with polyadenylic acid. The addition of increasing amounts of polyadenylic acid to an acridine orange solution in 0.5*M* KCL, 0.01*M* NaOAc, *p*H 6.7, results in a very marked decrease in extinction at $\lambda = 495$ mμ, which also decreases relative to the 465 mμ peak, as Figs. 1 and 2 show. Under the above conditions, the relative decrease in extinction at 495 mμ is almost linear with respect to the mole ratio of adenosine monophosphate to acridine orange, and reaches its limiting value at a mole ratio close to 1:1, as Fig. 1 indicates. If the addition of polyadenylic acid is continued until quite large mole ratios of adenosine monophosphate units to acridine orange (70:1) are attained, the visible spectrum gradually undergoes a second alteration, d_{495} increasing to a relative value of 0.75. In view of the altered shape of the absorption spectrum it is unlikely that the decrease in extinction can be attributed to optical shielding effects alone. It is more likely that it at least partially reflects an altered resonance state of the bound dye molecule.

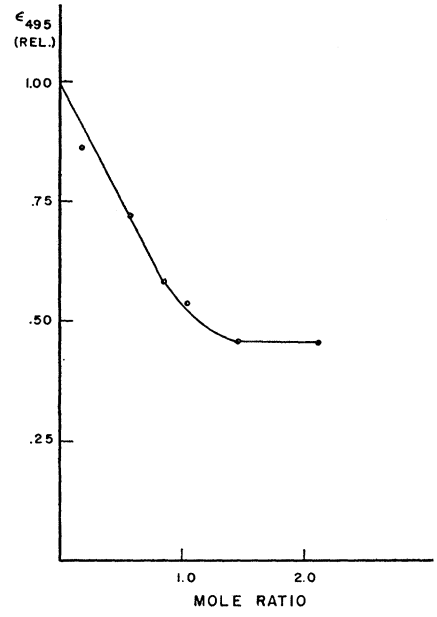

Fig. 1. Effect of addition of increasing amounts of polyadenylic acid to an acridine orange solution of molarity 4.7×10^{-6}. The *p*H is 6.7. The solvent is 0.01*M* NaOAc, 0.5 *M* KCl.

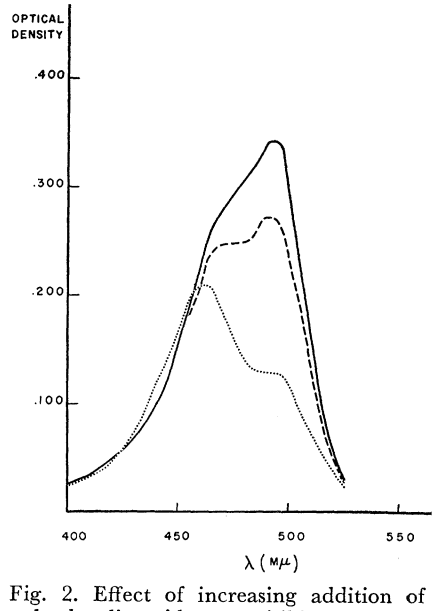

Fig. 2. Effect of increasing addition of polyadenylic acid upon visible spectra of acridine orange. The solvent is 0.01*M* NaOAc, 0.5*M* KCl. The *p*H is 6.7. The molarity of acridine orange is 6.7×10^{-6}. Solid line, no added polyadenylic acid; broken line, $2.6 \times 10^{-6}M$ (adenosine monophosphate units) polyadenylic acid; dotted line, $2.1 \times 10^{-5}M$ (adenosine monophosphate units) polyadenylic acid.

These phenomena were paralleled by a pronounced decrease in the intensity of the fluorescence of the acridine orange ($\lambda_{max.} = 550$ mμ). The intensity of fluorescence decreases to a value of 0.27 relative to that for free dye at about a 1:1 mole ratio of dye to monomer unit (for a total dye concentration of 6.2×10^{-6}) and slowly increases as the nucleotide-dye ratio becomes very large, reaching a relative value of 0.95 at a ratio of 70:1.

Pretreatment of the polyadenylic acid by formaldehyde according to the method of Fraenkel-Conrat results in abolition of both the spectrophotometric and fluorescence quenching effects (*4*).

The preceding results are consistent with the conclusion that acridine orange is bound in a one-to-one manner by the constituent adenosine monophosphate units of polyadenylic acid at low nucleotide-dye ratios. The subsequent alteration of the spectral and fluorescence behavior at high nucleotide-dye ratios reflects the competitive formation of a second complex, favored by the sharing of bound dye by an excess of polyadenylic macromolecules.

In view of the abolition of the spectral and fluorescence effects by blocking the 6-amino group of adenine with formaldehyde and by titration of the adenine groups, it appears that the adenine ring is probably involved in the interaction process. These results thus provide additional evidence for the availability of the adenine group of polyadenylic acid at alkaline *p*H's, as suggested earlier (*4*). In contrast, deoxyribonucleic acid isolated from *M. lysodeikticus* gave no spectral change.

These effects are of considerable interest in view of the known properties of acridine orange as a vital stain and bactericidal agent (*5*).

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5. The opinions expressed here are ours and are not to be construed as official or as reflecting the views of the Navy Department or the naval service at large.

23 October 1957

Use of Mononitrophenols Containing Halogens as Selective Sea Lamprey Larvicides

The invasion of the upper Great Lakes by the sea lamprey and its destructive attacks upon important fishes have engendered a considerable effort to control the numbers of this predator. Means for combating this pest may be applied effectively and economically during those parts of its life-cycle that are spent in streams (*1*). Destruction or diversion of the adults during their spawning runs (the terminal stage of the life-cycle) is a practicable procedure. This is being accomplished by electrical barriers which kill, or repel, the adults before they reach the spawning grounds (*2*). A serious shortcoming of this control technique is the time lag which occurs before the effect of such measures can become apparent. At least six generations of larval lampreys, already resident in stream habitats, continue to grow, transform into adults, and migrate into the lakes to begin their existence as parasites. Thus it may be as much as seven years before the lamprey population of a lake basin shows any marked decrease after the blocking of all streams suitable for spawning.

The life-cycle of the sea lamprey may also be broken effectively at the larval stage by the regulated application of selectively toxic chemicals. This treatment of streams inhabited by sea lampreys should produce more immediate control of the species than is being achieved by present techniques. In the course of screening various chemicals in a search for selective sea lamprey larvicides (*3*), we discovered six mononitrophenols containing halogens which are significantly more toxic to sea lamprey larvae than to other aquatic organisms (*4*). We also found that the conversion of four of these substances to their respective sodium salts increases their general utility without significantly affecting their useful biological activity (Table 1).

Laboratory experiments were conducted in 10-lit glass battery jars, each containing 5 lit of water. These vessels were aerated by means of standard stone air-breakers and were maintained at a constant temperature by immersion in

specially constructed troughs. Test animals in each experiment were larvae of the sea lamprey (*Petromyzon marinus*) and fingerlings of at least two of the following species of game fishes (choice of the species of game fishes depended on availability): rainbow trout (*Salmo gairdneri*), brown trout (*Salmo trutta*), and bluegill sunfish (*Lepomis macrochirus*). To each test container we added two specimens of the species chosen to be exposed to a chemical. Appropriate amounts of the compound were then added to the test jars to produce a desired concentration. Generally, 24 simultaneous replications were run with the same species at each such concentration. All laboratory tests were conducted for a 24-hour period at a water temperature of 55 ± 1°F.

The specific toxic effects displayed by the compounds studied under these controlled conditions are summarized in Table 1.

Table 1. Differential toxic effects among larval lampreys and fishes of certain mononitrophenols containing halogens. Sodium salt is expressed in parts per million of free phenol.

Name and form of compound	Concentration required to kill all lamprey larvae (ppm)	Concentration required to cause significant mortality† among fishes (ppm)		
		Rainbow trout	Brown trout	Bluegill sunfish
2-Bromo-4-nitrophenol				
Free phenol	5	13	11	
Na salt	7	15		
3-Bromo-4-nitrophenol				
Free phenol	5	11		15
5-Chloro-2-nitrophenol				
Free phenol	3	5	5	
2,5-Dichloro-4-nitrophenol				
Free phenol	3	13	7	
Na salt	5	17		
3,4,6-Trichloro-2-nitrophenol				
Free phenol	5	17	15	
Na salt	13	23		
3-Trifluormethyl-4-nitrophenol*				
Free phenol	2	9	7	
Na salt	2	7		

* α,α,α-trifluoro-4-nitro-*m*-cresol. † Mortality of approximately 10 percent of all test animals.

Our principal objective has been to locate chemicals which are acutely toxic to larval sea lampreys at low concentrations and which, at the same concentrations, are not toxic to other fishes inhabiting the same stream environments. A further requirement was that these selectively toxic materials should be capable of destroying the larvae in a relatively short period of time. We believe that the differential toxicities displayed by these mononitrophenols (with the exception of that exhibited by 5-chloro-2-nitrophenol) cover a sufficiently broad range of concentrations to permit their regulated application in natural streams to destroy lamprey larvae without concurrent damage to other fish. Requirements for a short treatment period are likewise met by these compounds. At minimum effective concentrations of each of the chemicals, all of the larvae were killed in less than 16 hours. At higher concentrations the time was much less. Several of the compounds killed all of the lampreys in less than 45 minutes without apparent harm to the game fish which remained exposed to these concentrations for 24 hours.

We have also tested 3,4,6-trichcloro-2-nitrophenol and 3-trifluormethyl-4-nitrophenol in a running water raceway in which natural stream conditions were simulated. In these cases aqueous solutions of the sodium salts of each of these phenols were applied under conditions where continuous flow required their regulated metering into the "stream" during the entire test treatment period.

The raceway utilized for this purpose was a rectangular concrete trough, 65 ft long, 6 ft wide, and 30 in. deep. Water for the "race" was pumped from Lake Huron; its flow into the experimental "stream" was regulated by a "V" weir. For each test treatment undertaken, an artificial stream bed was constructed on the floor of the raceway with materials taken from the beds of local rivers. Typical stream features such as pools and riffles were reproduced. Test animals were released in the raceway sufficiently in advance of a treatment to allow them to become adjusted to the "stream." Each experimental treatment (at a particular concentration) was conducted for a 24-hour period. The larvicidal compound was metered into the water by an electric, motor-driven, dual-piston, fluid-proportioning device. This machine pumped from a concentrated stock solution of the chemical and delivered the concentrate, slightly diluted with flush water, to a perforated pipe laid on the stream bed at the head of the test stream area.

Under conditions that simulated treatment of an actual stream, the toxic effects of each of the substances upon fishes seemed to be considerably less than the effects which were observed under laboratory-jar test conditions. On the other hand, concentrations of the materials lethal to all larval lampreys were essentially the same in the jar tests and in treatments of the simulated stream.

For example, in raceway tests of 3,4,6-trichloro-2-nitrophenol (applied as an aqueous solution of its sodium salt), all lamprey larvae were killed by concentrations as low as 12 parts per million. Sustained exposure to a concentration of 40 parts per million of the compound (the highest concentration that we applied experimentally) did not cause any significant toxic symptoms to appear among either rainbow trout or brown trout. Furthermore, exposure to this concentration did not cause any evident harm to any of the following additional test species: brook trout (*Salvelinus fontinalis*), rock bass (*Ambloplites rupestris*), bluegill sunfish, pumpkinseed sunfish (*Lepomis gibbosus*), lake chubs (*Couesius plumbeus*), northern creek chubs (*Semotilus atromaculatus*), logperch (*Percina caprodes*), crayfish (*Cambarus* spp.), dragonfly larvae (Anisoptera), and caddisfly larvae (Trichoptera). Several other species of fishes used in the raceway tests displayed some susceptibility to the higher concentrations of the chemical which we introduced. Yellow perch (*Perca flavescens*) and white suckers (*Catostomus commersoni*) were not affected by a concentration of 32 parts per million, but significant mortalities among these two species occurred during the application of a concentration of 40 parts per million. A considerable number of mortalities occurred among bullheads (*Ictulurus melas* and *I. nebulosus*) during all applications of concentrations of 20 parts per million or more of the phenol.

A comparable accentuation of the selectively toxic properties of 3-trifluormethyl-4-nitrophenol was observed when treatments with this chemical were conducted in our raceway. The results we obtained in our raceway tests with both compounds suggest that all of the mononitrophenols we have studied may be more useful for treatment of actual streams than could have been anticipated from our laboratory-jar tests (*5*).

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4. We are indebted to the following organizations for preparing and supplying chemical samples and for technical advice in the development of these materials for our specific use: The Dow Chemical Company; Progressive Color and Chemical Company; Diamond Alkali Company; Niagara Chemical Division, Food Machinery and Chemical Corporation; Monsanto Chemical Company; Michigan Chemical Company; chemistry department, Bucknell University; and chemistry department, University of Michigan.
5. This work was supported, in part, by the Great Lakes Fishery Commission.

21 October 1957

Convenient pH Stat Reaction Vessel for Small Volumes

In a series of studies in our laboratory with the *p*H stat of Jacobsen and Léonis (*1*), it became clear that the conventional beaker type of reaction vessel had several disadvantages, the main one being an irreducible minimum solution volume of about 5 to 6 ml, which is an important limiting factor in studies with precious proteins and peptides. The diameter of the beaker and, hence, its volume could not be reduced because of the large number of accessories dipping into it—the glass and calomel electrodes, external stirrer, nitrogen inlet, and others. We have overcome this problem by using a rotating test tube as the reaction vessel with a stationary, concentric glass and calomel electrode assembly (Radiometer No. GK2021). Excellent stirring, with a complete absence of foaming in protein solution, is achieved by the shearing forces between the test tube and the stationary electrode assembly, and volumes may be reduced to 1.0 ml.

As may be seen in Fig. 1, the glass test tube is housed in a Plexiglass rotor fitted with turbine blades. Both rotation of the turbine and temperature control are achieved by a small centrifugal pump which circulates water, from a thermostatically controlled bath, through a jet impinging on the turbine blades. The electrode assembly is raised and lowered by means of a rack and pinion (fitted with a stop to safeguard the electrode bulb). Only a single polyethylene capillary for delivery of acid or base dips into the reaction chamber, thus, we have a relatively unencumbered apparatus. In our work, the use of nitrogen has not been necessary because of the very small surface area of the solution, but in more critical work a polyethylene capillary for the delivery of nitrogen could easily be attached to the electrode assembly.

In summary, the following advantages have been found in the use of this reaction vessel: (i) By using a single size of test tube, volumes of 1 to 5 ml are easily accommodated, and with a larger size of tube, volumes can go to 10 ml. (ii) Stirring is rapid, smooth, and steady in rate for long periods of time, the rate being adjustable by restricting the flow through the jet. (iii) Precise temperature control is achieved. (iv) The glass test tubes are easily changed, giving convenience during a series of titrations and constantly clean glassware without the need for dismantling the apparatus.

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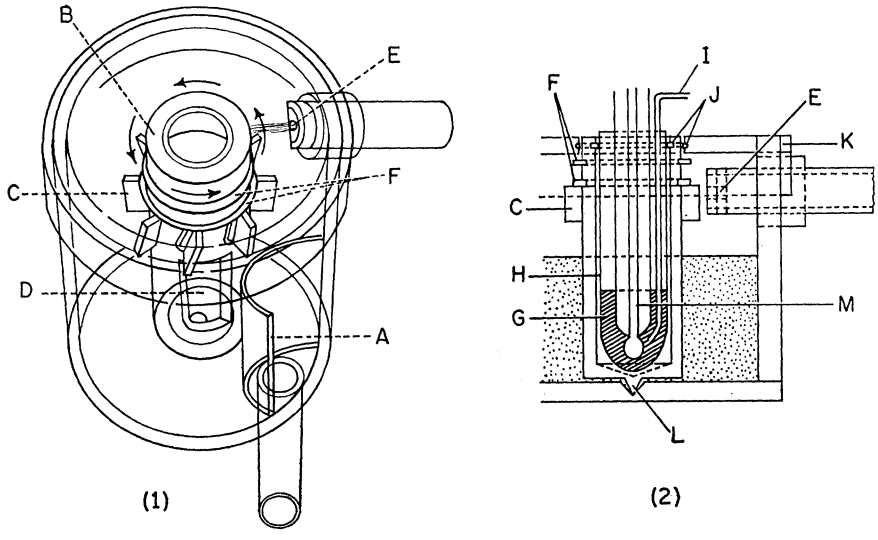

Fig. 1. Apparatus. *A*, Water outlet (designed to act both as a baffle plate and a constant-level device); *B*, rotor (Plexiglass); *C*, turbine blade; *D*, opening in rotor to facilitate rapid temperature equilibration; *E*, jet; *F*, baffle plates to prevent splashing; *G*, reaction mixture; *H*, test tube (glass); *I*, polyethylene capillary for delivery of acid and base from syringe; *J*, Teflon O-rings (the outer ring is a bearing and the inner one is an adaptor for use with various sizes of test tube); *K*, detachable lid; *L*, pivot (the pivot and its bearing should be constructed of stainless steel if extended periods of use are anticipated); *M*, radiometer electrode No. GK2021.

Reference

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31 October 1957

Correlation of Potassium-40 Concentration and Fat-Free Lean Content of Hams

A rapid, objective, and nondestructive method for determining the amount of lean meat present in live animals, carcasses, and cuts of meat should prove to be of considerable value in improving the pricing efficiency involved in the marketing of livestock and meat. Objective evaluation of the amount of lean present would make it possible to set a price that would better reflect the desirability of the product from the consumer's standpoint. In addition, such a technique should help to speed the development of better grade animals for butchering, since accurate nondestructive measurements of the lean content of live animals would aid in the selection of breeding stock.

The research of Woodward *et al.* (*1*) demonstrated that the potassium content of human beings, as determined by measurement of potassium-40 (K^{40}) gamma activity by means of the Los Alamos "human counter" (*2*), was related to the body water content and, therefore, to the lean body weight of the subjects. In view of these findings, it was considered desirable to evaluate the usefulness of the K^{40} concentration as an index of the amount of lean in meat products. Since Federal pork carcass grades (*3*) are based to a considerable extent upon the degree of fatness and the proportion of lean cuts, it was decided to use hams in the study described in this report.

Fresh hams were obtained from the Animal and Poultry Husbandry Research Division of the U.S. Department of Agriculture's Agricultural Research Center at Beltsville, Maryland. One pair of hams (group A) was selected on the basis of their rather fat appearance, while the other pair (group B) appeared to contain considerably less fat. The visible portion of external fat of the hams of group A ranged from 1.2 to 1.8 in. in thickness, while the corresponding range for group B was 0.9 to 1.0 in. The hams were frozen and transported to the Los Alamos Scientific Laboratory, where the following K^{40} measurements were made