was pushed at 30-40° angle directly through the mantle muscle to penetrate the giant fiber at some point in its course. Dye was injected by slight pressure through a closed water column attached to a 5 cc syringe. Injection was continued until the dye reached the nuclear mass in the stellate ganglion or, in the case of the second order fiber into the brain, until approximately 10 mm^3(2) of dye was injected. Injected specimens were fixed in 15% formalin, dehydrated in alcohol, cleared in xylene, and imbedded in paraffin to be sectioned later.

Results. 1) By injecting each of the giant fibers of the mantle, it was possible to outline the cells of origin of each in the stellate ganglion. The diagrams show a ventral view and side view of the squid's right-side stellate ganglion, and map out each of the cellular masses except the fifth and eighth, for which no satisfactory results were obtained. When the same fiber was injected in different specimens the nuclear mass was always constant in location.

2) The nature of the giant synapse in the stellate ganglion, although already known, is easily demonstrated by the intracellular injection method. By injecting one of the mantle axons with 0.5% Trypan Blue to its nuclear origin and then exerting slightly more pressure, the interaxonal plasma membranes can be ruptured and all the giant axons filled with bright blue dye. Then 1% Trypan Red is injected into the second order fiber, whose end feet form the giant synapse. When viewed through the low power dissecting microscope, the giant synapse is shown in brilliant red and blue. There is no admixture of dye, showing that the axon end feet are separated from the giant fiber axons by some form of membrane.

3) The cells of origin of the second order fiber in the brain, previously unknown, were easily located by gross inspection; however, finer localization must await microscopic sections. The second order fiber leaves the stellate ganglion and penetrates the muscular body wall, traveling in a nerve bundle along the dorsal side of the body cavity to pierce the cartilaginous brain case almost at the midline, just dorsal to the esophagus. Within the brain the fiber bends sharply laterally and ventrally and ends after completing a hemicircle in a small ventrolateral lobe behind the large optic lobe.

Summary. 1. A new intracellular staining method is proposed as a way of obtaining definitive knowledge as to the location of single fiber nerve tracts, their connections, branches and cells or origin. 2. The method is applied to the giant fibers of the squid with definitive results.

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Development of a Strain of Spontaneously Hypertensive Rabbits.* (21255)

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Essential hypertension develops in man without demonstrable renal, neural, or vascular involvement and becomes more severe in older age groups. Hamilton, *et al.* believe that essential hypertension is not a disease entity but merely a condition seen in that section of the population presenting arterial pressures higher than an arbitrarily selected value(1). Without a definite disease to which they can attribute the elevated pressure, in-

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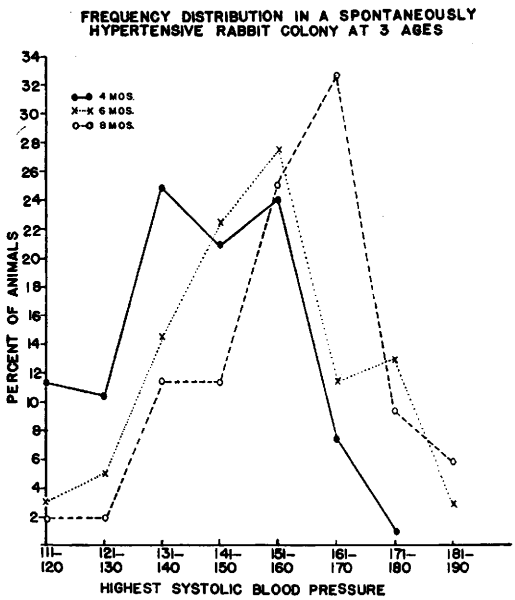

FIG. 1.

vestigators have looked to emotional factors and environmental stresses as possible causes for hypertension.

An hereditary or familial trait has long been considered a predisposing factor in the development of essential hypertension(2,3). Knowledge about this particular component of essential hypertension has come mostly from clinical data based on family histories. The possibilty of a controlled study of the inheritance of hypertension was presented to us when we noted that occasional stock rabbits had elevated pressures, *i.e.*, spontaneous hypertension. This paper deals with the results obtained from breeding such animals.

Method. New Zealand White, Dutch, and Californian rabbits were used. The incidence of spontaneous hypertension in these rabbits is low. This necessitated going directly to rabbitries in the Southern Californa area where large populations of rabbits could be screened. Out of 553 rabbits which were screened, 2.7% had systolic pressures of 160 mm Hg or more on 2 separate occasions and were selected as breeding stock. Their offspring cared for in a special rabbitry constructed for the purpose so that the environmental conditions were kept standard. We started with 8 separate matings, then interbred within the same family, and also introduced new spontaneously hypertensive animals into the original groups. Blood pressure from the abdominal aorta was determined with a sphygmomanometer cuff. This method has been described in detail elsewhere (4). The average of 4 or 5 systolic and diastolic determinations was recorded. We have obtained where possible the blood pressures on each rabbit that was born at our rabbitry for a period of at least 8 months. Readings can be obtained at 3 to 4 months of age (4 to 6 lb weight) and were taken as often as once or twice a month thereafter. For comparison with the cuff values, intra-arterial readings have been made on some of these animals. Two per cent procaine was infused locally and blood pressure obtained from the femoral artery with a No. 24 needle attached to a Statham strain gauge(5).

Results. Fig. 1 and Table I show the frequency distribution and mean values of the highest systolic pressure obtained from each animal bred from spontaneously hypertensive parents within the indicated age groups. The mean systolic pressures at 5 to 6 and 7 to 8 months of 155 and 160 mm Hg, respectively, are each statistically significant when compared to the mean of 145 mm Hg at 3 to 4 months. (The reason less animals were available for the 6 and 8 month groups is that they died from intercurrent diseases after 4 months. One family of 6 rabbits was eliminated after 6 months because of very low pressures; at the time, we did not wish to keep them. Otherwise, no selection was made for compilation of the statistical data.)

Data on normal rabbits have been reported by others. McGregor found the average abdominal systolic pressure to be 125 Hg with a range of 90 to 160 mm Hg in 1120 readings on 84 normal rabbits(4). These animals were probably 3 to 4 months old since that is the usual age of the ordinary laboratory rabbit. In our own laboratory, 4-month-old animals average 135 mm Hg with a range of 120 to 150 after one year. These animals show an average increase of about 10 mm Hg in systolic pressure(6).

Fig. 2 shows the relationship in 30 rabbits between systolic pressure obtained with the abdominal cuff and by direct femoral artery

TABLE I. Distribution of Highest Abdominal Systolic Blood Pressure for Entire Spontaneously Hypertensive Colony of Rabbits. (Some rabbits are represented in more than one of the age groups.)

Age (mo)	3-4		5-6		7-8	
No. of rabbits	96		62		52	
Systolic pressure, mm Hg	No. of rabbits	% of total No.	No. of rabbits	% of total No.	No. of rabbits	% of total No.
121-150	65	68	28	45	14	27
151-160	23	24	17	27	13	25
161-185	8	8	17	27	25	48
$\bar{X}$*	145		155		160	
σ †	± 15		± 16		± 15	

* $\bar{X}$—Arithmetic mean of systolic blood pressures obtained from frequency distribution. $\bar{X} = \frac{\Sigma fX}{N}$.

† σ—Stand. dev. (mm Hg) $= \sqrt{\frac{f(X^2)}{N}}$.

puncture. In each case, the cuff pressure was taken just preceding the dissection and puncture for the direct determination. The cuff pressures average 13 mm Hg above the direct pressure with a range from 0 mm Hg to 30 mm Hg. Only in 3 cases was the direct reading higher than the cuff value. This relationship exists in normal rabbits also(6).

The spontaneously hypertensive rabbits do not maintain a high steady pressure. An example of fluctuation in pressure is seen in

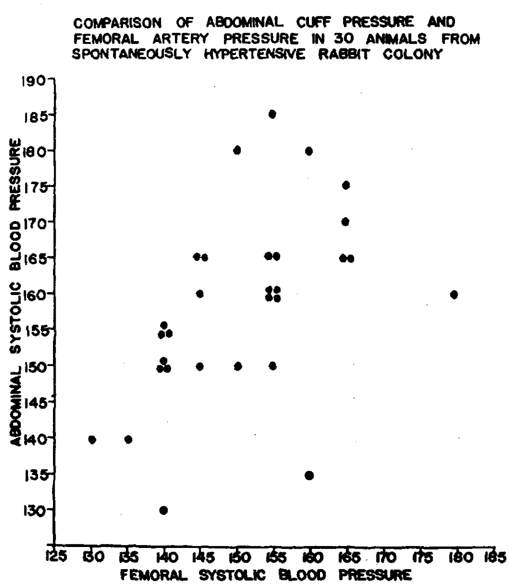

FIG. 2.

Table II. This is the protocol of a typical offspring of spontaneously hypertensive parents. Normal rabbits also show variations in abdominal systolic pressures(4).

A frequency distribution of abdominal systolic pressures was made for the 2 sexes. The mean values were not significantly different between the two sexes at any of the 3 age groups. However, the mean systolic pressures suggest that females may have a greater change of pressure than males over an 8 month period (Table III).

There was no correlation between body weight and systolic pressure in any single age group. Naturally, as the animals reached 8 months, the average body weights were higher than at 4 months of age. However, certain rabbits did not gain weight as rapidly as others, but still had high blood pressure at 8 months of age.

Discussion. Our results have shown that offspring of spontaneously hypertensive rabbits have a high incidence of elevated systolic pressures, compared to the normal rabbit population. Three characteristics of these spontaneously hypertensive rabbits are worthy of note. First of all, the mean systolic pressure values for each of the 3 age groups mentioned are not extremely high. When compared to animals made hypertensive by various experimental methods, our colony has only moderately elevated pressures(7). Secondly, the pressures tend to fluctuate in spontaneously hypertensive rabbits. This is also true of normal rabbits, whereas rabbits with experimentally induced hypertension have more stable pressures. Lastly, the mean systolic pressure for our colony becomes significantly higher at 6 or 8 months compared to the mean value at 4 months. Normal rabbits also show a tendency to have higher pressures as they get older. The ordinary New Zealand White laboratory rabbit is considered adult at 4 months at which time it can be reproductive. It continues to grow and gain weight for the first 6 months to a year of its life. However, it can be stated that some animals in our colony which did not continue to gain weight as rapidly as others, tending instead to level off at 4 or 6 months, nevertheless had increasing blood pressure with age.

Summary. We have bred and raised a colony of spontaneously hypertensive rabbits.

TABLE II. Typical Protocol of Spontaneously Hypertensive Rabbit.
Pd-3 (male) Born: 7/11/53

Date	Wt (lb)	1st reading	2nd reading (5 min. later)
10/15/53	5½	150/105	150/100
11/ 7	6¼	170/130	165/120
11/30	6⅛	170/135	160/125
1/13/54	6½	135/ 90	140/100
1/28		Cuff pressure:	160/110
		Femoral puncture:	150/115

TABLE III. Mean Systolic Pressures of Female and Male Rabbits in the Spontaneously Hypertensive Colony.

Age (mo)	3-4		5-6		7-8	
	♀	♂	♀	♂	♀	♂
No. of animals	43	53	29	33	23	29
Mean systolic pressure, mm Hg	145	150	155	160	165	160

It is concluded that there is a hereditary factor in hypertension and that the characteristic changes of blood pressure in spontaneously hypertensive animals are similar to the normal rabbit population. These findings would indicate that spontaneously hypertensive rabbits have characteristics in common with man in his development of essential hypertension.

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Effect of Dietary Aminopterin and Sulfasuxidine on Biosynthesis of Ascorbic Acid in the Rat.* (21256)

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Williams(1) reported that dietary aminopterin markedly reduced liver ascorbic acid concentrations in the rat. Subsequently, Schwartz and Williams(2) observed that the depression in liver ascorbic acid was not the result of an increased rate of urinary excretion of this vitamin. It has been further reported (3) that succinylsulfathiazole (sulfasuxidine) when fed at a 2% level in a purified diet also has a depressant action on rat liver ascorbic acid.

In the present work we have investigated various possible methods by which amino-

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