

animals blood pressure levels have been followed for as long as 2 years. The rather unexpected stability of systolic and diastolic blood pressures in rhesus monkeys makes this animal admirably suited for experiments in which blood pressure changes accompanying an experimental procedure are to be investigated.

Summary. Arterial blood pressures have been determined in unanesthetized monkeys by 2 indirect methods. Using a small "new-born" cuff (cloth cuff) on the upper arm and auscultation of the sounds in the brachial artery, mean blood pressure in 14 normal monkeys was found to range from 137/112 to 188/152 mm Hg with a group average of 159/127. With a cuff consisting of a rigid metal shell enclosing a rubber sleeve (metal cuff), mean pressures ranged from 103/78 to 132/101, the group average being 118/90 mm. In 27 splenectomized, but otherwise normal, monkeys the blood pressures were slightly but not significantly lower. Metal cuff pressure readings were found to be consistently less variable than corresponding cloth cuff values, the respective average standard deviations in normotensive animals being 7 and 12 mm. Blood pressures in untreated animals estimated by either indirect

method were relatively stable; in no instance did mean systolic or diastolic pressure during a 12-month period vary by as much as 10 mm Hg from the mean values obtained during an initial 2-month observation period. Simultaneous comparisons of indirect (cloth or metal cuff) and direct (strain gauge) blood pressure measurements in 46 anesthetized animals indicated that the metal cuff readings were lower and the cloth cuff readings higher than measurements obtained directly in the same artery with a strain gauge. The blood pressure as measured directly with a strain gauge fell about midway between the corresponding cloth and metal cuff readings.

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Experimental Hypertension in the Rhesus Monkey. (23500)

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There have been few reported studies on induction of experimental hypertension in the rhesus monkey. The investigation described here deals with attempts to provoke hypertension in this species by procedures which have been successful in rats, dogs, and other experimental animals. These procedures included temporary unilateral renal artery occlusion(1), unilateral or bilateral silk perinephritis(2), unilateral or bilateral constriction of the renal arteries with silk ligatures (3) or clamps(4), and oral administration of excessive amounts of sodium chloride(5).

Materials and methods. Animals. Sixty rhesus monkeys (*Macaca mulatta*), 26 males and 34 females, weighing from 3 to 12 kilos were used in this study. General care and feeding followed the pattern described by Schmidt and Genter(6). All animals had been infected previously with malaria, their infections cured by various chemotherapeutic agents, and this fact proved by absence of parasitemia following splenectomy. These monkeys were indistinguishable from intact control animals insofar as range and variability of systolic and diastolic blood pressures

are concerned(7). *Producing hypertension. Temporary unilateral renal artery occlusion* (1). In 7 monkeys blood flow to the right kidney was interrupted for one hour; in 2, flow was interrupted for 2 hours. After the prescribed time interval the clamp occluding the renal artery was removed and contralateral nephrectomy was performed immediately thereafter. *Silk perinephritis*(2). A. In 6 monkeys the right kidney was exposed through a lumbar incision and wrapped snugly in silk cloth, care being taken not to obstruct blood flow through the pedicle. B. In 6 monkeys the right kidney was wrapped as in A and contralateral nephrectomy was performed immediately thereafter. C. In 8 monkeys both kidneys were wrapped in silk as in A, the interval between the 2 wrappings varying from 7 to 21 days. *Stenosis of renal arteries with ligatures*(3,8). In 16 monkeys the renal arteries were exposed retroperitoneally and a loop of heavy silk thread placed around both the artery and a stylet held adjacent to it. After the tie was made, the stylet was withdrawn. Of the 16 monkeys, 12 had been operated upon 5 months previously (renal artery occlusion or silk perinephritis) but had remained normotensive. *Bilateral renal artery constriction with clamps* (4). In 15 animals not used previously in this investigation both renal arteries were constricted with small adjustable clamps. These clamps were constructed from one-inch strips of 1/16 inch thick methyl methacrylate (Lucite), 1/4 inch wide and bent in the shape of a flat "V", the arms of which could be closed by a stainless steel adjusting screw. In all instances the right renal artery was constricted first, the left renal artery 26 to 28 days later. Following the suggestions of Waklerlin(9), Schroeder and Goldman(10), and Goldblatt(4), attempts were made to reduce flow to one-third of normal. This point was difficult to judge even with transparent clamps. Seven of the animals developed uremia following application of the second clamp; in 5 of these, loosening or removing the second clamp failed to lower the uremia or to prolong life. *Sodium chloride administration*(5). The effect of excessive dosage of

NaCl was studied in 29 monkeys. Twenty of the group had been operated upon 4 to 6 months previously. Three of these had been subjected to temporary unilateral renal artery occlusion; 5 to unilateral silk perinephritis; 4 to unilateral silk perinephritis and contralateral nephrectomy; and 8 to bilateral silk perinephritis. Three others had had one kidney removed several months previously, while the remaining 6 monkeys were untreated controls. For the first 16 days NaCl was administered orally as a 4% solution at a daily dose of 400 mg/kg. For the following 14 days this same dose was supplemented by adding NaCl in a concentration of 0.5% to the diet and drinking water. For the final 22-day period the oral dose was raised to 800 mg/kg and the concentration in the diet to 1%. The average daily salt intake was 400 mg/kg for first 16 days, 1300 to 1800 mg/kg for the second period, and 2100 to 2600 mg/kg for the final 22-day period. *Blood pressure measurements and incidental studies.* Systolic and diastolic blood pressures were measured indirectly by the "newborn" cloth cuff and/or metal cuff procedures described by Smith and Ansevin(7). On each animal measurements were made 2 or 3 times each week during a control period of 2 or more months and at similar intervals during the experimental period. All monkeys were under close daily observation and were weighed weekly. The concentrations of sodium, potassium, and urea nitrogen in serum were determined biweekly or more frequently when indicated. Necropsies were performed immediately after death on all animals that succumbed. All surviving animals were sacrificed. In all cases weights of the heart, adrenals, liver, and kidneys were obtained. Segments of these and other organs were taken for histopathologic examination. The results of these studies will be reported separately.

Criteria of hypertension. The variation in the blood pressure of the normal rhesus monkey has been dealt with in the previous paper (7). As illustrated by the data in Fig. 1A, blood pressures of untreated animals were remarkably stable. Over a 12-month observation period average systolic and diastolic

TABLE I. Effectiveness of Various Experimental Procedures in Inducing Hypertension.

Exp. procedure	No. of monkeys used in exp. procedure						No. dying in acute re- nal failure
	Total	Blood pressure response					
		None	Hypertension			Severe	
			Slight	Moderate	Severe		
Unilateral renal artery occlusion	9	5					4
Silk perinephritis							
A. Unilateral	6	5		1			
B. Unilateral, contralateral nephrectomy	6	6					1
C. Bilateral	8	6	1	1			
Renal artery stenosis with silk ligu- tures	16	0	1	2		2	11
Bilateral renal artery constriction with plastic clamps	15	3	1	1		3	7

blood pressures never varied more than ± 10 mm from the mean pressure levels obtained during the initial 2-month standardizing period. This stability in the blood pressure of the normal monkey made it comparatively easy to establish criteria for hypertensive response. Monkeys whose blood pressures became consistently elevated above their pretreatment levels were considered to be hypertensive, the degree of elevation being classified as slight, moderate or severe. Elevations in mean pressure of 15 to 30 mm as measured with the metal cuff were classified as slight, elevations of 30 to 50 mm as moderate, and elevations greater than 50 mm as severe hypertension.

Results. Data in Table I show that restriction of the total blood supply to the kidney was by far the most effective method of inducing hypertension. Thus all 5 monkeys which survived constriction of the renal arteries through use of silk ligatures developed some degree of hypertension. In 2 the reaction was severe (Fig. 1D), in 2 moderate and in one slight. When constriction was achieved with plastic clamps, 5 of 8 surviving animals exhibited hypertensive changes, 3 being classified as severe and one each as moderate (Fig. 1B) or slight.

The hypertensive changes in the above animals were obtained at the price of a high overall mortality. Thus 18 of the 31 animals in the combined silk ligature-plastic clamp groups died in acute renal failure 3 to 23 days after the final operation. Two other animals with hypertensive responses also succumbed.

One animal developed severe hypertension 2 weeks after the second clamp had been applied and died 7 weeks later in renal failure (Fig. 1C). The remaining monkey presented an unusual blood pressure response which will be discussed subsequently.

Attempts to produce hypertensive changes by other procedures were relatively non-productive. Temporary unilateral renal artery occlusion produced no elevation in blood pressure. None of the animals subjected to silk perinephritis, either unilaterally or bilaterally, developed severe hypertension. One animal with one kidney wrapped in silk developed moderate hypertension. Necropsy 4 months after operation revealed gross suppuration in the area of the silk. Of the 8 animals with bilateral silk perinephritis, one developed moderate and another slight hypertension during an observation period of approximately 11 months.

In the monkeys fed excessive amounts of sodium chloride in their diet and drinking water, blood pressure responses were variable. Sustained elevations in pressure occurred in only 3 animals: one monkey with temporary unilateral renal occlusion; one unilaterally nephrectomized animal; and one control monkey. The experiment was terminated after 52 days when many of the monkeys became weak, began to lose weight and appetite, and showed evidence of enteric irritation.

Discussion. There have been few reports on production of experimental hypertension in the monkey although this condition has been studied extensively in other laboratory ani-

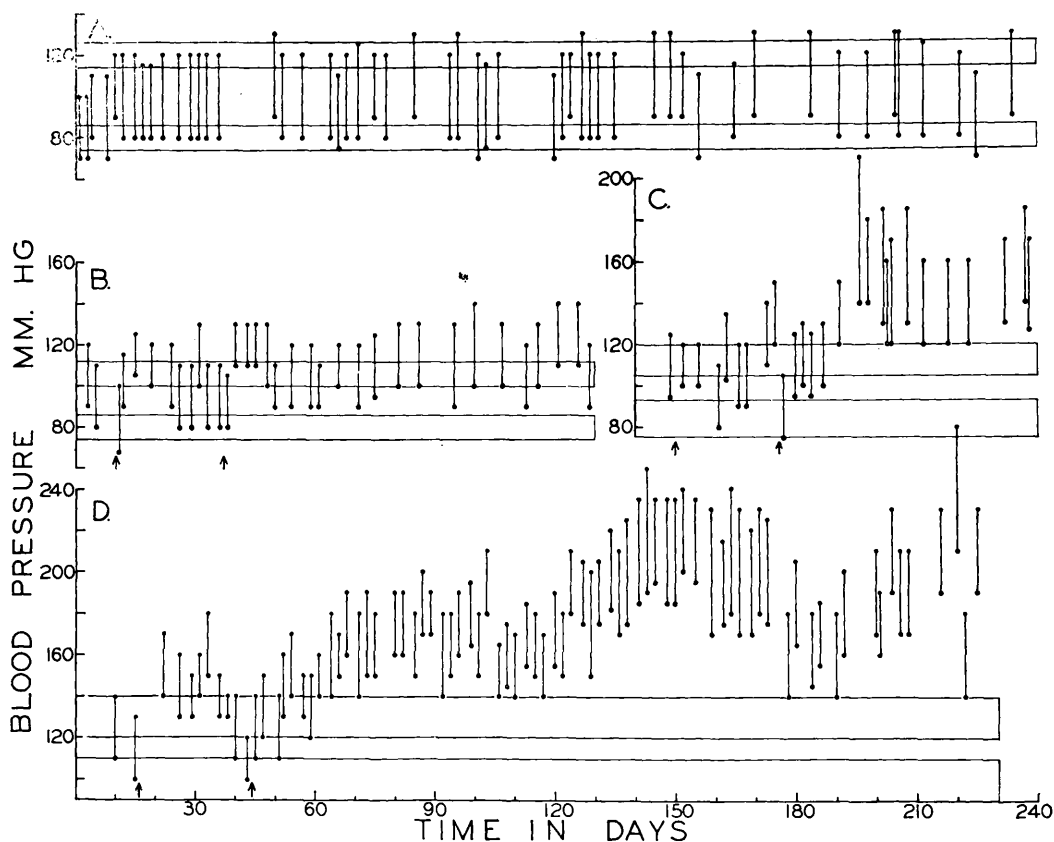


FIG. 1. Blood pressure readings in a control monkey and in monkeys with experimental hypertension; systolic (●) and diastolic (○). In each graph the upper box represents mean preoperative systolic pressure $\pm$ one standard deviation, the lower box, mean diastolic pressure $\pm$ one st. dev. Operative procedures are indicated by arrows. A. Control monkey; metal cuff readings. B. Slight to moderate hypertension following bilateral renal artery constriction with plastic clamps; metal cuff readings. C. Severe hypertension following bilateral renal artery constriction with plastic clamps. Monkey died in renal failure; metal cuff readings. D. Severe hypertension following bilateral renal artery stenosis with silk ligatures; cloth cuff readings.

imals. Goldblatt(4) described elevations in blood pressure in 3 of 5 monkeys following application of clamps to both renal arteries. In one animal indirect blood pressure measurements as high as 300 mm Hg were recorded; however, none of the animals had "malignant hypertension"(11). Frank and Wakerlin(12) dealt briefly with production of moderate hypertension in a larger series of monkeys using the Goldblatt method.

Of the 6 operative procedures compared in this study, only constriction of the renal arteries, either with silk ligatures or plastic clamps, induced significant elevation of blood pressure. These techniques invoked hypertensive changes in 10 of 13 animals which sur-

vived 3 or more weeks after operation. In 8 of these monkeys the elevations were moderate or severe. Although constriction of the renal arteries was an effective method for producing hypertension, degree of stenosis was critical; only a slight overconstriction led to acute renal failure and death.

The remaining 4 procedures, capable of producing hypertension in such laboratory animals as the rat, rabbit, or dog, were ineffective in producing elevations in blood pressure in the monkey. The observations of Koletsky(13) in rats suggest that temporary renal artery occlusion followed by contralateral nephrectomy might have been more effective had the normal kidney been removed

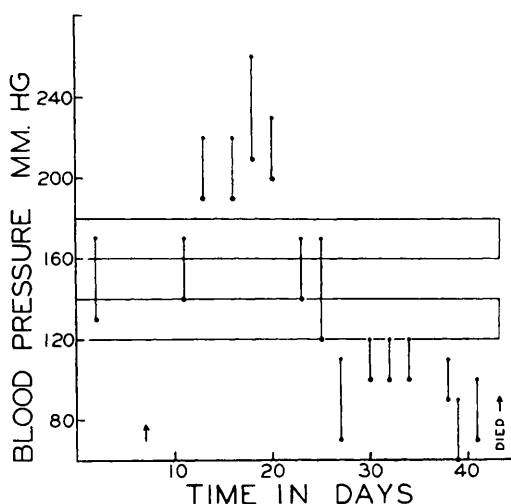


FIG. 2. Blood pressure readings following unilateral renal artery stenosis with silk ligature and contralateral nephrectomy. Cloth cuff readings; systolic (●) and diastolic (○).

at a later time when acute damage to the occluded kidney had been partially repaired. The 3 procedures employing silk perinephritis were ineffective, apparently because the silk caused very little inflammatory reaction except in those few instances in which it became infected accidentally. Examination of the wrapped kidneys at necropsy indicated that the silk had begun to disintegrate after it had been in the body about 6 months and after a year or more was discernible only as a small nodule where the purse-string tie had been made.

Blood pressure changes observed in Monkey 4900 (Fig. 2) are of interest in connection with the role of the liver in maintenance of hypertension. Raaschou and Trautner(14) found that ligation of the common bile duct in dogs with experimental renal hypertension caused a marked reduction in blood pressure often to normotensive levels. A similar series of events appears to have occurred by accident in Monkey 4900. Following application of a silk ligature to the single remaining renal artery, the monkey developed extreme hypertension. Apparently, at this point, there was bleeding into the gall bladder and bile ducts with clot formation producing complete biliary stasis. There was an abrupt drop in blood pressure to subnormotensive levels, and this suggests that in the monkey as in the dog,

and possibly also in man(15,16), certain functions of the liver are essential for maintenance of elevated blood pressure at least during early hypertension.

Summary. Six methods useful in producing experimental hypertension in other laboratory animals were studied in rhesus monkeys. Two methods involving direct constriction of the renal arteries either by silk ligatures or small transparent plastic clamps resulted in a significant incidence of hypertension. Of 13 animals which survived 23 days or longer after renal artery constriction, 10 developed hypertension. The degree of constriction was critical, and a significant proportion of the animals succumbed in acute post-operative renal failure. Four other methods used successfully in rats, rabbits, or dogs were ineffective in the monkey. These included: (1) unilateral temporary renal occlusion and contralateral nephrectomy; (2) unilateral silk perinephritis; (3) unilateral silk perinephritis and contralateral nephrectomy; and (4) bilateral silk perinephritis. Administration of large oral doses of sodium chloride *per se* did not induce hypertension, nor did it increase the effectiveness of any of the above 4 procedures. Attention has been called to some of the factors which may have contributed to the failure of certain of the methods to produce hypertension in the monkey.

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Porphyrin Synthesis by Mouse Harderian Gland Extracts: Sex, Age, and Strain Variation.* (23501)

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It has been observed that the Harderian glands of very young mice, 2 to 4 weeks of age, were not red fluorescent(1). It had also been shown that the red fluorescence of Harderian glands of mice of different strains varied in intensity. In some respects the Harderian glands resemble sebaceous glands in human subjects. Both of these glandular structures elaborate free porphyrins in the secretory product at some stage of development. In the adult human subject, the sebaceous glands of the skin of the face and forehead are seen as tiny red dots when illuminated with near ultra-violet light because of the red fluorescence of the sebum(2,3). However, no red fluorescent sebum was observed during the examination of the facial skin of more than 200 immature children. Thus, red fluorescent sebaceous secretions appear in the human subject at about the time of puberty, persist throughout the period of maturity, and usually decrease in older people. While the porphyrin content and red fluorescence of the Harderian glands of mice follow a similar pattern, variations in red fluorescence may occur as a result of dietary and other factors. It has been shown that the Harderian glands and the livers of mice contained an abundance of porphyrin-producing enzyme(4). Muscle-bone and brain tissues in mice contained some porphyrin-producing enzyme, but were less than 20% as effective in producing porphyrin(4).

In these experiments, an attempt was made to ascertain whether there was a variation in the porphyrin-producing enzyme content of the Harderian glands of mice of different ages and in different strains of mice of both sexes. It was found that the porphyrin-producing enzyme activity did vary with age and strain. The observations on the enzyme activity were in agreement with the previous observations on the fluorescence of Harderian glands(2,3).

Materials and methods. The Harderian glands used in this series of experiments were taken from 74 male and 78 female mice of the C₃H, C₅₇, A, and LCSa strains, varying in age from 2 to 52 weeks. The animals were killed with ether and the Harderian glands dissected out in a cold room (6°C). After removal of any excess blood, 100 mg of the tissue was weighed and placed in a mortar with a small amount of coarse grinding sand and 2 cc of 50:50 buffer-saline solution. After the tissue was homogenized, the residue was rinsed from the mortar with 3 cc of the buffer-saline solution. This was centrifuged at 2500 r.p.m. for 15 minutes and the supernatant solution was used as the enzyme extract. To each of 148 tubes (37 for each age series of tissues to be tested), 1.4 cc of 0.01 M δ -aminolevulinic acid hydrochloride buffer-saline solution was added to act as the substrate. Various amounts (0-0.8 cc) of enzyme extract, from each Harderian gland to be tested, were then placed in their respective Klett tubes. An amount of buffer-saline was added to each tube to bring the volume to 3 cc. The Klett tubes were then closed with cellophane covered corks. The tubes were incubated at 37°C for 24 hours. Concentrated HCl (6.5 cc) was then added to

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