by Franklin *et al*(9). However, this should not necessarily imply that the AB, *per se*, constitutes the CF antigen. To the contrary, antigens prepared by this method contained little or no AB, as revealed by electron microscopy of suspensions of such preparations. Examination of these antigens again revealed only amorphous stromatal material. These results indicate that the CF antigens may reside either on the outer matrix of the AB, which is stripped away during the purification process, or elsewhere in or on the infected erythrocyte. Ristic and Kreier(10) have reported that a capillary agglutinating (CA) antigen, prepared from *Anaplasma*-infected erythrocytes(11) consists of structures resembling the subunits (initial bodies) of *Anaplasma* marginal inclusion bodies. They likewise reported an outer envelope covering the marginal bodies. It is this outer matrix which apparently constitutes the CF antigen previously purified(1). The individual subunits do not appear to possess appreciable CF antigenicity.

A previous report(1) including the results of studies on partial and total lipid extractions of the CF antigen suggested that the CF antigen was a lipoprotein. Results presented herein confirm the earlier observation.

Summary. A CF antigen for *Anaplasma marginale* was prepared by differential centrifugation of the sonic lysate of infected bovine erythrocytes. Studies were conducted to determine the chemical and physical nature of the antigen. Qualitative biochemical tests and enzyme sensitivity studies indicated the antigen to be lipoprotein in nature. Electron microscopic observations showed that the preparation was not composed of *Anaplasma* bodies but of amorphous membranous material which probably constitutes the outer coating of the AB. Filtration studies were conducted and observations made on the effect of temperature on the antigen.

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Renal Vascular Responses to Drugs in Experimental Nephrosis in Rats. (30626)

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Animal models of nephrosis are characterized by morphologic abnormalities of glomerular epithelial cell foot processes and basement membranes as well as edema, proteinuria, hypoproteinemia and hyperlipemia(1-3). These symptoms are also typical of the human disease(2-5). Experimental models can be divided into two major groups: a) those produced by various antibodies to kidney proteins and b) those induced by chemical agents, *e.g.*, puromycin aminonucleoside (4,5). In view of the marked histological and

TABLE I. Body Weight, Urinary Protein and Hemodynamic Data Derived from Control and Nephrotic Rats.

	Nephrotoxic serum nephrosis		Aminonucleoside nephrosis	
	Control	Nephrotic	Control	Nephrotic
Body wt (g)	341 ± 7	309 ±17	282 ±15	265 ±12
Urinary protein (mg/18 hr)	8 ± 1	212 ±32 *	11 ± 2	201 ±31 *
Systemic blood pressure (mm Hg)	123 ± 5	124 ± 5	125 ± 4	110 ± 4 *
Perfusion pressure (mm Hg)	99 ± 2	102 ± 3	106 ± 3	101 ± 3
Renal blood flow (ml/min)	2.3 ± .3	1.8 ± .2	1.7 ± .1	1.8 ± .3
Resistance (pressure/flow)	47 ± 6	61 ± 8	65 ± 6	65 ± 7

* Significantly different from control; $P < .05$.

functional changes which occur in nephrotic rats, responses of the renal vasculature were investigated in these animals.

Methods. Nephrotoxic serum nephrosis was produced in one group of male Sprague-Dawley rats by intravenous (i.v.) injection of anti-rat kidney serum as described by Heymann *et al*(3,6). The serum containing heteronephrotoxic antibodies was obtained from rabbits after repeated intraperitoneal injections of a rat kidney homogenate. Rats received one-half of the dose of serum on 2 successive days. In a second group, nephrosis was induced by a single i.v. injection of an aminonucleoside of puromycin [6-dimethylamino-9-(3-amino-3-deoxy-D-ribofuranosyl)-purine] (Nutritional Biochemicals Corp.) at a dose of 100 mg/kg(7).

Two days prior to the experiments, rats were given 25 ml/kg of water and urine was collected for the next 18 hours. Urinary protein excretion was used as the criterion of nephrosis(3). Protein concentration was determined by the method of Kingsbury *et al* (8) as adapted for the photoelectric colorimeter(9). Rats with nephrotoxic serum and aminonucleoside nephrosis were used 6 to 10 and 14 to 18 days after treatment, respectively.

Animals were anesthetized with Dial-urethane solution* (0.65 ml/kg intraperitoneally). The aorta was cannulated above the renal arteries and blood was pumped (Sigmamotor pump model T8) at constant flow into a second cannula placed in a retrograde direction into the aorta below the renal arteries. All aortic branches between the cannulae, except the renal arteries, were ligated. Blood obtained from donor animals was used to fill the tubing. Systemic blood pressure and renal perfusion pressure were recorded from T-tubes located proximally and distally to the pump, respectively, by Statham pressure transducers and an Offner oscillograph. The output of the pump was adjusted to yield a perfusion pressure of approximately 100 mm Hg and flow remained at this level for the remainder of the experiment. Changes in perfusion pressure were thus proportional to changes in vascular resistance, which was calculated by dividing perfusion pressure (mm Hg) by flow (ml/min). Heparin (10 mg/kg) was used as the anticoagulant. Drugs were injected intra-arterially (i.a.) into the tubing between the pump and the distal cannula in volumes of 2 to 9 μl. Low doses of drugs were administered in random order followed by random injection of the higher doses.

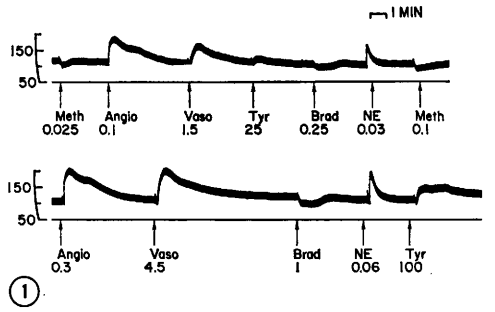
Each group consisted of 8 animals. Two control groups were included because the two series of nephrotic rats were not run concurrently. Data are presented as means ± standard errors. The Student t-test was used to determine statistically significant differences.

Results. Nephrotic rats excreted about 20 times as much protein during an 18-hour period as did controls (Table I, $P<0.05$). Systemic blood pressure was significantly reduced in the group with aminonucleoside nephrosis (Table I, $P<0.05$) but not in the nephrotoxic serum nephrosis group. However, neither type of nephrosis significantly altered renal blood flow or renal vascular resistance at the given perfusion pressure ($P>0.05$).

Intra-arterial injection of various vasoconstrictor and vasodilator agents at submaximal doses caused prompt changes in perfusion pressure (Fig. 1). Norepinephrine, angioten-

* Ciba Pharmaceutical Company. Each ml contains allobarbital 100 mg, urethane 400 mg and monoethylurea 400 mg.

sin (Hypertensin, Ciba) and vasopressin were capable of causing marked increases in perfusion pressure, *i.e.*, mean of over 100 mm Hg. The renal vasculature responded to relatively low doses of these agents but was comparatively insensitive to tyramine. The dilator agents methacholine and bradykinin (Calbiochem) caused only small decreases in perfusion pressure (mean of less than 30 mm Hg, Fig. 2 and 3). Little if any change in systemic blood pressure was observed as a consequence of intra-arterial drug injection.



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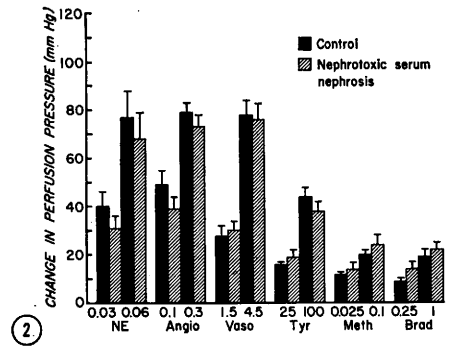

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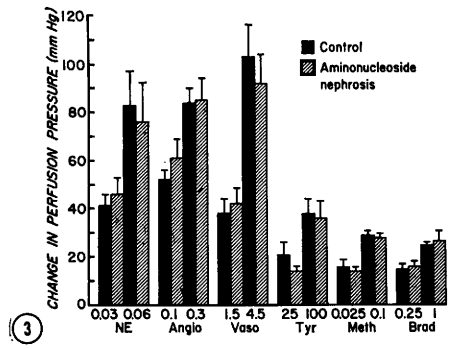

(3)

FIG. 1. Response of renal vasculature to intra-arterial injection of methacholine, angiotensin, vasopressin, tyramine, bradykinin and norepinephrine in a control rat. Renal perfusion pressure is expressed as mm Hg. All doses are in μg, except vasopressin which is in mu.

FIG. 2. Renal vascular response to intra-arterial injection of drugs in control and nephrotoxic serum nephrotic rats. Bars represent mean responses and vertical lines above the bars the standard error. Units as in Fig. 1. Norepinephrine, angiotensin, vasopressin and tyramine increased perfusion pressure and methacholine and bradykinin lowered perfusion pressure.

FIG. 3. Renal vascular response to intra-arterial injection of drugs in control and aminonucleoside nephrotic rats. Units as in Fig. 1.

Rats with either type of nephrosis responded to constrictor and dilator drugs in a manner similar to control animals. Thus, the magnitude of the effects produced in the 2 nephrotic groups did not differ significantly ($P>0.05$) from their respective controls (Fig. 2 and 3). The duration of the responses was also approximately the same in control and treated groups.

Discussion. In both models the 18-hour urinary protein excretion rate was about the same and approximately 20 times as great as in the control groups. Responses to 2 dose levels of a variety of vasoactive drugs including norepinephrine, pressor and dilator polypeptides (angiotensin, vasopressin and bradykinin), methacholine and tyramine were tested. Despite the severity of the disease, the vascular effects of all drugs investigated were similar in the nephrotic groups and their respective controls. Renal vascular resistance at the selected perfusion pressure was also unaltered.

Large responses (mean of up to 100 mm Hg) were obtained to norepinephrine, angiotensin and vasopressin in control and treated groups. However, tyramine exerted weak activity, *i.e.*, not only were large doses required but the magnitude of the vasoconstriction was relatively small. The small dilator responses to methacholine and bradykinin indicate that the kidney possesses little basal vascular tone.

Summary. The response of the renal vasculature to various intra-arterially administered drugs was determined in rats with nephrotoxic serum nephrosis and aminonucleoside nephrosis. Norepinephrine, angiotensin, vasopressin, tyramine, methacholine and bradykinin caused similar effects in control and nephrotic groups. Renal vascular resistance was also unaffected by the disease.

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Assessment of Vitamin B_6 Nutriture in Pregnancy in the Rat.* (30627)

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Assessment of vitamin B_6 nutriture in pregnancy in the rat. Erythrocyte transaminase activities, both glutamic-oxalacetic (GOT) and glutamic-pyruvic (GPT), have been shown to be suitable parameters for assessment of vit B_6 nutriture in the male rat(1-3). Long-term studies(3) of the vit B_6 requirement of male rats suggested that the apparent requirement as judged by these criteria was slightly higher than that based on the criterion of maximal body weight gain, the most widely accepted criterion of nutriture in animal studies. Since body weight gain cannot be used as a criterion of vit B_6 adequacy in pregnancy and since the interpretation of xanthurenic acid (XA) excretion following a tryptophan load has been questioned(4), it seemed desirable to investigate the application of the erythrocyte transaminase activities to the assessment of vit B_6 nutriture during pregnancy. Accordingly, the response of erythrocyte transaminase activity to varying doses of vit B_6 was determined in pregnant rats and compared with the response of the tryptophan load test and the response of the hepatic transaminases. Hepatic GPT activity has been shown to be sensitive to vit B_6 nutriture(5) but to be decreased by the state of pregnancy(6).

Materials and methods. In Experiment 1, 23 pregnant and 21 nonpregnant female Wistar rats (average weight, 189 g) were divided into 3 groups. These received 16, 80, and 400 μg pyridoxine · HCl in daily oral doses from day 0 to day 18 of pregnancy. These doses were selected to approximate (i) the suggested National Research Council recommendation(7), (ii) the level considered adequate on the basis of earlier studies in the male(3), and (iii) a dose well in excess of both. A 20% casein, 10% corn oil diet lacking in vit B_6† was fed *ad libitum*. The animals were killed on day 19 of pregnancy by which time no deliveries had occurred.

In Experiment 2, 48 pregnant and 43 control rats (average weight 187 g) were divided into 5 groups and given daily oral doses of 15, 30, 60, 90, and 120 μg pyridoxine · HCl. Feeding began on day 2 of pregnancy and continued to day 20, a total of 19 days. The tryptophan load test was carried out on days 18 and 19 in the pregnant rats and days 17 and 20 in the non-pregnant animals. The rats were given, by stomach tube, a solution of L-tryptophan which provided 0.5 mg trypto-

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† Diet composition per 100 g: vitamin-free casein, 20 g; sucrose, 64.5 g; corn oil, 10 g; salts mixture,‡ 3.5 g; Alphacel, 2.0 g; thiamine • HCl, 0.5 mg; riboflavin, 1.0 mg; nicotinamide, 5.0 mg; calcium pantothenate, 2.0 mg; *p*-aminobenzoic acid, 2.0 mg; folic acid, 0.1 mg; biotin, 0.1 mg; vitamin B_{12}, 2 μg; choline, 200 mg; inositol, 200 mg; menadione, 0.1 mg; α-tocopherol, 6.0 mg; vit. A, 3000 IU; vit. D, 750 IU.

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