resistant to polyoma virus cytolysis. Temporary virus carriage by cells may be mechanical in that the virus survives in an inert state, or it may be metabolic in that the virus participates biochemically in cell reactions. A cell-virus relationship that temporarily results in fewer mitoses and some cell loss occurs with polyoma and other viruses and is a factor in the selection of surviving cells. The persistent carrier state may involve the nuclei, the cytoplasm or both. It has not been possible to determine this by use of the fluorescent antibody technic probably because of the small amount of virus present. What effect the virus has, under this circumstance, on the behavior of the tumor is not known. In the case of a DNA virus such as polyoma, which has not been shown to affect the genome of cells (12), perhaps a persistent carrier state has relatively little effect on the cell population. Environmental effects on the virus such as changes in oncogenicity(13) require further study.

Summary. Tissue cultured cells of certain mouse and hamster tumors were classified according to their reactions to polyoma virus. *Group 1* cells had no morphologic changes and virus persisted only a few weeks in the culture. Murine RC carcinoma and ependymoma and HT6 fibrosarcoma (hamster) behaved in this way. *Group 2* cells had variable degrees of cytolysis and morphologic changes; virus persisted for several weeks. L2 and 1210 lymphomas, 6C3HED lymphosarcoma (Gardner) and the P815 mast cell (Dunn-Potter) gave this response. *Group 3* cells had no changes in cell morphology with persistent virus. A spontaneous adenocarcinoma (WT, Pearson) gave this response. *Group 4* cells had cytolysis and virus persisted for months in surviving cells. The unclassified mouse tumor (WM, Pearson) reacted in this way.

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Suppression of the Immune Response by Drugs in Combination. (27783)

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A search for agents capable of suppressing the immune response led to the adoption of a test system based on the formation of hemagglutinins to sheep red blood cells in mice(1). The demonstration that combinations of antimetabolites which affect biochemical systems related sequentially or concurrently show potentiation *in vitro*(2,3,4) and *in vivo*(5) suggested that the same principle might be applied to suppression of the immune response. The results of the experiments described herein demonstrate this to be the case.

Materials and methods. Charles River CD_1 male mice weighing 19-21 g were used for these experiments. Antigen administration, hemagglutinin determinations and scoring

TABLE I. Antibody Indices Obtained after Treatment with Combinations of Drugs with 6-Mercaptopurine.

	Dose, mg/kg	6-Mercaptopurine, mg/kg 0	8.33	25	75
		Antibody index			
5-Bromodeoxyuridine	0	1.00	.90	.52	.32
	3	.88	.63	.30	.34
	10	.62	.46	.28	.16
	30	.37	.43	.25	.09
5-Acetoxyuracil	0	1.00	.91	.61	.51
	33.3	.75	.65	.52	.45
	100	.57	.46	.34	.30
6-Azauracil + urethan	0	1.00	.74	.57	.48
	17 + 8	.64	.62	.57	.32
	50 + 25	.57	.55	.38	.28
	150 + 75	.37	.46	.42	.21

were carried out as previously described(1). In all instances of multiple drug administration the drugs were given separately by intraperitoneal injection. Four successive daily doses were given beginning with the day of antigen stimulation. The minimum effective dose (MED) of one compound and geometric decrements of it by thirds were tested in combination with a similar range of dosages of the second compound in a Latin Square arrangement.

The activities of several of the individual purine and pyrimidine analogues have been reported previously(1), but the activities of a number of pyrimidine analogues in the sheep cell-mouse system are reported for the first time here. These include 4-thiodeoxyuridine (TUDR), 5-acetoxyuracil and 5-bromodeoxyuridine (BUDR). The last named has been found by others(6,7) to interfere with the immune response in other systems. In addition, the combination of 6-azauracil and urethan, inhibitors of the biosynthesis of pyrimidines(5,8), was tested together with the purine analogues.

BUDR was obtained from Schwarz BioResearch Inc. The other materials were synthesized and characterized in these laboratories.

The results are recorded in terms of an antibody index (AI) which represents the ratio of hemagglutinin scores of treated to untreated mice(1).

Results. The results of representative experiments are presented in Tables I-III. A statistical analysis of more than 100 experiments indicates that an AI of 0.60 or lower denotes significant suppression of the immune response. It can be seen in Table I that the combination of 6-mercaptopurine (6-MP) and BUDR at 25 and 10 mg/kg respectively (essentially an MED of each drug) produced an AI lower than that obtained with 3 times the dose of each drug individually. Other dose combinations produced levels of suppression greater than that which can be accounted for by the sum of the individual drug activities. Somewhat less potentiation was observed when 5-acetoxyuracil or 6-azauracil plus urethan was used in combination with 6-mercaptopurine (Table I). Thioguanine (TGu) served equally as well as the purine component and TUDR could be substituted for BUDR (Table II). The derivative of 6-MP, B.W. 57-322 (6-(1-methyl-4-nitro-5-imidazolyl)thiopurine) did not show potentiation with BUDR. In fact at the higher levels of BUDR low doses of B.W. 57-322 appeared to interfere with the activity of BUDR (Table II) and the index never reached values significantly lower than those attained with one or the other drug alone.

Interference between drugs was also noted in experiments designed to arrive at the best ratio of doses to be used for the combination of 6-azauracil and urethan. Potentiation was obtained with intermediate dose levels of urethan (Table III). However, when the level of urethan was increased, there was an

TABLE II. Antibody Indices Obtained after Treatment of Combinations of Antagonists.

	Dose, mg/kg	Thioguanine, mg/kg 0	.3	1	3
		Antibody index			
4-Thiodeoxyuridine	0	1.00	.77	.33	.19
	50	.72	.37	.16	
	150	.52	.30	.13	
	450	.31			
		B.W. 57-322 mg/kg 0	7	20	60
5-Bromodeoxyuridine	0	1.00	.92	.72	.48
	3	.93	.72	.79	.48
	10	.56	.69	.58	.42
	30	.31	.56	.46	.30

TABLE III. Antibody Indices Obtained after Treatment with 6-Azauracil and Urethan.

		Urethan, mg/kg				
	Dose, mg/kg	0	25	75	225	675
		Antibody index				
6-Azauracil	0	1.00	.85	.70	.93	.89
	62.5	.96	.43	.63	.70	.64
	100	.85	.40	.35	.58	.76
	200	.93	.47	.32	.67	.87

apparent loss of activity. Representative data for these combinations are presented in Table III. The dependence of activity on urethan concentration received confirmation in many additional tests at intermediate doses and ratios. The dose-response relationship for BUDR is presented in Fig. 1. The chemotherapeutic index of this substance is higher than that of any other found to date. It will be seen that it shows significant activity at doses of the order of 7.5 mg/kg and becomes toxic only at 240 mg/kg under the conditions of this test.

Discussion. The present studies do not lend themselves readily to the demonstration mathematically of potentiation. Since the antibody index is a weighted index, proportionality between dosage and index is not to be expected. Moreover, with several of the drugs which were used here, increments of effect with increasing dosage could be demonstrated only at the lower levels with a tendency for the effect to level off at greater dosage. However it is apparent that a number

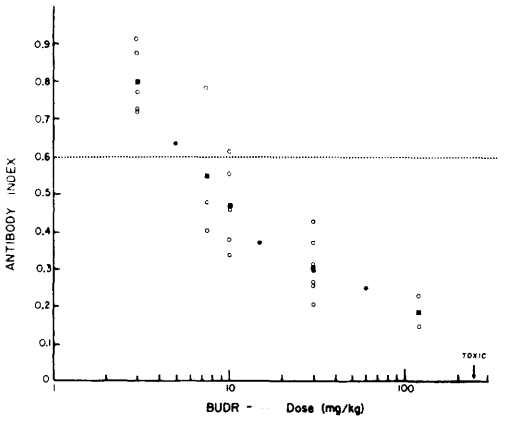

FIG. 1. Dose-response relationship for BUDR. Each open circle represents a test with a group of 5 mice. The squares represent averages where several tests were performed at the same dosage level.

of the combinations give rise to effects greater than those to be expected on the basis of arithmetic summation. Thus the combination of 6-MP at 25 mg/kg with BUDR at 3 mg/kg gave an index of 0.30 equal to that given by 6-MP at 75 mg/kg or BUDR at 30 mg/kg (Table I). Similarly, the combination of TUDR and TGu gave activity attained only with 3 to 9 times as much of the individual drugs (Table II).

The importance of the potentiations observed here is 2-fold. An improved chemotherapeutic index appears to result; in many instances each component of the binary mixture may be given at or close to its individual maximum tolerated dose. Possibly more importantly, several of the combinations appear capable of giving suppression of antibody formation greater than that achievable at any dosage of the individual drugs. These results suggest trials of such combinations in additional biological systems (*e.g.*, renal and skin homotransplants(9,10,11)) and autoimmune diseases(12,13).

The mechanisms involved in suppression of the immune response by antagonists of nucleic acid biosynthesis remain obscure. Both the cellular proliferative and inductive phases of the immune response involve nucleic acid biosynthesis, and either or both of these phases might be affected. Moreover there is accumulating evidence that several cell types may be involved in the immune response(14). The drug combinations which have shown the most potentiation in the present work are those in which a pyrimidine antagonist is combined with a purine antagonist. This may reflect the more effective inhibition of nucleic acid biosynthesis when the purine and pyrimidine pathways are blocked concurrently. However, preliminary histological evidence suggests that each of the two drug types may have a selective effect on a particular cell type(15) and that treatment with the combinations results in more balanced suppression of proliferative activity than is achievable by either type alone.

Summary. Combinations of purine with pyrimidine analogs were tested for their effects on formation in mice of hemagglutinins to red blood cells. Greater than additive ef-

fects were obtained with 6-mercaptopurine and thioguanine in combination with 5-bromodeoxyuridine, 4-thiodeoxyuridine, 5-acetoxyuracil and 6-azauracil plus urethan. 5-Bromodeoxyuridine is notable for the magnitude of the ratio of maximum tolerated dose to minimum effective dose.

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Susceptibility of Baboon (*Papio doguera*) Kidney Cells to Human Enteroviruses. (27784)

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The enterovirus flora of numerous animal species has been extensively investigated. Similarly, certain animal tissues have served as substrates for propagation of many viruses (1,2,3). The relative high incidence of native viruses in monkey kidney cell (*Macaca mulatta*) preparations suggested the need for studies on additional host cells for production of poliovirus vaccines and for allied viral investigations(4). The phylogenetic relationship of the baboon (*Papio doguera*) to the more commonly used laboratory primates recommends this animal for such purposes.

Hsiung and Melnick compared the susceptibility of kidney cell cultures of 13 monkey species and 4 baboon species to various enteroviruses(5). The use of various animal kidney cells as a "differential medium" for enterovirus isolation and identification was suggested.

In an attempt to develop a model animal system more closely related to man, preliminary investigations comparing the baboon kidney cell (BKC) and monkey kidney cell (MKC) for their propagative properties of known enteroviruses were undertaken. The ability of these 2 cell systems to isolate unknown enteroviruses from stool samples was also investigated.

Materials and methods. Preparation of kidney cell cultures. Kidneys were obtained from young, adult baboons by nephrectomy or by sacrificing of the animal. The kidneys were placed in Hanks' Balanced Salt Solution (BSS) maintained at 4°C, previously adjusted to pH 7.4-7.5 by addition of 7.5% $NaHCO_3$. After removal of the capsule, the kidney was minced and trypsinized in 0.25% trypsin containing 100 units penicillin and 100 μg streptomycin per ml. The trypsinization was performed by either the overnight method of Bodian(6) or the more rapid procedure recently reported by Wallis and Melnick(7). The cells, 3.0×10^5 per ml in 0.5%

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