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Experimental Tubular Nephritis Produced by Safranin O.

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Following the unexpected death of a rabbit 4 days after the intravenous administration of safranin O,‡ autopsy disclosed somewhat enlarged flabby kidneys with swollen yellowish-streaked cortices.§ Microscopic examination of the kidneys revealed severe, essentially selective necrosis of the renal tubular epithelium, involving predominantly the proximal convoluted tubules. The glomeruli appeared uninjured, and there were relatively minor degenerative changes and focal cellular necrobiosis in the loops of Henle and distal convoluted tubules; the collecting tubules appeared normal except for the presence of casts of necrotic material. No noteworthy changes were present in the other organs (brain not examined) except for moderate fatty change and sinusoidal congestion of the liver. The apparent relation of this renal damage to the administration of the dye, and the unexpectedness of this action of safranin, which has been apparently hitherto unreported, prompted a repetition of the procedure for verification and study.

Procedure. Healthy stock rabbits weighing about 2 kg were given a single intravenous administration of safranin O‖ in physiological saline or in aqueous solution, in a dose of 10, 20 or 40 mg per kg. The rabbits were then sacrificed at intervals for gross and microscopic study. In most instances determinations of blood NPN were made; these are recorded in Table I.

Observations. Immediately following injection of the dye, the tissues took on a distinctly pinkish tinge; this was especially noticeable in the ears, conjunctivae, and mucous and serous membranes. It was readily observed in the skin on parting the fur. The kidneys showed a diffuse staining of the cortex and boundary zone within a minute. The dye appeared in the urine within a few hours and was still present at 24 or even 48 hours, depending on the dose.

From Table I, it may be observed that none of 8 rabbits receiving 10 mg per kg showed any significant change in blood NPN. One animal sacrificed at 24 hours showed mild fatty changes and sinusoidal congestion of the liver; except for some excess of granular debris in the tubular lumens and apparent degenerative changes of somewhat questionable significance in the distal portions of the proximal convoluted tubules, the kidneys were not affected at this time. In 2 sacrificed at 48 hours, the greater portion of the renal parenchyma appeared entirely normal; here and there, however, a number of scattered proximal convoluted tubules presented one or several cells showing early but definite coagulation necrobiosis manifested by hypereosinophilia of the cytoplasm, and nuclear pyknosis, lysis and karyorrhexis. Many of these cells were in the process of being extruded into the lumen of the tubule, while adjacent cells appeared entirely normal. In all rabbits, at this dose, sacrificed subsequently, no significant lesions were observed, and any pre-existing damage had been repaired without remaining evidence.

At 20 mg per kg, 4 of 8 rabbits showed a distinct rise in blood NPN at the time of sacrifice. In all 4 animals in which this did

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‡ A basic nuclear stain; one of the azin group of quinone-imine dyes, a mixture of dimethyl and trimethyl diaminophenylphenazonium chloride.

§ The original observation was made by the author in 1942 as an incidental finding in a rabbit submitted for pathological examination by Captain Russel Bowers.

‖ Safranin O, for general staining purposes. Total dye content 85%. C. I. No. 841. National Aniline & Chemical Co., Inc., New York, N. Y.

TABLE I.
Blood NPN of Rabbits Following Intravenous Injection of Safranin O.

Dose (mg/kg)	NPN (mg/100 cc) Before injection	Days after injection 1	2	3	4	5	6	7	9	Fate
10	43	46								Sacrificed 1st day
	49		50							2
	51		59							2
	55			44						3
	47					35				5
	39					40				5
	45		48					56	50	9
	47		42					62	50	9
20	59	60								1
	39	82								1
	40		149							2
	49			46						3
	53		55		54					4
	44					181				5
	37					281				Died 5
	44		52				51			Sacrificed 12
	45		48				65			*
	*		138	98						" 4
40	—									Died 31-47 hr
	—									" ca. 48 "
	59		246							Sacrificed 2nd day
	40			258						" 3
	47			157						" 3
	—									Died 72-96 hr
	30				392					Sacrificed 4th day

* This animal received second injection of 20 mg per kg 12 days after first injection.

not occur, examination of the kidneys revealed relatively mild tubular necrosis, limited to the distal segment of the proximal convoluted tubules and involving apparently only a limited number of nephrons. In those in which the NPN rose, there was extension of the necrosis to the proximal as well as the distal portion of the proximal convoluted tubules, and involvement varying in extent from a moderate proportion to almost all of the nephrons (Fig. 1). In the most severe, there were also distinct degenerative changes and focal necrobiosis of cells in the ascending limb of Henle and in the distal convoluted tubules as well. In all, there was distinct sparing of the nephrons in the inner half of the cortex as compared with the outer half; I have noted this in experimental mercuric poisoning in rabbits also. Similar moderately severe damage was present in another rabbit which had had a previous injection of this same amount 12 days before; the first injection had resulted in but slight rise in NPN while a more marked elevation followed the second injection (Table I). In a dog which also received this amount of safranin, the kidneys presented a similar picture of extensive necrosis of the proximal convoluted cortical tubules.

The dose of 40 mg per kg was administered in 2 separate injections within a period of 2-4 hours, as the injection of this amount as a single dose led to slowing and cessation of respiration. With this amount, 3 of 7 rabbits died at 31-47, 48 and 72-96 hours, respectively. All the others showed marked elevation of NPN at the time of sacrifice. The kidneys of all of the animals of this group showed extensive severe tubular necrosis, even as early as 24 hours (not in Table I), the glomeruli remaining unaffected. It was apparent that the severity of renal damage would unquestionably have led to the death of the sacrificed rabbits as well, had they been permitted to continue. In this, as well as in the other groups, there were no noteworthy findings in the other organs, the brain not being examined, except for mild fatty degeneration and sinusoidal congestion of the liver.

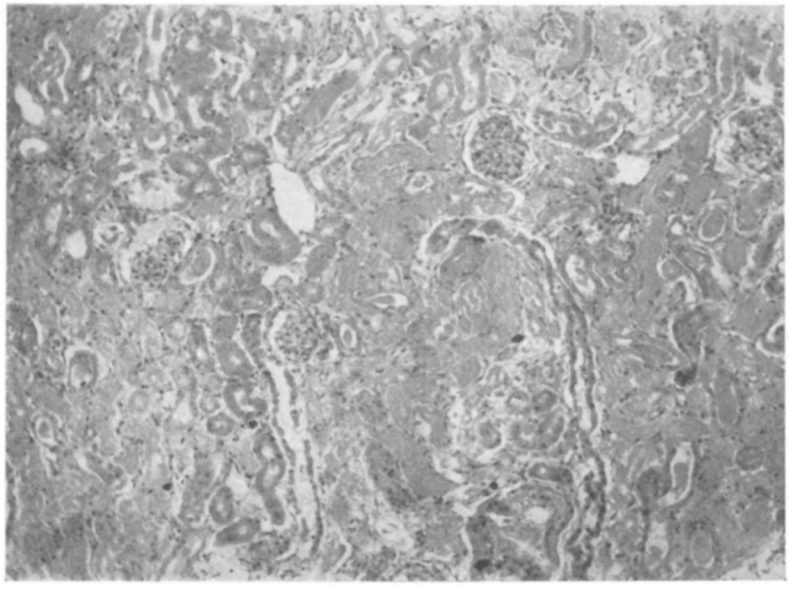

Fig. 1.
Extensive necrosis of epithelium of proximal convoluted tubules in renal cortex of rabbit following intravenous administration of 20 mg per kg of safranin O. On the left a small group of proximal convoluted tubules have escaped damage. The distal tubules are relatively intact except for the presence of casts in the lumen. The glomeruli appear uninjured except for the presence of some granular precipitate in the capsular space of the one at the left.

Discussion. The gross observation of the very rapid deposition of safranin in the kidneys has also been reported by Sheehan[1] who likewise administered this substance to rabbits, but failed to observe any renal or other damage, presumably because of the dose he employed which was apparently 5 mg per kg. Sheehan reported that in unfixed frozen sections of the kidney of rabbits sacrificed within one minute after injection of the dye, heavy staining of intermittent nephrons with the dye could be observed whereas the intervening ones might be altogether uncolored. He also showed that at low rates of renal circulation, the extraction ratio of the kidney for the dye is, for practical purposes, 100% and he has concluded that, whether or not there is glomerular filtration of the dye, a considerable proportion is secreted by the tubules from the peritubular capillaries.

The histologic picture of the renal injury observed in these animals closely resembles, indeed except perhaps for minor detail, is indistinguishable from that caused by mercuric chloride, chromates, uranium, bismuth, and, under certain conditions, cadmium.[2] It is of interest, in regard to biochemical mechanisms of tissue injury, that the pathologic-anatomical effects of heavy metal poisoning should be so closely paralleled by an organic compound such as safranin; in addition, essentially the identical histologic picture of renal tubular injury follows the nephrotoxic action of other organic compounds including oxalates and tartrates, and the dyes, the styrol quinoline dye No. 90, 2(p-acetylamino styryl)6-dimethylamino-quinoline methochloride[3] and acriflavine.[4] The question arises whether similar types of biochemical mechanism of intoxication may not be operative.

[1] Sheehan, H. L., *J. Physiol.*, 1931, **72**, 201.

[2] Ginzler, A. M., *et al.*, *Proc. Am. Assoc. Path. and Bact.*, *Am. J. Path.*, in press.

[3] Sheehan, H. L., *J. Path. and Bact.*, 1932, **35**, 589.

[4] Meleney, F. L., and Zau, Z., *J. A. M. A.*, 1925, **84**, 337.

Considerable evidence indicates that the toxic action of heavy metals may be due to combination with SH enzymes.[5,6] That at least some of the organic compounds mentioned above, specifically safranine, acriflavine and the styrol quinoline compound, may likewise act as inhibitors of enzyme systems and in this way exert their damaging action on the kidneys in a manner paralleling the action of the heavy metals biochemically as well as pathologically, is indicated by Dickens'[7] demonstration of a striking and selective inhibition of the normal aerobic inhibition of fermentation by extremely low concentrations of phenosafranine, the chemical structure of which is closely related to that of safranine, and he has demonstrated a like action of acriflavine as well as a quinoline relative of the styrol quinoline compound No. 90.

Summary. The intravenous administration of the dye, safranin O, to rabbits and dogs produces selective renal tubular necrosis, involving predominantly the proximal convoluted tubules. The histologic picture very closely resembles that observed in heavy metal poisoning.

Safranin O, in a dose of 10 mg per kg, produces minimal histologic injury in the kidney in rabbits, which is readily and rapidly repaired. The intravenous administration of 20 mg per kg is followed by more extensive and severe necrosis of the proximal convoluted cortical tubules, which is, in perhaps half the animals, reflected in elevation of the blood NPN. Increasing the dose to 40 mg per kg results in almost complete necrosis of the renal proximal convoluted tubular epithelium, as well as some damage to the distal tubules; this dose is probably uniformly fatal to rabbits. With all amounts of the substance, noteworthy lesions of other organs than the kidney, in the absence of examination of the brain, appear to be limited to a relatively mild degree of fatty degeneration and sinusoidal congestion of the liver.

The suggestion is made and evidence cited that the nephrotoxic action of safranin, as well as that of certain other nephrotoxic organic compounds, parallels the action of heavy metals biochemically as well as pathologically, and that these organic compounds exert their nephrotoxic effect by acting as inhibitors of vital enzyme systems.

[5] Peters, R. A., and Thompson, R. H. S., *Nature*, 1945, **156**, 616.

[6] Waters, L. L., and Stock, C., *Science*, 1945, **102**, 601.

[7] Dickens, F., *Biochem. J.*, 1936, **30**, 1233.

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Potassium Deficiency in the Dog.*

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Recent reports by Lambooy and Nasset[1] and Smith[2,3,4] have indicated that factors, in addition to the better known nutrients, are necessary for optimum growth, prevention of paralysis, maintenance of a healthy skin and prevention of anemia in the dog. We have found a basal ration containing only the 6 water soluble vitamins (thiamin, riboflavin,

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[1] Lambooy, J. P., and Nasset, E. S., *J. Nutrition*, 1943, **26**, 293.

[2] Smith, S. G., *Science*, 1944, **100**, 389.

[3] Smith, S. G., *Am. J. Physiol.*, 1944, **142**, 476.

[4] Smith, S. G., *Am. J. Physiol.*, 1945, **144**, 175.