on the basis of dissimilar species, the differences between rats remain unexplained.

From examination of Table I it is seen that the increase in hepatic capacity accompanying administration of o,p'-DDD is the result of both an increase in unit activity and the ratio of liver weight to body weight. The 300 mg dose of o,p'-DDD appears to have a smaller effect on liver size than does the 100 mg dose, but this apparent difference is due to the presence of older rats in the group receiving the highest dose level of the drug. Our data and those of others(10) suggest that the ratio of liver weight to body weight decreases with age.

Contrary to the findings of others(3,6), ethionine alone increased sleeping time and caused a significant decrease in hepatic metabolism of pentobarbital. These findings are in agreement with those reported for carbon tetrachloride and other liver poisons but not for ethionine, which is said to be a specific agent for inhibiting the induction of drug metabolizing enzymes(6). Both Conney(3) and Kato *et al*(6) used a single injection of ethionine (150 to 250 mg/kg) given 24 to 48 hours before determination of either sleeping time or enzyme assays and found no effect of ethionine alone. In the studies reported here, the rats were given daily injections of ethionine (150 mg/kg) for 2 days prior to determination of sleeping time and for 3 days prior to *in vitro* enzyme assays and it seems likely that this longer period of treatment with ethionine is responsible for the contradictory findings. This does not necessarily refute the claim that prevention of enzyme induction by ethionine is evidence for *de novo* synthesis but it does suggest that ethionine may possess a hepatotoxicity similar to carbon tetrachloride.

Summary. Both subcutaneous (100 mg/kg day) and oral (300 mg/kg day) treatment of rats for 2 days with the compound, o,p'-DDD, shortened pentobarbital sleeping time. Hepatic metabolism of pentobarbital was measured in liver slices taken from these animals after 3 days of treatment and found to be significantly increased. Concomitant treatment with ethionine (150 mg/kg), an inhibitor of protein synthesis, blocked the effects of o,p'-DDD on both sleeping time and *in vitro* hepatic metabolism of pentobarbital. On the basis of these criteria it is suggested that o,p'-DDD induces hepatic enzymes which metabolize pentobarbital.

1. Hart, L. E., Fouts, J. R., Proc. Soc. Exp. Biol. and Med., 1963, v114, 388.

2. Dyer, H. M., J. Biol. Chem., 1938, v124, 519.

3. Conney, A. H., Cancer Res., 1956, v76, 450.

4. Snedecor, G. W., Statistical Methods, Iowa State Univ. Press, Ames, 1956.

5. Brodie, B. B., Burns, J. J., Mark, L. C., Lief, P. A., Bernstein, E., Papper, E. M., J. Pharm. Exp. Therap., 1953, v104, 26.

6. Kato, R., Chissara, E., Vassanelli, P. V., Biochem. Pharmacol., 1962, v11, 211.

7. Serrone, D. M., Fujimoto, J. M., ibid., 1962, v11, 609.

8. Cazorla, A., Moncloa, F., Science, 1962, v136, 42.

9. Nichols, J., Kaye, S., Larson, P. S., Proc. Soc. Exp. Biol. and Med., 1958, v98, 239.

10. Addis, G., Gray, H., Growth, 1950, v14, 49.

Received November 2, 1964. P.S.E.B.M., 1965, v118.

Further Studies of Drug Combinations for Complete Cure of DNA Virus Infection in Cultured Cells.* (29854)

EIICHI FURUSAWA, SHINOBU FURUSAWA AND WINDSOR CUTTING
Pacific Biomedical Research Center, University of Hawaii

In previous papers(1,2) it was shown that 5-methyltryptophan (5-MTP) and noformicin (NOF), out of 60 selected compounds,

* This work was supported in part by the California Division of Am. Cancer Soc., National Inst. of Allergy and Infect. Dis. (AI-04433-02) and Cancer Chemotherapy National Service Center, Nat. Cancer Inst. (Contract PH43-63-616), of NIH. Contribution of the Pacific Biomedical Research Center.

TABLE I. Eradication of Vaccinia Virus from HeLa Cultures and Recovery of Normal Cells by Treatment with Drug Combinations.*

	Dosage per ml (½ MNTD)	Mitomycin .001 μg	5-BUDR .8	CA .01	NOF 2.5	5-MTP 400	ITSC .4	No drug
	(μg)							
Actinomycin D	.002	t†	11-15‡	t	9-15	9-16	9-16	7-11
5-BUDR	.8	14-18		t	R§	R	R	11-16
CA	.01	t	t		t	t	t	9-13
NOF	2.5	t	R	t		18-23	t	9-15
ITSC	.4	t	R	t	t	t		9-16
Mitomycin C	.001		14-18	t	t	11-18	t	7-11
No drug								5-9

* Drug mixtures were added just after inoculation of 100 $TCID_{100}$ of IHD strain of vaccinia virus; medium was changed every 2 or 3 days, cultures were incubated for 2 weeks with drugs and then for 2 further weeks without drugs.

† t indicates that the combinations were too toxic for cells to allow maintenance of the normal monolayer and original cell numbers.

‡ 11-15 indicates that virus-induced cytopathic effect started after 11 days and reached a maximum 15 days after infection.

§ R indicates that the combinations effectively eradicated the virus from the cultures and that normal cells could be recovered at the end of the incubation period.

showed remarkable activities against vaccinia propagation in HeLa cells, and that when enhanced by combination with 5-bromo-deoxyuridine (5-BUDR), could completely suppress vaccinia growth and eradicate the virus from the infected cultures. Normal cell population gradually returned when the drug combinations were removed from the culture medium. In this paper, we show that a combination of 5-BUDR and N-methylisatin thiosemicarbazone (ITSC) was also effective in eradicating vaccinia virus and allowing recovery of infected cultures. Comparisons were made with several anti-vaccinia chemicals whose mechanisms are already known. It was also found that none of these combinations could eradicate adeno virus type 12 from the infected cultures.

Materials and methods. Experimental methods were the same as described before (2). HeLa cells, freshly trypsinized and suspended at a concentration of 5×10^4 cells per 1 ml, were inoculated with 100 $TCID_{100}$ of virus, and one-half of the maximum nontoxic dose (MNTD) of agent was added immediately. Cultures were then kept stationary at 36°C for 2 weeks with drugs present in the medium, and 2 more weeks without drugs. The cells formed monolayers within 3 to 5 days after incubation. Medium was changed every 2 or 3 days. Drugs used were: mitomycin C, actinomycin D, ITSC, 1-β-D-arabinofuranosylcytosine (CA), 5-BUDR, 5-MTP and NOF.

Results. Table I shows the effects of drug combinations on vaccinia propagation. Mitomycin C and CA showed increased toxicities against cells when they were mixed with other drugs. Therefore, they were not suitable compounds for this experiment except for the combination of 5-BUDR and mitomycin C which suppressed the virus-induced cytopathic effect (CPE) for 9 days. ITSC also showed increased toxicity when it was mixed with other drugs except 5-BUDR. The combination of ITSC and 5-BUDR, as well as the combinations of 5-BUDR and NOF or 5-MTP, showed strong activity and could eliminate the virus from the infected cell population. Combinations of actinomycin D and other drugs showed only slightly increased activities, without complete elimination of virus. Table II shows the effects of drug combinations on adeno virus type 12 propagation. None showed sufficient activity to eradicate the virus from the cultures. Only the combination of 5-BUDR and NOF showed a strong inhibition for more than 2 weeks. Combination of actinomycin D and other drugs did not increase the activity to more than that of actinomycin D itself.

Discussion. 5-BUDR(3,4), CA(5) and mitomycin C(6,7) are well known as specific inhibitors of DNA synthesis. Actinomycin D(8,9,10) inhibits transfer of genetic infor-

TABLE II. Failure of Eradication of Adeno Virus Type 12 from Infected Cultures by Treatment with Drug Combinations.*

	Dosage per ml (½ MNTD)	Mitomycin .001 μg	5-BUDR .8	CA .01	NOF 2.5	5-MTP 400	ITSC .4	No drug
	(μg)							
Actinomycin D	.002	t	9-13	t	9-13	9-13	9-13	9-13
5-BUDR	.8	7-13		t	19-23	7-13	7-13	7-13
CA	.01	t	t		t	t	t	4-7
NOF	2.5	t	19-23	t		7-13	7-13	7-13
ITSC	.4	t	7-13	t	t	t		4-7
Mitomycin C	.001		7-13	t	t	4-7	4-7	4-7
No drug								4-7

* The method was the same as in the experiment with vaccinia (Table I), except for the inoculation of 100 $TCID_{100}$ of adeno virus type 12.

mation from the parent DNA. On the other hand, 5-MTP and ITSC seem to be inhibitors of protein synthesis(10,11), and NOF of the assembly of viral DNA and the protein coat (10). The experimental results suggest that combinations which provide action on both DNA and protein synthesis seem to produce maximum activity against virus reproduction at levels subtoxic to the host cells. Thus it was found that the combinations of 5-BUDR with 5-MTP, ITSC or NOF could eradicate vaccinia virus from infected cells and yet allow recovery of normal cell population. The difficulty of eradication of adeno virus type 12 suggests that such tumorigenic viruses may be similar to portions of the genome of cells, not only in location of propagation, but also functionally. There were no increased toxicities to host cells by combinations except for the combinations indicated by "t" in Table I or II. With the other combinations HeLa cells maintained normal monolayers, showing no pathological changes such as pycnosis or karyorexis, with decreased acid production, during the 2 weeks of incubation with the drugs, and after removal of the drugs the cells returned to a normal rate of growth within 2 more weeks of incubation. There was no countable loss of cell numbers during the incubation period with drug combinations.

Summary. In further screening tests of drug combinations for complete eradication of DNA viruses from cultured cells, it was found that a combination of 5-bromo-deoxyuridine and N-methyl-isatinthiosemicarbazone is effective in eradicating vaccinia virus with restoration of the normal cell population.

The authors are indebted to Dr. R. L. Thompson, Nat. Inst. Health, for supplying 5-BUDR, 5-MTP, NOF, CA and ITSC; to Dr. A. Terawaki, Palo Alto Med. Research Foundation, Calif., for supplying mitomycin C and actinomycin D and his generous advice.

1. Furusawa, E., Cutting, W., Furst, A., Chemotherapia, 1964, v8, 95.

2. Furusawa, E., Cutting, W., Buckley, P., Furusawa, S., Proc. Soc. Exp. Biol. and Med., 1964, v116, 938.

3. Simon, E. H., Exp. Cell Res., 1963, v9, 263.

4. Kaufman, H. E., Chemotherapia, 1963, v7, 1.

5. Buthala, D. A., Proc. Soc. Exp. Biol. and Med., 1964, v115, 69.

6. Shiba, S., Terawaki, A., Taguchi, T., Kawamata, J., Biken's J., 1958, v1, 79.

7. Oda, M., Virology, 1963, v21, 533.

8. Reich, E., Franklin, R. M., Shatkin, A. J., Tatum, E. L., Science, 1961, v134, 556.

9. ———, Proc. Nat. Acad. Sci. U. S., 1962, v48, 1238.

10. Furusawa, E., Furusawa, S., Cutting, W., Medicina Experimentalis, 1965, in press.

11. Bach, M. K., Magee, W. E., Proc. Soc. Exp. Biol. and Med., 1962, v110, 565.

Received September 2, 1964. P.S.E.B.M., 1965, v118.