mal with the protective nuclei fractions used by Cole *et al.* They used differential centrifugation in sucrose to obtain their protective nuclei fractions. The possibility that cells could be implanted in the present study is eliminated by the fact that all protective extracts were sterilized by both Seitz and Millipore filtration.

Cole and Ellis (8) reported that the protective activity of nuclei fractions was destroyed by DNase and trypsin. The exact chemical nature of the protective fraction in this study is unknown.

Summary. Fractions of Ellinger's cell-free splenic extract administered following a lethal X-ray exposure resulted in 94% survival. This increased survival is greater than has previously been reported with any cell-free preparations given after radiation exposure. The protective agent was found in the second protein peak obtained by gel filtration with Sephadex G-200. The molecular weight of the protective fraction as determined by gel filtration was approximately 70,000. The relative degree of protection induced by this cell-free factor decreased with increasing X-ray doses.

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Specificity of the Inhibitory Effect of "Uremic" Serum on *p*-Aminohippurate Transport* (32674)

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Several recent reports have demonstrated that addition of uremic serum from human patients with diseased kidneys or from bilaterally nephrectomized rats inhibits the *in vitro* accumulation of *p*-aminohippurate (PAH) by slices of rat renal cortex or isolated fragments of rabbit renal cortex (1–3). These data raised two important questions that required answering before a complete and thorough interpretation of these results could be made. First, preliminary work in our laboratory demonstrated that if rats were fasted for 24 hours prior to sacrifice and preparation of *in vitro* slices, the ability of these slices to accumulate PAH was significantly impaired. Inasmuch as bilaterally nephrectomized rats would not be expected to eat as much as normal rats, could the effect of nephrectomized serum on PAH accumulation merely be due to the fasting state of the donor animals rather than to the uremia produced by nephrectomy? Second, is the effect of nephrectomized serum on PAH accumulation specific for organic anion transport or is this a general depressant effect upon other functions of the *in vitro* slices as well? An attempt was made to answer these questions by measuring the simultaneous uptake of PAH and the organic cation *N*-methylnicotinamide (NMN). Experiments were first conducted on renal cortical slices taken from fasted and nonfasted rats. Subsequent experiments were then conducted by using renal cortical slices from nonfasted rats and determining the effect of addition of serum from nephrectomized or sham-nephrectomized rats on the uptake of PAH and NMN by these slices.

Methods. Male Sprague-Dawley Rats,

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weighing 150–200 gm, were used in all experiments. Animals were stunned by a blow to the head, and the kidneys were removed. Renal cortical slices (prepared free-hand) from any one animal were randomly distributed between two beakers, each containing 3 ml of Cross-Taggart medium (4) containing PAH, 7.4×10^{-4} *M*, and NMN-^{14}C, 1 μg/ml (7.7×10^{-3} μC/ml).[1] Slices were incubated in a Dubnoff apparatus for 1 hour at 25°C under an atmosphere of oxygen. After incubation the tissue was removed from the beakers, quickly blotted, and weighed. A 2-ml aliquot of medium was taken from each beaker. To both tissue and media 3 ml of 10% trichloroacetic acid was added, and after the tissue was ground, the samples were diluted to 10 ml with water. One ml of this final diluted sample was used for colorometric assay of PAH (5). Similarly, 1 ml was added to 10 ml of fluor reagent for the determination of NMN-^{14}C. Radioactivity in medium and tissue samples was determined in a Beckman DPM-100 ambient temperature liquid scintillation system, and external standardization was used. A simplified Bray's fluor solution as advised for the scintillation apparatus was used. Results were expressed as slice to medium (*S/M*) ratio, where *S* equals μg/gm tissue, or dpm/gm tissue, and *M* equals μg/ml medium or dpm/ml medium.

Bilateral nephrectomy was performed 48 hours before experimentation. The animals were lightly anesthetized with ether, and both kidneys were removed through an abdominal midline incision. Sham operations were conducted on an equal number of animals. After recovery from the operation the animals were given free access to water, but no food was allowed. At the time of experimentation blood was collected from nephrectomized and sham-nephrectomized animals by decapitation. The blood was allowed to clot and the serum was collected after centrifugation. Serum (0.5 or 1 ml) from both nephrectomized and sham-nephrectomized animals was added to incubation beakers containing 3 ml of the Cross-Taggart medium. The effect of this added serum was compared with control beakers to which had been added an equivalent amount of blank media (media containing no PAH or NMN). Both groups of beakers contained renal cortical slices from normal, nonfasted animals.

[1] NMN-^{14}C was obtained from New England Nuclear Company.

TABLE I. Effect of Fasting (24 Hours) on Simultaneous Uptake of PAH and NMN-^{14}C by Rat Renal Cortical Slices.

Sample	*N*	*S/M* Ratio (± SE)	
		Control	Fasted
PAH	12	8.7 (0.9)	5.8 (0.2)[a]
NMN	12	6.5 (0.4)	6.6 (0.2)

[a] Significantly different than control ($p<.05$), group *t* test.

The data obtained were submitted to statistical analysis by using either Student's *t* test, group comparison, or analysis of variance followed by Duncan's new multiple range test (6). A 0.05 level of probability was used as the criterion of significance in all statistical tests.

Results and Discussion. The ability of rat renal cortical slices to accumulate PAH was significantly depressed when the donor animals were fasted 24 hours prior to sacrifice (Table I). The accumulation of PAH could be raised to control levels, but not above, by adding 0.01 *M* sodium acetate to the incubation media. Possibly the depression of PAH uptake resulted from competitive inhibition by acidic metabolites which had accumulated in the kidney during the fasting period.

In the study by White (1), the effect of serum from nephrectomized animals on PAH uptake by normal kidney slices was compared with the effect of adding serum from "control" animals. In preliminary experiments, nephrectomized animals failed to eat during the 48 hours between surgery and sacrifice. Therefore, sham-nephrectomized animals were used as a source of control serum and food was withheld from both groups of animals for the 48-hour period. The effect of serum from nephrectomized and sham-nephrectomized animals was compared with control uptake in beakers to which was added a volume of blank media (no PAH or NMN) equal to the

TABLE II. Effect of Serum from Nephrectomized Rats on PAH and NMN-^{14}C Uptake by Renal Cortical Slices from Normal Rats.[a]

The data were analyzed by Duncan's new Multiple Range Test following analysis of variance. Any two means underscored by the same line are not significantly different, but any two means not underscored by the same line are significantly different ($p<.05$).

Sample	Serum conc.	N	S/M Ratio[b]			Coefficient of variability (%)
			N	C	S	
PAH	1:6	10	4.1	5.8	12.7	24.1
NMN			6.6	7.0	7.3	19.6
PAH	1:3	10	3.0	10.8	20.4	16.2
NMN			7.8	8.6	9.9	11.5

[a] Experiments were performed 48 hours after nephrectomy.

[b] N refers to serum from nephrectomized rats; C, to control media with no serum added; and S, to serum from sham-nephrectomized rats.

amount of serum added to maintain concentrations of these ions constant in all beakers. All beakers contained renal cortical slices from nonfasted animals. As shown in Table II, addition of serum from nephrectomized animals significantly inhibited PAH uptake, but serum from the sham-operated animas significantly enhanced PAH accumulation. Transport of NMN-^{14}C was not significantly altered by adding serum to the incubation beakers.

These data confirm the findings of others (1, 3) that serum from nephrectomized rats produces a concentration-dependent depression of *in vitro* PAH transport, whereas serum from control or sham-nephrectomized animals stimulates PAH uptake. This depressant effect of nephrectomized serum appears to be specific for organic anion transport and not a generalized depression of slice function in that simultaneously measured NMN-^{14}C transport was not affected. These observations complement those of Bourke *et al.* (3), who found that oxygen consumption was not affected in slices in which PAH uptake was depressed by the addition of nephrectomized serum. These authors also found that hippurates from intestinal bacteria were increased in serum after nephrectomy. Possibly these hippurates act as competitive inhibitors of PAH transport in the *in vitro* slice. This, then, would explain the inhibition of PAH transport which occurs in the absence of any effect on NMN-^{14}C transport or oxygen consumption. The ability of serum from control or sham-nephrectomized animals to stimulate PAH transport cannot as yet be explained.

Summary. Addition of serum from bilaterally nephrectomized rats 48 hours after nephrectomy produced a concentration-related inhibition of *in vitro* PAH uptake by rat renal cortical slices. Simultaneously measured NMN-^{14}C uptake was not significantly affected. Serum from sham-nephrectomized rats significantly enhanced PAH uptake without altering NMN-^{14}C transport. Thus, both inhibition of PAH transport produced by serum from nephrectomized rats and stimulation produced by control serum appear to be specific for the organic anion transport system. It is also concluded that the ability of rat renal cortical slices to accumulate PAH is impaired if the animals have been subjected to a 24-hour fast before the time of experimentation. Fasting produced no significant alteration in the transport of NMN-^{14}C.

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Human Follicular Conjunctivitis Caused by Infection with a Psittacosis Agent* (32675)

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The Bedsoniae (Chlamydiae or psittacosis-lymphogranuloma venereum-trachoma group of microorganisms) comprise a large number of parasites of both man and animals (1). It has been generally believed that within this group only the agents causing trachoma and inclusion conjunctivitis (TRIC) produce follicular conjunctivitis in man. When the lymphogranuloma venereum (LGV) agent infects the conjunctiva of man, the resulting conjunctivitis is nonfollicular. Infection of the human conjunctiva by psittacosis or ornithosis agents has not been reported. In subhuman primates, infection with TRIC agents produces an inclusion-positive follicular conjunctivitis. Psittacosis agents, on the other hand, are said to be incapable of producing conjunctivitis in the primate conjunctiva, although multiplication of the agent has been demonstrated (Thygeson, unpublished). This paper reports a case of human follicular conjunctivitis resulting from a laboratory infection with a Bedsonia apparently of psittacine origin.

The patient, a 27-year-old laboratory technician at the G.W. Hooper Foundation, was first seen June 8, 1966, after suffering for one week from foreign-body sensation, discharge, and redness in the left eye. On examination she had a small palpable left preauricular node, a slight ptosis in the left eye, conjunctival follicles in the lower fornix and on the upper tarsal plate, and moderate papillary hypertrophy. During the following week, discrete epithelial erosions on the cornea and small grey corneal infiltrates were observed.

On June 15, 1966, the patient was given 1 gm of tetracycline orally. This was continued daily for 2 months. The follicular conjunctivitis slowly subsided over the next month. She had epithelial keratitis and subepithelial infiltrates until late November, 1966.

Materials and Methods. Clinical specimens. The conjunctiva was anesthetized with 0.5% proparacaine topically. Specimens were obtained by scraping the conjunctival surface with a sterile platinum spatula. This material was spread on glass slides for cytologic studies or collected in antibotic broth for isolation attempts.

Cytology. Slides were air-dried and fixed in methanol for Giemsa stains. Immunofluorescent staining techniques were also applied to conjunctival smears. These techniques have been described elsewhere (2, 3).

Isolation attempts. Streptomycin-treated conjunctival scrapings were inoculated into the yolk sac (YS) of embryonated hens' eggs. Two different isolation techniques were performed by two different laboratories (JS and LH) in order to rule out any possibility of cross contamination. These techniques have been reported elsewhere (2, 4).

Serology. Complement fixation (CF) tests were performed as described by Meyer and

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