of 0.1% sodium acetate. The other was obtained as a lyophilized preparation (catalog number 7469) from the American Type Culture Collection. No dependent varieties were found among 60 isolates from each of these cultures. The question of whether our stock of *L. casei* contained a high proportion of dependent varieties in 1946 (when the Shankman stock originated from it) is apparently unanswerable, but it seems likely that this was true of our stock in 1952 on the basis of published data(4). It was reported(4) that 15 hour growth (but not later growth) of the "parent culture" was markedly stimulated by lactic acid, whereas we now know (unpublished data) that even the early growth of purified independent strains is not stimulated by α-hydroxy acids, and it may be inferred from this difference that the "parent culture"(4) contained a considerable proportion of dependent forms.

Summary. The stock culture of *L. casei* 7469 carried in this laboratory contained 6 to 45% (in different experiments) of forms resistant to inhibition by potassium acetate, yet inocula containing up to 12% of the resistant forms were markedly inhibited by potassium acetate apparently because of complex interactions between the resistant and sensitive varieties. Sensitive strains were found to be α-hydroxy acid-dependent (formerly this characteristic has been ascribed only to "mutant" strains). Sensitive strains, although predominating in the authors' stock culture, were not found among 60 isolates from either of two outside cultures of *L. casei* 7469.

1. Camien, M. N., Dunn, M. S., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 697.

2. Dunn, M. S., Shankman, S., Camien, M. N., Block, H., *J. Biol. Chem.*, 1947, v168, 1.

3. Camien, M. N., Dunn, M. S., *Science*, 1957, v125, 1149.

4. Camien, M. N., *J. Biol. Chem.*, 1953, v201, 621.

5. ———, *Science*, 1958, in press.

6. ———, *Arch. Biochem. Biophys.*, 1956, v60, 452.

Received December 9, 1957. P.S.E.B.M., 1958, v97.

Renal Response to Non-Shocking Hemorrhage. Sodium Retention at Constant Perfusion Pressure.* (23762)

ALLAN V. N. GOODYER,† LOUIS R. MATTIE,‡ AND ALLEN CHETRICK§
(Introduced by Philip K. Bondy)
Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn.

Non-shocking hemorrhage (20 cc/kg for 35 minutes) decreases renal excretion of sodium despite autonomic blockade or renal denervation(1). Excretion of sodium increases again *during retransfusion* of the shed blood even when this is accomplished at a constant renal arterial blood pressure by inducing extrarenal vasodilatation with hexamethonium. The present experiments were carried out in order to study renal excretion of sodium *during hemorrhage* at constant arterial pressure, under conditions where direct effects on the renal tubules of pharmacologic agents, or of the shed blood itself, were avoided.

Methods. Acute experiments were performed in dogs weighing from 10 to 20 kg anesthetized with 60 mg/kg of Dial intravenously. Inulin and para-aminohippurate in a solution of isotonic saline were administered via one femoral vein by means of a constant infusion pump. Renal arterial pressure was continuously recorded and monitored via a catheter inserted to the level of the renal vessels through the femoral artery. In some experiments the brachial artery pressure was recorded as an index of changes of the superior aortic blood pressure. A Sanborn Twin-Viso recorder and amplifiers were used with a Hathaway pressure capsule. Urine was col-

* Supported in part by grants from the Am. Heart Assn. and Nat. Heart Inst.

† During tenure of Established Investigatorship of Am. Heart Assn.

‡ During tenure of Traineeship of Nat. Heart Inst.

§ During tenure of Research Fellowship of Am. Heart Assn.

TABLE I. Changes in Functions of Innervated and Denervated Kidney Induced by Hemorrhage during Aortic Obstruction.

Exp. groups and procedures*	Kidney	No. of obs.	% changes from control to test periods† ENa Mean ± S.D.	P‡	EK Mean ± S.D.	P	FNa Mean ± S.D.	P	Cpah Mean ± S.D.	P
(A) *Controls*										
(1) (O–O)	Inn.§	20	+ 1 ± 40		+ 6 ± 23		+ 1 ± 78		− 4 ± 20	
	Den.§	9	− 5 ± 29		+ 5 ± 12		− 1 ± 18		− 8 ± 21	
(2) (O–BL)	Inn.§	14	−41 ± 37	.01	− 9 ± 63	.40	+ 2 ± 33	.90	−10 ± 17	.33
	Inn.‖	4	−36 ± 22	"	− 5 ± 10	.10	−13 ± 13	.40	+23 ± 37	.10
	Den.§	10	−48 ± 25	"	− 7 ± 20	.20	− 4 ± 16	.70	−15 ± 14	.42
(3) (O–CL)	Inn.‖	11	+34 ± 68	.10	+35 ± 62	.10	+ 4 ± 16	.80	− 1 ± 25	.70
	Den.‖	4	+25 ± 77	.40	−15 ± 46	.40	−16 ± 23	.20	−11 ± 22	.89
(B) *Exps. with hemorrhage at constant renal arterial pressure*										
(4) (O–BL/CL)	Inn.	7	−41 ± 23	.01	−10 ± 22	.10	−13 ± 14	.40	+ 8 ± 26	.20
	Den.	4	−33 ± 28	"	−23 ± 19	.02	−20 ± 24	.10	−14 ± 18	.60
(5) (O–BL/BaL)	Inn.	19	−24 ± 22	.03	0 ± 41	.90	−82 ± 23	.01	−10 ± 27	.40
	Den.	9	−59 ± 19	.01	−18 ± 29	.02	−18 ± 13	.10	−26 ± 25	.10
(BL/BaL–O)	Inn.	17	+90 ± 51	.01	+20 ± 54	.40	+16 ± 53	.50	+21 ± 68	.10
	Den.	9	+18 ± 50	.20	0 ± 20	"	0 ± 24	.90	+ 8 ± 25	"

* Letters and numbers designate exp. groups as described under *Methods*. Symbols designating procedures used are: O–O = Spontaneous changes; no test procedure. O–BL = Changes with hemorrhage. O–CL = Changes with aortic clamping. O–BL/CL = Changes with hemorrhage while aorta clamped. O–BL/BaL = Changes with hemorrhage while aortic balloon deflated. BL/BaL–O = Changes when shed blood retransfused while aortic balloon inflated.

† ENa = Excretion rate, sodium. EK = Excretion rate, potassium. FNa = Filtration rate, sodium. Cpah = Para-aminohippurate, clearance.

‡ P = Probability of chance diff. between test and control groups.

§ Control data derived from previous report(1). ‖ Control data from present experiments.

lected from each kidney by ureteral cannulation, each period of collection lasting 15 to 30 minutes. Aqueous heparin (0.2 mg/kg intravenously) was used as an anticoagulant. Bleeding, when desired, was performed by allowing blood to flow via a polyethylene cannula inserted into the brachial or femoral artery into an inverted capped silicone-coated bottle containing air at a pressure adjustable through a side arm connected to a manometer and pressure bulb. Bleeding volumes, (at 1.5 to 3% of the body weight), were sufficient to cause renal retention of sodium on the basis of past experience(1). Renal arterial pressure was maintained constant by appropriate adjustment of one of two types of mechanical aortic obstruction: 1. Compression of aorta by clamp applied just below renal arteries (O – BL/CL, Table I). Only small hemorrhages (< 2% of body weight) were possible by this procedure without a fall in renal arterial blood pressure. 2. Graded inflation of rubber balloon at end of catheter inserted via the left carotid artery to level of the diaphragm. Prior to bleeding, this balloon was inflated sufficiently to lower renal arterial pressure to about 90 to 100 mm Hg (mean), this level being controlled by constant monitoring with a direct-writing pressure recorder (see above). After at least one-half hour at this reduced arterial pressure, several control periods were obtained, following which bleeding was allowed up to 3% of the body weight, while the aortic balloon was gradually deflated so as to maintain renal arterial pressure constant ((O – BL/BaL), Table I). After several periods, the shed blood was retransfused while the balloon was appropriately reinflated ((BL/BaL – O), Table I). Large changes of blood volume were thereby possible without changing renal arterial perfusion pressure (and without using autonomic blocking agents, as in prior studies). In experiments designated by "Den", Table I, preliminary unilateral left renal denervation was carried out one to two weeks beforehand in 19 dogs by complete separation of each kidney from its attachments, except for the artery, veins and ureter, 1 to 2 cm of which were stripped of all visible nerve fibers and painted

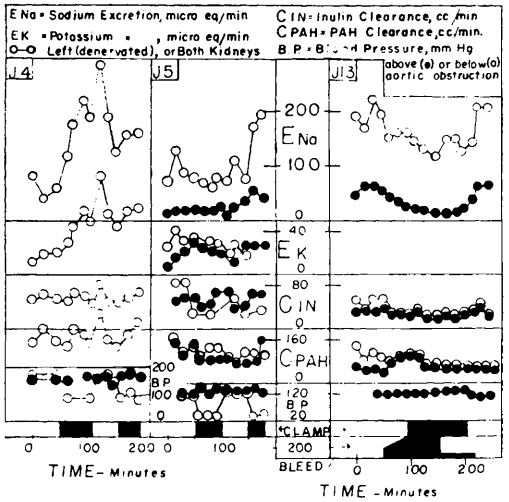

FIG. 1. Changes in functions of innervated and denervated kidneys induced by hemorrhage during aortic clamping, and by aortic clamping alone.

with absolute alcohol. The efficacy of this procedure has been previously verified. Clinical analyses and calculations were the same as those described in a previous report from this laboratory.

Results. A. Control Experiments (Summarized in Table I). Hemorrhage ((O – BL), Table I) of the degree used throughout our study (1.5 to 3% of body weight, representing about 20 to 35% of total blood volume) reduced excretion of sodium in 4 new experiments as had been observed previously (1). *Clamping of the aorta* just below the renal arteries ((O – CL), Table I) caused no significant change in sodium excretion or in other renal functions (Fig. 1) and only small, transient increases of superior aortic (brachial artery) blood pressure. *Inflation of the aortic balloon* until the renal arterial pressure was reduced to about 100 mm Hg decreased the excretion rate of sodium by both the denervated and the innervated kidney. The steady values of sodium excretion obtained after one-half hour at reduced renal arterial pressure were used as controls for the subsequent experimental periods.

B. *Experiments with hemorrhage at constant renal arterial pressure (Table I). Hemorrhage* at constant renal arterial pressure maintained either by aortic clamping ((O – BL/CL), Table I), or by deflation of an aortic balloon ((O – BL/BaL), Table I) reduced *excretion of sodium* by both the innervated and the denervated kidney Fig. 1 and 2). *Excretion of potassium* by the denervated kidney (but not by the innervated kidney) was also reduced. *Renal blood flow* was not significantly changed. *Glomerular filtration of sodium* was reduced significantly only in the innervated kidney when renal arterial pressure was controlled by an aortic balloon ((O – BL/BaL), Table I). Reflex changes of (? predominantly efferent) arteriolar tone may have changed glomerular filtration in these experiments, in response to the larger hemorrhages possible when an aortic balloon, rather than an aortic clamp, was used to control renal arterial pressure.

Retransfusion of the shed blood at constant renal arterial pressure, maintained by reinflation of the aortic balloon ((BL/BaL – O), Table I) increased sodium excretion in the innervated kidney (Fig. 2). Significant changes of other functions were absent. In several experiments, sodium excretion also increased in the denervated kidney (Fig. 2), but the average change of all experiments was not significant (Table I).

Discussion. The experiments of this report indicate that the excretion of sodium by the innervated or by the denervated kidney declines *during hemorrhage,* even though the

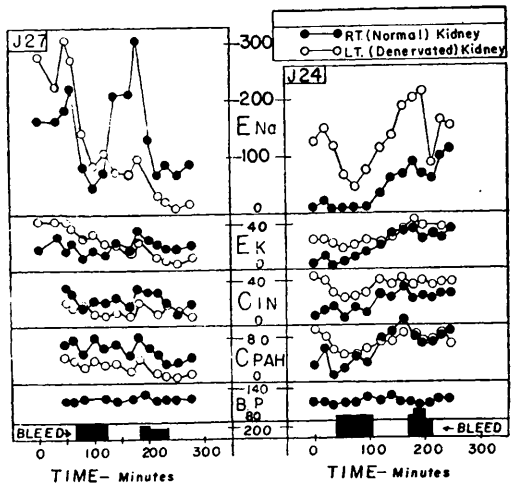

FIG. 2. Changes in function of innervated and denervated kidneys induced by hemorrhage during controlled deflation of an aortic balloon. (Symbols as of Fig. 1.)

renal arterial pressure is maintained at a constant value by mechanical means. These observations might be explained on the basis of an extrarenal receptor located in the upper aortic distribution, sensitive to diminished blood flow or volume, and regulating renal tubular reabsorption of sodium via humoral or endocrine secretion. Recent experiments have, in fact, disclosed that an increased secretion of aldosterone and hydrocortisone is induced by hemorrhage either directly or via ACTH(2,3). However, the renal retention of sodium elicited by hemorrhage in the present experiments was more rapid than might have been expected as a result of the secretion of presently known adrenal steroids. Furthermore, no changes of potassium excretion characteristic of adrenal steroid action(4) were observed (Table I). Consequently, the present experiments reinforce the previous proposal(1) that hemorrhage may cause an intrarenal redistribution of blood flow, perhaps toward vessels of less than average resistance perfusing tubules with more than average sodium reabsorbing activity. Current studies of the renal hematocrit during isobaric hemorrhage lend further support to this concept.

Summary. The renal excretion of sodium and other renal functions were studied in dogs during non-shocking hemorrhage, while renal arterial pressure was maintained constant by controlled partial mechanical obstruction of the aorta. In some experiments, one kidney was denervated beforehand. The excretion of sodium declined in the absence of measurable changes in glomerular filtration or renal plasma flow. The explanation is proposed that hemorrhage causes an intrarenal redistribution of blood flow which favors tubules with more than average sodium reabsorbing activity, although steroid effects on tubular function cannot be excluded.

The authors express their gratitude to Mr. Armen Negri, Miss Margaret Hetley, Miss Francis Korb, Miss Jacqueline McClave, and Miss Janice Pongonis for expert technical assistance.

1. Goodyer, A. V. N., Jaeger, C. A., *Am. J. Physiol.*, 1955, v180, 69.

2. Farrell, G. L., Rosnagle, R. S., Rauschkolb, E. W., *Cir. Res.*, 1956, v4, 606.

3. Fine, D., Meislas, L. E., Auerback, T., *Clin. Res. Proc.*, 1956, v4, 126.

4. Liddle, G. W., Cornfield, J., Casper, A. G. T., Bartter, F. C., *J. Clin. Invest.*, 1955, v34, 1410.

Received December 9, 1957. P.S.E.B.M., 1958, v97.

Yolk-sac Perfusion of Chick Embryos: Effects of Various Media on Survival.* (23763)

N. W. KLEIN, C. R. GRAU AND N. J. GREEN
Department of Poultry Husbandry, University of California, Davis

The difficulty of maintaining the delicate relationships of the chick embryo to its membranes and to its nutrient supply have until recently precluded direct studies of embryo nutrition in the intact egg. Important data (1) have been obtained indirectly by feeding the laying hen diets deficient in vitamins and minerals, and determining the subsequent effect on development of the embryo. This method cannot be used to study the embryo's supply of amino acids and most energy sources, however, because deficiencies of these nutrients in the hen's diet result in reduced egg production, not in the production of eggs containing lower concentrations of nutrients. One direct approach has been the *in vitro* cultivation technic, which Spratt(2) has used to study the nutrition of chick embryos during early development (18 to 58 hours of incubation). New(3) has recently developed a method of explantation which allows development to proceed for 60 hours, 40 hours of which are *in vitro*. The objectives of the methods developed in this laboratory have been to remove the embryo's nutrients, and

* This work was supported by grant from Nutrition Fn.