It has been proposed recently that angiotensin may be a specific aldosterone stimulating hormone(1,2). The evidence presented here suggests, however, that the action of the kidney in regulation of adrenal cortical secretion is not limited to aldosterone. Slater, Casper, Delea and Bartter(3) were unable to separate aldosterone stimulating and 17 OHCS stimulating effects of angiotensin, even at low doses, though Mulrow and Ganong(2) were able to separate these effects. It seems there must be some reservations concerning the specificity of the role of the kidney in control of aldosterone secretion.

In the burn experiments, 17 OHCS secretion fell to levels not distinguishable from normal after 30 minutes in nephrectomized dogs and 60 minutes in normal dogs. The more rapid return to control levels in nephrectomized dogs may be simply a reflection of the smaller rise after burn rather than a specific effect.

It has been suggested(16) on the basis of experiments on dogs with isolated pituitaries that there may be a humoral factor arising in the hind-brain mediating ACTH release. It is possible that a part of the increase in 17 OHCS secretion observed in those experiments results from extra-pituitary stimulation of the adrenal rather than from extra-hypothalamic stimulation of the pituitary. The present experiments suggest that the kidney may mediate such extra-pituitary stimulation.

Summary. Nephrectomized dogs respond to third degree burns with much less increase in 17 OHCS secretion than do normal dogs. ACTH injection also produces much less increase in 17 OHCS secretion in nephrectomized than in normal dogs. These diminished responses appear to result from decreased adrenal sensitivity to ACTH. It is suggested that the kidney plays an important role in the control of 17 OHCS secretion.

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Acridine Orange Staining of Purified Polyoma Virus.* (26858)

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Recent experimental evidence indicates that polyoma is a DNA virus. Di Mayorca *et al.*(1) extracted an infectious nucleic acid from the virus and demonstrated that infectivity was abolished by treatment with DNAase while RNAase had no effect. The studies

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TABLE I. Staining Properties of Viruses.

Virus preparation nucleic acid	Color developed on acridine orange staining	Nuclease susceptibility		
		DNAase	RNAase	Proteolytic enzyme necessary
Double-stranded animal virus DNA	Yellow green	+	—	+
Double-stranded bacteriophage DNA	" "	+	—	—
RNA	Flame red	—	+	—
Single stranded Φ × 174 DNA	" "	+	—	—

of Smith *et al.*(2) with purified P^{32} labelled virus and the inhibitors of DNA synthesis, aminopterin and bromodeoxyuridine, showed that polyoma virus contained DNA. The cytochemical studies of Allison and Armstrong (3), Williams and Sheinin(4) and Bereczky *et al.*(5) with the fluorochrome acridine orange revealed yellow green nuclear inclusions characteristic of DNA in infected cells. The latter two groups of workers noted that pretreatment with a proteolytic enzyme was necessary before the inclusions became susceptible to DNAase. Although these studies indicate a marked increase in nuclear DNA during polyoma production, they do not show how much of this DNA is actually viral in nature and in what state the viral DNA exists.

Mayor and Hill(6) demonstrated that the fluorochrome acridine orange provided a simple means for differentiating among RNA, double-stranded DNA and single-stranded DNA. The technic is particularly useful in identifying viral nucleic acids. Under carefully controlled conditions of dye concentration and pH the behavior of fixed preparations of animal viruses so far examined may be summarized from their data as in Table I.

The experiment reported here was carried out in an attempt to find where purified polyoma virus fits into this table.

Materials and methods. A stock of plaque purified polyoma virus was provided by Dr. Matilda Benyesh-Melnick, who also carried out all the infectivity and hemagglutination titers by methods already described(7). The titer after 3 passages in mouse embryo tissue culture was 6.3×10^7 PFU/ml and the hemagglutinating titer with guinea pig cells was 800/ml(7). Thirty-seven ml of this infected mouse embryo fluid was clarified at 5000 rpm in the Spinco 40 rotor for 30 minutes. The supernatant was removed and spun at 40,000 rpm for 2 hours. The resulting pellet was suspended in approximately 1.2 ml phosphate buffered saline (pH 7.2) by gentle overnight shaking at 4°C. The hemagglutinating titer of this material was 20,480/ml. One ml of this virus suspension was added to 4 ml of a cesium chloride solution to give a final density of 1.28. After centrifugation for 50 hours at 25,000 rpm in the Spinco SW 25 rotor, a sharp band appeared near the middle of the tube. This band was removed in 1 ml of fluid by puncture through the bottom of the tube and after dialysis against phosphate buffered saline had a hemagglutinating titer of 5120/ml.

Results and discussion. If a virus has a buoyant density different from that of contaminating material a purification of the virus can be obtained by isodensity centrifugation. Smith *et al.*(2) have shown that the sharp band appearing in CsCl at an approximate density of 1.29 coincides with maximum activity of the virus. By comparison in CsCl, DNA bands at 1.7, RNA at 2.0 or greater, while most proteins float at a density of 1.25 or less (Schaffer, personal communication). Our bands would appear to contain sufficiently purified polyoma virus. Table II gives results obtained in a second experiment

TABLE II. Assay of Polyoma Virus.*

Virus material	HA titer/ml	PFU/ml
Original virus fluid, 30 ml	160	1.1×10^7
Ultracentrifugal pellet resuspended in 1.5 ml	640	3×10^7
CsCl band, in 1 ml	80	2.5×10^6

* Assays kindly carried out by Dr. Matilda Benyesh-Melnick.

TABLE III. Staining Properties of Poliovirus Φ × 174 and Polyoma.

Fixed virus concentrate	Color developed with AO staining	Nuclease susceptibility: DNA-ase	Nuclease susceptibility: RNA-ase	Proteolytic enzyme necessary
Poliovirus	Flame red	—	+	—
Φ × 174	" "	+	—	—
Polyoma	Yellow green	+	—	+

which included both plaque and hemagglutination assays.

Droplet preparations of this purified polyoma virus suspension were fixed 30 minutes in Carnoy's fluid and stained with 0.01% acridine orange at pH 3.8. The staining technic and relevant enzyme digestion tests have been described in detail(8,6). Simultaneous staining of purified preparations of bacteriophage Φ × 174(6) and poliovirus (8) was carried out as a control. The results are shown in Table III. It would appear that the nucleic acid present in polyoma is DNA, and that in the mature virus particle it exists in the double-stranded form.

It may be argued that a DNA virus replicating in the nucleus could possibly adsorb to its surface sufficient cellular DNA to give a double-stranded color reaction in the standard acridine orange test. To clarify this point, 3 unfixed droplet preparations were first incubated in DNAase (0.01% in veronal buffer) for 10 minutes, 1 hour and 2 hours respectively. They were then rinsed in distilled water, fixed in Carnoy's and stained as above. All preparations developed an equally brilliant yellow green color only slightly less in intensity than the untreated specimens. It woud appear that there is some cellular DNA adsorbed to the virus particle but that this amount is readily and rapidly removed by nuclease digestion leaving the reaction of the intact virus particle essentially unchanged.

Summary. Preparations of polyoma virus purified by CsCl isodensity centrifugation stained yellow green at pH 3.8 with 0.01% acridine orange. Pretreatment with a proteolytic enzyme was necessary before development of the stain could be inhibited by DNAase. RNAase had no effect on similar preparations. These characteristics are consistent with the identification of the nucleic acid of polyoma virus as a double-stranded DNA.

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Vitamin D and Urinary Amino Acid Excretion in the Rat.* (26859)

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An elevated excretion of amino acids in the urine of rachitic children was first observed by Hottinger(1). Freudenberg and Goetz(2) later demonstrated that this could be reversed by a single massive dose of Vit. D. However, Jonxis *et al.*(3), who also reported

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