407937

Reports

Possible Relation between Marine Fungi and Limnoria Attack on Submerged Wood

Abstract. Wood submerged in the sea at Friday Harbor and at Naples contained only occasional hyphae, which showed no relationship to *Limnoria* burrows. Cultures of *Limnoria* established in "unconditioned" wood and in autoclaved wood were maintained in the absence of fungi. These results indicate that marine fungi have no significance for the activities of *Limnoria*.

In the 8 Nov. 1957 issue of *Science*, Meyers and Reynolds (*1*) reported that wood submerged in the sea is soon infested with marine fungi. Believing that fungal infection always occurs prior to attack by marine wood borers, especially *Limnoria*, they suggested that there might be a relationship between fungi and wood-destroying animals. Becker, Kampf, and Kohlmeyer (*2*), at about the same time, published the results of extensive observations and experiments that had led them to the same conclusion. Further work in support of this hypothesis was reported by Reynolds and Meyers (*3*) and by Schafer and Lane (*4*). In each case it was stated, suggested, or implied that marine wood-boring animals do not attack wood or become established in it unless the wood is first invaded and "conditioned" by marine fungi.

Because we believe that the evidence so far presented is insufficient to build a sound case, we undertook to examine the problem (*5*). From our studies, carried out at the Friday Harbor Laboratories of the University of Washington and at the Stazione Zoologica di Napoli, we have been unable to obtain results similar to those reported in the publications cited above. Since the suggestion that marine fungi may have a primary role in wood deterioration in the sea has serious economic implications, we feel that it is necessary to make our findings a matter of record.

The question of a possible relationship between marine fungi and marine wood borers was raised and discussed at the Friday Harbor Symposium on Marine Boring and Fouling Organisms, and much of the supporting evidence was published in the proceedings of that conference (*6*). The hypothesis rests mainly upon the following observations: (i) that wood when submerged in the sea is universally and rapidly attacked by marine fungi; (ii) that wood is not attacked by *Limnoria* until after it has been submerged for a period of time sufficient to "condition" the surface layers; (iii) that *Limnoria* is unable to survive in wood that has been sterilized by autoclaving.

Concerning the first observation, it was reported (*1, 3*) that fungi appeared on wooden test panels exposed in more than 63 stations in the Western Hemisphere, and that at Biscayne Bay the infection may be extensive in less than a week and vigorous sporulation by ascomycetous fungi may occur after 2 to 3 weeks' submergence. Working with laboratory cultures, Becker (*2*) recorded that all samples of both softwood and hardwood contained fungal mycelium in the surface layers and in the vicinity of *Limnoria* burrows after a submergence period of several weeks. It was also reported that wood samples collected from the North Sea, the Mediterranean, and the Indian Ocean regularly contained fungi.

We have examined, both at Friday Harbor and at Naples, wood that was collected at random from the sea, including pieces with and without *Limnoria* burrows, samples that appear to be relatively fresh, some that are water-soaked from long submergence, and others that are soft, spongy, and extensively deteriorated. We have also, again in both locations, placed blocks of fresh, fungus-free Douglas fir and western yellow pine into the sea at intervals so that they could be studied after known periods of submergence (up to 8 months at Friday Harbor; up to 5 months in the Bay of Naples). The procedure has been to make microscopic examination of thin freehand sections cut from the wood immediately upon its removal from the natural environment. Sections are cut parallel to the surface, tangential to the surface, and wherever possible, along the length of *Limnoria* burrows. In contrast to the authors of the previous reports, we do not find that marine fungi are universal inhabitants of submerged or floating wood; on the other hand, bacteria, some of which are undoubtedly cellulolytic, are nearly always present in the superficial wood fibers. That marine fungi do occasionally occur cannot be denied, but the presence of recognizable mycelium in wood freshly removed from the sea is uncommon indeed. Further, when fungi are present we find no evidence for a topographical relationship with *Limnoria* burrows. No significant differences could be seen between the cold temperate environment of the North Pacific and the warm subtropical conditions of the Mediterranean. It is our opinion that when a marine fungus does invade wood its presence is fortuitous so far as any relationship with *Limnoria* is concerned, and that these fungi have no significance for the activities of *Limnoria*.

Laboratory cultures of *Limnoria*, set up for breeding experiments, have been kept for 10 months in the sea-water system at Friday Harbor and show only four cases of fungal infestation out of 50 cultures of animals living in Douglas fir and western yellow pine. In an effort to obtain fungi in laboratory cultures, 10 Kolle flasks containing sterile sea water and autoclaved wood were each inoculated with 25 specimens of *Limnoria*, and to half of these a solution of penicillin-streptomycin was added. In the latter, bacteria have been suppressed, fungi have grown, and very few *Limnoria* have survived; in the controls there is no evidence of fungal infection, and the animals are healthy and vigorous and have established growing populations. These cultures have been maintained for 8 months.

The idea (observation ii) that *Limnoria* will not attack wood until its surface has been "conditioned" is based upon field observations (*1–3*) that there is a lag of some days, weeks, or even months between the time that wood is placed in the sea and the time when wood borers begin their invasion. During this interval microorganisms, especially bacteria, do indeed appear in the superficial wood fibers and may begin to deteriorate them; this is apparently what is meant by "conditioning." But these facts do not establish that *Limnoria* is unable to attack fresh wood or that such surface softening enhances the likelihood of *Limnoria* attack. It is not an unexpected finding that, in the natural environment, time should elapse before wood borers appear, since these animals are not constantly present, swimming

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper. (Since this requirement has only recently gone into effect, not all reports that are now being published as yet observe it.)

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].

freely in the sea water and in a position to exploit a new food supply. Whether attack on fresh wood is possible can be tested under controlled laboratory conditions. Accordingly, dry "unconditioned" blocks of lumber, including Douglas fir, elm, hemlock, redwood, western red cedar, and western yellow pine, were exposed to healthy *Limnoria*. Each of these wood species was attacked within 24 hours at Friday Harbor, and within 2 to 3 hours at Naples. If "conditioned" wood is unnecessary in laboratory cultures, it is unlikely to be essential in the sea.

Finally, with reference to observation (iii), it was reported that *Limnoria* is unable to attack sterilized wood (*3*) and that animals living in sterilized wood survive no longer than controls kept without a food source (*2*). We find that healthy animals are quite capable of attacking and living in wood sterilized by autoclaving. In these tests the same six species of wood were used and the animals attacked them all within the same period of time (24 hours at Friday Harbor; about 2 hours at Naples). In all of these cases, growing populations were established in the absence of marine fungi (*7*).

D. L. RAY
D. E. STUNTZ
Departments of Zoology and *Botany, University of Washington, Seattle*

References and Notes

1. S. P. Meyers and E. S. Reynolds, *Science* 126, 969 (1957).
2. G. Becker, W.-D. Kampf, J. Kohlmeyer, *Naturwissenschaften* 17, 473 (1957).
3. E. S. Reynolds and S. P. Meyers, *Office Naval Research, Research Revs.* (Dec. 1957), pp. 6–11.
4. R. D. Schafer and C. E. Lane, *Bull. Marine Sci. Gulf and Caribbean* 7, 289 (1957).
5. These studies were aided by a contract (NR 104-142) between the Office of Naval Research, Department of the Navy, and the University of Washington.
6. "Marine Boring and Fouling Organisms," *Proc. Friday Harbor Symposia in Marine Biology* (Univ. of Washington Press, 1958).
7. A full report on this whole problem, including consideration of marine wood-inhabiting bacteria and a discussion of the suggestion made by Becker and by Schafer and Lane that fungi might contribute to the nutrition of *Limnoria*, is in preparation.

8 September 1958

Zinc-65 in Foods and People

Abstract. Disposal of trace amounts of Zn^{65} is made in the Columbia River via Hanford reactor effluent water. The subsequent utilization of river water for irrigation permits the concentration of this radioisotope in farm produce and its eventual deposition in man. The Zn^{65} in irrigation water, in farm produce, and in individuals utilizing these materials has been measured.

Water from the Columbia River is used as a coolant for the Hanford reactors. The subsequent disposal of this water in the river introduces trace amounts of several induced radioisotopes, most of which have half-lives of the order of minutes to a few hours; however, the half-lives of some of these isotopes are sufficiently long to permit tracing the distribution of the isotopes into the food chains of the aquatic life in the river (*1*). Zinc-65 is the major long-lived radioisotope introduced into the river, and although it is present at a concentration far below the most conservative permissible limits, it exists in sufficient amounts to serve as a tracer; it is thus possible to follow its path from irrigation water through plants and animals to man.

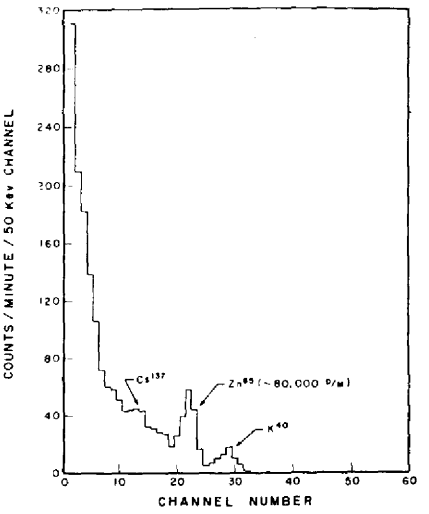

Fig. 1. Gamma-ray spectrum of an individual containing Zn^{65}.

Only a small fraction of the Columbia River water used for irrigation is obtained downstream from the Hanford project. The farm-produce and animal samples considered here were obtained from an irrigation project about 30 miles downstream from the Hanford reactors. By means of gamma-ray spectrometric techniques, measurable amounts of Zn^{65} were found in all the farm produce sampled from this location. The Zn^{65} concentrations found in milk, beef, and the various types of vegetables from this land are shown in Table 1. The concentration factor (Zn^{65} concentration in the sample/Zn^{65} concentration in the irrigation water) for each sample is also included.

With the exception of the beef, all of these samples were obtained during July and August 1957. The beef was obtained from an animal slaughtered in January 1957 after it had lived 1 year on the irrigation project. The fact that the pasture grass contained a relatively high Zn^{65} concentration as compared with the vegetables is probably related to both the manner and amount of irrigation as well as to the fact that some difference in uptake between the leaf and fruit portion of plants would be expected. The pasture grass was irrigated almost continuously, while the vegetables were irrigated only a few times during their growing season. In addition, the Zn^{65} may enter the grass by foliate absorption during irrigation as well as through the soil.

The relatively high Zn^{65} concentration in milk as compared with that in the pasture grass indicated that a large amount of Zn^{65} is taken from the feed into the blood stream of the cow and translocated into the milk. The low Zn^{65} concentration found in the beef samples (Table 1) may be explained by the fact that the animal was slaughtered in late winter and had been fed on essentially Zn^{65}-free foodstuffs for 3 to 4 months prior to that time. Measurements of Zn^{65} in the same milk supply during January and February of 1958 showed about 10 percent of the value listed in Table 1. This again can be explained by the animals' relatively Zn^{65}-free diet during the winter months.

A second animal which had spent its entire life in the same location was slaughtered in March of 1958 and was

Table 1. Concentrations of Zn^{65} in farm produce.

Sample	Concentration ($\mu\mu c/g$)	Concentration factor (produce/water)
Pasture grass	82.9	440
Beef, flesh	5.23	28
Beef, fat	1.48	7.9
Beef, bone	5.80	31
Milk (cow)	4.88	26
Black-eyed peas	0.55	2.9
Tomatoes	0.46	2.4
Okra	0.39	2.1
String beans	0.29	1.5
Corn	0.16	0.83
Grapes	0.089	0.47
Irrigation water	0.188	

Table 2. Concentrations of Zn^{65} observed in the various organs of a beef animal.

Sample	Concentration ($\mu\mu c/g$)
Flesh	10.7
Fat	2.22
Bone	13.4
Ovaries	4.07
Hide	3.91
Kidney	5.98
Lung	5.11
Brain	2.74
Pancreas	7.27
Blood	0.86
Hair	28.6
Thymus	3.79
Liver	11.5
Horn	3.59
Hoof	2.59

subjected to a much more extensive Zn^{65} analysis. The Zn^{65} concentrations found in the various organs of this animal are listed in Table 2. It may be noted that the Zn^{65} concentration in the flesh, fat, and bone samples are about twice those observed in the samples of the previous year (see Table 1). It is also apparent that hair, liver, and bone concentrate zinc to a greater extent than the other organs.

The observation of these Zn^{65} concentrations in farm-produce samples suggested the possibility that Zn^{65} could be measured in individuals obtaining their food supply from these irrigation projects. For the measurement of Zn^{65} in people, a 3- by 5-in. sodium iodide crystal detector operated in a shielded room similar to the installation developed at Argonne National Laboratory (*2*) was used. The gamma-ray spectrum of an individual who consumed approximately 0.1 and 0.7 kg, respectively, of the meat and milk per day from the sources listed in Table 1 is shown in Fig. 1. This Zn^{65} photopeak area represents about 3.6×10^{-2} μc or 80,000 disintegration/min.

Twelve other individuals were measured whose diet did not include food from the irrigation project but did in some cases include drinking water whose source was the Columbia River. The gamma-ray spectra of most of the individuals whose drinking water originated in the Columbia River show a small Zn^{65} photopeak. The Zn^{65} content of these individuals was estimated to be between 5000 and 10,000 disintegration/min. The gamma spectra of individuals who receive neither their water nor their food supply from the Columbia River or its irrigation projects showed no detectable Zn^{65} photopeaks. The Zn^{65} found in all samples of foods, in fodder, or in individuals could be traced either to Columbia River drinking water or to food from the lower irrigation projects. It is believed that, with a sensitive total-body counting device of the Argonne National Laboratory type (*2*) Zn^{65} could be measured in most individuals who receive their drinking water from the Columbia River downstream from Hanford, provided that some purification step in the local water treatment does not remove the isotope.

Zinc-65 has been reported (*3*) in individuals living near the Pacific Proving Grounds and was shown to be a result of contamination from nuclear tests; however, Zn^{65} from nuclear tests has not been observed in foods raised in this country. The presence of Zn^{65} in the amounts observed here in no way constitutes a hazard. Even the value of 3.6×10^{-2} μc of Zn^{65} in the individual whose diet included the meat, milk, and drinking water of Table 1, is less than 0.01 percent of the total permissible body burden for this isotope (*4*). Also, even the highest Zn^{65} value in Table 1 (for pasture grass) is less than 5 percent of the maximum permissible concentration for human foods (*4*).

Smaller amounts of the radioisotopes Cr^{51} and Sc^{46} have been detected in samples of pasture grass but have not been found in farm produce.

R. W. PERKINS
J. M. NIELSEN
Hanford Laboratories Operation, General Electric Company, Richland, Washington

References

1. J. J. Davis and R. W. Perkins, unpublished.
2. C. E. Miller *et al.*, *Nucleonics* 14, No. 4, 40 (1956).
3. ———, *Argonne National Laboratory Rept. No. ANL-5755* (July 1957), pp. 53–56.
4. "Recommendation of the International Commission on Radiological Protection," *Brit. J. Radiol. Suppl. No. 6* (1955).

23 September 1958

Studies on 4-Keto-L-Proline

Abstract. The administration of ketoproline to chick embryos resulted in an increase in the free hydroxyproline. This phenomenon is explained by the inhibitory action of ketoproline on the catabolism of hydroxyproline as well as by the conversion of the former to the latter. Ketoproline was found to be reduced to hydroxyproline by the supernatant fraction of rat-kidney homogenate in the presence of a reduced pyridine nucleotide.

Since hydroxyproline occurs uniquely in collagen in the animal, studies on the biogenesis and metabolism of this compound have a great significance in the understanding of the biochemistry of collagenous tissues. In the course of a survey of compounds structurally related to hydroxyproline for their ability to affect the metabolism of this imino acid, the administration of 4-keto-L-proline (*1*) to chick embryos was found to produce an increase in free hydroxyproline.

For these studies 5 mg of free 4-keto-L-proline in 0.2 ml of water was placed in the air space of a 12-day-old chick embryo through an opening in the shell. After 24 hours an 80-percent ethanol extract was prepared from the whole embryo and hydroxyproline was assayed colorimetrically by a modification (*2*) of the method of Neuman and Logan (*3*). The results are shown in Table 1. It can be seen that ketoproline caused a five- to sixfold increase in free hydroxyproline without affecting the proline level. Similarly, when 15 mg of ketoproline was administered subcutaneously to rats (200 to 250 g), a prolonged elevation of the blood hydroxyproline level was observed, an increase from 0.55 to 1.0 mg per 100 ml being maintained for a period of several hours. Although administration of 5 mg of hydroxyproline produced a comparable increase, this lasted for less than 2 hours. The mechanism by which ketoproline produces the increase in free hydroxyproline in vivo was found to be twofold: (i) inhibition of hydroxyproline destruction and (ii) conversion of ketoproline to hydroxyproline.

A strain of *Achromobacter* grown on hydroxyproline as the sole source of carbon metabolized hydroxyproline extensively. However, when increasing amounts of ketoproline were added to the incubation mixture, destruction of hydroxyproline was diminished (Table 2). Proline was also found to antagonize the metabolism of hydroxyproline under similar conditions. However, the metabolism of proline by proline-adapted *Achromobacter* was not appreciably affected by ketoproline. Similar observations were made when mammalian liver or kidney mitochondria were used in place of the bacteria. Neither the bacteria nor the mitochondria were able to convert ketoproline to hydroxyproline in any detectable amounts.

When ketoproline was incubated with a well-dialyzed soluble fraction of rat-kidney homogenate, it was reduced to

Table 1. Effect of ketoproline on free proline and hydroxyproline levels in chick embryos. Twelve-day-old embryos received either 0.2 ml of saline or 5 mg of ketoproline in 0.2 ml of water. After 24 hours, 80-percent ethanol extracts of the embryos were assayed for hydroxyproline (*3*) and proline (*6*).

Treatment	Wet wt. of embryo (g)	Free hydroxyproline (μg)	Free proline (μg)	Free hydroxyproline/proline free
Control (av. of 6)	5.2	112.1	206.2	1.84
Ketoproline administered (av. of 4)	6.3	627.2	218.7	0.35

Table 2. Effect of increasing concentrations of ketoproline on hydroxyproline metabolism by *Achromobacter*. One milligram of hydroxyproline was incubated for 30 minutes with 9.4 mg (dry weight) of hydroxyproline-adapted *Achromobacter* (*7*).

Ketoproline/hydroxyproline	Hydroxyproline remaining (μg)	Inhibition (%)
0	97	
1	340	29
2	420	36
3	585	54
4	690	66
5	780	76

Table 3. Enzymatic reduction of ketoproline to hydroxyproline. One milliliter of dialyzed supernatant fraction from a 1:2 KCl homogenate of rat kidney was used. All of the incubation beakers contained 0.5 ml of 0.5*M* phosphate buffer, *p*H 7.4; 1 mg of ketoproline; 5 μmole of nicotinamide, and 1 ml of rat-kidney preparation in a final volume of 3 ml. Where indicated, 0.5 μmole of DPN, 0.26 μmole of TPN, 200 μmole of glucose, and 250 units of glucose dehydrogenase were added. After 1.5 hours of incubation, hydroxyproline was assayed by a modification of the Wiss method (*2, 8*).

System	Hydroxyproline (μg)
DPN + glucose dehydrogenase system	42.7
TPN + glucose dehydrogenase system	52.5
DPN or TPN without glucose dehydrogenase	< 3.5

hydroxyproline. This reaction was found to require the presence of reduced pyridine nucleotides, either as such or generated in the incubation mixture by the glucose dehydrogenase system (*4*), as shown in Table 3. Reduced TPN (*5*) was found to be more active than reduced DPN. The rat-kidney preparation could not be replaced by purified commercial alcohol or lactic dehydrogenases. Neither reduced DPN nor reduced TPN was effective in the absence of the rat-kidney preparation.

The inhibitory effect of ketoproline on hydroxyproline metabolism is clearly established in these studies. The enzyme responsible for the reduction of ketoproline and the physiological significance of this reaction are under investigation.

CHOZO MITOMA, THOMAS E. SMITH, FRANCES M. DACOSTA, SIDNEY UDENFRIEND

National Heart Institute, National Institutes of Health, Bethesda, Maryland

ARTHUR A. PATCHETT, BERNHARD WITKOP

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland

References and Notes

1. A. A. Patchett and B. Witkop, *J. Am. Chem. Soc.* 79, 185 (1957).
2. A report on the modified procedure is in preparation.
3. R. E. Neuman and M. A. Logan, *J. Biol. Chem.* 184, 299 (1950).
4. H. J. Strecker and S. Korkes, *ibid.* 196, 769 (1952).
5. The following abbreviations are used: DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide.
6. W. Troll and J. Lindsley, *J. Biol. Chem.* 215, 655 (1955).
7. We are indebted to Dr. R. N. Doetsch of the University of Maryland for this strain.
8. O. Wiss, *Helv. Chim. Acta* 32, 149 (1949).

29 August 1958

Direct Observation of Evaporation from Quiescent Water

Abstract. The color change of a filter paper impregnated with cobaltous chloride and held just above the surface of water gives a good indication of the rate at which evaporation proceeds from individual regions of the surface. The marked effect of some monolayers on thermal convection currents within the liquid can be thus shown.

The usual technique of measuring the rate of evaporation of water from a quiescent surface and the effect of monolayers upon this is rather elaborate (*1*) yet does not provide any information about local conditions over small portions of the area studied. This report (*2*) presents a few observations based on a simple technique which gives qualitative but very direct visual information about the rate of evaporation and shows what happens over areas of the order of a few square millimeters. The technique is based on the color change produced by the vapor reaching a sheet of paper impregnated with cobaltous chloride and held very close to the surface.

Figure 1 shows the pattern—which was actually pink on blue—obtained when the indicator paper was placed above a square cell, 2 by 2 cm, filled with water whose surface was divided into two parts by a polyethylene barrier. To the left of the barrier the surface was clean, while some cetyl alcohol was sprinkled over the surface to the right of the barrier. In the photograph, taken 2 minutes after the paper was placed above the surface, the difference in the rates of evaporation from the two sides is strikingly apparent. Over the clean surface the paper is already pink, while over the monolayer it is still largely blue. In addition, the color change over the clean surface is uniform (it developed uniformly from the beginning), while over the protected part the change appears in spots, which gradually spread over the whole area.

Similar irregular development of the color, signifying uneven rate of evaporation in the presence of the monolayer, was observed with a variety of vessels. It is attributed to the presence of relatively large convection currents which rise warm, cause relatively rapid evaporation, and are thus cooled so that the rate of evaporation is reduced while they continue along the surface for a distance before finally sinking. On a clean surface the convection pattern is different, and local differences are much smaller. This interpretation is supported by observation of convection currents made visible by very slow injection of a very dilute solution of fluorescein into the surface. The convection currents, while irregular, seem to be more extended in the presence of the monolayer, and their general pattern corresponds to that of the spots on the indicator paper. Changes in the thermal resistance of the water, reported previously (*3*), are also in agreement with this observation.

This change in convection currents is connected with the well-known hysteretic resistance of a monolayer against extensions and contraction (*4*). When a cooled streamline of water detaches itself from the surface and sinks under the influence of gravity, the corresponding surface must shrink. Conversely, when a rising warm streamline reaches the surface it causes, necessarily, a local expansion of the surface. When the surface is clean, such expansions and contractions encounter no resistance, but when a monolayer is present they are impeded, and convection at the surface develops only over greater distances and when larger forces are present. The heat transport to the surface from the bulk of the water is thus necessarily affected and localized.

In the experiments under discussion (*5*), the filter paper was firmly attached to a glass plate, both to insure an even surface and to prevent access of vapor from the back. The plate was first covered with a thin layer of "rubber cement for pasting paper," and the filter paper was firmly pressed onto it. The whole was then submerged in a moderately concentrated solution of $CoCl_2$ (prepared without heating), excess liquid was pressed out between filter papers, and the assembly was dried in a vacuum desiccator. The rims of the vessels had to be coated with paraffin to prevent creeping of the water. After the rim had been adjusted to the horizontal, the vessel was filled with water to within about 1 mm of the top. The water surface was protected, when protection was desired, by manual sprinkling of a few specks of commercial cetyl alcohol upon it. A wait of a few minutes allowed the convection currents to develop and stabilize. The glass-backed indicator paper was then

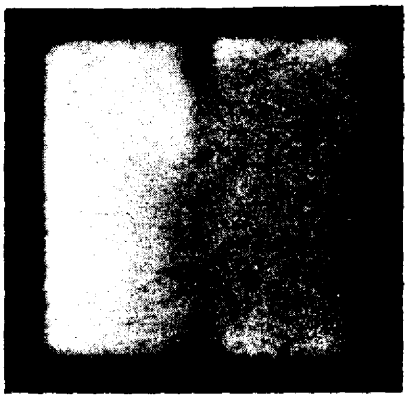

Fig. 1. The local pattern of evaporation from a water surface 2 cm square; the left side is clean, the right side is protected by cetyl alcohol. This photograph of cobaltous chloride indicator paper was taken 2 minutes after the paper was placed above the water surface.