false positive reactions can be given in this brief report. In general the false negatives fall into 3 classes: (1) patients with tumors of borderline malignancy; (2) patients with very small carcinomas; and (3) those with extensive terminal malignant disease, or rapidly spreading metastases. The false positives fall almost entirely into 3 groups: (1) patients with advanced tuberculosis; (2) patients who have been operated upon very recently; and (3) patients with active infections. Of the last, those in whom the streptococcus is the agent can be ascertained by the use of the streptococcal antifibrinolysin test (Christensen,[8] and Boisvert[9]). Reactions in the doubtful range occur frequently with serum from patients with other diseases (such as diabetes and lues) which gave false positive reactions with previous antifibrinolysin tests.

A non-specific antiproteolytic reaction has been outlined, the results of which when carefully analyzed give a high correlation between a positive test reaction and the presence of malignancy in the patient whose serum is tested. Certain false negative and positive reactions occur and careful study will be necessary to define these. It may be hoped that methods to exclude the effect of other specific antifibrinolytic factors, such as the antistreptokinase, will be developed with the ultimate goal of a specific reaction for malignancy. For the present, the reaction is of value as a screening mechanism with a degree of correlation warranting extremely careful observation in all patients with a consistently elevated titre.

[8] Christensen, L. R., *J. Gen. Physiol.*, 1945, **28**, 363.

[9] Boisvert, P. L., *J. Clin. Invest.*, 1940, **19**, 65.

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Streptomycin-Induced Chlorophyll-less Races of *Euglena*.

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In the course of attempts to obtain with the aid of antibiotics pure cultures of algal flagellates and related colorless forms, it was noticed that after exposure to streptomycin (STM), normal green pure cultures of several strains of *Euglena gracilis*, used for orientation experiments, became colorless and remained so on serial transfer in light on STM-free media. The theoretical implications of this observation prompted further study.

A study was made of this bleaching as a function of (1) concentration of STM, (2) duration of exposure to STM, (3) exposure under proliferating and non-proliferating conditions, and (4) exposure to STM in light and darkness.

Methods. The organism used for most of these studies is classified as *Euglena gracilis* var. *bacillaris*.[1] It has the advantage over the widely used Pringsheim strain of growing rapidly up to 32°C, while the Pringsheim strain does not grow above 28°C. Illumination for growth was supplied by 3500°K white or 6500°K "daylight" fluorescent lamps, at intensity levels of 150 to 300 foot candles. This was sufficient for practical light saturation of moderately dense cultures.

Streptomycin solutions were compounded from crystalline salts and were sterilized by filtration through sintered glass.

It has been found previously in unpublished

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[1] Pringsheim, E. G., personal communication.

experiments that *E. gracilis*, and the other green and colorless euglenoids available in pure culture, were stimulated several hundred-fold by, or required absolutely, an unidentified growth factor present in relatively very high concentration in liver concentrates refined for parenteral treatment of pernicious anemia. It was thenceforth simple to devise reproducible media virtually devoid of utilizable carbon and energy sources ("substrate"), but which allowed very heavy growth in light or darkness when appropriate substrates were added. A medium used in initial studies of bleaching is shown in Table I. This medium, supplemented with good substrates, gives green cells only a slight advantage in light over colorless cells.

TABLE I.

Basal Medium.	Concentration (%)
KH_2PO_4	.1
K_2HPO_4	.1
NH_4NO_3	.1
$MgSO_4 \cdot 7H_2O$	.02
K_3 citrate $\cdot H_2O$	.08

Liver concentrate (Lederle, 1 ml ≅ 3.3 U.S.P. units), 0.02 ml/100 ml.

Trace elements (mg %): Ca 2.0, Fe 0.3, B 0.02, Co 0.01, Cu 0.001, Mn 0.1. Mo 0.01, V 0.001, Zn 0.02.

pH 6.8-7.0. Media distributed 10 ml/25 ml Erlenmeyer flask.

Notes: 1. Citrate, added as a metal carrier,[2] is not utilized by *E. gracilis*.

2. The organism grows through a remarkably wide pH range, even in the presence of acetate or 0.25% Na butyrate. In later work the initial pH was 6.5.

3. Liver concentrates from 2 other commercial sources gave similar results.

Results. The effect of STM as influenced by concentration, substrates, and illumination was observed in the experiment summarized in Table II, using the medium of Table I. The observations on color refer to the macroscopic aspect of the cultures. *E. gracilis* strains normally become colorless when placed in darkness and become green again rapidly when restored to light. The following conclusions were drawn from the experiment of Table II and similar experiments:

1. STM reduced growth in light to a level equal to or lower than that in darkness.

2. Growth in darkness was unaffected. As a corollary, STM did not interfere with the utilization of externally supplied substrates.

3. Certain amino acids (alanine and glutamate) were good substrates for growth in darkness, although they accelerated growth in light only slightly; they were inferior however to certain lower fatty acids (represented in Table II by butyrate).

TABLE II.

Effect of Varying Concentrations of Streptomycin (STM) and of Various Substrates in Light and in Darkness. Growth expressed as cells/mm³

	Light				Darkness			
	No STM	STM 100 μg/ml	STM 1000 μg/ml	STM 5000 μg/ml	No STM	STM 100 μg/ml	STM 1000 μg/ml	STM 5000 μg/ml
	G	G	W					
No substrate	1300	410	8	0	8	10	13	51
Na butyrate	G	W	W	W				
0.15%	1900	760	480	238	950	680	720	880
Na H glutamate	G	G	W	W				
0.3%	1950	750	13	43	204	178	190	189
DL-Alanine	G	G	W	W				
0.25%	980	300	190	132	360	420	320	410
Glucose	G	G	W	W				
1.0%	1380	240	24	42	430	158	147	147

W—white, G—green. All cells in darkness were white.

In later experiments, a comparison was made of the action of STM upon cells grown under conditions permitting active proliferation as contrasted with non-proliferating cells. To cope with the possibility that STM-treated cells might acquire additional nutritional requirements, or that STM-dependent individuals might arise, the experiments were designed as follows: (a) Proliferating conditions were obtained by using a medium containing

[2] Hutner, S. H., *Trans. N. Y. Acad. Sci., Ser. II*, 1948, **10**, 136.

butyrate, glutamate, tryptic digest of casein (Sheffield's "N-Z-Case") 0.3%, solubilized liver (Wilson's fraction "L") 0.02%, and yeast extract (Difco) 0.04%. This portion of the experiment was conducted in light and darkness with varying time of exposure to a constant concentration of STM (100 μg/ml), and with varying concentrations of STM with constant time of treatment (15 days). Flasks were inoculated with one drop of a normal green culture. At the end of the experimental periods, 1-drop transfers were made into duplicate flasks containing the same medium, one set having 100 μg/ml STM, the other set without STM. The original cultures and the transfer cultures were then incubated in light for observation of the degree of bleaching. (b) Exposure to STM under non-proliferating conditions was obtained by first growing the cultures in light for 2 weeks in a substrate-free medium similar to that in Table I, thus making the cells wholly dependent on photosynthesis for energy and carbon. The cultures were then moved into darkness, and, after a 24-hour period to allow the dissipation of reserve food, treatment with STM was begun and carried out in darkness. At the end of the periods of exposure, 1-drop seedings were made as before into the complex medium, again with and without STM, and the cultures incubated in light. The salient observations from these experiments were:

1. There was a smoothly progressive bleaching of the cultures with increasing duration of exposure and increasing concentration of STM. The secondary cultures in STM-free media accurately reproduced the gross aspect which the primary culture had at the moment of transfer, if a small allowance be made for the slight selective advantage of the green cells in light.

2. There was no difference in the degree of bleaching between proliferating and non-proliferating cultures, either in respect to the necessary duration of exposure to STM or to the concentration of STM required. There was no difference in these respects between light- and dark-treated cultures under proliferating conditions.

3. There was no evidence of development of STM-dependency, even in cultures exposed for 2 weeks to 1000 μg/ml.

4. With STM constant at 100 μg/ml, an exposure of 1 to 8 hours was required for loss of roughly 50% of the color, and 4 or more days for apparently complete bleaching.

5. With constant time of exposure (15 days), 1 μg/ml gave roughly 50% loss of color, and bleaching was nearly complete with 40 μg/ml.

6. Preliminary microscopic examination of partly bleached mass cultures showed them to consist of both pale and of almost fully green individuals. However a few green cells had only 1-2 somewhat ill-defined chloroplasts instead of the normal number (about 10). Also, some green cells did not have chloroplasts of normal thickness. Aged white cells, derived both from primary treated cultures and from subcultures, accumulated an unusually large number of red granules as compared with aged untreated green cells or with cells of *Astasia*. The stigma and paramylon grains remained permanently unaltered in all colorless cultures.

Similar experiments on a restricted scale were carried out with *E. gracilis* var. *urophora* and the classical Pringsheim strain. Bleached cells of *E. gracilis* var. *urophora* have now been carried through 5 transfers in light on STM-free media, and *E. gracilis* var. *bacillaris* through 9, and the Pringsheim strain through 2, without the least resumption of color. Parallel tests on several species of *Astasia* showed that like *E. gracilis* in the dark they were not inhibited by 5000 μg/ml STM, the highest concentration tested.

Discussion. "Directed evolution" has so far been achieved only in bacteria, as in the interconversion of types of *Pneumococcus*[3] and of *Escherichia coli*[4] under the influence of specific nucleoproteins. The change to the non-chlorophyll condition (apochlorosis) described here falls rather into the special class of "loss mutations" ("pertes de fonctions")

[3] McCarty, M., Taylor, H. E., and Avery, O. T., *Cold Spring Harbor Symposia Quant. Biol.*, 1946, **11**, 177.

[4] Boivin, A., *Cold Spring Harbor Symposia Quant. Biol.*, 1946, **12**, 7.

discussed by Lwoff.[5] That this change follows a well-established evolutionary path is suggested by the following considerations:

1. Permanently colorless euglenoids similar to *E. gracilis* are abundant in nature, and are referred to the genus *Astasia*.[6] Some species of *Astasia* have a stigma like that in the colorless strains here derived from *E. gracilis*.

2. The euglenoids, as compared with other algae, have one of the richest arrays of colorless counterparts of green species.[5]

3. As already mentioned, the strains of *E. gracilis* tested all grow well in darkness when provided with suitable substrates in a good basal medium and permitted free access to atmospheric oxygen, indicating that the loss of photosynthesis does not here involve a profound alteration in metabolism.

4. Colorless euglenas have been reported to arise spontaneously in artificial culture (see [5,6] for references).

Sexuality is lacking in euglenoids and therefore it is impossible to determine here by the methods used for higher plants whether bleaching is a purely cytoplasmic phenomenon or involves a certain measure of conventional genic control—the classic problem of plastid autonomy. Another related question is whether bleaching, permanent or temporary, can be induced in forms which retain chlorophyll when grown in the dark.

In photosynthetic bacteria, as in blue-green algae, chlorophyll is not localized in plastids. Strains of purple bacteria (*Athiorhodaceae*) adapted to aerobic conditions, grow well in darkness aerobically, with retention of characteristic color, although the pigments are diminished in amount.[7] When 5 strains of purple bacteria, representing 5 species, were treated with STM, the sharp inhibition of growth was the same in light and darkness, and there were no obvious changes in color in partially inhibited cultures.

During the preparation of this paper, articles by von Euler and Bracco[8] were received. They found that seeds germinated in STM solutions developed into seedlings whose leaves showed chlorophyll deficiencies associated with colorless or structurally defective plastids. They did not report observations beyond the seedling stage.

The effectiveness in respect to *Euglena*-bleaching of portions of the STM molecule, and of other antibiotics, actinomycete and non-actinomycete in origin, is being studied; such data should determine whether the bleaching phenomenon may be used as a specific test for streptomycin, and should also furnish some indication as to the mode of action of STM.

Summary. When acted upon by streptomycin, certain strains of *Euglena gracilis* undergo a permanent loss of chlorophyll, without being inhibited in growth if good energy and carbon sources are supplied to make up for the loss of photosynthesis. The degree of bleaching is proportional to the degree of exposure to streptomycin. Proliferating and non-proliferating cells, and cells exposed in the light and in the dark, appear equally susceptible to bleaching.

[5] Lwoff, A., L'Évolution Physiologique. Étude des Pertes de Fonctions Chez les Microorganismes. Paris: Hermann et Cie, 1943, pp. 308.

[6] Pringsheim, E. G., *Biol. Rev. Camb.*, 1948, **23**, 46.

[7] van Niel, C. B., *Bact. Rev.*, 1944, **8**, 1.

[8] Euler, H. v., *Arkiv f. Kem., Min., Geol.*, 1948, **25A**, 1; Bracco, M., and Euler, H. v., *Kem. Arb. II*, 1948, **10**, 1; Bracco, M., and Euler, H. v., *Kem. Arb. II*, 1947, **10**, 1.