havior of the enzyme separated in acrylamide gels or present in crude supernatant material. Tetrazole reduction did not occur in sections immersed in reaction mixtures containing all components except substrate. Inclusion of quinacrin or an α-keto acid in cytochemical reaction mixtures decreased tetrazole reduction (Table 1). Substitution of succinic acid for α-hydroxy acid in cytochemical reaction mixtures resulted in typical mitochondrial staining (Fig. 3). We feel that the cytochemical reaction system used to produce these results yields a valid image of the cellular distribution of α-hydroxy acid oxidase and, hence, of those renal microbodies which contain the enzyme.

The functional significance of microbodies is obscure. The identification of yet another hydrogen peroxide–producing enzyme in microbodies strongly implicates peroxide metabolism as a significant functional feature of these structures. The disparate catalytic functions of D-amino acid oxidase, urate oxidase, and α-hydroxy acid oxidase appear to preclude any unified metabolic role other than an as yet undefined association with catalatic oxidation. Perhaps microbodies will be found to contain diverse catalysts which have in common only the production of hydrogen peroxide. In this case, microbodies would serve to degrade hydrogen peroxide by catalatic oxidation and would be a device to protect cell components from indiscriminate oxidative attack by this molecule. The high concentration of catalase in hepatic microbodies (*8*), and presumably in those of other cells, would be admirably adapted to this role in cellular decontamination.

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References and Notes

1. H. Beaufay, P. Jacques, P. Baudhuin, O. Sellinger, J. Berthet, C. deDuve, *Biochem. J.* **92**, 184 (1964).
2. M. Blanchard, D. Green, V. Nocito-Carroll, S. Ratner, *J. Biol. Chem.* **163**, 137 (1964). Direct confirmation of hydrogen peroxide production by α-hydroxy acid oxidase separated by gel electrophoresis was made by incubating these gels in a reaction mixture patterned after that proposed by R. Graham and M. Karnovsky at the meeting of the Histochemical Society, Philadelphia, 1965. Sites of hydrogen peroxide liberation in the gels were marked by the formation of a red-brown reaction product. This pigment corresponded exactly in its position in the gel to that produced by the reduction of nitro blue tetrazolium.
3. α-Hydroxy acid oxidase in supernatant preparations was assayed by 2,6-dichlorophenolindophenol reduction according to J. Robinson *et al.* (*4*).
4. J. Robinson, L. Keay, R. Molinari, I. Sizer, *J. Biol. Chem.* **237**, 2001 (1962).
5. Electrophoresis in acrylamide gels was done according to B. Davis, *Ann. N.Y. Acad. Sci.* **121**, 404 (1964). Reaction mixtures for identification of α-hydroxy acid oxidase after electrophoresis contained the following final concentrations of reactants: 0.05*M* Sörenson's phosphate buffer, *p*H 7.5; 0.5*M* sodium L-lactate or 0.1*M* any other D, L-α-hydroxy acid; 78 μ*M* phenazine methosulfate; 2 mg nitro blue tetrazolium per milliliter. Photometric scanning of developed gels was done according to J. Allen and J. Gockerman, *Ann. N.Y. Acad. Sci.* **121**, 616 (1964).
6. Fractions in differential centrifugation experiments were obtained by the following schedule: nuclear fraction, 270*g* for 10 minutes followed by two washes at 270*g* for 10 minutes; mitochondrial fraction, 3020*g* for 10 minutes followed by one wash at 2600*g* for 10 minutes; lysosomal fraction, 22,000*g* for 20 minutes; microsomal fraction, $10^6 g$ for 1 hour. Density equilibrium centrifugation was done according to H. Beaufay *et al.* (*1*), with the Spinco SW 39 rotor operated at 39,000 rev/min for 2.25 hours exclusive of acceleration and deceleration times. Gradients were prepared and fractions were removed by the procedure of R. Martin and B. Ames, *J. Biol. Chem.* **236**, 1372 (1961). Enzymatic activity in fractions was determined as follows: α-hyroxy acid oxidase and D-amino acid oxidase by determination of α-keto acid formation, by the method of J. Robinson *et al.* (*4*) in the presence of 0.1*M* D, L-α-hydroxy valeric acid or 0.05*M* D-alanine; succinic dehydrogenase by 2,6-dichlorophenolindophenol coupled to phenazine methosulfate reduction as suggested by T. Singer and E. Kearney, in *Methods of Biochemical Analysis*, vol 4, D. Glick, Ed. (Interscience, New York, 1957), p. 307; acid phosphatase by determination of α-naphthol liberated from α-naphthyl acid phosphate according to J. Allen and J. Gockerman, *Ann. N.Y. Acad. Sci.* **121**, 616 (1964).
7. The reaction system used for cytochemical localization in sections was identical to that used for visualization of α-hydroxy acid oxidase after electrophoresis (*5*). All cytochemical results mentioned in this report were obtained with 2- or 4-μ cryostat sections of rat kidney fixed according to D. Walker and A. Seligman, *J. Cell Biol.* **16**, 455 (1963).
8. C. deDuve, in *The Harvey Lectures*, Series 59 (Academic Press, New York, 1965), p. 49; C. Bruni and K. Porter, *Amer. J. Pathol.* **46**, 691 (1965).
9. Supported by grants from PHS, AM 5731 C-2, and from the University of Michigan Cancer Research Institute, American Cancer Society, Michigan Division Account.

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Algal Cultures: Ability To Reduce Turbulent Friction in Flow

Abstract. *Liquid cultures of several freshwater and marine algae required less pressure to flow through a pipe at a given rate than the pure liquid medium before algal growth. This increased ease of flow can be attributed to long-chain polysaccharides produced in the medium during growth. Measurements of friction were used to estimate the molecular weight of an algal polysaccharide and to show the effect of bacterial action on the polymer.*

In tanks for towing model ships, the occasional drastic decrease in the normal drag associated with a given model has been of great concern (*1*). Because solutions of many natural and synthetic high-polymers are effective in damping turbulence and reducing friction (*2*), dissolved algal polysaccharides have been proposed as the cause of the variations in drag in test tanks. It has been shown (*2*) that solutions of carrageenin, derived from *Chondrus crispus*, have lower friction in turbulent flow than does pure water.

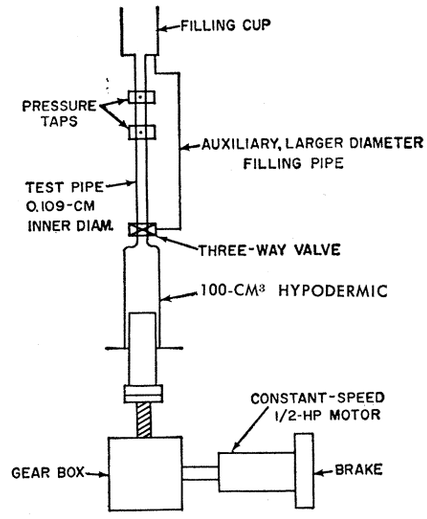

Fig. 1. Diagram of apparatus for measuring pressure losses by turbulent friction, caused by flow of algal cultures through a test pipe.

In order to show that algal growth can influence turbulent friction drag, we have examined liquid cultures of several freshwater and marine algae in an apparatus that measures the loss of pressure by friction in a short length of small-bore pipe. The instrument is designed to give a constant rate of flow of a sample of test solution (initially contained in a 100-cm^3 hypodermic syringe) through a measuring pipe, regardless of the magnitude of friction loss in the pipe; a fully turbulent flow is obtained, with the Reynolds number in the pipe approximately 14,000. The test consists in measuring pressures, during a run, at the two points marked "pressure taps" in Fig. 1. Strain-gage pressure transducers together with an oscillograph are used

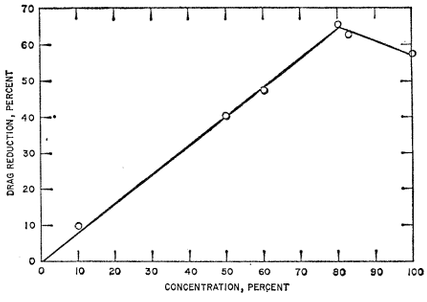

Fig. 2. Effect of dilution of culture of *Anabaena flos-aquae* with distilled water on observed reduction of friction.

Table 1. Reduction of friction by algae in flowing liquid media. For each effective species, determinations were made on each of several cultures; the reduction shown is the highest recorded. Reproducibility from any given culture was within 1 percent; variations between cultures were as great as 20 percent. Other marine species such as the diatoms *Nitzschia closterium* and *Biddulphia*, the dinoflagellates *Peridinium trochoideum* and *Gyrodinium*, and the flagellate *Cryptomonas erosa*, similarly grown and tested, did not reduce drag.

Alga	Culture		Age (days)	Incubation temp (°C)	Drag reduction (%)
	Method	Medium			
		Freshwater species			
Anabaena flos-aquae	Tube	Knops	12	40	59.5
	Tube	Bristol	28	40	57.3
	Flask	Soli*	26	40	6.4
Anabaena cylindrica	Tube	Bristol	12	23	0
Chlamydomonas peterfii†	Tube	Bristol	12	23	14.6
Ankistrodesmus sp.	Tube	Bristol	12	23	0
		Marine species			
Chactoceros didymus	Flask	Soli	11	18	16.0
	Tube	Soli	10	18	35.9
Porphyridium cruentum‡	Flask	Soli	15	23	65.0

* Reduced ionic strength. † No. 728 in the Indiana University collection. ‡ No. 161 in the Indiana University collection.

to measure the pressure, since the run lasts approximately 8 seconds. Friction loss can then be calculated from the following equation:

$$\text{Reduction in drag } (\%) = 100\,(1-\Delta P_t/\Delta P_w)$$

where ΔP_t is pressure difference using test fluid, and ΔP_w is pressure difference using distilled water.

Results obtained with various species of algae appear in Table 1. Some algae were cultured by the method of Moore and Tischer (*3*), in which agitation is maintained by bubbling air and CO_2 into the bottom of long glass tubes containing the medium ("tube" method). Algae cultured in flasks received either gentle aeration or none. Freshwater algae were grown under continuous illumination by fluorescent lamps; marine algae were grown on a cycle of 12 hours of light and 12 hours of darkness. Tests of the culture media (*4*) for possible reduction of drag showed no significant difference from distilled water. All friction tests were made at 23°C.

Both freshwater and marine species of algae caused large reductions in turbulent friction (Table 1), especially when they were grown by the tube method. Dilution of the tube culture of *Anabaena flos-aquae* with up to 20 percent of distilled water further reduced the drag to 66 percent (Fig. 2); still further dilution linearly decreased this reduction in drag. The drag reductions shown in Table 1 were increased a few tenths of 1 percent when the cells were removed by centrifuge; this indicates that the drag-reducing substance is contained in the medium (after algal growth) and that the cell bodies play no part in reducing drag.

Both of the *Anabaena* species examined produce copious polysaccharides in their culture media (*3*, *5*) but only *A. flos-aquae* reduced the drag. This difference in friction-reducing properties probably reflects differences in the molecular weights of the polysaccharides. Tests of other polymers have shown no reduction in friction with molecular weights under about 50,000. Key features of a good drag-reducing polymer seem to be linearity of molecular structure, high molecular weight, and good solubility. Our experience with species of *Anabaena* suggests that the friction-reduction test may assist in settling taxonomic questions in closely related species.

One may estimate the molecular weight of the *A. flos-aquae* polysaccharide from its effect in reducing friction. This is done by use of a chart derived from systematic friction-reduction tests on the linear water-soluble polymer poly(ethylene oxide) in a range of molecular weights (*6*) from 0.2 to 8×10^6. The reductions in drag shown in Fig. 3 were measured in the same apparatus used to test the algal cultures.

Lines of reduction in drag for constant polymer concentrations (in parts per million) are shown, with the zero of drag reduction arbitrarily put at 50,000 molecular weight. Running systematic tests of a polymer of unknown molecular weight at various concentrations in the same apparatus and plotting the data points obtained on the corresponding concentration curves in Fig. 3 will show that a vertical line passing through the new data yields a good estimate of the molecular weight. Although the method is gen-

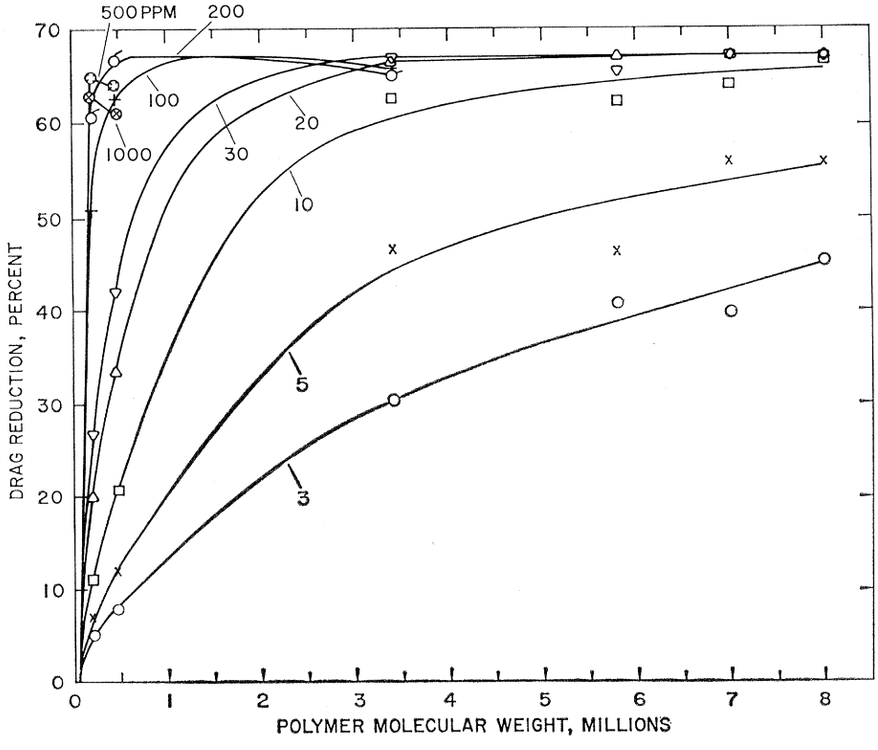

Fig. 3. Chart for estimating molecular weight of polysaccharide, based on test data on poly(ethylene oxide).

eral, the drag-reduction effect itself depends upon the Reynolds number of the flow through the measuring pipe of the apparatus; thus a "calibration curve" such as that shown in Fig. 3 must be drawn for each solvent and, for each test pipe of different dimensions.

The concentration of polysaccharide in the *A. flos-aquae* medium at the time of testing was over 500 ppm, which corresponds well with other similar measurements of concentration (*3*). The only possible fit of the data of Fig. 2 to the chart of Fig. 3, assuming 100-percent culture concentration of *A. flos-aquae* to be 500 ppm polysaccharide, yields a molecular weight of about 100,000.

To investigate by its effect on reduction of friction the nature of bacterial action on the extracellular polysaccharide, we grew two cultures of *Chaetoceros didymus* Ehrenberg simultaneously, one inoculated with syntrophic bacteria, the other bacteria-free (*7*). The culture containing bacteria effected only half as much reduction in friction as the axenic one (*8*). Available data are insufficient to determine whether the effect of bacteria is to lower the molecular weight, reduce the concentration of polysaccharide, or partially block synthesis of polysaccharide. Cultures of *Anabaena flos-aquae* effected considerable reduction in drag even with bacteria present.

Thus it seems entirely possible that algal polysaccharides can influence measurements in tow-tanks and other large-scale hydrodynamic facilities.

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References and Notes

1. R. N. Newton, *Trans. Roy. Inst. Naval Architects* **102**, 435 (1960); K. C. Barnaby and A. L. Dorey, *ibid.* **107** (1965).
2. J. W. Hoyt and A. G. Fabula, in *Proc. ONR 5th Symp. Naval Hyrodynamics, Bergen, Norway, 1964* (Fed. Clearinghouse Sci. Tech. Inform., AD-612 056).
3. B. G. Moore and R. G. Tischer, *Science* **145**, 586 (1964).
4. J. N. Phillips, Jr., and J. Meyers, *Plant Physiol.* **29**, 148 (1954); R. C. Starr, *Am. J. Bot.* **51**, 1013 (1964); G. Soli, *Limnol. Oceanogr.* **9**, 265 (1964).
5. C. T. Bishop, G. A. Adams, E. O. Hughes, *Can. J. Chem.* **32**, 999 (1954).
6. Determined by the manufacturer, Union Carbide Chemicals Co.
7. G. Soli, in *Symposium on Marine Microbiology*, C. H. Oppenheimer, Ed. (Thomas, Springfield, Ill., 1963), chap. 12.
8. Standard sterile techniques were used to grow all algae, even in handling nonaxenic cultures.
9. We thank R. H. Wade, H. I. Scribner, and Carol Ann Flanders for technical assistance, and R. G. Tischer (Mississippi State University) and P. Wold (California Institute of Technology) for gifts of algae.

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Malignant Lymphomas Following Allogenic Disease: Transition from an Immunological to a Neoplastic Disorder

Abstract. *The graft versus host reaction which occurs in F_1 hybrid mice injected with parental spleen cells was used to examine several immunological theories of neoplasia. Long-term survivors of this reaction developed lymphoid neoplasms which resembled Hodgkin's disease and lymphosarcoma. Mice with these tumors were chimeras, but the parental cells present within their spleens had specific immunological tolerance toward host antigens. This, together with the finding that the tumors were transplantable only to isogenic recipients, indicates that the tumors were of host rather than donor origin.*

Chronic lymphocytic leukemia, lymphosarcoma, and Hodgkin's disease are frequently associated with immunological abnormalities. Defects of immunoglobulin synthesis, autoimmunity, and anergy are commonly encountered in patients with these diseases. It is not known whether these aberrations of immunity represent cause or effect of the malignancy. Nevertheless, their elucidation has stimulated considerable interest in immunological theories of neoplasia.

One of these theories (*1*) has as its basis the cellular proliferation that characterizes the response of lymphoid tissue to antigens. It proposes that a continuous or repetitive exposure to antigens could result in a sustained proliferative response which culminates in neoplasia. This concept is supported by Metcalf's (*2*) experiments in which an increased incidence of reticulum cell sarcomas and plasma cell tumors was found in mice which had been given repeated injections of bovine serum albumin or *Salmonella adelaide* vaccine.

Tyler (*3*) has elaborated a theory that neoplasms of lymphoid tissue develop in two steps. In the first step, an immunologically competent cell undergoes a mutation which results in the loss of a histocompatibility antigen. In the second step, normal cells having a full complement of histocompatibility antigens serve as antigenic, and hence proliferative, stimuli for the abnormal, immunologically competent cell. The result is a constant, unrestrained growth leading to what is recognized as a neoplasm of lymphoid tissue.

Tyler further proposed that the reaction between graft and host produced in hybrid mice by the injection of parental lymphoid cells into F_1 hybrids represents a potentially useful model for studying the etiology of cancer. Since the transplanted parental lymphoid cells lack one or more histocompatibility antigens present in the F_1 hybrid host, they are analogous to the hypothetical antigen-deleted, immunologically competent cell of lymphoid neoplasms. Kaplan and Smithers (*4*) and Green *et al.* (*5*) have also advanced the idea that the graft versus host reaction is analogous to malignant lymphomas, but on somewhat different grounds.

In our experiments the graft versus host reaction (runt disease, allogenic disease, homologous disease) (*6*) was studied with particular reference to the development of neoplasms. An important technical drawback to the use of this reaction for testing immunological theories of neoplasia is that ordinarily it is rapidly fatal. This objection was overcome in two ways: (i) The recipients were 4- to 6-week-old F_1 hybrids rather than infant mice, which are customarily used in studies of the graft versus host reaction. The mortality due to the graft versus host reaction was therefore reduced from 100 to 70 percent. (ii) The F_1 hybrid recipients of parental spleen cells were treated with amethopterin, which reduces the mortality due to the graft versus host reaction to 30 percent (*7*). It was possible by these methods to accumulate adequate numbers of mice for a long-term study.

The graft versus host reaction was induced in 6-week-old male (C57Bl/$6 \times$ DBA/2)F_1 mice (hereafter referred to as BDF_1) by four weekly injections of approximately 80×10^6 male C57Bl/6 spleen cells. The cells were prepared by mincing whole spleens with fine scissors and gently pressing the fragments through tanta-