Modification of Grollman's Method for Induction of Renal Hypertension.* (25755)

S. CHARLES FREED (Introduced by Meyer Friedman)
Harold Brunn Inst., Mount Zion Hospital and Medical Center, San Francisco, Calif.

Grollman's method for producing hypertension in rats is probably the simplest and most convenient procedure available(1). As is well known, this method consists of uninephrectomy and constriction of the remaining kidney by a figure-of-eight ligature. However, the yield of hypertensive rats is relatively small. In our hands, about 15 to 20% of the rats are found hypertensive 4 to 5 weeks following this operation. Efforts to increase the yield by a moderate increase in tension of the ligature resulted in a mortality of about 80% within 3 days, compared to about 40% usually encountered with the routine procedure after this interval. The cause of death following renal ligation was suspected to be uremia. Thus, the increase in intra-renal tissue pressure from ligature compression, combined with the swelling from manipulation of the kidney, could result in anuria by obliteration of the tubules. It was surmised that if such an increased intra-renal pressure could be reduced, many potentially hypertensive rats might survive the operative effects and become useful experimental animals. It seemed worthwhile to administer the anti-inflammatory steroid, prednisolone, postoperatively, in an effort to suppress the suspected fatal renal swelling in order to increase the yield of hypertensive rats.

Method. One hundred Long-Evans male rats, 4 to 5 weeks old, were subjected to Grollman's procedure as usually done in this laboratory. Another group of 100 similar rats were similarly operated upon, but in addition were injected subcutaneously with 5 mg prednisolone† in aqueous suspension. The animals of both groups were observed for fatalities for 5 to 5 weeks. Blood pressure determinations were made at this time(2), and those rats whose blood pressures were over 140 mm Hg systolic were classified as hypertensives.

Results. Table I presents results. The data obtained show that administration of prednisolone, postoperatively, reduces immediate mortality from 42% to 11%. In conjunction with improvement in survival rate, yield of hypertensive rats 4 to 5 weeks later was more than doubled. Apparently many of the rats which ordinarily succumb to renal ligation were potentially hypertensive. Prednisolone treatment protected many of these animals from the immediate effects of operation, resulting in a salvage of significant numbers of hypertensive rats.

It is readily acknowledged that the mechanism by which prednisolone prevented death of many of the rats following renal ligation has not been demonstrated. Direct evidence was not obtained to indicate that the anti-inflammatory steroid prevented death from anuria by reducing the suspected renal swelling. On the basis that this steroid produced results which conformed to its well-known physiological activity, such a deduction is not unreasonable. Regardless of the actual mechanism, this modification of Grollman's procedure appears to be effective in increasing yield of hypertensive rats.

These results provoke the thought that in certain cases of anuria encountered clinically, where increased intra-renal pressure is suspected to be a major factor, some benefit might be obtained from corticosteroid therapy.

Summary. Administration of prednisolone

TABLE I. Effect of Prednisolone on Induction of Hypertension by Renal Ligation.

Group	No. of rats	72-hr mortality	Subsequent mortality	No. of non-hypertensive	No. of hypertensive
Untreated	100	42	10	32	16
Treated with prednisolone	100	11	16	36	37

* Aided by grant from Nat. Inst. Health, Nat. Heart Inst.

† Prednisolone was supplied through courtesy of Schering Corp., Bloomfield, N. J.

to rats subjected to Grollman's method for inducing renal hypertension lowered immediate mortality rate. It was deduced that the steroid was effective in combating intra-renal pressure suspected to be a frequent cause of death, resulting in a higher yield of hypertensive rats.

Addendum. According to Grollman, there is a low mortality and a high yield of hypertensive rats in a two-stage operation, the renal ligation first, and several weeks later the nephrectomy. The above report is significant, therefore, only when a one-stage operation is desired.

1. Grollman, A., PROC. SOC. EXP. BIOL. AND MED., 1944, v57, 102.

2. Friedman, M., Freed, S. C., *ibid.*, 1949, v70, 670.

Received March 21, 1960. P.S.E.B.M., 1960, v104.

Fluorescence of Tetracyclines in Filarial Worms.* (25756)

JOHN E. TOBIE AND HENRY K. BEYE (Introduced by V. Knight)
Laboratories of Immunology and Clinical Investigations, Nat. Inst. of Allergy and Infectious Diseases, N.I.H., Bethesda, Md.

Antibiotics of the tetracycline series fluoresce a yellow-gold color when stimulated with long wavelength ultraviolet light. Following administration of these antibiotics to animals, a diffuse, induced fluorescence has been observed throughout soft tissues with no special affinity for these tissues(1,2). However, studies have shown (3,4) that these drugs localize *in vivo* in newly proliferated normal bone tissue or new bone formations associated with certain neoplasms (unpublished). Recently, we have demonstrated that tetracyclines are deposited in filarial worms *in vitro* following exposure to drug and that yellow fluorescence of worms is sufficiently pronounced to permit their visualization *in vivo* following administration of drug to human or animal hosts. The following report describes these studies with special emphasis on fluorescent detection of worms in subcutaneous lesions of a patient with filariasis.

Materials and methods. Patient study. The patient was a 15-year-old white female who had spent most of her life in French Equatorial Africa, an area endemic for infection with the "eye worm," *Loa loa*. On admission, she had "fugitive swellings" on trunk and extremities and throughout her hospital course there was often seen in the center of these swellings a white, elevated band which moved about and appeared to be a subcutaneous migrating worm. On one occasion by application of heat to the forearm, a migrating worm was made to appear subcutaneously, then made to disappear by application of ethyl chloride spray. On 6 occasions, both night and day, blood samples from the patient were examined by Knott's technic(5) and in one daytime specimen the unsheathed microfilariae of *Acanthocheilonema perstans* were demonstrated. Although sheathed microfilariae were not detected, it is likely that the patient also had *Loa loa* infection since, in Africa, infection with *A. perstans* is frequently associated in patients with loaiasis(6). The patient proved markedly sensitive to dog heartworm (*Dirofilaria immitis*) antigen, giving a 3+ intradermal reaction to a 1:48,000 dilution of antigen. Tetracycline, in a dosage of 1 g daily by mouth, was given to the patient. At intervals thereafter, the patient's skin was fluoresced with a 9-watt hand lamp emitting ultraviolet at 3660 Å. *In vitro experiment with D. immitis.* Approximately 0.25 ml of heparinized dog blood containing living microfilariae of the dog heart-worm was pipetted into 2.5 ml of a buffered solution of chlortetracycline containing 1 mg/ml. After 5 minutes exposure to the antibiotic, a sample was taken and a slide prepared. At the end of 45 minutes exposure, remaining microfilariae in the drug solution were washed by centrifugation 2 times with buffered saline, pH 7.0, and a slide prepared from the sediment

* The authors thank Charles B. Evans for valuable technical assistance.