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Virucidal Efficacy of Physico-chemical Treatments Against Coronaviruses and Parvoviruses of Laboratory Animals

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Virucidal efficacy of chemical disinfectants, heating and ultraviolet radiation against mouse hepatitis virus (MHV), canine coronavirus (CCV), Kilham rat virus (KRV) and canine parvovirus (CPV) were examined. Coronaviruses (MHV and CCV) were inactivated by ethanol, isopropanol, benzalkonium chloride, iodophor, sodium hypochlorite, sodium chlorite, cresol soap and formaldehyde as well as by heating at 60°C for 15 minutes, whereas parvoviruses (KRV and CPV) appeared to be inactivated by disinfectants such as formaldehyde, iodophor, sodium hypochlorite and sodium chlorite. Parvoviruses were stable under heating of up to 80°C for 30 minutes. Ultraviolet radiation inactivated all viruses within 15 minutes. No significant differences in stability against physico-chemical treatments were seen between viruses in the same group.

Various physico-chemical treatments, such as disinfectants, heating and ultraviolet radiation, have been commonly used in laboratory animal facilities to prevent invasion and transmission of infectious agents. However, little information about these treatments has been presented, especially concerning their efficacy against viruses. Only few reports [3, 4] have been published because of the difficulty of removing the cytotoxicity from the disinfectant-virus mixture. In this study, we determined the virucidal efficacy of various physico-chemical treatments against coronaviruses and parvoviruses of laboratory animals, using gel filtration for the detoxification of the disinfectant-virus mixture.

A total of five strains of four viruses, classified as coronavirus and parvovirus, were used in these experiments. Two strains of a mouse hepatitis virus (MHV-2 and MHV-N) were kindly supplied by Dr. N. Hirano, Iwate University. Canine coronavirus (CCV) I-71 strain was originally obtained by Dr. K. Hirai, Gifu University from Dr. L. E. Carmichael, Cornell University, U.S.A. Canine parvovirus (CPV) SP-80 strain and Kilham rat virus (KRV) RV-13 strain were obtained from the Chemo-sero Therapeutic Research Institute, Kumamoto, and the American Type Culture Collection, U.S.A., respectively. Delayed brain tumor (DBT) and Crandeal feline kidney (CRFK)cells were routinely grown in Eagle's minimum essential medium (MEM) containing 10% fetal calf serum (FCS), 10% tryptose phosphate broth (TPB) and antibiotics. Primary rat embryo (RE) cells were prepared from 15-day embryos of Sprague-Dawley rats (Crj: CD) confirmed to be seronegative to common murine

Table 1. Chemical disinfectants and concentrations

Chemical disinfectant		Concentration
Ethanol	(J. P.)	70%
Isopropanol	(G. R., Kanto Chem.)	50%
Benzalkonium chloride	(J. P.)	0.05%
Iodophor	(Lindres®, Taito Pfizer.)	5ppm[a)]
〃		50ppm[a)]
Sodium hypochlorite	(E. P. R., Kanto Chem.)	10ppm[b)]
〃		100ppm[b)]
Sodium chlorite	(Expor®, Alcide Co.)	0.23%
Cresol soap	(J. P.)	1.0%
Formaldehyde	(G. R., Kanto Chem.)	0.7%
Chlorhexidine digluconate	(Hibitane gluconate®, Sumitomo Chem.)	0.02%

[a)]: Titratable iodine concentration
[b)]: Titratable chlorine concentration
J. P.: Japane pharmacopenia
G. R.: Guaranteed reagent
E. P. R.: Extra-pure reagent

viruses, including KRV, and grown in MEM containing 5% FCS, 10% TPB and antibiotics. CRFK cells were employed for propagation and assay of CCV and CPV and DBT and RE cells were used for MHV and KRV, respectively. Each propagated virus suspension used in these experiments was prepared with MEM containing 2% FCS, and stored at −80°C.

Table 1 lists the various disinfectants evaluated in this study. The manufacturer's recommended levels of concentration were generally tested. Each disinfectant was diluted with distilled water to obtain the double strength of the final concentrations, and was mixed with an equal volume of stock virus. Four milliliters of disinfectant-virus mixture was allowed to react for 10 minutes at room temperature (approximately 23°C), then immediately detoxified by Sephadex LH-20 (Pharmacia) gel filtration according to the method developed by Blackwell and Chen [1]. Heating was carried out on 1 m*l* of virus suspension in a test tube using water bath controlled temperatures of 40, 60, 80 and 100°C. For ultraviolet radiation, a Petri dish (9 cm in diameter) containing 1 m*l* of virus suspension was placed 1 meter away from a 15W ultraviolet lamp (GL-15, Toshiba). The infectivity titer of the gel filtrate or treated virus suspension was assayed by plaque formation in MHV and CCV, observation of inclusion bodies in CPV, and hemoadsorption activity in KRV. The virucidal efficacy of various treatments was estimated from the difference between infectivity titers of treated virus suspension and untreated control, except for the distilled water-treated control in the disinfectant treatment.

The virucidal efficacies of nine chemical disinfectants are shown in Table 2. For simple comparison, disinfectants which decreased viral infectivity titer beyond 2 logs were regarded as having sufficient virucidal efficacy. The disinfectants tested in this study could be divided into three separate groups, depending upon their virucidal activity against coronaviruses and parvoviruses. The first group had sufficient efficacy against both virus groups (iodophor, sodium hypochlorite, sodium chlorite and formaldehyde). The second group had sufficient efficacy against the coronaviruses but not the parvoviruses (ethanol, isopropanol, benzalkonium chloride and cresol soap), and the third group had nearly no efficacy against either virus group (chlorhexidine digluconate). Sodium hypochlorite and iodophor are usually applied to disinfect animal cages and equip-

Table 2. Virucidal efficacy of disinfectants against parvoviruses and coronaviruses

Disinfectant	Parvovirus		Coronavirus		
	CPV[a]	KRV[b]	MHV-2[b]	MHV-N[b]	CCV[a]
Ethanol	1.33[c]	1.05[c]	>4.20[d]	>3.91[d]	>3.28[d]
Isopropanol	1.00	0.88	>3.70	>4.10	>3.74
Benzalkonium chloride	0.67	1.46	>3.70	>4.10	>3.74
Iodophor (5 ppm)	0.50	0.96	0.66	0.54	0.44
〃 (50 ppm)	>2.83	3.25	>3.70	>4.10	>3.28
Sodium hypochlorite (10 ppm)	0.83	0.68	0.57	0.26	0.90
〃 (100 ppm)	>2.83	2.38	2.82	2.26	1.05
Sodium chlorite	NT	>3.55	>3.70	>3.91	>4.00
Cresol soap	0.40	0.46	>3.18	>3.23	>2.74
Formaldehyde	>2.83	2.18	>3.68	>3.45	>3.74
Chlorhexidine digluconate	0.40	0.39	0.80	0.66	0.28

[a]: The value obtained from one experiment is presented.
[b]: The average value obtained from two separate experiments is presented.
[c]: Decrease of infectivity titer (log $TCID_{50}$/0.1 m*l*)
[d]: Decrease of infectivity titer (log PFU/0.1 m*l*)
NT: Not tested

Table 3. Virucidal efficacy of heating against parvoviruses and coronaviruses

Heating temperature	Time (min.)	Parvovirus		Coronavirus		
		CPV[a]	KRV[b]	MHV-2[b]	MHV-N[b]	CCV[a]
40℃	1	NT	0.25[c]	0.16[d]	NT	NT
	5	NT	0.75	0.33	0.18[d]	NT
	15	NT	0.75	0.34	0.05	NT
	30	NT	0.75	0.26	0.28	NT
60℃	1	NT	1.02	2.60	2.87	1.56[d]
	5	0.83[c]	1.58	3.55	>3.88	2.64
	15	0.83	1.42	>4.51	>3.88	>4.04
	30	1.50	1.42	>4.51	>3.88	>4.04
80℃	1	NT	1.02	>4.51	>3.88	>4.04
	5	0.73	2.02	NT	NT	NT
	15	1.83	1.75	NT	NT	NT
	30	1.83	2.02	NT	NT	NT
100℃	1	>2.00	>4.75	NT	NT	>4.04

NT: Not tested
[a], [b], [c], [d]: See footnote to Table 2.

ment at concentrations of 100 to 200 ppm and 50 to 100 ppm, respectively. Moreover, 10 to 20 ppm of sodium hypochlorite is often added to drinking water to prevent *Pseudomonas aeruginosa* infection of laboratory rodents [2]. However, the data presented in this report show that chlorination of drinking water is not effective for the inactivation of viruses.

The results of inactivation by heating are given in Table 3. Both coronaviruses, MHV

Table 4. Virucidal efficacy of ultraviolet radiation aganst parvoviruses and coronaviruses

Radiation time (min.)	Parvovirus		Coronavirus		
	CPV[a]	KRV[b]	MHV-2[b]	MHV-N[b]	CCV[a]
0	0	0	0	0	0
5	0.66[c]	2.66[c]	0.33[d]	1.87[d]	NT
15	>2.00	>4.00	>4.67	>3.34	3.84[d]
30	>2.00	>4.00	>4.67	>3.34	>4.68

NT: Not tested

[a], [b], [c], [d]: See footnote to Table 2.

and CCV, were completely inactivated by heating at 60°C for 15 minutes and at 80°C for one minute. On the contrary, both parvoviruses, CPV and KRV, were not inactivated by heating even at 80°C for 30 minutes. Only by heating at 100°C could the parvoviruses be inactivated within one minute. Ultraviolet radiation offered similar virucidal efficacy against all the viruses tested, as shown in Table 4. Each virus strain appeared to be inactivated completely by radiation of 15 minutes' duration. It has been well documented that nonenveloped viruses are usually more stable against physico-chemical treatments than enveloped viruses, and that parvoviruses are among the most stable animal viruses [3]. The results of this report also indicate that parvovirus, which has no envelope, is more stable against chemical disinfectants and heating than coronaviruses containing a lipoprotein envelope.

In conclusion, the virucidal efficacy of chemical disinfectants and heating against coronaviruses and parvoviruses was markedly different in the two virus groups. Nevertheless, there was apparently no difference between viruses in the same group. In this experiment, however, both virus groups showed similar stability against ultraviolet radiation.

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References

[1] Blackwell, J. H., and Chen, J. H. S. (1970). *J. Assoc. Offic. Anal. Chem.*, **53**, 1229-1236.
[2] Bywater, J. E. C., and Kellett, B. S. (1977). *Lab. Anim.*, **11**, 215-217.
[3] Scott, F. W. (1980). *Am. J. Vet. Res.*, **41**, 410-414.
[4] Yagami, K., Ando, S., Omata, Y., Furukawa, T., and Fukui, M. (1982). *Exp. Anim.*, **31**, 27-35.

コロナウイルスおよびパルボウイルスに対する物理・化学的処置の殺ウイルス効果の検討

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マウス肝炎ウイルス（MHV），イヌコロナウイルス（CCV），Kilham ラットウイルス（KRV）およびイヌパルボウイルス（CPV）に対する消毒薬，加熱，紫外線の殺ウイルス効果を検討した。コロナウイルス（MHV および CCV）に対しては，ほとんどの消毒薬，60℃，15分の加熱で不活化ができたが，パルボウイルス（KRV

および CPV）に対しては，ホルムアルデヒド，ヨードホール，次亜塩素酸ナトリウム，亜塩素酸ナトリウム以外に有効な消毒薬はなく，80℃，30分の加熱でも不活化できなかった。紫外線は，いずれのウイルスに対しても，15分の照射で不活化できた。また，同一ウイルス群に属するウイルスは各処置に対して同程度の反応を示し，ウイルス種，株による差は認められなかった。