TABLE III. Eight Groups of Cockerels Fed .5% Cholesterol Atherogenic Diet—8 Weeks Experimental Period—With *Severe* Atherosclerotic Plaque Development.

Series 3 (57 birds)		Blood serum cholesterol				
		Not incubated		Incubated 30 hr at 37 to 38°C		
	Atherosclerotic plaques (% area)	Total (mg %)	Free (mg %)	Free to combined form (mg %)	Free remaining (mg %)	% original free remaining after incubation
	14.1	219	59.5	25.8	33.7	56.6
	20.1	297	76.1	26.5	49.6	65.2
	19.6	416	104.6	35.1	69.5	66.4
	10.8	325	82.0	24.6	57.4	70.0
	26.8	314	84.5	21.0	63.5	75.1
	18.0	456	121.7	23.7	98.0	80.5
	19.1	396	102.1	14.7	87.4	85.6
	16.6	328	90.2	11.8	78.4	86.9
Mean values	18.1	344	90.1	22.9	67.2	73.3
Range	10.8–26.8	219–456	59.5–121.7	35.1–11.8	33.7–98.0	56.6–86.9

atherosclerotic birds is 9, 14, and 67 mean mg % respectively. Amounts combined upon incubation show smaller differences (17, 13, and 23 mean mg %) for the 3 series. Corresponding ranges of per cent original free cholesterol not combined are 17 to 45 for normal, 45 to 56 for mild spontaneous, and 57 to 87% for severe cholesterol-induced atherosclerosis. Results of this study indicate that there is a relationship between serum free cholesterol not combined upon incubation and development of atheroma in young cockerels.

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Mitochondrial α-Glycerophosphate Dehydrogenase Activity of Juxtaglomerular Cells in Experimental Hypertension and Adrenal Insufficiency. (26514)

R. HESS AND A. G. E. PEARSE (Introduced by D. G. Scarpelli)
Department of Pathology, Postgraduate Medical School, London, England

It has been established in both man and experimental animals that the juxtaglomerular (JG) cells are related to formation of extractable renin in the kidney, that they respond to alterations of sodium metabolism and, possibly, participate in mechanisms maintaining systemic arterial pressure. The physiological state of these cells, which form part of the preglomerular arterioles, is usually assessed by estimating the amount of stainable cytoplasmic granules(1), which are believed to be secretory in nature and may contain renin (2). The type of metabolic pathway operating in JG cells is unknown.

Using a histochemical technic, mitochondrial α-glycerophosphate dehydrogenase (GPD, Meyerhof-Green enzyme) can be demonstrated to be particularly active in the JG cells. This activity changes in the same direction as renin content in both experimental hypertension and adrenal insufficiency and seems to indicate one of the major metabolic steps which govern the functional state of the juxtaglomerular complex.

TABLE I. α-Glycerophosphate Dehydrogenase Activity* of Rat Kidney Mitochondria.

Addition	Cortex	Medulla
None	.37	.72
Amytal (5×10^{-3}M)	.36	.70
Menadione (10^{-4}M)	1.50	4.10
" + amytal (5×10^{-3}M)	1.44	3.90
" + antimycin A, 10 μg/ml	1.53	4.30
" + dicoumarol (10^{-5}M)	.12	.24

* Expressed as μg of formazan/mg protein/min. (mean values of 2 exp.).

Materials and methods. A first group of 11 male albino rats (mean initial weight 158 g) were rendered hypertensive by unilateral clamping of the renal artery. Their blood pressure increased from 120 ± 2.9 mm Hg to 184 ± 7.4 mm Hg 36 days after operation. A second group of unilaterally nephrectomised animals (mean initial weight 106 g) were overdosed with desoxycorticosterone acetate (DOCA) and sodium chloride; after 36 days of treatment, systolic pressure had risen from 96 ± 1.8 mm Hg to 145 ± 5.1 mm Hg. Details of the experimental procedure have been reported(3). A comparable group of 6 animals were adrenalectomised and put on a sodium deficient diet for 7 days. Untreated animals served as controls.

For histochemical examination, 5 to 8 μ fresh frozen cryostat sections were used from slices of the middle parts of the kidneys which had been quenched in liquid oxygen. The sections, mounted on coverslips, were incubated for 30 minutes at 37° in a medium containing substrate, 10^{-1}M, (sodium L-α-glycerophosphate); 2-methyl-1,4-naphthoquinone (menadione), 10^{-4}M; tris (hydroxymethyl) aminomethane-HCl buffer, 5×10^{-2} M, pH 7.4; 3-(4,5-dimethyl thiazolyl-2) 2,5 diphenyl tetrazolium bromide (MTT), 5×10^{-3}M; $CoCl_2$, 10^{-3}M. For demonstration of lactate and malate dehydrogenase activity, menadione was omitted and the sodium salt of L-lactic acid or L-malic acid was used as substrate, in presence of diphosphopyridine nucleotide (DPN), 5×10^{-3}M.

In vitro, mitochondria from cortex and medulla of normal rat kidneys isolated in 0.25M sucrose and frozen-thawed, were incubated in the α-glycerophosphate medium at 37° and the cobalt-formazan formed was measured spectrophotometrically at 660 mμ in ethyl acetate.

Results. The *in vitro* effect of some activators and inhibitors on specific GPD activity of cortical and medullary mitochondria is shown in Table I. Menadione is a more efficient primary electron acceptor than tetrazolium in this system and formazan production is increased 4- to 5-fold in its presence. Hydroquinone reduces tetrazolium non-enzymically(4). GPD is highly sensitive to dicoumarol but, in contrast to the menadione-dependent GPD of rat brain(5), it is not inhibited by amytal.

Distribution of GPD in normal rat kidney. High activity was found in the tubules of the outer medulla (descending and ascending limb) and in the cortical collecting ducts. Moderately strong activity was displayed by JG cells, distal convolutions (including the macula densa segment), vascular endothelium and medullary collecting duct epithelium. Both glomerular epithelium and interstitial cells were weakly active. No reaction occurred in the proximal convolutions or in vascular smooth muscle.

Renal hypertension. Five weeks after unilateral clamping of the renal artery, GPD activity was strongly enhanced in JG cells (Fig. 1). In the untouched contralateral kidney JG-cell activity was lower than normal and in some areas, decreased to zero (Fig. 2). These changes were more pronounced in outer cortical areas than in zones adjacent to the medulla.

DOCA and saline-induced hypertension. JG cells throughout the cortex were very low in activity and in many areas no GPD reaction could be obtained.

Adrenal insufficiency. Seven days after adrenalectomy an increased activity of GPD was observed in JG cells throughout the cortex. The appearance of the cells in adrenal insufficiency was very similar to that found in the ischemic kidney of animals bearing a Goldblatt clamp. In contrast to JG cells, GPD activity in the other renal structures was not significantly altered by the experimental procedures. After DOCA-saline overdosage, GPD activity was increased in col-

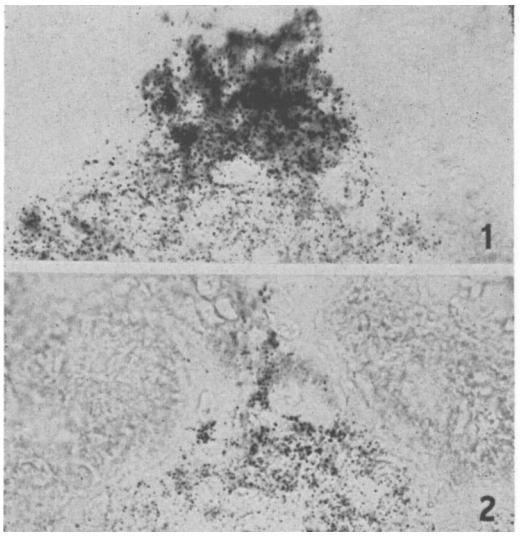

FIG. 1. High activity of mitochondrial GPD in JG cells (ischemic kidney from a rat bearing a Goldblatt clamp). 580 ×.

FIG. 2. Same animal as Fig. 1. Low GPD activity in JG cells of unclamped kidney. 580 ×.

lecting duct epithelium of the papilla in about half the animals tested.

Lactate and malate dehydrogenase activity in JG cells, in muscle cells of the afferent arteriole, and in cells of the glomerular tuft, was increased in areas showing hypertrophic vascular changes in hypertensive animals. However, no deviation from normal activity occurred in adrenal insufficient rats.

Discussion. McManus' concept that JG cells and the macula densa segment of the distal tubule form a functional unit (*viz.* the "juxtaglomerular complex")(6) is well supported by observations on the relationship of enzymic activity present in these structures. Although a different enzyme system is shown to prevail in the two parts composing the JG complex (glucose-6-phosphate dehydrogenase (G6PD) in macular cells(3,7,8) and mitochondrial GPD in JG cells), both kinds of activities have been demonstrated to change in the same way as renin content.

Metabolic activity of the macular segment seems to a large extent to depend on the function of the hexosemonophosphate (HMP) shunt which, by supplying reduced triphosphopyridine nucleotide, is linked to biological synthetic systems(9). The non-coenzyme linked GPD(10) on the other hand, participates in the α-glycerophosphate cycle(11) which, in certain tissues (insect flight muscle) provides the main energy-yielding mechanism. Histochemical proof for the existence of the complete GP-cycle is lacking, however, since we found it impossible to separate DPN-dependent cytoplasmic GPD activity (von Euler-Baranowski enzyme) from its mitochondrial counterpart by means of tetrazolium reduction. The equilibrium of the cytoplasmic GPD is shifted far toward GP(12). It may be significant that changes in GPD activity in JG cells were not accompanied by similar alterations of lactate dehydrogenase. Dissociation of these two activities together with low levels of lactate dehydrogenase has been found to occur in insect flight muscle biochemically(13) and histochemically (Hess and Pearse, unpublished). Furthermore, it is unlikely that the glycogen cycle is operating in JG cells. We were unable to demonstrate glucosan phosphorylase activity(14) in them, in contrast to muscle cells of the afferent arterioles which were strongly active.

If we postulate that triose phosphate isomerase is present in the JG complex, then glyceraldehyde-3-phosphate generated by the HMP pathway(9) could provide the dihydroxyacetone phosphate necessary for operation of the GP-cycle. A metabolic pathway of this sort might represent a biochemical link between the two major parts of the JG complex which have been shown recently to be in intimate communication by means of membranous structures(15).

Summary. Non-coenzyme linked α-glycerophosphate dehydrogenase activity has been demonstrated histochemically in juxtaglomerular cells of rats. Menadione, which increased the activity of rat kidney mitochondria 4- to 5-fold *in vitro,* was required as intermediate electron carrier when a thiazol substituted monotetrazole was used as final H-acceptor in tissue sections. α-Glycerophosphate dehydrogenase activity of juxtaglomerular cells increased both in ischemic kidneys from renal hypertensive rats and in kidneys from adrenal insufficient animals. Decrease in activity of juxtaglomerular cells was noted both in untouched kidneys from ani-

mals bearing a unilateral arterial clamp and in the renal cortex of rats overdosed with DOCA on a high sodium diet.

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Trachoma Viruses Isolated in the United States.* 4. Infectivity and Immunogenicity for Monkeys. (26515)

CHANDLER DAWSON, ERNEST JAWETZ, PHILLIPS THYGESON AND LAVELLE HANNA
Francis I. Proctor Foundation for Research in Ophthalmology and Departments of Ophthalmology and Microbiology, University of California, Medical Center, San Francisco

Monkeys and apes have been the only laboratory animals useful in trachoma research. Baboons and apes have been more susceptible to experimental infection than the lower primates, but even in these animals the experimental disease, produced irregularly by transfer of conjunctival scrapings, has been equivocal—at best a mild follicular conjunctivitis without inclusions. The primate eye has shown more reaction to transfer of material from the closely related disease, inclusion conjunctivitis, developing an experimental disease often acute in onset and more closely resembling the human disease(1).

With egg-grown trachoma virus it became possible to produce regularly in rhesus and cynomolgus monkeys a clear-cut clinical disease with an exudate presenting a characteristic cytologic picture and with inclusion bodies in the conjunctival epithelial cells. It was soon apparent that isolates differed in their pathogenicity for these monkeys, and we have reported experiments on the *qualitative* aspects of this difference as displayed by 2 strains of trachoma virus isolated in the United States(2,3). This report deals with attempts to *quantitate* the infectivity of these strains and to induce resistance to challenge infection.

Materials and methods. Viruses: Three strains of trachoma virus(4) and one strain of inclusion conjunctivitis virus(5) isolated in this laboratory were employed. Several yolk sac passage levels of each strain with the following egg LD_{50} titers (ELD_{50}/ml) were used: Strain BOUR, YS3 to YS12, $10^{4.4}$ to $10^{6.4}$ ELD_{50}/ml; Strain ASGH, YS4 to YS8, $10^{5.8}$ to $10^{7.4}$ ELD_{50}/ml; Strain APACHE #1, YS8, $10^{6.4}$ ELD_{50}/ml; Strain IC-Cal 1 of inclusion conjunctivitis virus, YS9, $10^{5.6}$ ELD_{50}/ml. Stock virus was prepared, stored and titrated as described(6).

Monkey inoculations. For eye challenge, stock virus was diluted in skim milk. Small cotton swabs were soaked in the inoculum and rubbed back and forth 10 times in the upper and lower conjunctival fornices. An

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