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From Haskins Laboratories, 305 East 43rd St., New York 17, N. Y., U.S.A.

Bleaching Euglena gracilis with Antihistamines and Streptomycin-Type Antibiotics*

By

ARTHUR C. ZAHALSKY, S. H. HUTNER, MARY KEANE and RICHARD M. BURGER

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Some strains of *Euglena gracilis* grow equally vigorously photoautotrophically or heterotrophically, with reversible disappearance of chloroplasts in dark-grown cultures. Such strains are highly sensitive to permanent bleaching ("apochlorosis") by streptomycin. The molecular mechanism of bleaching is unknown, and the absence of sex in *Euglena* rules out Mendelian experiments. The increasing use of *E. gracilis* for studying development and function of the photosynthetic apparatus (reviewed by WOLKEN 1961) increases the need to understand irreversible bleaching. The observation by the PRINGSHEIMS (1952) that exposure to elevated temperatures also bleached, compounded the puzzle of the exceptional lability of the photosynthetic apparatus in *E. gracilis* coupled with its high efficiency. We studied the effectiveness as permanent bleachers of several antibiotics of the streptomycin family in the hope of discerning a molecular configuration governing bleaching activity. As some antihistamines also bleach (see WOLKEN 1961) we likewise compared several antihistamines. With certain of the antibiotics we observed independent annulment of bleaching and gross toxicity. Another aim was to see whether bleaching activity of the streptomycin-family antibiotics correlated with ability to damage the 8th cranial nerve; such a correlation turned out to be highly likely.

Methods

Euglena gracilis Z was used. The general culture methods have been described (HUTNER et al. 1957). The basal medium had the following components (g/100 ml of final medium unless otherwise mentioned): K_3 citrate · H_2O 0.1; Na acetate · $3H_2O$ 0.1; monoethylglycine ethylester HCl 0.1; monoethylglutamate HCl 0.1; L-asparagine · H_2O 0.15; Na_2 glycerophosphate · $5H_2O$ 0.05; Ca 0.001; $MgSO_4 \cdot 7H_2O$ 0.05; trace elements (mg) Fe 1.0, Mn 0.8, Zn 0.5, Mo 0.05, Cu 0.05, Co 0.05, B 0.01; V 0.005, I 0.004, Se 0.002; $(NH_4)_2SO_4$ 0.002; B_{12} 0.4 μg; thiamine HCl 1.0 mg, adjusted with "Quadrol" (PACKER et al. 1961) to p_H 6.8. Experimental media were distributed in 10-ml amounts in 12-ml (in Kimble catalog "10-ml") micro-

* This paper is dedicated to Professor Dr. E. G. PRINGSHEIM on the occasion of his 80th birthday.

Fernbach flasks covered with translucent autoclavable polypropylene caps. Inocula were prepared from cells grown in the basal medium; one drop of inoculum contained 14—30 viable cells as determined by plating.

Stock solutions of antibiotics and antihistamines were sterilized by Seitz filtration and were added aseptically in appropriate dilution to experimental flasks. Dark cultures were grown at 28° C. Light-grown cultures were placed ~70 cm below a bank of "cool-white" fluorescent lamps which provided the cultures with 200 ft.-candles of fairly even illumination. Growth was recorded as optical density (O.D.) with a Welch Densichron (Chicago, Illinois, U.S.A.) equipped with a red-sensitive light probe.

Photosynthetic pigments were estimated by extraction of a 10-ml culture with 5 ml of cold absolute methanol. After 12 hours the extraction was considered complete. Pigment concentrations were estimated as units in a Klett-Summerson colorimeter equipped with nos. 42 (400—500 mμ), 62 (600—640 mμ), and 66 (640 to 680 mμ) filters.

Bleached organisms were routinely transferred to inhibitor-free liquid and plate media to assess permanence of bleaching.

Kanamycin sulfate was a gift from Bristol Laboratories (courtesy of Dr. A. R. MENOTTI). Paromomycin sulfate was a gift from Parke, Davis & Co. (courtesy of Dr. JOHN EHRLICH). Streptomycin and neomycin were used as the sulfates. Antihistamines: Antazoline HCl ("Antistine") and tripelennamine HCl ("Pyribenzamine") were gifts from Ciba; diphenylhydramine ("Benadryl") Parke, Davis; doxylamine succinate ("Decapryn") W. S. Merrell; methapyrilene HCl ("Histadyl") Eli Lilly and Co.; phenindamine tartrate ("Thephorin") Hoffman-LaRoche; pheniramine maleate ("Trimeton") Schering Corp.; promethazine HCl ("Pyrrolazote") Upjohn; and pyrilamine maleate ("Neo-Antergan") Merck Sharp & Dohme.

Results

a) Streptomycin-Family Antibiotics

Table 1 shows the concentration of each antibiotic causing permanent bleaching and growth inhibition. The virtual nontoxicity of kanamycin and streptomycin and their high potency as bleachers is clear; as bleaching agents they had a high margin of safety compared with neomycin and paromomycin. Histidine appeared to oppose both bleaching and killing. Urocanic acid likewise protected against toxicity and bleaching by streptomycin and kanamycin at ~ 70% the effectiveness of histidine. Arginine and lysine showed weak protection against bleaching but only at levels > 50 mg-%.

PRESCOTT et al. (1959) reported that a combination of pantothenate, nicotinic acid, and ascorbic acid permitted 90% survival of mice treated with toxic doses of streptomycin while controls showed 7—12% survival. A combination of pantothenate, nicotinic acid, and thiamine did afford *E. gracilis* some protection against lethality and bleaching (Table 2). A mixture of amino acids gave even greater protection; whether this activity is all attributable to histidine, and whether protection by vitamins plus amino acids and Mg is additive or synergistic, was not determined.

Table 1. *Annulment of antibiotic bleaching and toxicity by histidine (free base)*

Concentration mg-%		0 O.D.	0 Color	5 O.D.	5 Color	10 O.D.	10 Color	25 O.D.	25 Color
No antibiotic		1.48	G	1.57	G	1.61	G	1.62	G
Neomycin	0.3	1.10	G	1.38	G	1.39	G	1.42	G
	0.1	0.89	G	1.20	G	1.23	G	1.36	G
	0.3	0.62	G	1.06	G	1.18	G	1.25	G
	1.0	0.41	W	0.94	G	1.13	G	1.10	G
	3.0	0		0.89	G	0.98	G	1.10	G
Paromomycin	0.003	1.20	G	1.28	G	1.30	G	1.34	G
	0.01	0.90	G	1.24	G	1.27	G	1.29	G
	0.03	0.66	G	1.20	G	1.18	G	1.21	G
	0.1	0.26	W	0.44	G	1.02	G	1.12	G
	0.3	0		0.37	G	0.88	G	1.07	G
	1.0	0		0	G	0.26	G	0.84	G
Kanamycin	1.0	1.18	G	1.30	G	1.33	G	1.32	G
	3.0	1.07	W	1.32	G	1.30	G	1.40	G
	10.0	0.98	W	1.24	G	1.20	G	1.32	G
	30.0	0.84	W	1.00	W	1.00	W	1.19	W
Streptomycin	1.0	1.37	W	1.34	G	1.34	G	1.49	G
	3.0	1.11	W	1.22	G	1.31	G	1.33	G
	10.0	1.06	W	1.07	W	1.15	W	1.12	W
	30.0	0.94	W	0.95	W	1.06	W	1.10	W

Table 2. *Partial annulment of bleaching and toxicity by vitamins*

	Concentration mg-%	Control O.D.	Control Color	Pantothenate 1 mg-% + nicotinic acid 1 mg-% + thiamine 1 mg-% O.D.	Pantothenate 1 mg-% + nicotinic acid 1 mg-% + thiamine 1 mg-% Color
No antibiotic		1.49	G	1.51	G
Neomycin	0.03	1.10	G	1.12	G
	0.3	0.32	G	0.98	G
	1.0	0.11	W	0.55	G
	3.0	0		0	
Paromomycin	0.001	1.45	G	1.40	G
	0.003	1.30	G	1.17	G
	0.01	0.90	G	0.89	G
	0.03	0.66	G	0.66	G
	0.1	0.28	W	0.25	W
	0.3	0		0	
Kanamycin	1.0	1.11	G	1.24	G
	3.0	0.91	W	1.04	G
	10.0	0.88	W	0.89	W
	30.0	0.82	W	0.83	W
Streptomycin	1.0	1.29	W	1,43	G
	3.0	1.07	W	1,21	G
	10.0	1.02	W	1.12	W
	30.0	0.97	W	1.05	W

b) Antihistamines

Tripelennamine (previously observed by GROSS et al. 1955) and some other antihistamines (Table 3) bleached. Disconcertingly, some other antihistamines not radically different in structure (Formula-summary) did not bleach even in concentrations as high as 0.1%; these

Table 3. *Bleaching by antihistamines*

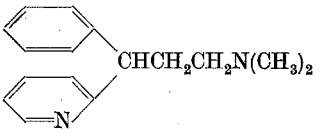
	Concentration mg-%	O. D.	Color		Concentration mg-%	O. D.	Color
No antihistamine		1.25	G	Antazoline	10.0	0.97	G
					30.0	0.85	G
Diphenyl-					60.0	0.76	G
hydramine	1.0	1.21	G		100.0	0.56	G
	3.0	1.13	G				
	10.0	0.97	G	Pyrathiazine	1.0	0.86	G
	30.0	0.84	W*		3.0	0.84	G
	60.0	0.72	W*		10.0	0.66	G
	100.0	0.43	W*		30.0	0	
Tripelennamine	3.0	1.27	G	Pheniramine	3.0	1.19	G
	10.0	1.09	G		10.0	1.03	G
	30.0	0.88	W		30.0	0.99	G
	60.0	0.79	W		60.0	0.49	G
	100.0	0.67	W		100.0	0.32	G
Methapyrilene	30.0	1.05	G	Phenindamine	1.0	1.15	G
	60.0	0.82	G		3.0	1.11	G
	100.0	0.69	W		10.0	0.96	G
Pyrilamine	3.0	1.24	G		30.0	0.76	W*
	10.0	0.95	G		60.0	0.42	W*
	30.0	0.78	G		100.0	0	
	60.0	0.76	W	Promethazine	1.0	1.08	G
	100.0	0.65	W		3.0	0.95	G
Antazoline	1.0	1.13	G		10.0	0.85	G
	3.0	1.08	G		30.0	0	

* Regreened in drug-free media; other bleachings were permanent.

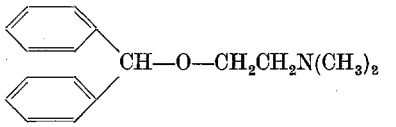
were antazoline, doxylamine, and prophenpyridamine. The bleached strains, unlike some described by GROSS et al., had no obvious impairment of vigor while being maintained in the defined medium used throughout this work.

It seems tacitly assumed that streptomycin and heat damage the same site. As seen in Table 4, streptomycin and heat act additively, not synergistically, which points to a common site of action. In these experiments the cultures were held at the indicated temperatures for 6 days in the dark, then put in light.

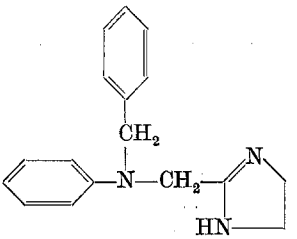
Formula-summary. Antihistamines used in this study



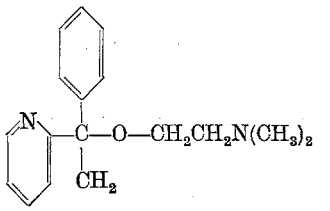
Prophenpyridamine (Trimeton)



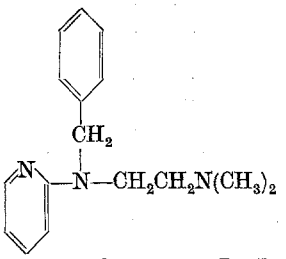
Diphenylhydramine (Benadryl)



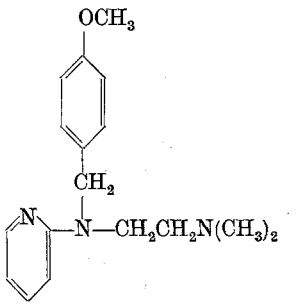
Antazoline (Antistine)



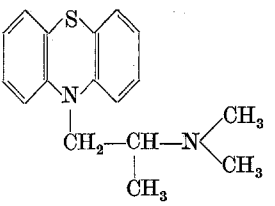
Doxylamine (Decapryn)



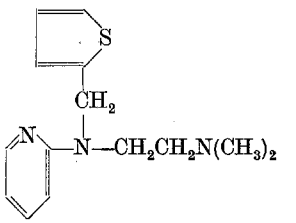
Tripelennamine (Pyribenzamine)



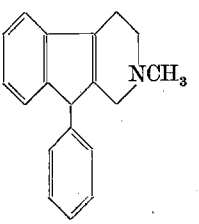
Pyrilamine (Neo-Antergan)



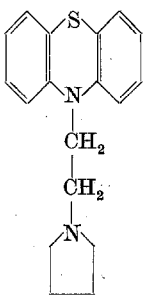
Promethazine (Phenergan)



Methapyrilene (Thenylene)



Phenindamine (Thephorin)



Pyrathiazine (Pyrrolazote)

Table 4. *Combined effect of streptomycin and heat*

	Conc. mg-%	Temperature in °C									
		28°		31.4°		33.5°		34.8°		35.6°	
		O.D.	Color	O.D.	Color	O.D.	Color	O.D.	Color	O.D.	Color
No antibiotic		1.42	G	1.13	G	0.91	W	0.72	W	0.53	W
Streptomycin	0.1	1.41	G	1.05	G	0.86	W	0.66	W	0.48	W
	0.25	1.40	G	1.00	G	0.83	W	0.66	W	0.49	W
	0.5	1.42	G	0.90	W	0.80	W	0.64	W	0.44	W
	1.0	1.35	W	0.88	W	0.74	W	0.66	W	0.35	W
	2.0	1.18	W	0.80	W	0.71	W	0.58	W	0.33	W

Discussion

Histidine and Mg oppose bleaching and killing by streptomycin perhaps merely by slowing uptake and transport. PRAMER (1956) and LITWACK and PRAMER (1957) showed that Mg slowed uptake of streptomycin by *Nitella*, and adduced evidence for an ion-binding system which transported streptomycin across the cell wall. Histidine and Mg may, then, act peripherally (Mg was studied in detail for *E. gracilis* by ROSEN and GAWLIK 1961), revealing little about the target.

That protoplastids are the site of action is made likely by the proof by EPSTEIN et al. (in press) of the persistence in dark-grown euglenas of cytoplasmic bodies which in light develop into proplastids and then chloroplasts. This leaves untouched the problem of the extreme sensitivity of these entities—if these proplastids are indeed the sensitive sites—to antibiotics, antihistamines, heat, and ultraviolet radiation.

E. gracilis in this respect may not be so different from higher plants, whose bleaching by streptomycin is long known. The guard cells of bean seedlings are bleached by UV (BLAKELY and CHESSIN 1959). SATO (1960) showed Mg-streptomycin antagonism in timothy *(Phleum pratense)*, also bleaching activity by viomycin (a streptomycin-family antibiotic—see Table 5).

No clear molecular common denominator for bleaching activity has emerged for antihistamines. Some antihistamines bleached only temporarily. Antihistamines have side effects, e.g. drowsiness, nausea, and local anaesthesia (DRAGSTEDT 1958). More antihistamines and related drugs must be tested to see which if any of these side effects correlates with bleaching.

A limitation in our experiments was the use of defined media. Whether the antihistamines would show the same order of activity in media containing complex natural products, and whether cultures bleached in these media could then be maintained in the standard defined media, are being investigated.

Attempts to define a common molecular target for these diverse physical and chemical agents are complicated by the discovery of another permanent bleacher, *o*-methylthreonine (AARONSON and BENSKY; GRAY and HENDLIN 1961); here the mode of action is interference with isoleucine anabolism, presumably in the chloroplast. The resemblance between photosynthetic function in *E. gracilis* and higher plants is further borne out by the temporary bleaching of *E. gracilis* by the herbicide 1,2,4-aminotriazole (AARONSON and SCHER 1960); aminotriazole bleaching in higher plants seems reversible (SUND et al. 1960) and, interestingly, is annuled by histidine and adenine (WEYTEK and BROQUIST 1960).

Table 5. *Composition and side-effects of bleaching antibiotics*

Antibiotic	Moieties	Side-effects
Streptomycin	Streptidine (1,3-diguanido-2,4,5,6-tetrahydrocyclohexane) Streptose (3-C-formyl-5-deoxy-L-lyxose) N-Methyl-L-glucosamine	nephrotoxicity; neurotoxicity, especially to 8th cranial nerve
Kanamycin	6-Deoxy-6-amino-D-glucose 3-Deoxy-3-amino-D-glucose 1,3-Diamino-4,6-dihydroxycyclohexane	Same
Neomycin	D-Ribose Deoxystreptamine	Same
Erythromycin	Desosamine (a dimethylaminodeoxy sugar) Cladinose (a methoxydesoxymethylase) Erythronolide (a macrocyclic lactone)	Few
Viomycin	Incompletely known; strongly basic	8th nerve damage
Paromomycin	Deoxystreptamine An amino sugar An aminomethylamino sugar	?

Acriflavin, which inactivates the sex factor (F^+) in *Escherichia coli* (reviewed by JACOB and WOLLMAN 1961), deprives trypanosomes of their kinetosomes, and induces petite mutants in yeasts, had no bleaching activity as repeatedly found in light and dark cultures in gradient plates at concentrations up to lethal.

The changes in nucleotide excretion in streptomycin-treated *E. coli* (ROSANO et al. 1960) and claims of changes in RNA or DNA composition in heat (BRAWERMANN et al. 1960) or streptomycin-bleached euglenas (NEFF 1960), hardly permit a comprehensive theory.

Permanent bleaching may reflect deranged chloroplast nucleic acids, as inferred from the demonstration by LYMAN et al. (1961) that the

action spectrum for bleaching by UV shows peaks at 260 and 280 mμ—a characteristic of nucleoproteins. This in turn invites the speculation that the unusual sugars in bleaching antibiotics interfere with nucleic acid ribose and deoxyribose.

For an antibiotic to behave as an antibiotic it must have some special property that permits such frequently bulky, predominantly highly polar molecules, to penetrate the cell and reach vulnerable sites. One must assume that antibiotics resemble molecules for which there are activated-transport systems. The restricted distribution of bleaching antibiotics may therefore follow from the low probability that the same molecule will embody a configuration making for transport and a configuration selectively destructive to chloroplasts and proplastids.

Were only the streptomycin family of antibiotics taken into account, one might conclude that the prerequisite for bleaching was a ring with basic substituents linked to one or more unusual sugars. Bleaching by erythromycin (ERBINGER 1961a, b) puts emphasis on the sugars, as the erythromycin family of antibiotics—the macrolides—lacks the strongly basic rings of the streptomycins. One may now postulate two independent prerequisites for bleaching: a peculiarity in the sugar moiety and another peculiarity elsewhere in the molecule favoring transport of this sugar. Studies of the macrolide antibiotics and the cardiac glucosides may provide the critical test of this idea.

Streptomycin antibiotics are so designated because of common molecular architecture (Table 5) as well as biological resemblances such as cross-resistance (MILLER et al. 1959). Thus paromomycin was included in this study before its structure was known because paromomycin-resistant *E. coli* was also resistant to streptomycin, kanamycin, and neomycin (FINLAND 1959).

The correlation between ototoxicity and bleaching activity suggests that new members of the streptomycin family should be tested for bleaching activity, the hope being that lack of bleaching activity might mean lack of ototoxicity. Antibiotics with an antimicrobial spectrum like streptomycin's but free of ototoxicity are urgently needed (DUNLOP and MURDOCH 1960; FROST et al. 1960).

Summary

Kanamycin, paromomycin, and neomycin, like streptomycin, permanently bleach *Euglena gracilis*. This effect, along with general toxicity, is opposed by Mg, histidine or a combination of pantothenate, nicotinic acid, and threonine. Such opposition is thought to be peripheral effects centered on uptake and transport.

Certain antihistamines, notably tripelennamine, methapyrilene, and pyrilamine induce permanent bleaching. Diphenylhydramine and

phenindamine induced temporary bleaching. Doxylamine, antazoline, pyrathiazine, pheniramine, prophenpyridamine, and promethazine did not bleach when tested up to inhibitory concentrations.

Bleaching by streptomycin + heat was additive, not synergistic.

The evidence at hand for the mode of action of ultraviolet irradiation, streptomycin antibiotics, and erythromycin suggests, as a working hypothesis, that the common factor may be interference with nucleic acid metabolism; the common factor in bleaching by antibiotics may be simultaneous provision of a molecular grouping favoring uptake and transport of the active moiety, which in turn may be rare sugars interfering with ribose and desoxyribose in the photosynthetic apparatus.

New antibiotics of the streptomycin family might well be screened for bleaching activity as a possible index of damage to the 8th cranial nerve, for so far the correlation is excellent for this class of antibiotic.

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References

AARONSON, S., and B. BENSKY: O-methylthreonine, a new bleaching agent for *Euglena gracilis*. J. gen. Microbiol. (in press).

AARONSON, S., and S. SCHER: Effect of aminotriazole and streptomycin on multiplication and pigment production of photosynthetic microorganisms. J. Protozool. **7**, 156—158 (1960).

BLAKELEY, L. M., and M. CHESSIN: Disappearance of guard cell chloroplasts in ultra-violet irradiated leaves. Science **130**, 500—501 (1959).

BRAWERMAN, G., C. A. REBMAN and E. CHARGAFF: A bleached variant of *Euglena gracilis* showing a doubling of the content of deoxyribonucleic acid. Nature (Lond.) **137**, 1037—1038 (1960).

DRAGSTEDT, C. A.: Histamine and antihistaminics. In: Pharmacology in Medicine, pp. 619—634. V. A. DRILL, ed. New York: McGraw-Hill 1958.

DUNLOP, D. M., and J. M. MURDOCH: The dangers of antibiotic treatment. Brit. med. Bull. **16**, 67—72 (1960).

EPSTEIN, H. T., and J. A. SCHIFF: Studies of chloroplast development in *Euglena*. IV. Electron and fluorescence microscopy of the proplastid and its development into a mature chloroplast. J. Protozool. (in press).

ERBINGER, L.: Die Erythromycinwirkung auf die Flagellaten Euglena gracilis Klebs. Naturwissenschaften **18**, 606—607 (1961a).

ERBINGER, L.: Some effects of antibiotics on protozoa. Abstracts 1st Int. Conf. Protozoologists, Prague, pp. 267—268 (1961b).

FINLAND, M.: Cross-resistance among four antibiotics: neomycin, paromycin, kanamycin and streptomycin. In Symposium V: Biochemistry of Antibiotics, Proc. 4th Int. Congr. Biochem., pp. 85—90. London: Pergamon Press 1959.

FROST, J. O., J. E. HAWKINS jr., and J. F. DALY: Kanamycin. II. Ototoxicity. Amer. Rev. Resp. Dis. **82**, 23—30 (1960).

GRAY, R. A., and D. HENDLIN: Growth inhibition and bleaching in higher plants and *Euglena* by *o*-methylthreonine and reversal by isoleucine. Plant Physiol. **37**, 20 (1956).

GROSS, J. A., T. L. JAHN and E. BERNSTEIN: The effect of antihistamines on the pigments of green protista. J. Protozool. **2**, 71—75 (1955).

HUTNER, S. H., H. BAKER, S. AARONSON, H. A. NATHAN, E. RODRIGUEZ, S. LOCKWOOD, M. SANDERS and R. A. PETERSEN: Growing *Ochromonas malhamensis* above 35° C. J. Protozool. **4**, 259—269 (1957).

JACOB, F., and E. L. WOLLMAN: Sexuality and the Genetics of Bacteria. New York: Academic Press 1961.

LITWACK, G., and D. PRAMER: Absorption of antibiotics by plant cells. III. Kinetics of streptomycin uptake. Arch. Biochem. **68**, 396—405 (1957).

LYMAN, H., H. T. EPSTEIN and J. A. SCHIFF: Studies of chloroplast development in *Euglena*. I. Inactivation of green colony formation by u.v. light. Biochim. biophys. Acta **50**, 301—309 (1961).

MILLER, A. K., M. E. VALIANT and B. M. FROST: Laboratory studies on the antibacterial activity of kanamycin. Antibiot. and Chemother. **19**, 475—484 (1959).

NEFF, R. H.: Volume, nucleic acid and nitrogen contents of strains of green and colorless *Euglena gracilis* and *Astasia longa*. J. Protozool. **7**, 69—74 (1960).

PACKER, E. L., S. H. HUTNER, D. COX, M. N. MENDELOW, H. BAKER, O. FRANK and D. AMSTERDAM: Use of amine buffers in protozoan nutrition. Ann. N.Y. Acad. Sci. **92**, 486—490 (1961).

PRAMER, D.: Absorption of streptomycin by *Nitella clavata*. Arch. Biochem. **62**, 265—301 (1956).

PRESCOTT, B., T. KAUFFMANN, W. D. JAMES and M. J. STONE: Means of increasing the tolerated dose of streptomycin in mice. II. Certain vitamins. Antibiot. and Chemother. **9**, 369—375 (1959).

PRINGSHEIM, E. G., and O. PRINGSHEIM: Experimental elimination of chromatophores and eye-spot in *Euglena gracilis*. New Phytologist **51**, 65—76 (1952).

ROSANO, C. L., R. A. PEABODY and C. HURWITZ: Studies on the mechanism of action of streptomycin. Effect of streptomycin on the excretion of nucleotides by *Escherichia coli*. Biochim. biophys. Acta **37**, 380—387 (1960).

ROSEN, W. G., and S. R. GAWLIK: Effect of streptomycin on chlorophyll accumulation in *Euglena gracilis*. J. Protozool. **8**, 90—96 (1961).

SATO, Y.: II. Influences of inorganic salts upon the streptomycin effect on the timothy seed (under resting conditions). Keiô J. Med. **9**, 25—32 (1960).

WEYTEK, F. W., and H. P. BROQUIST: Interference with adenine and histidine metabolism of microorganisms with aminotriazole. Biochim. biophys. Acta **40**, 576—579 (1960).

WOLKEN, J. J.: Euglena: an Experimental Organism for Biochemical and Biophysical Studies. New Brunswick: Rutgers Univ. Press 1961.

Professor Dr. ARTHUR C. ZAHALSKY
Haskins Laboratories, 305 East 43rd St., New York 17, N.Y./U.S.A.