than the guaiac test for detection of known minute amounts of blood in the stool.

Discussion. Not all of the injected radioactive iron is incorporated into red blood cells. Hahn and associates(4) have shown that 35 to 70% of injected isotope becomes incorporated in peripheral circulating red blood cells. However, if a sufficient period of time is allowed to elapse following injection of radioactive iron, the excretion pattern of the isotope in the stool may subsequently be utilized for detection of bleeding from the alimentary canal, since elevated levels of stool radioactivity after 21 days are indicative of abnormal sources of iron loss.

The method appears to be considerably more sensitive than the guaiac test, utilizing amounts of the isotope and the technic for determination of stool radioactivity described herein.

It is conceivable that the sensitivity of the method might even be enhanced somewhat by modifying the technic of examination by ashing and counting the entire 24-hour stool. If proved correct, this modification could permit administration of smaller amounts of the isotope. In the presence of a positive test, a comparison of esophageal, gastric and colonic washings could prove to be of real help in localizing the source of the bleeding from the tract. It is proposed presently to undertake clinical investigation of the efficacy of the method in the detection and localization of silent malignancies of the gastrointestinal canal.

Summary. 1. Excretion patterns of intravenously administered radioactive iron in the stool of normal dogs and in dogs with histamine induced bleeding of the gastrointestinal tract have been compared. 2. Levels of stool radioactivity have been measured following intragastric administration of known amounts of isotope-labelled blood. 3. Radioactivity in the stool can be utilized for detection of occult bleeding. 4. Comparative evaluation following administration of known small quantities of isotope-labelled blood (Fe^{59}) indicates definitely that this test is considerably more sensitive than the guaiac test.

1. Hahn, P. F., Bale, W. F., Ross, J. F., Hettig, R. A., Whipple, G. H., *Science,* 1940, v92, 131.

2. Ledlie, E. M., Baxter, C. F., *Proc. Second Radioactive Isotope Conf.,* p97, 1954, Butterworth, London.

3. Varco, R. L., Code, C. D., Walpole, S. H., Wangensteen, O. H., *Am. J. Physiol.,* 1941, v133, 475.

4. Hahn, P. F., Granich, S., Bale, W. F., Michelcis, L., *J. Biol. Chem.,* 1943, v150, 407.

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Pressor Substances in Kidneys of Renal Hypertensive Rats with and Without Adrenals. (24037)

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In the rat, sustained hypertension develops after clamping the artery of one kidney, leaving contralateral kidney untouched(1). It has also been demonstrated that the content of pressor substances (renin) in aqueous extracts prepared from kidneys is normal or enhanced in the clamped kidney, while it is reduced or absent in the contralateral untouched kidney which, however, shows typical morphological changes(2). Furthermore, it is well known that experimental renal hypertension of the Goldblatt type is dependent on presence of functioning adrenal cortex. In the adrenalectomized animal, hypertension does not develop or blood pressure falls to normal values if the adrenals are extirpated after establishment of hypertension(3). In former experiments we observed that in rats in which hypertension has been produced by overdosage of cortexone and salt, pressor active substances disappeared from the kidneys (4). The experiments reported here were

* We are indebted to Mr. R. Doebelin for technical assistance.

performed in order to evaluate the significance of unequal distribution of renin in the clamped and unclamped kidney in development of renal hypertension and to study the influence of adrenalectomy on the renin content of the kidneys.

Methods. Male Wistar rats with average initial weight of 150-200 g were used. Food consisted of standardized pellets containing 0.57% of salt. Tap water or 0.5% saline was given as drinking fluid. A. *Production of renal hypertension.* Two methods were used: clamping of left renal artery or encapsulation of left kidney by cellulose acetate. Clamps were prepared from silver strip, bent over metal plate of 0.2-0.3 mm thickness, the space within the clamp being made proportional to size of animal. For the encapsulation, animals were anesthetized with ether, the left kidney exteriorized through a paravertebral incision. A thin leaf of cellulose acetate (0.1 mm thickness) bearing a hollow for kidney pedicle was placed around kidney and kept together by 2 rings of rubber. After hypertension had developed in the majority of animals in both groups, they were killed by ether. Kidneys were removed and the pressor material determined by the method described below. B. *Adrenalectomized animals.* Two groups of 7 animals each were used. In all animals the left renal artery was clamped. In one group the adrenals were removed a fortnight after clamping and after hypertension had developed. The other 7 animals were sham operated. After adrenalectomy, animals received 0.1 mg cortisol acetate subcutaneously/day. Five weeks after beginning of experiment, *i.e.,* 3 weeks after adrenalectomy or sham operation, all animals were killed and kidneys assayed for their pressor activity. Blood pressure was determined by tail plethysmograph method under light ether anesthesia. C. *Bioassay of pressor material.* Kidney extracts were prepared by grinding the minced kidney with sand and isotonic saline. Ground tissue was centrifuged 30 minutes at 6000 rpm. Such extracts contain the pressor active material in the supernatant fluid. The pressor activity was determined semiquantitatively in nephrectomized rats anesthetized with urethane (1.4 g/kg s.c.) from which kidneys were removed about 20 hours before. A standard dose of 0.1 ml of aqueous kidney extract was injected into jugular vein. Results were judged by maximum blood pressure and the integral of blood pressure curve over 30 minutes expressed in cm^2.

Results. A. Pressor activity in clamped or encapsulated and in contralateral intact kidney. In nephrectomized test animals, average increase in blood pressure produced by 32 extracts of an equal number of normal kidneys was 64.8 ± 2.1 mm Hg. Blood pressure did not return within several hours to the level before injection. In the same animals intravenous injection of 0.02 mg/kg of adrenaline gave a short hypertensive response averaging 50.5 ± 2.2 mm Hg.

Hypertensive activity of extracts from

TABLE I. Pressor Activity of Freshly Prepared Extracts from the Unclamped (Right) and Clamped (Left) Kidney of Rats with Hypertension and with Normal Blood Pressure on 152nd Day after Operation. Maximal pressor response in mm Hg and integral of blood pressure curve in cm^2.

No. of rats	Final body wt (g)	Final blood pressure (mm Hg)	Kidney wt (mg)		Pressor activity (mm Hg) of extracts from unclamped (right) and clamped (left) kidney		Planimeter (cm^2)	
			Right	Left	Right	Left	Right	Left
12	198.7 ± 9.4	205.0 ± 6.9	955.0 ± 80.0	487.0 ± 38.0	17.4‡ ± 3.1	71.8† ± 4.6	13.4 ± 2.8	39.9 ± 2.9
19	202.1 ± 11.2	123.4 ± 4.9	1027.0 ± 44.8	358.0 ± 21.1	29.1‡ ± 4.2	68.2* ± 3.4	20.2 ± 3.2	35.6 ± 2.2

* $p < 0.3$
† $p < 0.2$
‡ $p < 0.001$
} compared with content of pressor material of normal kidneys leading to rise of 64.7 ± 2.1 mm Hg (37 rats).

TABLE II. Pressor Activity of Freshly Prepared Extracts from Unclamped (Right) and Encapsulated (Left) Kidney at 80th Day after Operation. Maximal pressor response in mm Hg and integral of blood pressure curve in cm². In 2 groups the contralateral kidney was removed.

No. of rats	Final body wt (g)	Final blood pressure (mm Hg)	Kidney wt (mg)		Pressor activity (mm Hg) of extracts from unclamped (right) and encapsulated (left) kidney		Planimeter (cm²)		Drinking fluid	
			Right	Left	Right	Left	Right	Left		
6	164.7 ± 6.6	137.5 ± 14.5	681.0 ± 58.0	431.0 ±122.0	10.5‡ ± 6.0	61.0* ± 7.0	7.9 ± 4.0	41.0 ± 9.0	Water	
5	168.0 ± 4.0	118.0 ± 8.0	731.0 ± 26.2	455.0 ± 84.6	20.0‡ ± 5.8	47.2† ± 5.0	14.1 ± 6.8	29.5 ± 3.2	.5% NaCl	
6	159.0 ± 3.7	126.0 ± 7.1		736.0 ± 37.8		36.0‡ ± 12.6		25.0 ± 8.8	Water	**
6	150.0 ± 17.6	153.3 ± 4.4		826.0 ± 26.8		44.0‡ ± 2.4		33.0 ± 1.8	.5% NaCl	

* $p < .7$
† $p < .01$
‡ $p < .001$
} Compared with content of pressor material of normal kidneys.

** Unilaterally nephrectomized.

clamped kidneys was either normal or slightly enhanced, it was significantly below normal in extracts prepared from unclamped kidneys (Tables II and III). This difference in the content of pressor material in clamped and in contralateral kidney was more pronounced in animals in which hypertension developed (Table I).

Encapsulation of kidney produced results like those of renal arterial clamping. Administration of 0.5% saline as drinking fluid instead of tap water, did not lead to more pronounced decrease of pressor substances in the intact kidney but to a slight diminution of pressor activity in the encapsulated kidney. After nephrectomy the remaining encapsulated kidney contained less pressor material than a normal kidney, regardless of whether tap water or saline was given as drinking fluid.

B. *Influence of adrenalectomy.* In confirmation of results of others, adrenalectomy in renal hypertensive rats was followed by a

TABLE III. Body Weight, Blood Pressure, Kidney Weight and Pressor Activity of Freshly Prepared Extracts from Unclamped (Right) and Clamped (Left) Kidney of Renal Hypertensive Rats with and without Adrenals, Assayed in Nephrectomized Rats. Maximal pressor response in mm Hg and integral of blood pressure curve in cm².

No. of rats	Body wt (g)			Avg blood pressure (mm Hg)			Kidney wt (mg)		Pressor activity (mm Hg) of extracts from unclamped (right) and clamped (left) kidney		Planimeter (cm²)	
	Initial	14th day	35th day	Initial	14th day	35th day	Right	Left	Right	Left	Right	Left
7	205.1 ± 3.8	218.4 ± 3.4	256.5 ± 4.8	112.1 ± 4.2	177.1 ±10.5	210.7 ±10.2	1040.0 ±54.9	840.0 ±31.3	14.0§ ± 6.6	71.6* ± 6.9	16.3 ±10.0	46.3 ±5.5
6‖	204.3 ± 5.5	219.8 ±11.3	175.2 ±12.8	108.3 ± 3.1	172.5 ± 7.8	105.8 ± 4.2	897.0 ±34.5	393.0 ±45.8	54.8† ± 7.1	77.7‡ ± 3.9	39.8 ± 5.2	42.0 ±2.3

* $p < .3$
† $p < .2$
‡ $p < .02$
§ $p < .001$
} compared with content of pressor material of normal kidneys.

‖ Rat No. 3897 on autopsy showed surviving remnants of adrenal tissue. The following values for this rat were not included in calculation of the mean: Final blood pressure: 165 mm Hg; kidney wt: left, 949 mg; right, 1327 mg; rise in blood pressure of extract of left kidney: 58 mm Hg; of right kidney: 6 mm Hg. Final body wt: 236 g.

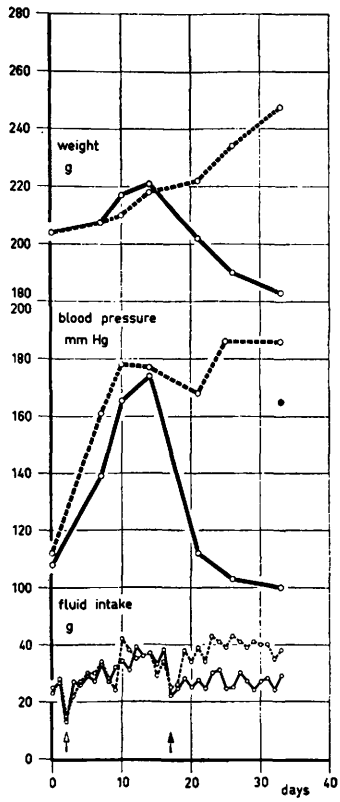

FIG. 1. Mean values of body wt (above), blood pressure (middle) and water intake (below) in rats with unilateral clamping of renal artery (dotted line) and in a second group adrenalectomized 17 days after clamping and treated with .1 mg of cortisol acetate daily. The point in blood pressure curve at 32nd day marks blood pressure of one animal of the adrenalectomized group with regenerated remnants of right adrenal. White arrow: clamping of left renal artery. Black arrow: bilateral adrenalectomy.

fall of blood pressure to normal values (Fig. 1). The content of pressor material in the clamped kidney was almost the same as in non-adrenalectomized animals, while in the intact kidney it was only slightly reduced and significantly higher than in kidneys from the corresponding sham-operated animals or from unilaterally clamped animals with intact adrenals. One animal of the adrenalectomized group (Table III) not only showed no fall in blood pressure but also complete depletion of pressor material from its right kidney. Autopsy revealed that adrenalectomy was incomplete in this animal.

The clamped left kidneys of the adrenalectomized animals were only about 50% of the weight of those with adrenals. The unclamped kidney, however, was only slightly smaller in the adrenalectomized animals than in animals from which adrenals were not removed. In spite of this difference in size, the pressor activity of kidney extracts prepared from these 2 groups are in the same range (Table III).

Discussion. In rats in which the left renal artery is clamped, in all animals, regardless of whether hypertension resulted or not, the content of pressor material in the clamped kidney was found to be either normal or slightly enhanced, while it was significantly reduced in the unclamped kidney. The same change in the distribution of pressor material was also observed if one kidney was encapsulated. The decrease of pressor material (renin ?) in the intact kidney is not strictly correlated with sustained high blood pressure. In hypertensive animals the removal of the adrenals not only produces a fall in blood pressure to normal values but is also followed by a restoration of the content of pressor substances in the right kidney. Whether higher doses of cortisol or other cortical steroids can prevent not only the fall in blood pressure but also the return to normal levels of pressor substances in the unclamped kidney has not been investigated. The fact that both blood pressure and the renin content of the unclamped kidney are simultaneously restored to normal by adrenalectomy makes it probable that the reduction of renal pressor activity is involved in pathogenesis of renal hypertension. It is, however, not a prerequisite, since sustained renal hypertension develops even more regularly in unilaterally nephrectomized animals bearing one clamped kidney. Hence it may be that depletion of renin-like substances and sustained renal hypertension are independent and unrelated phenomena.

Summary. 1. In rats, clamping of one renal artery or encapsulation of one kidney leads to a significant decrease in pressor material (renin ?) in the contralateral "untouched" kidney, while in the clamped kidney pressor activity is normal or only slightly enhanced. 2. These changes in content of renal pressor material are not necessarily accompanied by increase in blood pressure but were observed in every case of renal hypertension.

3. When hypertensive rats were adrenalectomized and maintained on small doses of cortisol, there followed a decrease in blood pressure and restoration of level of pressor material in the unclamped kidney. 4. It is suggested that diminution of pressor material in the unclamped kidney is not a prerequisite but a consequence of the pathological situation leading to renal hypertension.

1. Wilson, C., Byrom, F. B., *Lancet*, 1939, v236, 136.
2. Wilson, C., *ibid.*, 1953, v265, 579, 632.
3. Floyer, M. A., *Clin. Sc.*, 1951, v10, 405.
4. Gross, F., Sulser, F., *Arch. exp. Path. Pharmakol.*, 1956, v229, 374.

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Use of Dyes with Rapid Bloodstream Clearance for Serial Determination of Cardiac Output. (24038)

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The T-1824 dye dilution method has been widely used for measurement of the cardiac output. Because of albumin binding the dye leaves the bloodstream very slowly. This causes serious technical and clinical difficulties when repeated injections of the dye are given for serial estimation of the cardiac output, for example, appearance of bluish coloration of skin and subcutaneous tissues, and development of excessively high concentrations of dye in the plasma above the range of accurate determination with a spectrophotometer. These facts suggested that a dye with rapid bloodstream clearance might be more suitable than T-1824 dye for frequent serial determination of cardiac output. The present study is concerned with investigation from this viewpoint of a group of 45 blue dyes.

Materials and methods. Healthy dogs weighing 10 to 25 kg were fasted 48 hours before each experiment. Anesthesia was produced by intraperitoneal injection of sodium pentobarbital (30 mg/kg body weight), after which an endotracheal tube with inflatable cuff was placed in the trachea. The dyes dissolved in distilled water and sterilized by heating were injected (0.25 mg/kg body weight) into one exposed femoral vein. Venous blood samples were removed from the opposite femoral vein before injection and at intervals after, *viz.* 1, 5, 10, 15, 20, 25, 30, 45, 60, 90, and 120 minutes. Optical density readings of dye-plasma samples were made at point of maximum spectral absorption with Beckman Spectrophotometer, DU model. Spectral absorption curves and disappearance rates of 38 of the 45 blue dyes were investigated. The remaining 7 blue dyes were either partially soluble in water (Acetin Blue) or insoluble (Alcian Blue, Sudan Blue, Ultramarine Blue, Oil Blue, Galamine Blue, Alazarin Blue S) and were, therefore, unsuitable for further study. In the cardiac output experiments, the selected dyes were injected (0.15 mg/kg or 0.25 mg/kg body weight) into one femoral vein. Following injection, arterial blood samples were collected (from a polyethylene tube inserted through one femoral artery into the aorta) at 2-second intervals into cuvettes of motor-driven sample collector. Cardiac output values were calculated from formula of Stewart(1), of Hamilton and associates(2) as modified by Nicholson and Wood(3).

Results. Dyes cleared within one min. The following dyes were cleared from the bloodstream in less than one minute; Anthracene Blue, Toluidin Blue O, Victoria Blue R, Night Blue, Poirrer Blue, Prussian Blue, Luxon Fast Blue MBS, Niagara Blue 2B, Delphine Blue, Isamin Blue, Cyanin Blue G, Fast Acid Blue, Brilliant Cresyl Blue, Brilliant Alazarin Blue, and Azure Blue B.

Dyes cleared within 5 min. The following dyes were cleared between one and 5 minutes; Acid Alazarin Blue 2B, Nile Blue, Toluylene