their vector is believed to be certain species of *Culicoides*. It would seem to be of some importance to determine whether bluetongue virus represents a bona fide arbovirus, different from those generally accepted at present, or whether it belongs elsewhere among the animal viruses.

Summary. It has been demonstrated that a Cyprus (BT-Cy) and a California (BT-8) strain of bluetongue virus are not sensitive to inactivation by treatment with ether and sodium deoxycholate. Two methods for assaying the sensitivity of viruses to sodium deoxycholate in cell cultures are described. Some aspects of these findings are considered in light of what is known of bluetongue as an arthropod-borne virus disease.

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1. Du Toit, R. M., Onderst. J. Vet. Sci., 1944, v19, 7.

2. Price, D. A., Hardy, W. T., J. Am. Vet. Med. Assn., 1954, v124, 255.

3. Foster, N. M., Jones, R. H., McCrory, B. R., Am. J. Vet. Res., 1963, v24, 1195.

4. Livingstone, C. W., Moore, R. W., ibid., 1962, v23, 701.

5. Andrewes, C. H., Viruses of Vertebrates, Williams & Wilkins Co., Baltimore, 1964.

6. Gambles, R. M., J. Comp. Path. Therap., 1949, v59, 176.

7. McKercher, D. G., McGowan, B., Saito, J. K., Proc. Am. Vet. Med. Assn., 1954, p167.

8. Wada, E. M., Thesis, Univ. of Wisconsin, 1961.

9. Studdert, M. J., Wada, E. M., Kortum, W. M., Groverman, F. A., J. Am. Vet. Med. Assn., 1964, v144, 615.

10. Madin, S. H., Andriese, P. C., Darby, N. B., Am. J. Vet. Res., 1957, v18, 932.

11. Andrewes, C. H., Horstman, D. M., J. Gen. Microbiol., 1949, v3, 290.

12. Dulbecco, R., Vogt, M., J. Exp. Med., 1954, v99, 167.

13. Anderson, S. G., Ada, G. L., Virology, 1959, v8, 270.

14. Morgan, C., Howe, C., Rose, H. M., J. Exp. Med., 1961, v113, 219.

15. Mussgay, M., Rott, R., Virology, 1964, v23, 573.

16. Mackerras, I. M., Mackerras, M. J., Burnet, F. M., Bull. Coun. Sci. Indust. Res. Australia, 1940, No. 136, 116.

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Protection Against Cardiovascular Collapse in Acute Adrenalectomy by Autonomic Blocking Agents.* (30031)

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Although the long term cardiovascular consequences of adrenalectomy are well documented(1,2,3,4), little information is available about the changes occurring shortly after adrenalectomy. Harrison and coworkers(5) reported that cardiac output progressively decreased as heart rate increased in the first 3 hours after occlusion of the adrenal veins. Brand(6) found that arterial blood pressure fell rapidly after adrenal venous occlusion. Recently, Zekert *et al*(7) ligated and rapidly removed the adrenals in anesthetized dogs. They found that blood pressure and cardiac output rapidly fell after adrenalectomy, whereas total peripheral resistance increased. The purposes of the present study were (1) to determine the time course of hemodynamic events following acute bilateral adrenalectomy leading up to the cardiovascular collapse and (2) to obtain data concerning the mechanism(s) responsible for these effects.

Methods. Male mongrel dogs (15.0 to 23.5 kg), fasted for 24 hours, were anesthetized

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with 30 mg/kg pentobarbital injected intravenously. The left carotid artery and right external jugular vein were cannulated for measurement of mean arterial blood pressure (MABP) and central venous pressure (CVP), respectively. The cannula in the jugular vein was advanced to the level of the superior vena cava. An electromagnetic flowmeter probe(8) was placed around the intact left femoral artery for measurement of femoral blood flow (FBF). Electrocardiographic limb electrodes were inserted under the skin in each limb, and heart rate (HR) was determined by use of a cardiotachometer circuit. In some experiments, left ventricular contractile force (LVCF) was measured by suturing a strain gage arch(9) to the epicardial surface of the left ventricle. All variables were continuously recorded on a 6-channel Offner Type R Dynograph.

In several experiments, blood samples were drawn at timed intervals for determination of plasma glucose and total plasma catecholamines. Total plasma catecholamines were determined by a modification of the trihydroxyindole method of Cohen and Goldenberg(10). Potassium ferricyanide was used instead of manganese oxide as the oxidizing agent.

Adrenalectomy was performed through a flank incision on both sides. Total surgery time averaged about 40 minutes. No fluid was administered, except 0.9% NaCl (isotonic saline) to replace the blood withdrawn for chemical analysis. All incisions were sutured, thus hemodynamic data were obtained in closed-chest, closed-abdomen animals. All drugs were prepared in isotonic saline and administered intravenously.

Results. Fig. 1 shows the time course of the hemodynamic and chemical events in a typical adrenalectomized dog. LVCF, MABP, and FBF rapidly declined soon after the second adrenal was removed and reached a level which we term a state of cardiovascular collapse (or shock) approximately 30 to 60 minutes after removal of the second adrenal. Heart rate increased progressively over the same period. Central venous pressure did not change significantly. Three hours after adrenalectomy, the values of these variables had stabilized. Although not shown in the

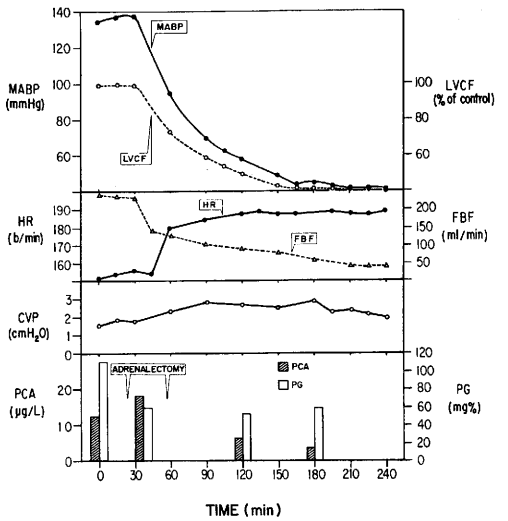

FIG. 1. Typical hemodynamic and chemical changes occurring after acute bilateral adrenalectomy in the dog. MABP, mean arterial blood pressure; LVCF, left ventricular contractile force; HR, heart rate; FBF, femoral blood flow; CVP, central venous pressure; PCA, plasma catecholamines; PG, plasma glucose. Adrenalectomy was performed over a period of 30 min. The left adrenal was removed at 20 min on the time scale, the right at 50 min.

Figure, the values at 270 and 300 minutes were essentially the same as those obtained at 240 minutes. The bar graphs in Fig. 1 show that plasma glucose (PG) concentration markedly decreased after adrenalectomy, but did not approach hypoglycemic shock levels. Plasma catecholamine (PCA) concentration increased just after the first adrenal was removed, but later decreased considerably below the initial value. Three of the 21 adrenalectomized dogs expired 3 to 4 hours after adrenalectomy. The other 18 survived for at least 4 hours after adrenalectomy and were eventually sacrificed.

Table I shows a summary of the hemodynamic data obtained in sham operated, in adrenalectomized, in cortisol treated, and in autonomic drug treated groups. It is evident from the sham operated controls that the surgical manipulations result in slight, but not significant alterations in LVCF, MABP and HR. However, FBF significantly decreased in the sham operated dogs, perhaps as a result of local vasoconstriction. The untreated adrenalectomized dogs exhibited precipitous and severe declines in LVCF, MABP

TABLE I. Hemodynamic Changes in Sham Operated, in Adrenalectomized, in Cortisol Treated, and in Autonomic Drug Treated Dogs.

Treatment	LVCF	MABP	FBF	HR
	% of control			(Δ in beats/min)
Sham (6)	91.0 ± 4.6 (4)*	89.5 ± 5.1	78.6 ± 4.1	+ 4.6 ± 3.9
Adrenex (21)	35.4 ± 2.8 (12)	34.6 ± 2.6	38.2 ± 3.1	+35.2 ± 5.3
Cortisol + adrenex (5)	—	87.0 ± 2.7	72.2 ± 4.3	+11.2 ± 7.1
Hexamethonium alone (3)	86.5 ± 6.1 (2)	89.4 ± 4.2	95.4 ± 7.6	+ 8.7 ± 8.5
" + adrenex (12)	88.3 ± 6.6 (6)	81.5 ± 3.6	87.0 ± 5.4	+ 5.5 ± 4.6
Pentolinium alone (3)	—	83.9 ± 7.2	91.6 ± 8.6	+ 9.6 ±13.8
" + adrenex (6)	87.6 ± 8.6 (3)	79.9 ± 6.8	73.1 ± 6.2	+ 7.8 ± 5.8
Phenoxybenzamine alone (3)	—	86.0 ± 6.7	78.8 ± 6.8	+ 9.3 ± 4.3
" + adrenex (6)	84.5 ± 8.5 (3)	78.5 ± 9.9	81.1 ± 5.6	+ 9.0 ±11.1

All values are means ± SE, using 0 time and 180 min after adrenalectomy or sham adrenalectomy as the two reference points. No. in parentheses under Treatment are total No. of dogs used in each group.

Adrenex = Bilateral adrenalectomized.

* LVCF was not measured in every experiment; No. in parentheses in this column indicate No. of animals in which LVCF was measured.

See Fig. 1 for explanation of other abbreviations.

and FBF, and a significant degree of tachycardia 3 hours after adrenalectomy. Cortisol, infused at a rate of 500 μg/min starting 30 minutes prior to adrenalectomy and continuing for 3 hours, completely protected against cardiovascular collapse. However, neither aldosterone (2 μg to 2 mg) nor cortisol (2 μg to 200 mg), administered after the development of cardiovascular collapse, had any detectable effect on the variables measured.

The ganglionic blocking agents, hexamethonium (5 mg/kg) and pentolinium (1 mg/kg) intravenously infused over a 20-minute period just prior to adrenalectomy, completely protected against the cardiovascular collapse which followed adrenalectomy. The adrenergic blocking agent phenoxybenzamine (4 mg/kg) similarly protected.

The protection conferred by pretreatment with the autonomic blocking agents was a very consistent finding. Cardiovascular collapse was prevented by pretreatment with hexamethonium in 12 of 12 dogs, with pentolinium in 6 of 6 dogs, and with phenoxybenzamine in 5 of 6 dogs. The same doses of these agents had no effect when administered after the development of the cardiovascular collapse. These autonomic blocking agents, when given to dogs with intact adrenals, exerted transient hemodynamic effects, but recovery was complete 3 hours later (Table I.)

Just prior to the onset of cardiovascular collapse (*i.e.*, immediately preceding removal of the second adrenal), there was a significant increase ($P<0.01$) in total plasma catecholamine concentration from a mean value of 14.9 ± 1.1 μg/l to 20.3 ± 1.7 μg/l in 8 untreated dogs. In 6 dogs treated with hexamethonium, catecholamine concentration decreased from 15.6 ± 2.2 μg/l to 12.6 ± 1.8 μg/l.

Table II shows the major findings in dogs infused intravenously with a mixture of 1 μg/min each of epinephrine and norepinephrine for one hour, during which time the adrenals were removed. The second group received in addition an infusion of hexamethonium (5 mg/kg) prior to the catecholamine infusion. Both groups showed similar results. During the catecholamine infusion, MABP, FBF and HR were maintained at normal levels. However, when the infusion was terminated, the animals regressed into a collapse which was comparable to that observed in the untreated adrenalectomized dogs shown in Table I. This cardiovascular collapse occurred within 30 minutes after withdrawal of the catecholamines.

Discussion. Immediately following bilateral adrenalectomy the dog exhibits a state of cardiovascular collapse; *i.e.*, arterial blood pressure, peripheral blood flow and myocardial contractility are severely decreased, whereas heart rate is markedly increased. Plasma glucose decreases and total plasma

TABLE II. Hemodynamic Data in Adrenalectomized Dogs During and After Infusion with Catecholamines.

	MABP (% of control)	FBF (% of control)	HR (Δ in beats/min)
Adrenex + catecholamine infusion (5)			
During CA infusion	85.5 ± 3.9	86.2 ± 2.8	— 1.0 ± 4.1
180 min after infusion	35.7 ± 4.7	55.8 ± 9.1	+21.0 ± 8.5
Adrenex + catecholamine infusion + hexamethonium (5)			
During CA infusion	80.1 ± 4.5	80.4 ± 3.9	—10.5 ± 4.5
180 min after infusion	48.8 ± 6.3	52.4 ± 8.6	+36.0 ±11.2

All values are means ± SE, and refer to the pretreated, preadrenalectomized condition which is taken as 100%.
CA = catecholamine.

catecholamines temporarily increase.

The shock state is reached about 30 to 60 minutes after adrenalectomy, at which time the peripheral plasma corticosteroid concentration undoubtedly is quite low(11,12,13, 14). Cortisol(13), corticosterone(12) and aldosterone(11) have biological half-times of 20-30 minutes in the dog. These biological half-times coincide reasonably well with the onset of cardiovascular collapse. The condition of the animal progressively deteriorates until it reaches a maximal shock state about 2 hours later, a circumstance which is essentially in agreement with the findings of Remington(1).

The factors responsible for development of the cardiovascular collapse may be an excessive secretion or release of norepinephrine from adrenergic nerve endings(15) or storage sites concomitant with a precipitous fall in plasma corticosteroid and epinephrine concentrations. The combination of these 3 factors would offer an explanation for the effectiveness of either corticosteroids or adrenergic blocking agents in preventing the occurrence of the cardiovascular collapse. These concepts were originally developed by Ramey and Goldstein(16). These workers(17) have previously reported that autonomic blocking agents (*e.g.*, atropine, banthine and dibenamine) protected against the adverse effects of a variety of stresses (*e.g.*, cold, hemorrhage, formalin) in adrenalectomized dogs maintained on deoxycorticosterone. However, they did not study the effect of these agents on non-steroid supported adrenalectomized animals subjected to no additional stress other than the surgery of adrenalectomy.

The ineffectiveness of autonomic blocking agents, after cardiovascular collapse has occurred, may be attributed to the sudden onset and irreversibility of the sympathetic hyperactivity, a situation which would require prior blockade. The corticosteroids were ineffective in the present study when given after removal of the second adrenal. The effects of such treatment were followed for a relatively short period. After a longer period, salutary effects may have become manifest. Swingle *et al*(18) have been unable to revive animals from adrenal insufficiency with intravenously administered aldosterone, and Gross(19) found that aldosterone had a 6-hour delay in acting in the adrenally insufficient dog. Furthermore, Brand(6) found that cortisol and cortisone were likewise ineffective in adrenally insufficient dogs.

The fundamental mechanism responsible for the protection by autonomic blocking agents may not be simply due to their blockade of catecholamines, but may be in part due to metabolic actions such as described by Baez *et al*(20). These workers found that phenoxybenzamine protected the hepatic ferritin systems against hypoxic damage.

Summary. Acute adrenalectomy in the anesthetized dog resulted in cardiovascular collapse which developed over a 30-60-minute period and was characterized by a decrease in left ventricular contractile force, mean arterial blood pressure, and femoral blood flow and the occurrence of prominent tachycardia. Pretreatment with appropriate doses of cortisol, hexamethonium, pentolinium and phenoxybenzamine prevented these hemodynamic alterations. Treatment with these

agents after the development of the collapse had no beneficial effect.

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1. Remington, J. W., Am. J. Physiol., 1951, v165, 306.
2. Swingle, W. W., Parkins, W. M., Taylor, A. R., Hays, H. W., ibid., 1938, v123, 659.
3. Lefer, A. M., Sutfin, D. C., ibid., 1964, v206, 1151.
4. Reidenberg, M. M., Ohler, E. A., Sevy, R. W., Harakal, C., Endocrinology, 1963, v72, 918.
5. Harrison, T. R., Blalock, A., Pilcher, C., Wilson, C. P., Am. J. Physiol., 1927, v83, 284.
6. Brand, E. D., Fed. Proc., 1954, v13, 338.
7. Zekert, H., Entrup, R. W., Walter, K. E., Paiewonsky, D., Caciolo, C., Muelheims, G., Wegria, R., Proc. Soc. Exp. Biol. and Med., 1964, v115, 491.
8. Olmsted, F., Aldrich, F. D., J. Appl. Physiol., 1961, v16, 197.
9. Sutfin, D. C., Lefer, A. M., Med. Electron. Biol. Eng., 1963, v1, 371.
10. Cohen, G., Goldenberg, M., J. Neurochem., 1957, v2, 58.
11. Ayers, C. R., Davis, J. O., Lieberman, F., Carpenter, C. C. J., Berman, M., J. Clin. Invest., 1962, v41, 884.
12. Willoughby, H. W., Chen, C., Freeman, S., Endocrinology, 1959, v65, 539.
13. Hechter, O., Frank, E., Caspi, E., Frank, H., ibid., 1957, v60, 705.
14. Bojesen, E., Degn, H., Acta Endocrinol., 1961, v37, 541.
15. Kleinberg, W., Remington, J. W., Drill, V. A., Swingle, W. W., Am. J. Physiol., 1942, v137, 362.
16. Ramey, E. R., Goldstein, M. S., Physiol. Rev., 1957, v37, 155.
17. Goldstein, M. S., Ramey, E. R., Fritz, I., Levine, R., Am. J. Physiol., 1952, v171, 92.
18. Swingle, W. W., Da Vanzo, J. P., Grossfield, H. C., Glenister, D., Osborne, M., Wagle, G., Proc. Soc. Exp. Biol. and Med., 1958, v99, 75.
19. Gross, F., Experientia, 1961, v17, 57.
20. Baez, S., Srikantia, S. G., Burack, B., Am. J. Physiol., 1958, v192, 175.

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Effect of FUDR on Interferon Production. (30032)

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Two recent papers present conflicting data concerning the need for new DNA synthesis for the production of interferon. Holmes *et al*(1) using iododeoxyuridine (IUDR) as an inhibitor of DNA synthesis, indicated that when DNA synthesis was stopped, interferon production was also blocked. On the other hand, Donikian and Stewart(2) found that IUDR increased the amount of interferon produced by chick embryo (CE) cells infected with irradiated influenza virus under certain circumstances, or had no effect under others. As part of a larger study on the control of interferon production, we have studied the effect of fluorodeoxyuridine (FUDR) on interferon production, using both an RNA virus and a DNA virus as stimulating agents, and these studies are reported here. Both FUDR and IUDR block the synthesis of DNA by inhibiting the conversion of deoxyuridylic acid to deoxythymidylic acid.

Experimental. Chickungunya virus was assayed by plaque method on primary hamster embryo cells, as previously described(3). Interferon was assayed by the plaque reduction method on CE cells using vesicular stomatitis virus as challenge. The titer of interferon is expressed as the reciprocal of the dilution of interferon that caused a reduction of 50% in number of plaques produced. Vaccinia virus was assayed by the fluid plaque method of Postlethwaite(4), using HeLa cells.

FUDR at concentrations of 3×10^{-6} to 1.8×10^{-5} molar inhibited DNA synthesis in CE cells by 90-95%, as measured by the incorporation of H^3-uridine into the DNA of the cells. Table I demonstrates the lack of effect of these concentrations of FUDR on