21. Langdon, R. G., Weakly, D. B., *J. Biol. Chem.*, 1955, v214, 167.

22. Ashmore, J., Hastings, H. B., Nesbett, F. B., Ronold, A. E., *ibid.*, 1956, v218, 77.

23. Weber, G., Allard, C., de Lamirande, G., Cantero, A., *Biochim. Biophys. Acta,* 1955, v16, 618.

24. Shull, K. H., Cahill, G. F., Jr., Godsden, E. L., Mayer, J., *J. Biol. Chem.,* 1956, v222, 415.

25. Ashmore, S., Cahill, G. F., Jr., Hastings, A. B., *Metabolism,* 1956, v5, 774.

26. Kuether, C. A., Clark, M. R., Scott, E. G., Lee, H. M., Pettinga, C. W., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 215.

Received July 1, 1959. P.S.E.B.M., 1959, v102.

Purine Antagonists and Growth of Tetrahymena.* (25228)

VIRGINIA C. DEWEY, G. W. KIDDER AND D. G. MARKEES

Biol. Lab., Amherst College, and Dept. of Chemistry, Wells College, Aurora, N. Y.

The demonstration that a purine analog interferes with the metabolism of acetate and with sterol synthesis in *Tetrahymena*(1) led to a reexamination of the effects of other structural analogs of purines. These compounds may be divided into groups on the basis of the nature of structural changes embodied in them, nature of substances bringing about reversal and whether or not the inhibitors are able to bring about death of the cultures. These features can be shown to have a certain degree of correlation to one another.

Material and methods. Strain W of *Tetrahymena pyriformis* was grown in medium A (2) containing Tween 80 at a concentration of 10 mg/ml. From this medium acetate and adenylic acid were omitted as indicated. In certain experiments β-sitosterol or adenine was added. Conditions of growth and incubation were as previously described(2). The purine analogs 8-azaguanine (8AG), 8-azaadenine (8AA), 6-methylpurine (6MeP), 6-chloropurine (6ClP) and purine (Pu) were commercial products. One sample of 6-methoxypurine (6MeOP) was prepared in the Biological Laboratory at Amherst College and another obtained commercially. The sample of 6-methyl-1-deazapurine (6MeDAP) was also prepared in the Amherst College laboratory. Through the kindness of Dr. K. Pfister of Merck and Co. we obtained a sample of 2-azaadenine (2AA).

* Supported by research grants (A-1005 and CY-2924) from the National Institutes of Health, U. S. Public Health Service and a grant (CH-27) from the American Cancer Society.

Results. Table I shows the response of *Tetrahymena* to the various inhibitors in the basal medium and also with addition of acetate, β-sitosterol or adenine or combinations of these substances. The first 3 inhibitors represent the group of analogs in which changes in ring structure have been made and all (Figures in parentheses) are most readily reversed by adenine. Addition of acetate, sterol or both have relatively little influence on the inhibition. The next group of 4 analogs differs from adenine only in the nature of the substituent replacing the amino group in the 6 position. Two of these compounds, 6MeP and 6ClP are reversed best by sterol and synergistic reversing effects are shown by combinations of adenine with sterol or acetate. The other 2 compounds, Pu and 6MeOP, are almost equally well reversed by either adenine or sterol and the effects of combinations of material are not so striking. The last analog (6MeDAP) has changes both in the ring and in the substituent group. It resembles the first group of analogs in its reversal by adenine. With regard to 1-deazaadenine (DAA) it can only be said that it is reversed by adenine(3). There are no data on the effects of the other compounds considered here.

Certain of these purine analogs have another peculiarity in common. They permit normal growth of the organisms up to a point. The organisms then lose motility, settle to the bottom of the tubes and autolyze. This process releases fat droplets, glycogen granules and other particulate material into the medium, thus making it more, rather than

TABLE I. Inhibition of *Tetrahymena* by Purine Analogs.

Inhibitor	8-azaguanine				2-azaadenine				8-azaadenine				Purine			
	γ/ml															
Additions	.2	.4	.8	2	5	10	20	50	50	100	200	500	100	200	500	1000
	% inhibition															
None	17	44	60	70	33	46	54	90	37	44	50	89	31	35	44	60
Acetate, 1 mg/ml	33	49	52	58	33	46	62	90	32	38	50	90	30	33	39	48
Sterol, 25 γ/ml	22	43	51	55	37	49	57	70	39	48	53	71	39	41	43	50
Adenine, 20 γ/ml	11	22	(25)	30	13	17	(37)	89	3.5	10	(19)	93	19	26	(39)	46
+ acetate	11	20	28	36	7	21	40	88	2	6	17	87	11	22	30	41
+ sterol	17	25	28	35	13	25	35	81	4	9	16	68	18	23	28	32
+ acet.	17	24	30	36	10	20	32	75	1	3.5	8.5	47	13	19	19	27

Inhibitor	6-methylpurine				6-chloropurine				6-methoxypurine				1-deaza-6-methylpurine			
	γ/ml															
Additions	.2	.4	.8	2	50	100	200	500	100	200	500	1000	100	200	500	1000
	% inhibition															
None	28	42	71	93	1	12	46	93	0	4	56	97	6	24	53	93
Acetate	31	36	51	76	0	0	17	92	0	4	41	96	8	22	52	91
Sterol	26	34	(44)	79	2	8	(17)	44	0	0	34	96	18	34	60	93
Adenine	10	11	68	95	4	10	33	84	2	3	(31)	95	4	5	(10)	86
+ acetate	8	10	25	71	2	5	13	74	1	0	24	93	4	5	11	85
+ sterol	3	5	14	60	3	10	21	64	0	0	15	92	4	6	12	86
+ acet.	4	7	11	26	1	7	16	74	0	0	12	86	2	3	9	86

less, turbid. Table II shows the various properties of the analogs and their correlation with structural changes. While reversibility by adenine appears to be correlated with changes in ring structure and reversibility by sterols with the substituent in the 6 position, ability to cause death of the cultures is not clearly related to one type of structural change rather than the other.

An apparently obvious omission from this list of analogs is 6-mercaptopurine. This compound is inactive as a growth inhibitor for *Tetrahymena*.

Discussion. The fact that acetate and/or sterols are active to some degree in reversal of all of a series of 8 purine analogs and in certain cases are the most active compounds in this respect, indicates an involvement of purines at some point in synthesis of sterols and other lipids. This point does not appear to be in activation of acetate to form acetyl adenylate (prepared by M. R. Heinrich by published methods(4,5,6)), since the latter was no more active than an equimolar mixture of acetate and adenosine-5′-phosphate in reversing inhibition by 6MeP. The data presented by Wright(7), however, suggest that a certain type of ribonucleic acid or polyribonucleotide is necessary for sterol synthesis and the explanation of the results presented above may lie in a requirement for synthesis of such polynucleotides from precursors such as adenine.

TABLE II. Characteristics of Certain Purine Analogs.

Analog	Structural change		Reversing compound		
	Ring	Substituent	Adenine	Sterol	Death
8AG	+	—	+	—	—
2AA	+	—	+	—	—
8AA	+	—	+	—	—
DAA	+	—	+	?	+
6MeP	—	+	—	+	—
6ClP	—	+	—	+	—
Pu	—	+	+	+	—
6MeOP	—	+	+	+	+
6MeDAP	+	+	+	—	+

It is obvious from the large variation in amounts of the analogs required to produce equal degrees of inhibition that there is also a large variation in affinity of these inhibitors for the enzymes metabolizing purines. It would also appear that there is a differential affinity among the various enzymes such that different analogs are not equally reversible by all of the active compounds.

Since *Tetrahymena* is not inhibited by 6-mercaptopurine, this interesting compound could not be included in this series. With *Escherichia coli* it has been shown, however,

that 6-mercaptopurine decreases incorporation of radioactive acetate(8). This would indicate that the effect of purine analogs on acetate metabolism is not confined to *Tetrahymena*. Also 6-mercaptopurine may act in much the same way as the analogs described above.

No explanation is yet available for the death of cultures containing certain of the purine analogs. It cannot be accounted for on the basis of accumulation of acid as is the case in cultures deficient in either thioctic acid (9) or thiamine(10). Among other compounds which cause "suicide" of *Tetrahymena* are protopine and α-phenylbutyric acid, an analog of acetate (unpublished observations). So far it has not been possible to reverse the inhibition caused by protopine, but all the other "suicidal" compounds or conditions are in some way involved in acetate metabolism.

Summary. Eight purine analogs were compared as to their effects on growth of *Tetrahymena*. A certain degree of correlation was found between type of structural change present in the analog and ability of adenine, acetate, sterol or combinations of these compounds to reverse the inhibition. Analogs which cause "suicide" of cultures do not fit the pattern of other inhibitors.

1. Dewey, V. C., Heinrich, M. R., Kidder, G. W., *Suppl. Internat. Abst. Biol. Sci.*, 1958.
2. Dewey, V. C., Parks, R. E., Jr., Kidder, G. W., *Arch. Biochem.*, 1950, v29, 281.
3. Kidder, G. W., Dewey, V. C., *Arch. Biochem. Biophys.*, 1952, v66, 486.
4. Whitehouse, M., Moeski, H., Gurin, S., *J. Biol. Chem.*, 1957, v226, 813.
5. Berg, P., *ibid.*, 1956, v222, 1015.
6. Lee Peng, C. H., *Biochim. Biophys. Acta*, 1956, v22, 42.
7. Wright, L. D., Cleland, M., Dutta, B. N., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 425.
8. Mandel, H. G., Inscoe, J. K., Maling, H. M., Smith, P. K., *J. Pharm. Exp. Therap.*, 1957, 120, 195.
9. Dewey, V. C., Kidder, G. W., *Fed. Proc.*, 1953, v12, 196.
10. Tittler, I. A., Belsky, M. M., Hutner, S., *J. Gen. Microbiol.*, 1952, v6, 85.

Received July 1, 1959. P.S.E.B.M., 1959, v102.

An Activator of Plasminogen Produced by Cell Culture. (25229)

EUGENE V. BARNETT* AND SAMUEL BARON
(Introduced by K. Habel)
U. S. Dept. of HEW, NIH, Division of Biologics Standards, Bethesda, Md.

A simplified scheme, modified from Astrup (1) for activation of plasminogen by "activators" or protease, and for demonstration of such activation by clot lysis or casein hydrolysis is shown in Fig. 1. There are many activators of plasminogen. Those of note are streptokinase, particulate "tissue activators," potassium thiocyanate extracted "tissue activators," microsome fractions of animal tissues, urokinase, human milk, trypsin, and chloroform. In addition, spontaneous activation may occur(1). This study describes materials released by metabolizing cell cultures which activate plasminogen(2) or which are proteolytic in the absence of plasminogen(3,4).

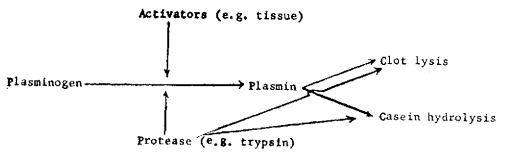

FIG. 1.

Materials and methods. Clarified supernatant medium from cell cultures was used as a source of plasminogen activator and of caseinolytic protease. The supernatant medium was prepared by feeding Medium 199(5) to cell cultures which had been previously washed 4 times with balanced salt solution (b.s.s.). After incubation for 96 hours at 36°C it was decanted and clarified by low speed centrifugation. Supernatant medium

* Strong Memorial Hospital, Rochester, N. Y.