the term mouse hemopexin to identify this protein constituent of mouse serum.

Summary. A method for purification of mouse Beta 2-III globulin is described. The purified protein is perchloric acid soluble and contains hexose, hexosamine and sialic acid. It is soluble in rivanol and binds hematin. It has a sedimentation constant of 4.3 S, indicating a molecular weight of about 65,000. It is suggested that this protein henceforth be called mouse hemopexin.

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Sensitivity of Bluetongue Virus to Ether and Sodium Deoxycholate.* (30030)

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Arboviruses as they are presently defined, in addition to biologic transmission by arthropod vectors, have other properties in common. All are believed to contain RNA as their genetic material and all so far examined are inactivated by treatment with ether or sodium deoxycholate (SDC). Evidence that bluetongue virus is transmitted by arthropod vectors is compelling(1-3). Evidence that this transmission is biologic in the sense that the virus infects and multiplies within the cells of the insect before it is transmissible to susceptible sheep, while convincing (3), is not definitive. The genome of bluetongue virus is believed to be RNA(4). Andrewes(5) has indicated that bluetongue virus is not inactivated by treatment with ether or SDC. In the present study the sensitivity of 2 strains of bluetongue virus to ether or SDC was examined.

Materials and methods. A strain of bluetongue virus, BT-Cy, recovered from infected sheep in Cyprus(6) and a strain, BT-8, recovered from infected sheep in California(7) were examined for sensitivity to ether or SDC. Both viruses were originally isolated and passaged by yolk sac inoculation of chicken embryos (CE). BT-Cy at the 82nd CE passage and BT-8 at the 78th CE pas-

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TABLE I. Inactivation of Bluetongue Virus by Ethyl Ether.

Virus	HRS treated	Treated	Control
BT-Cy	7	4.63*	6.15
"	24	4.68	5.50
"	26†	5.20	6.00
"	44	3.50	5.50
"	48	4.00	4.50
BT-8	7	4.57	5.00
"	26†	4.83	5.00
IPV-55	26†	<1	5.50

* Titer expressed as negative logarithm to the base 10 of the $TCID_{50}$ dilution/ml.
† Same experiment.

sage were adapted to lamb embryo kidney (LEK) cell cultures(8). Stocks of each virus (BT-Cy at the 32nd and 33rd LEK passage and BT-8 at the 20th LEK passage) were prepared and stored in sealed, glass ampoules at 4°C. Infectious pustular vulvovaginitis (IPV-55) virus(9), a member of the herpesvirus group, after 5 passages in bovine embryo kidney cell cultures, was used as an ether and SDC sensitive control virus.

Viruses were assayed in LEK cell monolayers prepared using methods outlined by Madin *et al*(10). Cells were used between the 2nd and 5th passages. The media used (LE media) consisted of Earle's balanced salt solution containing 0.5% lactalbumin hydrolysate and 500 units penicillin and 50 μg streptomycin per ml. For cell growth 10% lamb serum was added; when monolayers were complete, the lamb serum was reduced to 3%. Virus titers were expressed as 50% tissue culture infective dose ($TCID_{50}$) per ml or per 0.1 ml calculated by the method of Reed and Muench.

Ether sensitivity was examined using methods similar to those described by Andrewes and Horstman(11). To a 10^{-1} dilution of stock virus in phosphate buffered saline (PBS)(12), 20% ethyl ether† was added. The ether-virus mixtures and virus controls were held at 4°C for 7 to 48 hours (Table I), during which time they were intermittently shaken. After treatment the ether was evaporated and serial 10-fold dilutions of each preparation were made using LE media plus 3% lamb serum as diluent One ml of each dilution was inoculated into each of 4 or 5 cell culture tubes.

Sensitivity of the viruses to SDC was ascertained using 2 different procedures. Anderson and Ada(13) working with Murray Valley encephalitis (MVE) virus indicated that treatment with SDC resulted in the release of infectious RNA which was destroyed by ribonuclease. In the present experiments infectious RNA would probably not have been detected if it were released from bluetongue virus by SDC treatment; nonetheless, ribonuclease‡ was included in the test mixtures. The protocol for this procedure is indicated in Table II. Viruses were exposed to a final concentration of 1 or 0.1% SDC for 1 hour at room temperature. Because SDC was toxic to LEK cells SDC-virus mixtures and control preparations were dialyzed overnight at 4°C against several changes of PBS. After dialysis serial 10-fold dilutions were made in LE media containing 3% lamb serum and 1 ml of each dilution was inoculated into each of 4 or 5 cell culture tubes.

When it was realized that probably neither BT-Cy nor BT-8 was inactivated by SDC, a simpler procedure was adopted. A 1:5 dilution of stock virus in PBS was mixed with an equal amount of 0.2% SDC in PBS and after treating at room temperature for one hour serial 10-fold dilutions were made using PBS as diluent. One-tenth ml of each dilution was inoculated into each of 4 or 5 cell culture tubes. Virus was allowed to adsorb for one hour at 37°C before addition of 0.9 ml LE media containing 3% lamb serum to each tube.

Results. Twenty-four hours is considered a reasonable time for ether to inactivate sensitive viruses. In several experiments this period was extended (Table I) and in each instance it was shown that neither strain of bluetongue virus was inactivated by treatment sufficient to completely destroy the infectivity of IPV-55 virus.

In each of the experiments designed to demonstrate the sensitivity of bluetongue virus to SDC, no inactivation was demonstrated. Results of typical experiments are shown in Tables II and III. In the assay

† Ether USP: E. R. Squibb & Sons, New York.

‡ Worthington Biochemical Corp., N. J.

TABLE II. Effect of Sodium Deoxycholate on Bluetongue Virus (Procedure 1).

Experiment	Amount in ml				Result
	Virus*	SDC†	RNase‡	PBS	$TCID_{50}$
BT-Cy virus + 1% SDC + RNase	1.5	1.5	3	0	5.4§
BT-Cy virus + 0.1% SDC + RNase	1.5	1.5	3	0	5.5
IPV-55 virus + 0.1% SDC + RNase	1.5	1.5	3	0	<1
BT-Cy virus titration	1.5	0	0	4.5	5.2
IPV-55 " "	1.5	0	0	4.5	4.7
1% SDC control	0	1.5	0	4.5	toxic
0.1% " "	0	1.5	0	4.5	—‖
RNase "	0	0	3	3.0	<1

* Stock virus diluted 1:2.5 in PBS, hence final dilution 10^{-1}.

† 4% SDC in PBS was added where 1% was required. 0.4% SDC was added where 0.1% was required.

‡ 40 μg/ml in PBS to give final concentration of 20 μg/ml.

§ Titer expressed as negative logarithm to the base 10 of the $TCID_{50}$ dilution/ml.

‖ In cell cultures receiving 0.01% SDC some toxic change was present but this was distinguishable from cytopathic changes produced by either virus.

method shown in Table II SDC was toxic for cells at concentrations of 0.001%. The toxic effect was largely removed by dialysis of the test mixtures without appreciably interfering with the titer of the virus. Using the method outlined in Table III, 0.1 ml of a test mixture containing 0.1% SDC followed one hour later by 0.9 ml media was also toxic for cells; the next dilution representing a final concentration of SDC of 0.001% was not toxic and hence there was no difficulty in ascertaining the endpoint of the titration. SDC resulted in almost total inactivation of IPV-55 (Tables II and III).

Discussion. Not all viruses presently included in the arbovirus group have been thoroughly investigated and many are included on the basis of serologic and epidemiologic data. Certain misgivings have been expressed about the arbovirus group as it is presently defined (*e.g.*, 5). It has been tacitly assumed that bluetongue virus belongs to the arbovirus group. The present findings support the statement of Andrewes(5) and show that unlike other known members of the arbovirus group bluetongue virus is not inactivated by lipid solvents.

TABLE III. Effect of Sodium Deoxycholate on Bluetongue Virus (Procedure 2).

Virus	.1% SDC	Control
BT-Cy	5.0*	5.15
IPV-55	<1	4.5
BT-8	4.0	4.33
IPV-55	<1	4.66

* Titer expressed as negative logarithm to the base 10 of the $TCID_{50}$ dilution/0.1 ml.

Viruses which are sensitive to ether and SDC are believed to contain essential structural lipid and as far as is known all belong to the myxo-, herpes-, and arbovirus groups. There is ample evidence that myxo- and herpesviruses possess an envelope. Good electron micrographs of arboviruses which clearly reveal their architectural detail are unknown to the author. However, examination of thin sections of cells infected with western equine encephalomyelitis virus(14) and negatively stained preparations of Sindbis virus(15) indicate that an envelope may be a general property of arboviruses. Electron micrographs of negatively stained preparations of the Cyprus strain of bluetongue virus indicate that no envelope is present (Studdert, Pangborn and Addison, unpublished). It seems reasonable to propose that sensitivity to ether and SDC is a property of those viruses which have an envelope. This, however, does not *a priori* exclude the possibility that essential lipid may also be present in the capsid.

The present findings are not commensurate with the inclusion of bluetongue virus in the arbovirus group, at least as this group is presently defined. Bluetongue virus together with the virus of African horsesickness (1) and perhaps ephemeral fever virus of cattle(16) are unique among "arboviruses" in at least one other respect, namely, that

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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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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