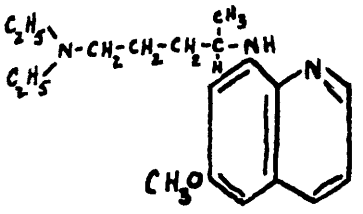
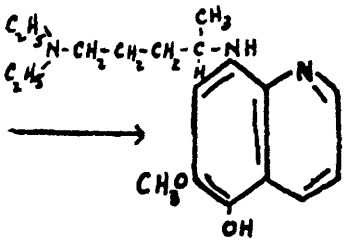
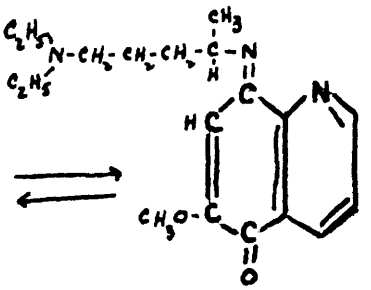


Pamaquine



5-Hydroxy Pamaquine



Quinonimine of Pamaquine

The possibility exists that both the toxicity and therapeutic effects of pamaquine are mediated through the same metabolic derivative. This is an important consideration in assaying the chances of obtaining an 8-aminoquinoline which is curative in malaria without being toxic. It is of interest that Greenberg, Taylor and Josephson have found that whereas pentaquine, (8-(Isopropylaminoamylamino)-6-methoxyquinoline) exerts a negligible effect on the erythrocytic form *P. gallinaceum in vitro,* its 5-hydroxy derivative exerts considerable activity on this parasite(10).

Summary. Two metabolites of pamaquine have been demonstrated in the plasma and urine of humans and dogs receiving pamaquine. One of these is an unstable compound which can, *in vitro,* convert hemoglobin to methemoglobin and lyse erythrocytes. The other is a stable compound which is highly fluorescent. Neither of these substances has been isolated in crystalline form.

It is possible that the substance with the oxidant and lytic properties is responsible for the toxic actions of the parent drug. The answer as to whether it also accounts for its therapeutic effect awaits definition of its antimalarial activity.

A homologue of pamaquine which differs only in its substitution on the side chain, yields metabolic products with properties similar to those obtained with pamaquine.

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Histochemical Alterations Revealed by Tetrazolium Chloride in Hypertensive Kidneys in Relation to Renal VEM Mechanisms.* (18066)

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Tetrazolium salts provide a new tool for the study of metabolic activity of living cells (1-3). The compound used in the present

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study, 2,3,5-triphenyl tetrazolium chloride (TTC), is a colorless, water soluble substance which yields on reduction a deep red, water insoluble formazan. The intracellular reduction of this compound results from its interaction as a hydrogen acceptor with tissue dehydrogenases, the insoluble formazan being deposited locally in the cells in patterns which vary in different tissues. There is as well an excellent correlation between the oxygen consumption of tissue as measured by conventional Warburg technic and the extent of deposition of formazan. The formazan can be extracted from the cells by solvents such as acetone and measured quantitatively in a photoelectric colorimeter.

The present study is concerned with the histochemical appearance of kidneys of normal and hypertensive animals and human subjects after treatment of the tissues with TTC. Our findings indicate that a correlation exists between the microscopic pattern and the alterations in kidney VEM metabolism which occur in human and experimental renal hypertension. In view of the potential value of this method as an indication of the participation of a renal factor in different hypertensive syndromes, these studies have been extended to other hypertensive states, including that produced by DCA, and will be reported elsewhere.

In the normal kidney the renal vasoexcitor, VEM, is produced consistently by the cortex only under conditions of anaerobiosis(4). Under aerobic conditions no VEM formation is demonstrable and in addition an inactivation of its angiotropic properties occurs. The hypertensive kidney uniformly shows a specific alteration in VEM metabolism as a result of which VEM is formed under both aerobic and anaerobic conditions(5). This occurs *in vivo* as well as *in vitro*, the VEM being demonstrable in the renal vein and peripheral blood throughout the hypertensive syndrome. This metabolic defect is usually unaccompanied by any changes in overall oxygen consumption of the kidney. These alterations in renal VEM metabolism occur independently of any pathological changes demonstrable by the usual staining technics.

Materials and methods. The data here presented were obtained from normotensive and hypertensive rats, dogs and man. Hypertension was induced in the rat by perinephric caps on both kidneys, and in the dog by Goldblatt clamps on the renal arteries of both kidneys. Included in this series were kidneys from 6 control and 4 hypertensive rats, as well as 4 control and 3 hypertensive dogs. In the human subjects the renal cortical tissue consisted of biopsies obtained at operation from 2 hypertensives and 2 normotensives, and from 2 normotensive subjects very soon after death.

The tetrazolium studies were carried out as follows. Thin slices from the renal cortex were made as for microrespiration determinations and incubation performed within 15 minutes of receiving the specimen. To insure uniformity the sections were made at right angles to the long axis of the kidney. Two or 3 slices weighing on the average 150-200 mg were placed in an Erlenmeyer flask containing 5 ml physiological saline or Ringer phosphate (pH 7.4) and 0.5% TTC. The tissues were incubated at 37.5°C for 1 hour in air with constant shaking in the Warburg bath. Incubations were carried out with and without sodium succinate (0.4%), the latter substrate having been shown to intensify the TTC reduction and formazan deposition. The histochemical patterns were qualitatively similar with both solutions. At the end of 1 hour, the tissue slices were fixed in 10% neutral formalin. Frozen sections were made and mounted in glycerol for histochemical study. In accordance with the technic previously described, a portion of each set of slices was

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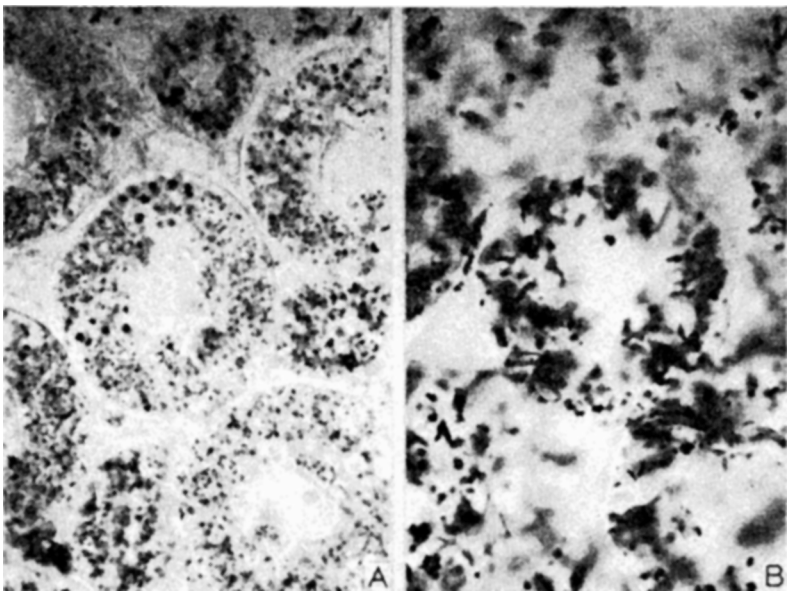

Normotensive Hypertensive

FIG. 1.

Kidney cortex of dog after incubation for 1 hour at 37.5°C in saline solution containing 0.5% tetrazolium (TTC) and 0.4% sodium succinate. Reduced TTC (formazan) deposited in cells. (a) Section from normotensive dog showing dust-like formazan granules in proximal convoluted tubules. (b) Section from hypertensive dog showing coarse plaques and clusters of formazan needles in proximal tubular cells. (300×).

extracted with acetone to remove the formazan for colorimetric determinations(6). The acetone extracted tissue was then dried in air and weighed to determine the color reading per mg of dried tissue. These values were related to oxygen consumption of slices from the same kidney as measured in Warburg manometers under similar substrate conditions.

Concurrent with the TTC studies, slices prepared from each kidney were examined for their ability to form VEM under aerobic and anaerobic conditions, utilizing the rat mesoappendix technic for the bioassay of VEM activity(7). The incubation procedures were as previously described(8).

Our previous studies have shown that prior anaerobic incubation of normal kidney tissue for 60 to 90 minutes will transform the *in vitro* VEM metabolism of the normal kidney to that of the hypertensive kidney. On subsequent restoration to oxidative conditions, the pretreated normal kidney now forms VEM in oxygen as well as in nitrogen. This procedure was also utilized in this study to determine whether this metabolic alteration might be associated with changes in the pattern of formazan deposition in these kidneys. Such experiments were carried out on the kidneys of 4 normal dogs, 1 normotensive human, and 6 normal rats.

Experimental results. (a) *Kidneys of Normal and Hypertensive Dogs.* The kidneys of normotensive dogs uniformly show a regular pattern of formazan deposition characterized by fine dust-like granules throughout the cytoplasm of cells of the proximal convoluted tubules (Fig. 1a). The nuclei remain unstained. The granules have a purplish cast and are almost entirely intracellular. Other tubular structures in the

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7. Zweifach, B. W., *Methods in Medical Research*, V. R. Potter, Editor, 1948, v1, 131, Year Book Publishers, Chicago.

8. Shorr, E., Zweifach, B. W., Furchgott, R. F., and Baez, S., *Circulation*, in press.

TABLE I.

Total Reduced Tetrazolium (Formazan) and Oxygen Consumption Measured by Warburg Technic. Formazan (reduced TTC) expressed as color units per mg of acetone dried tissue per hour. These values may be expressed in terms of micrograms of reduced tetrazolium by comparison with a standard curve, *e.g.*, 133 = 4.7 γ, 121 = 4.2 γ, 96 = 3.2 γ, etc. Q_{O_2} = cc/g/hr wet weight.

		Formazan		Q_{O_2}	
Group	No.	Mean	Range	Mean	Range
		Dog			
Control	4	121	60-148	1.9	1.6-2.3
Hypertensive	3	118	107-129	2.3	1.8-2.7
		Human			
Control	4	96	79-123	1.6*	—
Hypertensive	2	106	—	1.5	—
		Rat			
Control	6	133	115-150	3.1	2.9-3.3
Hypertensive	4	121	87-157	2.8	2.6-3.2

* 2 subjects.

cortex also take up the formazan but less intensively and with a different pattern. The formazan deposits in the collecting and distal convoluted tubules are needle-like or polyhedral and have a more reddish hue. The glomeruli show minimal or no staining.

The staining characteristics of the hypertensive kidney are strikingly different, especially in the proximal convoluted tubules (Fig. 1b). Formazan deposition is much coarser with angular crystals, coarse needles and plate-like deposits in these cells. There is a distinct tendency for the dye to deposit extracellularly as well. The crystals are usually deposited towards the base and borders of the proximal convoluted tubular cells and may appear in the lumen of the tubules. There are no appreciable differences in staining of glomeruli, distal convoluted and collecting tubules.

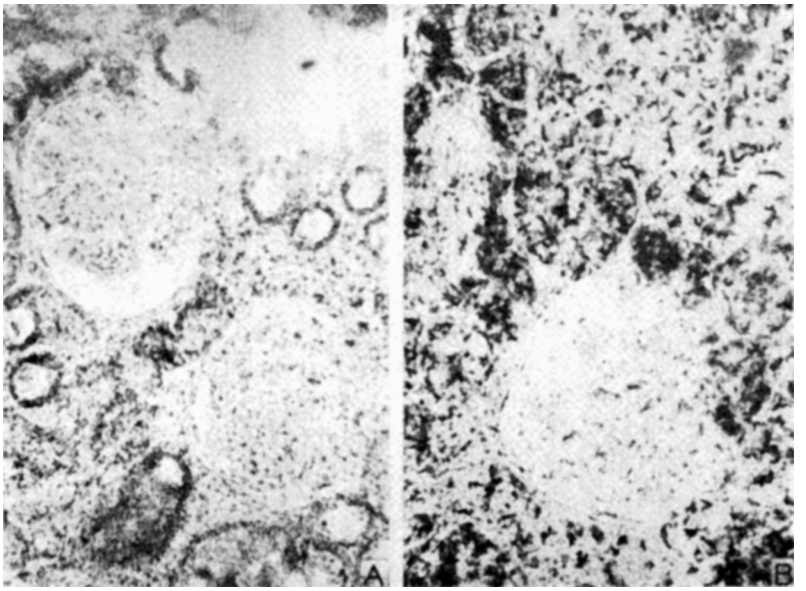
In spite of the differences in the staining pattern with TTC, the total amount of formazan per mg of tissue did not differ in normal and hypertensive kidneys, nor did the respiratory data reveal any significant differences between the oxygen consumption of the normal and hypertensive kidneys. (Table I) This was true for the kidney slices both in the presence and absence of succinate in the medium. An inference that can be drawn from the intensity of the stain in different parts of the kidney cortex is that maximal respiratory activity is localized in the proximal convoluted tubules.

Studies of the renal VEM mechanism gave results in conformity with our previous observations. VEM was formed by kidneys from normotensive dogs only under anaerobic conditions, whereas this vasoexcitor material was formed by the hypertensive kidney in approximately equal amounts under both aerobic and anaerobic conditions.

Thus, there is a parallelism between the disorganization in the proximal convoluted tubules of the hypertensive dog kidney indicated by the altered formazan pattern and the metabolic derangement as a result of which the hypertensive kidney loses its ability to restrict VEM formation to the anaerobic state. The possibility of a direct relationship between these two disturbances is under present investigation.

(b) *Kidneys from Normotensive and Hypertensive Human Subjects.* The same types of observations were carried out on biopsy specimens of renal cortex from kidneys of 4 normotensive and 2 hypertensive subjects. In one of the hypertensive subjects the occasion for surgery was a sympathectomy; in the other, an associated hydronephrosis. Of the 4 normotensive kidneys, 2 were obtained at operation, the specimens chilled immediately on removal and incubated within 30 minutes. The remaining 2 normotensive kidneys were obtained shortly after exitus. With these latter kidneys Warburg respiratory and VEM measurements were not carried out.

The results corresponded in every respect with those previously described for the dog. It is of interest that in both the hypertensive dog and human not all of the proximal convoluted tubules exhibited an alteration in the normal granular type of formazan deposition. In the dog, about 80% of the tubules were involved; in the human, about 60-70%. Sections of the normal and hypertensive human kidney under low power magnification (67×) are shown in Fig. 2 to illustrate the type of formazan distribution characteristic of both conditions. Under higher magnification the details are indistinguishable from



Normotensive Hypertensive

FIG. 2.

Human kidney cortex after incubation for 1 hour at 37.5°C in saline solution containing 0.5% tetrazolium (TTC) and 0.4% sodium succinate. (a) Normotensive patient; dust-like deposition of formazan; glomerulus unstained. (b) Patient with essential hypertension; coarse, heavy deposition of formazan in proximal convoluted tubules. Note tendency for dye to be deposited extracellularly as well. (67×).

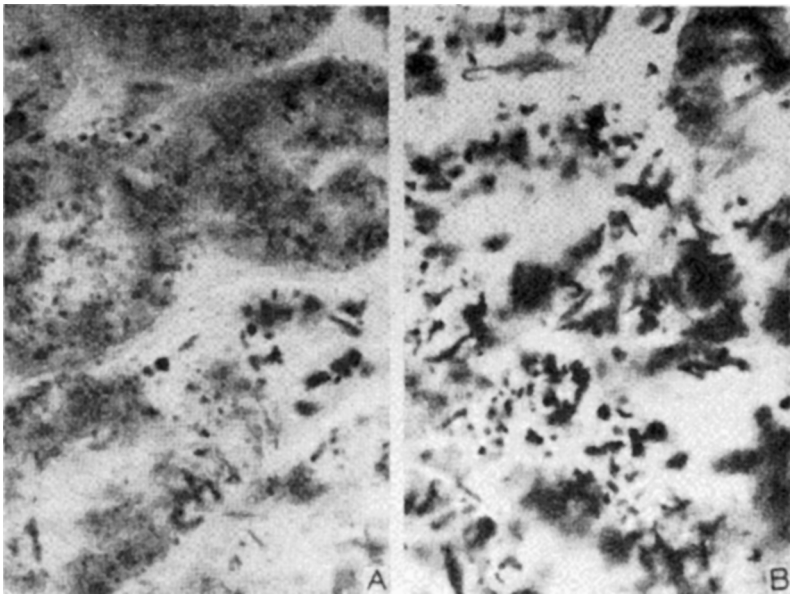
those seen in the dog in Fig. 1.

VEM production took place in both the hypertensive kidneys under aerobic and anaerobic conditions. The kidneys from normotensive patients formed VEM only under anaerobic conditions. The biopsy obtained from the hydronephrotic infected kidney exhibited considerable interstitial inflammatory reaction and had a low oxygen consumption. The other from the patient undergoing sympathectomy presented a normal histologic picture by the usual staining method and oxygen consumption was in the normal range for human kidney cortex. The total formazan content of the hypertensive kidney was also of the same order as that for kidneys from normotensive patients, despite a difference in the staining pattern visible under the microscope.

A biopsy from one of the normotensive kidneys came from an area adjacent to an infected cyst and showed an extensive interstitial inflammatory reaction. Nevertheless, the formazan pattern and VEM formation were of an entirely normal character.

(c) *Kidneys from normal and hypertensive rats.* The results on the kidneys of normal and hypertensive rats corresponded closely with those obtained in the dog and human. One precaution, prompt chilling and incubation, should be observed in carrying out these studies on the rat, in view of the greater susceptibility of rat kidney to anaerobiosis and of the consequent development of the metabolic and histologic alterations to be described.

(d) *Alterations in VEM metabolism and TTC staining of normal kidneys after anaerobic exposure.* We have previously demonstrated that after a prior exposure to anaerobiosis *in vitro* the normal kidney behaves like a hypertensive kidney in that it continues to form VEM when re-exposed to oxidative conditions(8). It was of interest to determine whether this metabolic derangement was accompanied by the development of formazan deposition similar to that observed in hypertensives. These experi-



Aerobic control After 90 min., anaerobiosis

FIG. 3.

Kidney cortex of dog before and after pretreatment under anaerobic conditions for 90 minutes. Treated with TTC and succinate as in previous illustration. (a) Usual appearance of formazan deposits in cells of proximal tubules; fine, dust-like and uniform. (b) After kidney has been pretreated under N_2 and returned to acrobic conditions. Formazan deposition transformed to pattern like that in hypertensive kidneys—coarse plaques, heavy needles. (300×).

ments were carried out on kidneys from 4 normal dogs, 1 normotensive human and 6 normal rats. In all instances the development of aerobic VEM formation was accompanied by the development of the coarse, plaque-like deposition of formazan crystals in the proximal convoluted tubules as illustrated in Fig. 3. This is indistinguishable from the pattern in the kidney of the hypertensive animal or human. The period of anaerobic exposure required for this transformation was 45 minutes for the rat, and 60-90 minutes for the human and dog. No differences in formazan deposition could be distinguished in the other structural units of the kidney.

The parallel appearance of an altered formazan pattern in the proximal convoluted tubules and of the derangement in VEM metabolism after prior anoxia suggests that the proximal convoluted tubules may be the site of VEM formation. A further extension of this inference would be that this defect in VEM metabolism might be related to the derangements in cellular intermediary metabolism of which the formazan deposition is a reflection. It should be mentioned that occasionally some of the proximal convoluted tubules in kidneys from normotensive subjects may show deviations from the normal type of formazan deposition. However, in no case of hypertension was the tubular pattern of formazan deposition normal.

Summary and conclusions. The incubation of slices of renal cortex with tetrazolium chloride (TTC) has disclosed patterns of formazan deposition in the proximal convoluted tubules with striking differences between normal and hypertensive kidneys obtained from dogs, rats and man. In the proximal convoluted tubules of kidneys from normotensive animals and man, the formazan is deposited as fine dust-like granules evenly distributed within the cytoplasm. In the hypertensive kidney the formazan is deposited in coarse clumps, plaques or needles, commonly at the cell margins and often extracellularly as well.

Despite the difference in formazan patterns the total amount of reduced TTC per mg of kidney tissue is approximately the same in normal and hypertensive kidneys, as is the oxygen consumption determined by the conventional micro-respiration technic. Concurrent studies of VEM metabolism show the uniform restriction of VEM production to anaerobiosis in normal kidneys, and the formation of VEM under both anaerobic and aerobic conditions by the hypertensive kidneys of animal and human subjects. When the VEM metabolism of a kidney from a normotensive rat, dog or human is transformed to the hypertensive type by a period of prior anaerobiosis *in vitro,* there is a parallel development of the characteristic hypertensive formazan pattern in the proximal convoluted tubules.

The reduction of TTC to the insoluble formazan results from an interaction between TTC and enzyme systems within the cell. The observations reported would indicate some alteration in the metabolic characteristics of the proximal convoluted tubules in experimental renal and essential hypertension. The association of these histochemical changes in the proximal convoluted tubules with alterations of the VEM mechanisms in experimental and human hypertension suggests, 1) that the proximal tubules may be the site of VEM formation, and 2) that the failure of the hypertensive kidney to limit VEM formation to anaerobiosis may be related to the enzymatic disorganization revealed by TTC.

Finally, the data presented provide specific evidence for a similarity between experimental renal hypertension and essential hypertension in man.

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Procarcinogenic Effect of Vitamin B_{12} on p-Dimethylaminoazobenzene-Fed Rats.* (18067)

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Diet is known to influence the carcinogenicity of p-dimethylaminoazobenzene (DAB). Riboflavin has been shown to exert a protective effect(1) while it has been reported that biotin is procarcinogenic(2). The experiments to be reported in this paper indicate that vitamin B_{12} exerts a marked procarcinogenic effect on DAB-fed rats.

Experimental. Weanling female Sprague-Dawley rats were fed a purified diet consisting of the following: isolated soybean protein,† 18 g; sucrose, 67.4 g; hydrogenated vegetable oil, 8 g; cod liver oil, 2 g; salt mixture(3), 4 g; choline chloride, 0.1 g; inositol, 10 mg; thiamine chloride, 1.5 mg; riboflavin, 0.5 mg; nicotinamide, 2 mg; calcium pantothenate, 1.0 mg; pyridoxine hydrochloride, 0.5 mg; vitamin K, 0.025 mg; biotin, 0.005 mg; folic acid, 0.5 mg; and p-dimethylaminoazobenzene, 70 mg. This diet contains approximately 0.27% methionine. One group of rats received this diet unsupplemented, one group received this diet plus 5 micrograms of vitamin B_{12} (Rubramin) per 100 g, a third group received this diet plus 0.6% DL-methionine, and a fourth group received the

* Research paper No. 910, Journal Series, University of Arkansas. This investigation was supported in part by a research grant from the National Institutes of Health, Public Health Service.

† "Alpha Protein," obtained from the Glidden Company, Chicago.

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