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.

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Fluorescence of Tetracyclines in Filarial Worms.* (25756)

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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localize *in vivo* in newly proliferated normal bone tissue or new bone formations associated with certain neoplasms (unpublished).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

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after the last centrifugation. Both 5 and 45 minute wet-mount preparations were examined at fluorescence microscopy using a Leitz Ortholux microscope equipped with a 1000-watt General Electric AH-6 mercury arc lamp(7). Using a Corning No. 5840 transmitting filter (2.25 mm thick), living microfilariae were stimulated with ultraviolet light at a wavelength of 3650 Å. A Wratten 2A ultraviolet absorbing filter was used in the eyepiece of the microscope. *In vivo experiment with D. immitis.* A 25 kilo dog infected with *D. immitis* was given a single I.V. injection of tetracycline in a dosage of 40 mg/kilo. Samples of heparinized blood taken ½, 1½, 3, 24, and 96 hours later were examined at fluorescence microscopy to determine uptake of drug by microfilariae. At a later date, the same dog was given the same I.V. dose of tetracycline. The animal was killed 30 minutes after cessation of injection, the adult *D. immitis* worms recovered from the heart, and grossly fluoresced with a 9-watt ultraviolet hand lamp. Subsequently, the worms were quick-frozen in petroleum ether at −65°C(8), fresh-frozen sections cut in a cryostat, and the sections mounted in a medium containing 9 parts of glycerine and 1 part of buffered saline, pH 7.0.

Results. Patient study. Twenty-four hours after initial dose of tetracycline, the patient was placed in a dark room and the skin fluoresced with a UV hand lamp. Linear, yellow-fluorescent streaks were seen on many parts of the body. When traced at intervals over a 6-hour period, streaks were seen to migrate in a circular fashion over a 5 to 6-inch area. A photograph of the patient's forearm, taken under ultraviolet light, localized the subcutaneous worms as yellow-fluorescent tracts (Fig. 1). From morning to afternoon or to the following day, the fluorescent tracts changed in location. For example, one day a fluorescent tract was observed behind the patient's left ear and the following day behind the right ear. This may have represented the same worm in its subcutaneous migrations. From these observations, it appeared evident that the worms had selectively taken up a considerably larger amount of drug than the surrounding host tissues since

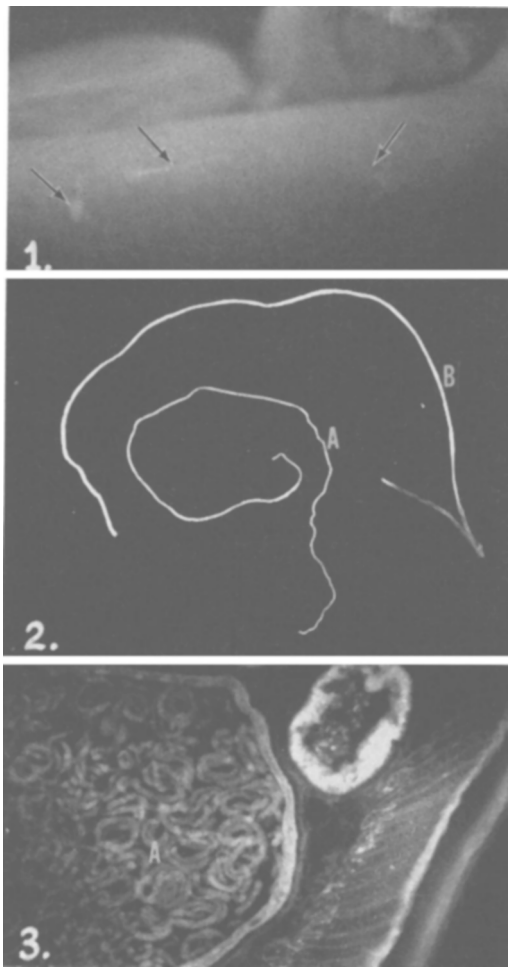

FIG. 1. Photograph of patient's forearm under ultraviolet light showing 3 fluorescent tracts (arrows).

FIG. 2. Fluorescence photograph of adult *Dirofilaria immitis* worms after *in vivo* exposure to tetracycline. A. Male. B. Female.

FIG. 3. Fluorescence photomicrograph of fresh-frozen cross section of adult female *Dirofilaria immitis* after exposure to tetracycline. A. Fluorescence of microfilariae *in utero*. × 172.

only discrete tracts on the skin showed induced yellow fluorescence in contrast to blue-gray autofluorescence of uninvolved skin areas. At times during tetracycline therapy, no fluorescent tracts were observed on the skin. This was probably a function of the depth of the worms in subcutaneous tissue and, therefore, lack of ultraviolet stimulation.

In vitro experiment with D. immitis. The microfilariae of *D. immitis* which had been exposed to a solution of chlortetracycline for

5 minutes were examined with a fluorescence microscope. They fluoresced a yellow color at approximately a 3+ intensity. In each embryo, the drug had been deposited in the central column of nuclei so the internal structure of microfilaria was clearly visible. During examinations, yellow fluorescence of microfilariae showed rapid fatiguing of an irreversible nature, a process frequently due to a photochemical reaction accompanied by oxidation(8). The embryos exposed to antibiotic for 45 minutes, then washed, fluoresced brilliantly (4+) but the yellow color fatigued at the same rate as in those exposed for 5 minutes. Microfilariae not exposed to drug exhibited blue-gray autofluorescence. Living microfilariae which first had been exposed to chlortetracycline, then stimulated with UV light, were killed in about 1 minute. However, without drug exposure, it took about 10 minutes for them to die.

In vivo experiment with D. immitis. To obtain more direct evidence of uptake of tetracycline by adults and embryos of a filarial worm, *in vivo* studies were conducted on a dog infected with dog heartworm. One-half hour and 1½ hours after I.V. administration of tetracycline, blood samples were taken and examined at fluorescence microscopy. As in the case of the *in vitro* studies, the central column of nuclei in each microfilaria obtained from the dog fluoresced a bright yellow color. In blood drawn 3 and 24 hours after drug was given, the microfilariae still contained drug but in somewhat smaller amounts, as shown by their being slightly less fluorescent. Microfilariae examined 96 hours after tetracycline administration showed no fluorescence. Fluorescence of the parasites observed in the ½, 1½, 3, and 24 hour samples fatigued quite rapidly under ultraviolet stimulation and the light was lethal to them in about 1 minute.

To demonstrate that tetracycline was specifically deposited in adult worms, the dog was given a single I.V. injection at a later date. The adult worms recovered from the heart and fluoresced with a UV hand lamp were a brilliant yellow-gold color, demonstrating presence of considerable amounts of drug. Some of the worms were quick-frozen and after 4 months were still brilliantly fluorescent (Fig. 2). Microscopically, in frozen sections of the adult worms, the drug was localized by its yellow fluorescence throughout most of the internal structures (Fig. 3). Of particular interest was the observation that the drug had been deposited in the uterus of the female as well as in the embryos contained within the uterus. Cuticula of the worms contained no drug, as was evidenced by a blue autofluorescence. Adult specimens of *D. immitis* not exposed to tetracycline exhibited a blue autofluorescence.

The selective concentration of tetracyclines by microfilariae and adult worms in host tissues has at least 2 therapeutic implications. First, tetracyclines themselves may have some antifilarial effect since treated microfilariae survived less well under ultraviolet stimulation than did untreated specimens. Second, if it were possible to combine some active antifilarial compound with tetracycline, it might permit a greater and more rapid concentration of antifilarial drug in worms within the tissues of the host. Since the tetracyclines can be localized and visualized in worms by fluorescent methods, this might serve as an effective tool in study of mode of action of these drugs. The observation that filarial worms can be visualized by a simple fluorescent method in a human subject after administration of tetracycline offers certain diagnostic possibilities.

Summary. 1. After administration of tetracycline to a patient having migrating, subcutaneous filarial worms, parasites were localized and visualized as yellow-fluorescent tracts beneath the skin following stimulation with UV light. 2. Exposure of microfilariae of *D. immitis in vitro* to a solution of chlortetracycline resulted in uptake of antibiotic by embryos in amounts which were readily visible by fluorescent methods. 3. *In vivo* studies on a dog infected with *D. immitis* revealed that the central column of nuclei of each microfilaria specifically took up tetracycline. The drug also was deposited in adult worms making them brilliantly fluorescent. In fresh-frozen sections of an adult female worm, the drug was localized throughout internal structures including uterus with its contained embryos.

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Congenital Malformations in Offspring of Mice Treated with Caffeine.* (25757)

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Certain substances which act as mitotic poisons have been reported to have teratogenic effects in mammalian embryos(1,2). However, many chemicals present in foods, beverages or drugs have not been tested or have been insufficiently studied for possible teratogenic effects. A few studies on caffeine have been reported. Druckrey and Schreiber(3) observed cessation or irregularity in mitosis in fertilized eggs of sea urchin after these were dipped in a caffeine solution. Eichler and Mügge(4) found no deleterious effects on embryonic development when pregnant rats were injected subcutaneously with large doses of caffeine. However, continuous treatment of pregnant rabbits with high doses of caffeine resulted in occasional early embryonic death or retarded development; the newborn young appeared to have "poor resistance"(5-7). Since no congenital malformations have been reported, we have investigated the effects of high doses of caffeine injected in pregnant mice.

Methods. Approximately 100 pregnant mice of the SMA strain (originating from Japanese Nat. Inst. of Genetics) were given a single intraperitoneal injection of 1% caffeine once during 7th to 15th days of pregnancy. A dosage level of 0.25 mg/g body weight, considered to be approximately the maximum dose tolerated, was used. The animals were sacrificed near term or in mid-pregnancy and fetuses weighed, examined for anomalies, and fixed in 20% formalin solution. Fetuses obtained from 25 normal pregnant mice were used as controls.

Results. Table I shows that caffeine administration may result in embryonic death or in malformed fetuses. Incidence of embryonic death was highest for injections given during 7th-12th days, when resorption of all embryos of a litter was sometimes induced. However, any injection given between 7th-14th days resulted in some dead or macerated fetuses. Malformations were observed in 18 to 43% of fetuses when injections were given between 10th and 14th days; a single malformation was found for injections on 9th day

TABLE I. Effects of Intraperitoneal Injection of Caffeine* in Pregnant Mice Observed at Term.

Day of inj.	No. of mice	No. of complete resorptions	Young		
			Total No.	% dead	% abnormal
7	4	2	11	18	0
8	5	0	36	6	0
9	11	2	45	11	2
10	6	0	40	3	38
11	10	0	57	12	19
12	8	1	41	17	41
13	7	0	51	8	43
14	7	1	44	18	18
15	3	0	24	0	0
Uninjected controls	25	0	225	2	0

* 1% aqueous solution, 0.25 mg/g body wt.

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