitude than the normotensive animal.

Kidney changes were found in 36 of 86 autopsies of our colony animals, sacrificed or dead from intercurrent disease; in 50 animals the kidneys appeared normal. In the majority renal changes consisted of tiny, congested areas, 1-2 mm in diameter and depressed

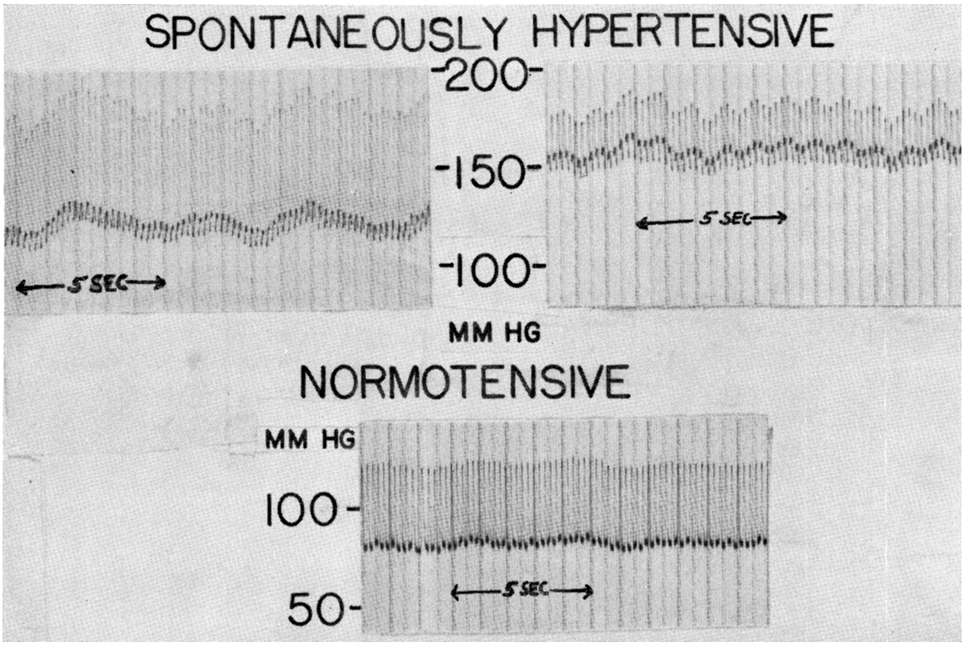

FIG. 2. Recordings from the carotid arteries of two spontaneously hypertensive and one normotensive rabbit showing Traube-Hering waves (see text).

about 0.5 mm, scattered over the surface of normal sized kidneys. The cut surface showed mild tissue striation of the medullary region. In others the kidney surface was knobby, and the tissue tough to cut. Marked medullary striation and a narrowed cortex was seen. Twenty-eight of the 36 abnormal kidneys were examined microscopically, and the findings presented in Table IV. Eighteen of the 28 animals (64%) had been hypertensive and 10 (36%) normotensive during life. These percentages are representative of pressure distribution in our colony (Table I). The significant fact shown in Table IV is that interstitial nephritis was found in normotensive animals. Although in this series none of the normotensives had the more marked degree of nephritis, an earlier report from our laboratory showed marked interstitial nephritis to be present in animals with normal blood pressure(8). Lesions, described in detail in that report, are identical to those found in these colony rabbits. The same paper also noted that if normal laboratory animals were subjected to laparotomy or intravenous procedures, the incidence of interstitial nephritis reached 66.6%. (*Staphylococcus albus* was cultured from some of the kidneys implicating the hematogenous route.) Twenty-four of our 36 cases were known to have had infections, surgery or unsterile intramuscular injections during life.

Presence of interstitial nephritis in normotensive animals indicates that these renal changes are incidental and not a causative

TABLE IV. Microscopic Findings in 18 Hypertensive and 10 Normotensive Animals Which Showed Renal Pathology at Gross Autopsy Examination.

	Hypertensive		Normotensive	
	No.	%	No.	%
Normal	4	22.2	1	10
Interstitial nephritis:*				
Minimal to mild	8	44.4	9	90
Moderate to marked	3	16.6		
Moderate + amyloidosis	1	5.6		
Unilateral ascending pyelonephritis†	1	5.6		
Amyloidosis	1	5.6		
Totals	18	100.	10	100

* The term, interstitial nephritis, is synonymous with pyelonephritis arising by the hematogenous route. Degree of interstitial nephritis based on scars per low power field: minimal, less than 1; mild, 1; moderate, 2; marked, 3 or more.

† Associated with unilateral hydronephrosis.

factor in production of spontaneous hypertension.

Eleven of the 50 normal appearing kidneys were examined microscopically. One of these had a focal glomerulitis and came from a normotensive animal. The other 10 were normal microscopically and were from hypertensive animals.

Calcification of large and medium size arteries was found in the first family of rabbits raised in our colony, but this condition died out the following generation and has only shown up occasionally in other animals. Enlarged hearts have not been found at autopsies. One probable reason is that spontaneous hypertension is a relatively mild labile type of hypertension and, in addition, autopsies have been done in the early part of the animal's life span.

Summary. (1) Two new generations of spontaneously hypertensive rabbits showed about the same incidence of elevated systolic pressures as their progenitors at 6 and 8 months of age, 58 and 71% respectively. At 4 months, mean systolic pressure in the 2 new generations was significantly higher than in their progenitors at same age. (2) Male rabbits of the new generations in all 3 age groups had higher mean systolic values than females and than their male or female progenitors. (3) Direct arterial pressure measurements in a group of spontaneously hypertensive rabbits showed average diastolic pressure to be 10 mm Hg higher and systolic pressure 30 mm Hg higher than in normotensive stock rabbits. (4) Direct arterial recordings from unanesthetized animals demonstrated spontaneously hypertensive rabbits to have larger Traube-Hering waves than normotensives. (5) The presence of interstitial nephritis in normotensive as well as spontaneously hypertensive animals precludes it as an etiological factor in the development of spontaneous hypertension.

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1. Alexander, N., Hinshaw, L. B., and Drury, D. R., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 855.

2. McGregor, L., *Arch. Path.*, 1928, v5, 630.

3. Sturkie, P. D., Weiss, H. S., and Ringer, R. K., *Am. J. Physiol.*, 1953, v174, 405.

4. Medoff, H. S., and Borgiovanni, A. M., *ibid.*, 1945, v143, 297.

5. Hamilton, M., Pickering, G. W., Robert, J. A. F., and Sowry, G. S. C., *Clin. Sci.*, 1954, v13, 11.

6. Aalkjaer, V., *Skand. Arch. f. Physiol.*, 1935-36, v71-73, 301.

7. Wiggers, C. J., *Physiol. in Health and Disease*, 1949, Philadelphia, Lea & Febiger.

8. Flasher, J., Edmondson, H. A., and Drury, D. R., *Arch. Path.*, 1951, v51, 53.

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Effects of Corticosterone, Cortisone and Hydrocortisone on Fat Metabolism in the Chick. (22444)

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The influence of glucocorticoids on fat metabolism is well documented in many species(1-5). There are, however, several published reports which indicate that there are some qualitative differences exhibited by these hormones on lipid metabolism(3,6). This is particularly interesting since these steroids are considered to be qualitatively similar to their physiological effects and any differences are primarily quantitative(7). It was therefore of interest to determine the effects of crystalline adrenal cortical hormones on fat metabolism in the chick. Accordingly, the ability of corticosterone (compound B), cortisone (compound E), and hydrocortisone (compound F) to produce changes in fat deposition in the chick was studied.

Materials and methods. Single-comb white leghorn cockerels were used throughout these