Viruria and Viraliquoria in the Dog After Intravenous Injection of T5 Bacteriophage.* (29778)

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Several reports have appeared which indicate that macromolecules, such as viruses, pass across anatomical barriers previously considered impermeable to much smaller particles. The studies of Petrov(1), and Keller and Zatzman(2) indicated that sufficiently high blood levels of bacteriophages result in the transfer of particles into the urine. Other workers have shown transfer of viruses across the skin(3), GI tract(4), and placenta(5). The studies reported herein demonstrate that in the dog, blood levels of virus associated with viruria may also result in virus gaining access to the spinal fluid. The term viraliquoria has been suggested for this phenomenon(5).

Materials and methods. Animals. Mongrel dogs (of either sex), weighing *10* to *20* kg were anesthetized with intravenously administered pentobarbital, 35 mg per kg. The femoral artery and vein were exposed and the abdomen entered through a mid-line incision. Heparin sodium, 5 mg/kg, was then injected intravenously. A polyethylene catheter (I.D. 0.106″) was inserted into the abdominal aorta through the femoral artery and a control blood sample was obtained. A control urine specimen was obtained by stimulating bladder contraction manually, or by direct withdrawal with a sterile needle and syringe, if gentle pressure was ineffective. The phage suspension, in phosphate buffered saline (PBS), was injected intravenously in one 2 ml bolus through the femoral vein. Either a "high dose" inoculum, containing 1 to 12 $\times$ 10^{10} phage, or a "low dose" inoculum, containing 1 to 5 $\times$ 10^{8} phage, was used. Arterial blood samples were obtained from the aortic catheter at appropriate time intervals, taking particular care to discard the blood contained in the catheter dead space. A final urine sample was obtained by direct bladder aspiration in each experiment. Spinal fluid was withdrawn by cisternal tap. All urine and spinal fluid samples were examined microscopically within minutes after they were obtained. Samples that were free of blood contamination were included in analysis of data except as noted.

Phage. T5 bacteriophage† was purified by a modification of the method described by Adams using differential ultracentrifugation with a resultant pool containing 10^{11} to 10^{12} plaque forming particles per ml as determined using the host *E. coli B* in the soft agar assay(5). The blood, urine, and spinal fluid specimens were tested undiluted or following appropriate $\log_{10}$ dilutions in PBS after storage at 4° for periods up to 24 hours. T5 phage was selected because it was found not to be inactivated in normal dog urine. Each animal's serum was examined for T5 phage inactivating factors 2 to 3 days prior to each experiment, and dogs whose serum was shown to contain them were not used. The result of the phage titrations are based on at least duplicate, and often 4 to 6 replicate samples, run simultaneously.

Results. Ten minutes after rapid intravenous injection of 2 cc of phage suspension, relatively constant blood levels were obtained 5-6 $\log_{10}$ below the concentration of the inoculum. The data observed in experiments in which it was possible to obtain urine and/or spinal fluid for adequate testing are shown in Table I. It was not possible to detect phage in either urine or spinal fluid if the "low dose" inoculum resulted in blood levels of a concentration less than 10^{2} particles per ml. This was strikingly evident in the 2 experiments in which blood tinged spinal fluid was tested. As much as 1 or 2 ml of these fluids were tested in 0.5 ml aliquots and were negative for phage. Following injection of

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TABLE I. Viruria and Viraliquoria in Dogs After Intravenous Injection of T5 Coliphage.

	Dog No.	Time samples obtained (min)	No. of phage particles per ml of: Arterial blood	Bladder urine	Cerebrospinal fluid
Group I, "low dose" ($1\text{-}5 \times 10^8$ phage)	1	30	2.6×10^2	0*	0†
	2	20	7.0×10^2	0	0
	3	30	2.0×10^3	23	—
	4	20	2.3×10^3	0	0†
	5	35	3.5×10^3	—	55
	6	30	5.6×10^3	42	2
Group II, "high dose" ($1\text{-}12 \times 10^{10}$ phage)	1	20	6.3×10^3	176	—
	2	30	1.3×10^4	10	10
	3	30	1.3×10^4	5	4
	4	34	1.4×10^4	—	153
	5	32	2.0×10^4	—	56
	6	24	7.5×10^4	376	—
	7	20	8.6×10^4	—	4
	8	30	1.1×10^5	82	—
	9	30	3.6×10^5	113	—
	10	30	2.0×10^6	301	358

— = Not done.

* A zero indicates that at least 1 ml of material was tested and was negative for phage.

† Indicated experiments in which grossly bloody cerebrospinal fluid was, nevertheless, negative for phage.

"high dose" inocula, however, viruria and viraliquoria were consistently observed. When urine and spinal fluid were obtained from the same animal (7 dogs) phage concentrations were usually the same in both fluids. In these experiments particular care was taken to be certain that specimens were uncontaminated by the peripheral blood which contained large amounts of phage.

Discussion. The data reported were obtained during broader studies relative to the distribution of virus particles in different vascular beds after intravenous injection. Passage of phage from blood to urine and spinal fluid was observed. The quantitative ratio of particles in blood to the number in either of these body fluids was similar (*i.e.*, approximately 10^2 or 10^3:1). Phage was found in the urine or spinal fluid in each of the 10 "high dose" inoculum experiments, when specimens were obtained that were free of microscopic blood contamination. Mere traumatic contamination cannot explain the results, since the amount of blood required to result in the quantity of phage cultured would necessitate obvious gross bleeding into the specimens.

Utz *et al* first suggested the term viraliquoria when they recovered Coxsackie B-3 virus in the spinal fluid of a patient who also excreted virus in the urine(5). We are unaware of the use of virus particles or bacteriophage in experimental animals for the study of viraliquoria, although there have been recent reports of the passage of virus particles from blood to urine(2,7) and across the placenta(4). In guinea pigs, a ratio of 10^6:1 of maternal to fetal blood levels of bacteriophage ϕX 174 has been reported. Flanagan and Schultz noted a u/p ratio of 10^{-2} to 10^{-3}:1 for Coxsackie B-1 virus in the dog(7). Using ferritin molecules in electron microscopic studies, Bondareff(8) has shown a rapid egress from the blood to cells of the brain; and Keller and Zatzman reported passage of phage through the skin and across the GI tract of mice, although the amounts were very small in relation to the amounts available for passage(2). Thus, it is apparent from several studies that anatomical barriers are relatively effective filters; it is equally apparent that small numbers of large particles, such as viruses, are capable of passing through these barriers. The significance of these findings in the pathogenesis of virus infections remains to be investigated.

Summary. After intravenous injection of T5 bacteriophage, small amounts of virus were recovered in urine (viruria) and cerebrospinal fluid (viraliquoria) of anesthetized

dogs. The quantitative ratio of virus particles in blood to that in either of these fluids was similar (approximately 10^2 or 10^3:1).

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Esterification of Cholesterol and Hydrolysis of Cholesterol Oleate by Homogenates of Bovine Adrenal Glands and Their Subcellular Components.* (29779)

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In an earlier study(1) rats were injected intravenously with a very low-density lipoprotein fraction prepared from the chyle of donor rats that had been fed ^{14}C-labeled cholesterol. About 75% of the labeled cholesterol in this chyle preparation was in the esterified form. Twenty-four hours after the injection about 90% of the ^{14}C-sterol in liver, small intestine, adipose tissue, muscle, bone and spleen of the recipient rats was in the free form. The situation in the adrenal glands was unique: the proportion of the injected ^{14}C-sterol in the free form was higher than that of the injected preparation at the earliest intervals; later, however, the proportion in the free form decreased, and by 24 hours about 90% of the sterol ^{14}C in the gland was esterified. This finding suggested that the adrenal gland first hydrolyzed the cholesterol esters of the chyle lipoproteins, but subsequently resynthesized cholesterol esters characteristic of the gland. In a later study(2) in which chyle lipoproteins were incubated with a variety of rat tissues, it was shown that the adrenal gland was among the tissues possessing a very high hydrolytic activity. Cholesterol esterifying activity has been demonstrated in adrenal homogenates of dogs(3), hogs(4) and rats(5). The present report deals with some characteristics of the esterifying and hydrolytic reactions in the bovine adrenal gland and with their intracellular localization.

Materials and methods. Preparation of tissue homogenates and subcellular fractions. Bovine adrenal glands, obtained from local abattoirs, were cleared of visible adipose tissue and homogenized with three volumes of an ice-cold 0.9% NaCl† solution in a motor-driven, Potter-Elvehjem type homogenizer equipped with a tight-fitting teflon pestle. The homogenate was first centrifuged at 350 $\times$ g for 15 minutes to sediment the cellular debris, which was discarded. The supernatant fraction was designated supernatant A. Supernatant A was centrifuged at 10,000 $\times$ g for 10 minutes to sediment Pellet I, and the supernatant from this centrifugation was again centrifuged at 100,000 $\times$ g for 45 minutes to sediment Pellet II. The supernatant fraction obtained by centrifugation at 100,000 $\times$ g for 45 minutes was designated cell sap. Pellets I and II were separately resuspended by homogenizing them gently by hand in the Potter-Elvehjem homogenizer in a volume of 0.9% NaCl equal to the original volume of supernatant A. En-

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† In some experiments 0.25 M sucrose was used in place of 0.9% NaCl and the same results were obtained.