by cortisol as well as its potentiation in adrenalectomized animals is paralleled by the known general antiendotoxic effect of cortisol (15), and the dramatic increased susceptibility of adrenalectomized animals to endotoxin death(16).

Summary. The administration of 1 to 5 mg of cortisol to 1 kg rabbits markedly suppressed interferon production by *E. coli* endotoxin. Single doses of cortisol, up to 25 mg, did not decrease circulating interferon levels following inoculations with Newcastle disease virus. This type of interferon was only partially suppressed with multiple injections of 250 mg. Thus, administered cortisol inhibited more readily the production of endotoxin-induced interferon than the virus-induced counterpart. Adrenalectomy markedly potentiated the production of interferon by endotoxin. Adrenalectomized rabbits inoculated with endotoxin produced serum interferon to a mean titer of 1:388, while the mean titer in control animals was 1:28, suggesting that endogenous steroids suppress the interferon esponse to endotoxin.

Mrs. Linda Carcione offered excellent technical assistance. One of us (Miss Catherine DeAngelis) was a recipient of a summer research fellowship under NIH grant 5416-05.

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Received January 9, 1967. P.S.E.B.M., 1967, v125.

Comparative Viral Sensitivity of Bovine and Avian Cells Before and After Storage in Liquid Nitrogen.* (32022)

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Dimethyl sulfoxide (DMSO) has been found useful for minimizing the loss of cell viability on freezing and storage in the frozen state and is gaining wide acceptance for routine storage of mammalian and avian cells for virus studies (1-8). The procedure enables one to have on hand pre-tested cell stocks suitable for routine or investigational use and assures the safety of such cultures by preventing loss by accidental microbial contamination or by chromosomal mutation which frequently occur when cultures are repeatedly transferred *in vitro*. Many commercial manufacturers of tissue cultures depend on frozen cell stocks for production of various types of primary, diploid, and heteroploid cultures.

Although cultures derived from frozen cell stocks are in wide use, adequate information

* This work was supported by National Cancer Institute Field Studies, Contract PH 43-64-941, Nat. Inst. Health, Bethesda, Md.

is not available on the relative viral sensitivity of such cultures. Beem *et al*(9) recently reported that secondary monkey kidney cultures derived from frozen cell bank of primary monkey kidney cells equalled the sensitivity of primary kidney cultures for the isolation and assay of para-influenza viruses. We report herein studies of bovine embryonic kidney (BEK) and chicken embryo fibroblast (CEF) cultures which indicated that the viral sensitivity of these cultures to selected viruses was not altered by the DMSO storage technique.

Materials and methods. Tissue cultures. BEK and CEF culture suspensions were prepared by a procedure described previously (10). Each culture lot was prepared by using one or two pairs of kidneys or 3 to 5 leukosis virus-free chicken embryos of the same hen. Growth medium for CEF was EGM(10), consisting of Eagle's minimum essential medium(11), supplemented with 5% calf serum, 5% tryptose phosphate broth, L-glutamine, and antibiotics. Agamma calf serum was substituted for calf serum for BEK cells. Culture media were replaced at 3 to 5 day intervals. The serum concentration was reduced to 1% when confluent cell growth was obtained.

Primary cultures were planted at 6×10^5 cells per ml; cell suspension was inoculated in 1.5 ml amounts in 16 × 125 mm screw-capped tissue culture tubes and in 40 ml amounts in 32 oz bottles. Secondary BEK cells were similarly planted at 10^5 cells per ml and secondary CEF cells at 3×10^5 cells per ml in tubes and petri dishes (5 ml per 60 mm Falcon dish). The effect of freezing and storage on viral sensitivity of cells was determined by assaying viruses in parallel in primary and secondary cultures grown prior to and after freezing and storage of trypsinized cells for 24 hours. Frozen stocks of BEK and CEF cells were also similarly tested after 3 months and 6 months of storage.

Cell storage and reconstitution. Trypsinized cells were sedimented and resuspended in a concentration of 5 to 10 million cells per ml in a freezing medium consisting of EMEM supplemented with 15% agamma calf serum and 7.5% DMSO (Fisher). The cell suspensions were added to sterile 5 ml ampoules in 4 to 5 ml amounts. The ampoules were sealed and cells frozen in a Linde BF-3 freezer which lowered the temperature of the contents of vials at the rate of 1°C a minute to —120°C and then at 5°C a minute to —180°C. The frozen samples were then stored in liquid nitrogen vapor of a Cryenco liquid nitrogen freezer whose average vapor temperature was maintained below —170°C by periodical additions of liquid nitrogen.

Frozen cells were reconstituted and planted as follows. The frozen cells were rapidly thawed at 37°C, centrifuged at 250 g for 10 minutes, and the supernatant medium containing DMSO was discarded. Cells were resuspended in fresh medium and viable cell count was made by the use of trypan blue. Cells were planted in tubes or in petri dishes.

Virus assay. Virus assays were performed with cultures grown in screw-capped tubes. Avian leukosis and sarcoma viruses were assayed using 60 mm disposable plastic dishes (Falcon).

A bovine enterovirus, isolated from an outbreak of acute, fatal respiratory disease(12), and infectious bovine rhinotracheitis virus were assayed in BEK tubes. GB Texas strain of Newcastle disease virus (NDV), vesicular stomatitis virus (VSV), RPL-12 strain of visceral lymphomatosis virus, and Bryan and Schmidt-Ruppin (S-R) strains of Rous sarcoma virus RSV were assayed in CEF cultures.

NDV stock was prepared as clarified allantoic fluids of embryonated chicken eggs. VSV was grown in a serial line of rabbit kidney cells (MA 111, Microbiological Associates, Inc.). Other virus stocks were grown as described elsewhere (10,12,13). All virus stocks were stored at —70°C. The bovine virus, NDV, and VSV titers reported are 50% tissue culture infectivity endpoints calculated by the method of Reed and Muench (14). Each virus dilution was inoculated in 0.2 ml amounts into each of 4 culture tubes. The avian leukosis and Rous sarcoma viruses were assayed by determining the highest virus dilution which induced the leukosis group-specific CF antigen (COFAL test)(13) and foci of transformed cells (Rous sarcoma virus).

TABLE I. Cell Viability and Viral Sensitivity of Bovine Embryonic Kidney Cells Grown After Storage at Below —170°C for Varying Lengths of Time.

Exp No.	Culture	Storage prior to growth (days)	% Cell viability	Virus titer* Enterovirus	Virus titer* IBR†
1	Primary	none	53	6.7	6.4
	"	1	44	7.0	6.7
	"	90	35	6.7	7.4
	"	180	34	7.2	7.0
	Secondary	none	90	6.5	5.7
	"	1	75	6.7	6.4
	"	90	75	6.7	6.4
	"	180	74	6.7	7.0
2	Primary	none	64	6.7	6.7
	"	1	36	7.0	7.7
	"	90	38	7.0	7.4
	"	180	35	7.0	7.4
	Secondary	none	94	7.7	7.0
	"	1	78	7.4	6.7
	"	90	76	6.7	7.4
	"	180	80	7.0	7.2
3	Primary	none	66	6.4	6.4
	"	90	41	6.4	7.0
	"	180	30	6.7	7.0
	Secondary	none	83	6.7	7.4
	"	1	70	7.0	7.4
	"	90	66	7.0	7.4
	"	180	70	7.8	7.2
4	Primary	none	61	6.7	7.7
	"	1	45	6.4	7.4
	"	90	36	7.0	7.2
	"	180	35	7.4	7.2
	Secondary	none	93	6.7	6.7
	"	1	74	6.7	7.0
	"	90	76	6.7	7.4
5	Primary	none	76	6.0	7.0
	"	1	58	5.7	7.4
	"	90	50	8.0	7.0
	Secondary	none	87	6.0	6.7
	"	1	71	7.7	7.0
	"	90	68	7.4	7.7

* Log_{10} $TCID_{50}$/ml. † IBR = infectious bovine rhinotracheitis virus.

Results. Cell viability and growth characteristics. Cells freshly derived from bovine kidneys generally yielded viable counts of between 53% to 76%; cell freezing and storage at below —170°C in the presence of DMSO lowered the viable count to between 36% and 58% (Table I). There appeared to be a slight but progressive drop in viability with increasing length of storage in liquid nitrogen (46% to 54% in 6 months; Exp. 3, 4, Table I). It generally took 5 to 8 days to prepare confluent monolayer cultures from freshly trypsinized and frozen cell stocks. On the other hand, frozen cell stocks derived after initial growth as primary cultures yielded higher and fairly uniform viable counts (66% to 78%) over a 6 month storage period and monolayer cultures were obtained within a short interval of 2 to 4 days.

As usually observed with secondary cultures, the cellular morphology and monolayer growth of secondary BEK cultures were more uniform than those of primary BEK cultures.

Freshly trypsinized and frozen stocks of trypsinized CEF cells yielded viable counts of $\geq$71% and provided monolayer cultures within 1 to 3 days of planting cultures (Table II). As noted with BEK, CEF cells stored after initial growth as monolayer cultures gave higher viability counts than those directly procured from tissues.

Virus assay. Freezing and storage of the BEK cells for periods up to 6 months did not alter the sensitivity of these cells to bovine enterovirus and infectious bovine rhinotracheitis virus (Table I). Similarly CEF cells fully retained their sensitivity to NDV, VSV, RPL-12, and 2 strains of RSV after freezing and storage in liquid nitrogen (Table II).

TABLE II. Cell Viability and Viral Sensitivity of Chick Embryo Fibroblast Cell Grown After Freezing and Storage at Below —170°C.

Exp No.	Culture	Storage prior to growth (days)	% Cell viability	Virus titers						
				NDV*	VSV*	RPL-12† COFAL	RSV – Bryan† Foci	RSV – Bryan† COFAL	RSV – S-R† Foci	RSV – S-R† COFAL
1	Primary	none	73	9.4						
	"	1	71	9.0		6.7	6.7	7.7	4.7	5.7
	Secondary	none	95	9.4		6.7	6.7	7.7	5.7	5.7
	"	1	83	9.4		6.7	6.7	7.7	4.7	4.7
2	Primary	none	94	9.7	7.7					
	"	1	71	9.4	7.7	7.7				
	"	180	60	8.4	8.4					
	Secondary	none	98	9.7	8.0	7.7				
	"	1	83	9.7	7.7	7.7				
	"	180	87	9.2	8.7					

* Log_{10} $TCID_{50}$/ml.
† Log_{10} titer/ml obtained by determining the highest dilution which induced RSV foci and avian leukosis group CF antigen in CEF culture (COFAL test).

Discussion. Results obtained in these experiments indicated that freezing and storage of BEK and CEF cells in liquid nitrogen in the presence of DMSO did not diminish their growth potential or alter their sensitivity to viral effects (cytopathic effect, cell transformation). Since such storage offers the advantage of ready availability of cells of known status, purity and viral sensitivity, this procedure should find uses in situations where the availability of fresh tissues is meager and/or unpredictable. These experiments indicated that cells frozen after initial growth as primary monolayer cultures were superior to cells frozen after fresh procurement from tissues.

Summary. Satisfactory monolayer cultures were produced after freezing bovine embryonic kidney (BEK) and chicken embryo fibroblast cells in the presence of 7.5% DMSO in the storage medium and storage for varying lengths of time in liquid nitrogen vapor. The sensitivity of such cultures to selected viruses remained undiminished after 6 months of such storage. BEK cells frozen after initial growth as primary monolayer cultures generally provided satisfactory monolayer cultures within a relatively short time (2 to 4 days) as compared with BEK cells which were frozen after fresh procurement from tissues (5 to 8 days).

The authors wish to acknowledge the excellent technical assistance of Mr. Horace C. Turner and Mr. Monty Bell.

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Received January 23, 1967. P.S.E.B.M., 1967, v125.

Relation of Renal Arterial Pressure to Activity of Renin-Angiotensin System in Renal Hypertension.* (32023)

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Previous studies in both the rat(1,2) and the dog(3) demonstrate clearly that experimental renal hypertension can be divided into two distinct phases. There is an acute phase characterized by the presence in both the renal vein blood and the general circulation of a potent vasopressor agent with properties similar to angiotensin. After approximately 2 weeks a chronic phase ensues in which, despite the persistence of the hypertensive state, blood from the renal vein and from the general circulation ceases to show vasopressor activity. This is in accord with reports that the levels of plasma renin(4,5) or angiotensin (6), are elevated in dogs with early renal hypertension and are normal in the chronic stage.

Since renal arterial pressure(7,8,9,10,11) is probably the factor which governs the release of renin by the juxtaglomerular apparatus of the kidney, the present study was undertaken to determine whether the change in activity of the renin-angiotensin system during the course of renal hypertension could be correlated with the levels of renal arterial pressure.

Method. Production of unilateral hypertension. One hundred ninety-five white male rats weighing about 200 g were used. Under nembutal anesthesia the abdominal aorta just above the ostium of the left renal artery was reduced to a diameter of 0.25 mm by ligating the vessel around a stylet of this diameter and then removing the stylet. This led to rapid ischemic atrophy of the left kidney while the right kidney remained intact.

Determination of systemic blood pressure and the left renal arterial blood pressure. Mean systemic blood pressure was obtained under anesthesia by inserting a polyethylene catheter into the brachial artery and connecting it to a Hg manometer. Control readings were made prior to the operative procedure. The mean pressure in the left main renal artery was determined in a similar manner by inserting a catheter into this vessel and directing it toward the aorta. Since the artery had to be ligated on withdrawing the catheter, only one reading could be obtained for each animal and the rat was then discarded. Determinations of both the systemic and the left renal arterial pressure were made at intervals of stat to 6 months after constriction of the aorta.

From past experience in our laboratory it was known that following constriction of the aorta the pressures in both the femoral and the left renal artery are quite similar or even identical. This observation was useful since the renal arterial pressure could be taken only once, while arterial pressure could be obtained several times in the same femoral vessel. By following the femoral arterial pressure serially in all rats, especially during the first 2 weeks after operation, the animals could be divided into groups with either a rapid, or average, or slow return to normal of the arterial pressure distal to the aortic constriction. With these

* Supported by Grant H-6304 from Nat. Inst. Health USPHS.