tain BV constant, the proportional changes in BV being estimated by the ratio: calculated Hct/measured Hct.

Long term study. By way of contrast with the previous 2- and 6-hour trials, Fig. 1-C depicts the Hcts observed in an 8-day series. The 10 birds here were on a feed deprivation test and had lost 21% of their initial 2326 ± 60 g BW before feed was restored on the 8th day. Sample size was 1½ ml, taken at 12, 18, and 24 hours, and daily thereafter. Initial Hct was 46.2 ± 1.0. Except for the period between 18 and 60 hours, there was little agreement between measured and calculated Hcts. At 12 hours, after a single 1½ ml sample, measured Hct was lower than expected, very suggestive of the hemodilution noted by Sturkie and Newman(3) under similar conditions. From the 3rd day on, measured Hcts were significantly larger than calculated values (pooled SE of differences = ± 0.8) and, in general, had returned close to the initial level. While this is highly suggestive of addition of cells to the vascular compartment, it must not be overlooked that inanition itself can raise Hct due to relative loss of plasma(8). In any event, the equation obviously cannot be used to predict Hcts under these conditions.

It should be noted that effects on Hct of such factors as size of tube, force of centrifugation, trapped plasma, and site of sampling should not alter the relationship between measured and calculated values as long as the test conditions remain uniform within any one series of measurements.

Summary. A formula has been derived for calculating the hematocrit of successive blood samples based on the assumption that blood volume is maintained constant through replacement of lost cells and plasma by extravascular fluid. The formula predicted, with an error of 2% or less, decreasing hematocrits which followed a removal of 7-11 successive blood samples of 2½-3½ ml each, within a period of 6 hours or less in the adult male chicken. The formula failed to predict the Hcts observed in an 8-day test on fasting chickens in which 1½ ml blood samples were obtained at 12-24-hour intervals. In the course of this study, the blood volume of the 2.5 kg White Leghorn male was determined to be 6.6 ± 0.25 ml/100 g body weight.

1. Newell, G. W., Shaffner, C. S., *Poultry Sci.,* 1950, v29, 78.

2. Bond, C. F., Gilbert, P. W., *Am. J. Physiol.,* 1958, v194, 519.

3. Sturkie, P. D., Newman, H. J., *Poultry Sci.,* 1951, v30, 240.

4. Pino, J. A., Weiss, H. S., Sturkie, P. D., de Falco, R. J., *IX World's Poultry Congress,* 1951, v3, 25.

5. Allen, T. H., Semple, R. E., *Am. J. Physiol.,* 1951, v165, 205.

6. Rodnan, G. P., Ebaugh, F. G., Fox, M. R. S., *Blood,* 1957, v12, 355.

7. Sturkie, P. D., *Am. J. Physiol.,* 1943, v138, 599.

8. Pareira, M. D., Sicher, N., Lang, S., *A.M.A. Arch. Surg.,* 1958, v77, 191.

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Adaptation of Columbia SK Virus to Sarcoma 180 Ascites Tumor Cells *in vivo* and Passage in the Cells *in vitro.* (26904)

Eiichi Furusawa and Windsor Cutting
Department of Medical Microbiology, Stanford University, Calif.

Viruses growing in tissue culture provide a desirable means for testing the effects of antiviral compounds. Unfortunately, the Columbia SK virus has not in the past been successfully cultured serially in established cell cultures(1), although we have reported (2,3) that the virus could be adapted to Ehrlich ascites tumor cells *in vivo.* We now show that Columbia SK virus adapted to Ehrlich cells *in vivo* can also be adapted to Sarcoma 180 ascites tumor cells *in vivo* after serial passage in mixtures of the 2 cells, and that the new strain can propagate efficiently even in *in vitro* cultures of Sarcoma 180 as-

cites cells. Clear cytopathic effects (CPE) and high titers of hemagglutinins (HA) are produced. The new virus-host system is proving useful, in parallel with the usual studies in mice, to study the effect of the antiviral substance "propionin"(4).

Materials and methods. 1). *Virus passage* in vivo. The original virus source was our 43rd passage of Columbia SK virus in Ehrlich ascites tumor cells (E43) in mice. This had a potency of about 10^6 LD_{50}/0.1 ml and 1:32 HA just before use. Two-tenths ml of a 10^{-3} dilution of the E43 ascites fluid was added to a mixture of 0.2 ml of Ehrlich ascites cells and 0.2 ml of Sarcoma 180 ascites cells both freshly harvested from ascites tumor-carrying mice. The infected mixture was immediately inoculated into the peritoneal cavities of 3 to 5 mice. Before the mice died, at 3 to 5 days after inoculation, ascites fluid was aspirated and the supernatant after centrifugation was used for the next virus passage. Microscopic observation was also made, after Giemsa staining, for detection of oncolysis.

2). *Virus passage* in vitro. After washing, 2 to 4 $\times$ 10^6 Sarcoma 180 ascites cells harvested freshly from mice were suspended in 1 ml of culture medium per tube. The medium was composed of 80% of Medium 199 and 20% of S180 ascites serum from which the fibrin had been removed by standing at 4°C for 1 week. One-tenth ml of a definite dilution of source virus with Medium 199 was inoculated into each tube. The culture was stationary at 36°C. After 2 to 4 days incubation, the cultures were shaken by hand to remove the cells from the glass wall, and then centrifuged. The supernatant was used for the next passage, and also for titration of the infectivity and for HA. The cells were resuspended in saline and stained with 1 drop of 0.2% of erythrosin, which colors the damaged cells red but does not stain living cells. With this staining method, the CPE of the virus can be calculated quantitatively. For the HA test, 0.2% sheep red cells suspended in potassium barbital buffer were used(5). The infectivity of the culture fluids was determined in mice injected with Sarcoma 180 cells, and *in vitro* in culture tubes.

Results. 1). *Adaptation of Columbia SK virus from Ehrlich ascites cells* in vivo *to Sarcoma 180 ascites cells* in vivo. Several preliminary trials showed that the Ehrlich-adapted strain of virus could not propagate directly in Sarcoma 180 ascites cells. One of the trials is shown in the top 2 lines in Table I. Two-tenths ml of 10^{-3} dilution of the 43rd passage of Ehrlich-adapted strain of virus (E43), mixed with 0.2 ml of Sarcoma 180 ascites cells, resulted in the death of each of 4 mice in 4 to 5 days, without oncolysis. Then a 10^{-2} dilution of the infected ascites harvested just before death (named S_1) was again inoculated into mice with fresh S180 ascites cells by the same method, and the 4 mice died at 7 to 8 days without oncolysis. The harvest (S_2) was not infectious when 10^0 to 10^{-3} dilutions were inoculated along with S180 ascites cells into mice. This indicates that the Ehrlich-adapted virus cannot adapt to Sarcoma 180 cells directly.

In contrast to the results with S180 cells alone, a mixture of Ehrlich cells and S180 cells supported virus propagation; that is, E43 propagated in the mixture with severe oncolysis of both types of cells, and the harvest $(SE)_1$ showed a clear hemagglutination. This harvest $(SE)_1$ could be passaged serially in the mixtures of both cells but not in the S-180 cells alone. At each generation, $(SE)_1$, $(SE)_2$, $(SE)_3$ and $(SE)_4$, attempted passage to Sarcoma 180 ascites cells alone was unsuccessful as shown further in Table I. Unexpectedly, at the 5th generation, it was found that $(SE)_5$ could propagate in S180 ascites cells alone with a clear oncolysis, and the harvest $(SE)_5S_1$ showed a definite HA indicating the existence of mature virus of at least 10^6 LD_{50}. The following passages from $(SE)_5S_1$ grew easily in Sarcoma 180 ascites cells alone. Further passage *in vivo* was stopped after 5 more generations (S_5). The newly adapted strain also propagated in Ehrlich ascites cells alone.

2). *Virus passage* in vitro. The *in vivo* harvest $(SE)_5S_2$ was centrifuged and 0.1 ml of 10^{-1} dilution of the supernatant was inoculated into culture tubes which contained Sarcoma 180 ascites cells. After 3 days of culture complete CPE had appeared, and the

TABLE I. Adaptation of Columbia SK Virus from Ehrlich Ascites Tumor Cells *In Vivo* to Sarcoma 180 Ascites Tumor Cells *In Vivo*.

Inoculum	Dilution	Cells	Oncolysis	Day mouse died	Harvests	
					No.	HA titer
E43	10^{-3}	Ehrlich	+	4, 4, 4	E44	64
E43	10^{-3}	S180	—	4, 5, 5, 5	S_1	0
E43	10^{-3}	Ehr + S*	+	4, 4, 4	$(SE)_1$	16
S_1	10^{-2}	S180	—	7, 7, 7, 8	S_2	0
S_1	10^{-3}	"	—	All survived†		
S_2	10^{0} to 10^{-3}	"	—	All survived		
$(SE)_1$	10^{-3}	Ehr + S	+	4, 4, 4	$(SE)_2$	16
$(SE)_1$	10^{-3}	S180	—	All survived		
$(SE)_1$	10^{-2}	"	—	5, 5, 6	$(SE)_1S_1$	0
$(SE)_1S_1$	10^{0} to 10^{-3}	"	—	All survived		
$(SE)_2$	10^{-3}	Ehr + S	+	4, 4, 4	$(SE)_3$	8
$(SE)_2$	10^{-2}	S180	—	6, 6, 6	$(SE)_2S_1$	0
$(SE)_2S_1$	10^{0} to 10^{-3}	"	—	All survived		
$(SE)_3$	10^{-3}	Ehr + S	+	3, 4, 4	$(SE)_4$	16
$(SE)_3$	10^{-2}	S180	—	5, 6, 6	$(SE)_3S_1$	0
$(SE)_3S_1$	10^{0} to 10^{-3}	"	—	All survived		
$(SE)_4$	10^{-3}	Ehr + S	+	3, 3, 3	$(SE)_5$	32
$(SE)_4$	10^{-2}	S180	—	5, 6, 7	$(SE)_4S_1$	0
$(SE)_4S_1$	10^{0} to 10^{-3}	"	—	All survived		
$(SE)_5$	10^{-3}	Ehr + S	+	3, 3, 3	$(SE)_6$	32
$(SE)_5$	10^{-2}	S180	+	4, 4, 4	$(SE)_5S_1$	32
$(SE)_5S_1$	10^{0}	"	+	3, 3, 3		
$(SE)_5S_1$	10^{-2}	"	+	4, 4, 4	$(SE)_5S_2$	32
$(SE)_5S_2$	10^{-3}	"	+	4, 4, 4	S_3	32
S_3	10^{-3}	"	+	3, 3, 3	S_4	64
S_4	10^{-3}	"	+	3, 3, 3	S_5	64
S_4	10^{-3}	Ehrlich	+	3, 3, 3	S_4E_1	64
S_5	10^{-3}	S180	+	3, 3, 3	S_6	64
S_5	10^{-3}	Ehrlich	+	3, 3, 3	S_5E_1	64

* Ehr + S = mixture of Ehrlich ascites cells and Sarcoma 180 ascites cells.
† All the mice inoculated survived.

supernatant of the infected medium (harvest T1) contained $10^{6.5}$ LD_{50}/0.1 ml in mice and 1024 HA units/0.5 ml. Table II shows the subsequent passages in Sarcoma 180 ascites cells *in vitro*. From these results the serial passage of the virus appeared to be established with a stable virus titer but rather variable HA.

3). *Comparative development of CPE in control and infected cells.* "Non-established" strains of Sarcoma 180 ascites tumor cells cannot propagate *in vitro* but survive for several days. The proportion of living cells may be determined at any time by erythrosin staining. Although the viability seems to be rather variable in each case, on the average in our medium 50% of the total cells are alive at 1 day after incubation, 30% at 2 days, 20% at 3 days, 15% at 4 days and 8% at 5 days. Fortunately virus growth in the cells is more rapid than the degeneration of cells themselves, so that the virus infectivity titration can be performed *in vitro* by the criteria of CPE and detection of HA in the infected tubes.

Ten-fold dilutions of the 16th virus passage (T16), from 10^{0} to 10^{-8}, were each inoculated into 10 tubes, and percentage of live cells and HA titers were calculated daily from 2 tubes. The cells inoculated with 10^{0} to 10^{-3} dilutions were all stained (dead) at 2 days after incubation, the cells inoculated with 10^{-4} to 10^{-6} were all stained at 3 days, while those at 10^{-7} to 10^{-8} were the same as the control (Fig. 1). The stained cells in the infected tubes were more pyknotic and of more irregular shape than those of controls. HA tests also confirmed the CPE (Fig. 2). The infective titer of T16 in mice was also about 10^{6} LD_{50}/0.1 ml, the same as *in vitro*.

4). *Neutralization and anti-hemagglutination tests with an immune serum prepared against original brain-Columbia SK virus.* With an immune rabbit serum against the original brain-Columbia SK virus possessing an antihemagglutinin titer of 1:1280 and a neu-

TABLE II. Virus Passage in Sarcoma 180 Ascites Tumor Cells *In Vitro*.

Inoculum	Dilution	Harvests: Day	No.	HA titer	In mice LD_{50}	*In vitro* ID_{50}
$(SE)_5S_2$	10^{-1}	3	T1	1024	$10^{6.5}$ per 0.1 ml	
T1	10^{-1}	4	T2	512		
T2	10^{-1}	2	T3	512		
T3	10^{-3}	3	T4	2048	$10^{7.2}$	$10^{7.2}$
T4	10^{-3}	3	T5	512		
T5	10^{-3}	3	T6	512	$10^{6.5}$	
T6	10^{-1}	2	T7	256		
T7	10^{-3}	3	T8	256		
T8	10^{-1}	3	T9	128	$10^{6.5}$	$10^{6.2}$
T9	10^{-1}	3	T10	32		
T10	10^{-2}	3	T11	2		
T11	10^{-2}	3	T12	32		
T12	10^{-2}	3	T13	64	$10^{6.2}$	$10^{6.2}$
T13	10^{-2}	3	T14	512		
T14	10^{-1}	3	T15	256		
T15	10^{-1}	3	T16	512	$10^{6.5}$	$10^{6.5}$
T16	10^{-2}	3	T17	1024		
T17	10^{-2}	2	T18	256		
T18	10^{-2}	3	T19	512	$10^{6.2}$	$10^{6.5}$
T19	10^{-1}	3	T20	256		
T20	10^{-2}	2	T21	1024		
T21	10^{-2}	2	T22	512		
T22	10^{-2}	3	T23	64		
T23	10^{-2}	2	T24	128		$10^{6.5}$
T24	10^{-2}	3	T25	128		
T25	10^{-2}	2	T26	64		
T26	10^{-2}	3	T27	128		

tralizing index of log 4, and the T18 passage of virus, *in vivo* neutralization and anti-hemagglutination tests were performed by usual methods(2). The results obtained were the same as those against the brain virus. This indicates that the adapted virus maintains the same antigenicity as the original virus.

5). *Pathogenicity of the adapted virus in mice, albino rats and guinea pigs.* The T18 passage-virus showed about 10^6 LD_{50}/0.1 ml of infectivity when the virus was inoculated intraperitoneally with Sarcoma 180 ascites cells, but only 10^3 LD_{50} without S180 cells. The virus showed no pathogenicity in albino rats, which are susceptible to EMC and Mengo viruses, or in guinea pigs, which are susceptible to brain-Columbia SK virus, even when the undiluted fluid was inoculated intraperitoneally.

Discussion. The purpose of this experiment was to obtain an adapted strain of Columbia SK virus which could be passaged in established cell cultures for *in vitro* screening of antiviral drugs. As reported previously (2,3) we had obtained an adapted strain which could propagate in Ehrlich ascites tumor cells *in vivo* with oncolysis, but did not propagate in established Sarcoma 180 cells and HeLa cell cultures *in vitro*. Because there are no established Ehrlich tumor cells which can be cultured serially, we tried to adapt the Ehrlich-adapted Columbia SK virus to Sarcoma 180 ascites tumor cells *in vivo* with a hope that the new strain would then be able to propagate in the "established" or "non-established" S180 cells *in vitro*. It was found that the adaptation of the virus from Ehrlich to S180 ascites cells *in vivo* was possible only through the use of mixtures of Ehrlich and S180 cells. Such an adaptation method has been described by several workers. Sato *et al.*(6) reported that an egg-strain of vaccinia virus produced inclusion bodies in Ehrlich ascites cells which were propagating on chick chorioallantoic membranes. Takahashi *et al.*(7) also re-

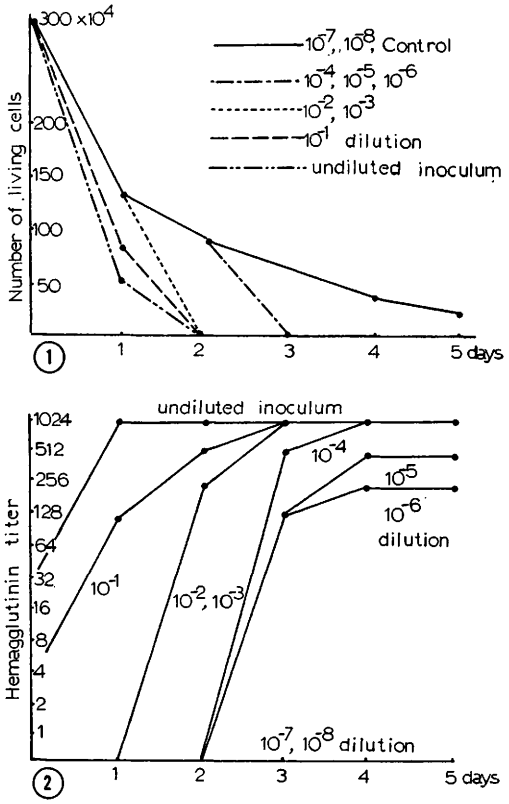

FIG. 1. Cytopathic effect of adapted Columbia SK virus against Sarcoma 180 ascites cells *in vitro*.

FIG. 2. Hemagglutinin of Columbia SK virus in Sarcoma 180 ascites cells *in vitro*.

ported that adaptation of poliovirus type I from HeLa cells to the egg was successful only through inoculation of the virus with HeLa cells but did not succeed with cell-free infected culture fluid. At present it is not certain that our new adapted strain can be cultured serially in established S180 cells. Among the Columbia SK virus group, propagation of Mengo and EMC viruses in mouse ascites cells was reported by Koprowska and Koprowski(8), Flanagan and Colter(9) and Hoskins and Sanders(10). These viruses grew easily from the original brain viruses without necessity of adaptation, but this was not possible with the Columbia SK virus. They did not describe HA in their systems. We have found HA a useful addition to CPE and infectivity in studying the effects of antiviral drugs because it tends to be more specific than CPE and more rapid and quantitative than infectivity titration.

Summary. Adaptation of Columbia SK virus from Ehrlich ascites cells to Sarcoma 180 ascites cells *in vivo*, and the serial passage *in vitro* are described. The adaptation was successful only through the use of mixtures of both cells. The new adapted virus propagates efficiently in Sarcoma 180 ascites cells *in vitro* with CPE and production of HA. This virus-host system has been used for screening tests of antiviral drugs.

1. Jungeblut, C. W., Kodza, H., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 133.

2. Furusawa, E., Cutting, W., *ibid.*, 1960, v103, 618.

3. ———, *ibid.*, 1960, v103, 851.

4. Cutting, W., Furst, A., Read, D., Grant, D., Cords, H., Megna, J., Butterworth, E., *Antibiot. and Chemother.*, 1960, v10, 623.

5. Horvath, B., Jungeblut, C. W., *J. Immunol.*, 1952, v68, 627.

6. Sato, Y., Kameyama, S., Takahashi, M., *Med. J. Osaka Univ.*, 1956, v7, 589.

7. Takahashi, M., Yamamura, T., Toyoshima, K., Miki, T., Okuno, Y., *Biken's J.*, 1960, v3, 249.

8. Koprowska, I. Koprowski, H., *J. Nat. Cancer Inst.*, 1953, v14, 627.

9. Flanagan, A., Colter, J., *Cancer Res.*, 1955, v15, 657.

10. Hoskins, J. M., Sanders, F. K., *Brit. J. Exp. Path.*, 1957, v38, 268.

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Relation of Potassium and Glucose Release from the Liver in the Unanesthetized Dog.* (26905)

WILLIAM C. SHOEMAKER AND ANTHONY G. FINDER
Department of Surgical Research, Michael Reese Hospital and Medical Center, Chicago, Ill.

An interrelationship between potassium and glucose movement has long been noted. Fenn(1) has described the deposition of glycogen in a matrix of potassium and phosphate ions, and has shown that glycogen can not be deposited unless accompanied by appropriate amounts of potassium and water. D'Silva(2) noted transitory increases in plasma potassium with glycogenolysis induced by epinephrine in the intact animal and in the perfused cat liver(3). This has been confirmed(4,5,6,7). However, it has also been demonstrated that epinephrine produces a small increase in blood potassium when the liver is out of the circulation(8,9). Moreover, epinephrine also causes the perfused dog lungs to release potassium(10).

Since glycogen deposition is accompanied by potassium accumulation in the liver, D'Silva(11) suggested that glycogenolysis must result in potassium release from the liver.

In the present study concentrations of potassium and glucose of plasma entering and leaving liver were measured in unanesthetized dogs before and after glucagon and epinephrine administration. Concentration differences across the organ were multiplied by the simultaneously measured hepatic plasma flow rate to give an approximation of net

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