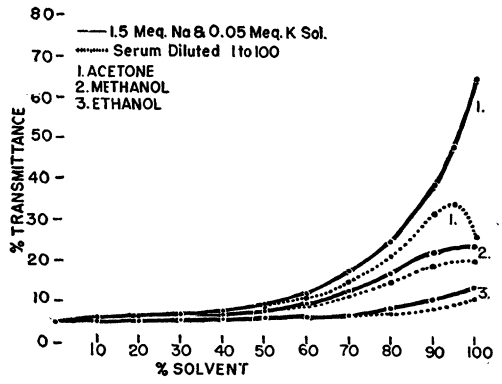

FIG. 2. Comparison of the effect of different concentrations of organic solvents on the flame spectra of potassium at 770.0 μ in diluted serum and aqueous solutions, as measured with a Beckman Spectrophotometer with flame attachment #9200, acetylene-oxygen-burner assembly. Ordinate, relative light intensity read as % T.

flame spectrum of potassium. All aqueous-organic solvent solutions of pure standards studied (Figs. 1 and 2) were not centrifuged and showed complete solubility of the sodium or potassium salts, except the 100% acetone solutions, which were turbid.

The enhancing effect of acetone on the flame spectrum of Na and K was also observed with the Weichselbaum-Varney (*1*) flame photometer.

The effect of protein on the flame spectrum of sodium and potassium was studied by using a 7.0% bovine albumin[4] solution which contained a trace of sodium and no potassium. This protein solution was diluted to 0.14% in 0–100% acetone containing 0.3 mEq sodium and 0.01 mEq potassium, for study of the flame spectra of sodium and 1.5 mEq sodium and 0.05 mEq potassium for the study of the flame spectra of potassium (Table 1). Acetone solutions containing 0.3 mEq Na and 0.01 mEq K with and without 0.014% bovine albumin read against an aqueous solution containing 0.3 mEq Na and 0.01 mEq K set at 5% T indicated that bovine albumin had no effect on the flame spectra of sodium (Table 1). Although bovine albumin was precipitated with 50–90% acetone, no loss of sodium with this precipitate occurred at these concentrations of solvent. No effect of bovine albumin on the flame spectrum of potassium (Table 1) was observed. Loss of potassium with bovine albumin precipitate occurred, beginning at 60% acetone.

TABLE 1

EFFECT OF ALBUMIN ON FLAME SPECTRA OF SODIUM AND POTASSIUM IN PRESENCE OF DIFFERENT CONCENTRATIONS OF ACETONE

Acetone (%)	Na spectra at 589.6 μ			K spectra at 770 μ		
	0.3 mEq Na 0.01 mEq K (% T)	0.3 mEq Na 0.01 mEq K 0.014% Albumin (% T)	0.014% Albumin (% T)	1.5 mEq Na 0.05 mEq K (% T)	1.5 mEq Na 0.05 mEq K 0.07% Albumin (% T)	0.07% Albumin (% T)
0	5.0	5.5	0.5	5.0	5.0	0.0
10	8.0	8.5	.5	5.0	5.0	.0
20	9.5	10.0	.5	6.0	6.0	.0
30	11.0	11.5	.5	7.5	7.5	.0
40	13.0	13.5	.5	9.0	9.0	.0
50	15.8	16.3	0.5	11.5	11.5	.0
60	18.5	19.5	1.0	15.0	15.0	.0
70	25.0	26.0	2.0	20.5	20.0	.0
80	34.0	35.0	2.0	29.0	28.0	.0
90	50.0	51.0	4.0	42.0	39.0	.0
95	59.0	58.0	3.0	53.0	43.0	.0
100	63.0	26.0	1.0	52.0	28.0	0.0

[4] Fraction V, Armour Laboratories, Chicago, Ill.

We have observed losses of sodium (7%) and potassium (10%) when trichloracetic acid is used to precipitate proteins from serum diluted 1/500 and 1/100. The use of trichloracetic acid (*2*) or other protein-precipitating agents to obtain protein-free filtrates for assay of Na and K by flame photometry is not recommended.

The observations reported here of the effect of a few common organic solvents on increasing the intensity of flame spectra from sodium and potassium have indicated the possibility of bringing more trace elements within the range of measurement of the flame photometer and the use of smaller samples for analyses. A further investigation is being made of the effect of other organic solvents, mixed organic solvents, and water on the emission spectra of sodium, potassium, calcium, magnesium, lead, and other elements.

References

1. WEICHSELBAUM, T. E., and VARNEY, P. L. *Proc. Soc. Exptl. Biol. Med.*, **71**, 570 (1949).
2. OVERMAN, R. R., and DAVIS, A. K. *J. Biol. Chem.*, **168**, 641 (1947).

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A Culture Technique for the Diagnosis of Infections with *Dermocystidium marinum* Mackin, Owen, and Collier in Oysters[1]

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A simple technique has been devised for the rapid and accurate diagnosis of infections with *Dermocystidium marinum* Mackin, Owen, and Collier, 1950 (*1*). This organism is now known to be widely distributed in oysters on the Gulf and South Atlantic coasts of the United States. Its pathological significance is discussed by Mackin (*2*). The presence of *D. marinum* has been demonstrated by this method in oysters from localities in Florida, South Carolina, and Louisiana.

A satisfactory culture medium is Difco's fluid

[1] Grateful acknowledgment is made to Asa C. Chandler for direction of this research, and to Mrs. E. K. Hake for histological assistance. Photomicrographs are by F. C. Breckenridge, Baylor Medical School, Houston.

thioglycollate rehydrated with sea water, and tubed in 10-ml amounts. Streptomycin and penicillin solutions are added shortly before inoculation to give concentrations of 250 units of each/ml. Up to 1000 units of each can be used, however. Complete sterility is not necessary; retardation of heavy bacterial growth during the early period of incubation is sufficient. The anaerobiosis of the medium suppresses the growth of molds and protozoa, but is not essential for the parasites. Incubation temperatures from 18°C to 25°C are satisfactory; above 25°C bacterial growth is less easily suppressed.

D. marinum has been grown in all oyster tissues cultured, even from decomposing dead oysters. Routinely, heart, rectum, and pieces of gill and mantle (about 5 × 10 mm) are cultured. In general, the distribution of the parasites closely parallels Mackin's (*2*) findings in histological sections; that is, there is sometimes a marked difference in the concentration of parasites in different tissues and even in different areas of the same tissue. This has been observed more often in living oysters than in dead or gaping ones. The parasites in cultured tissue were followed in histological sections during the early hours of cultivation; by 10–12 hr they have enlarged enough to be seen in whole mounts, but are not easily detected until after appoximately 18 hr, when they appear as thin-walled, spherical, cystlike bodies, a few measuring up to 35 μ in diameter. As the parasites continue to grow, the walls thicken, the cytoplasm becomes more and more vacuolated, and fat droplets accumulate. The maximum size increases to about 90 μ at 72 hr and to 100 μ–150 μ in cultures a week or more old. In a single oyster from South Carolina the majority of the parasites exceeded 200 μ, some attaining a diameter of 280 μ. The total volume of the heavily infected pieces of tissue is greatly increased, and the original tissue is practically obliterated by the mass of the cystlike bodies (Fig. 1 *A*). Many become free in the tube or on the slide (Fig. 1 *B*).

If numerous and well developed, the parasites are conspicuous and readily identifiable without further treatment. Their recognition is, however, rendered easy and certain, even if they are scanty or small, by the characteristic manner in which they react when treated with iodine. A 1:25 aqueous dilution of Lugol's is satisfactory. After about 18 hr of cultivation the walls of the parasites begin to stain a faint blue, the color becoming a clear blue in 24–36 hr, and deepening until the organisms appear as opaque blue-black bodies after a week or more. Best results are obtained by staining the parasites left on a slide after removal of the tissue. Enzymatic and other tests indicate that the blue color is not due to starch; possibly it is due to a glucoside such as saponarin (*3*).

A germination of the cystlike bodies, with the production of a short thick hypha several times as long as the diameter of the body producing it, has been observed (Fig. 1 *C, D*). The production of hyphae and budding forms definitely establishes *D. marinum* as a fungus, and supports Mackin's idea that *D. marinum* might be related to such organisms as *Blastomyces* and *Cryptococcus*.

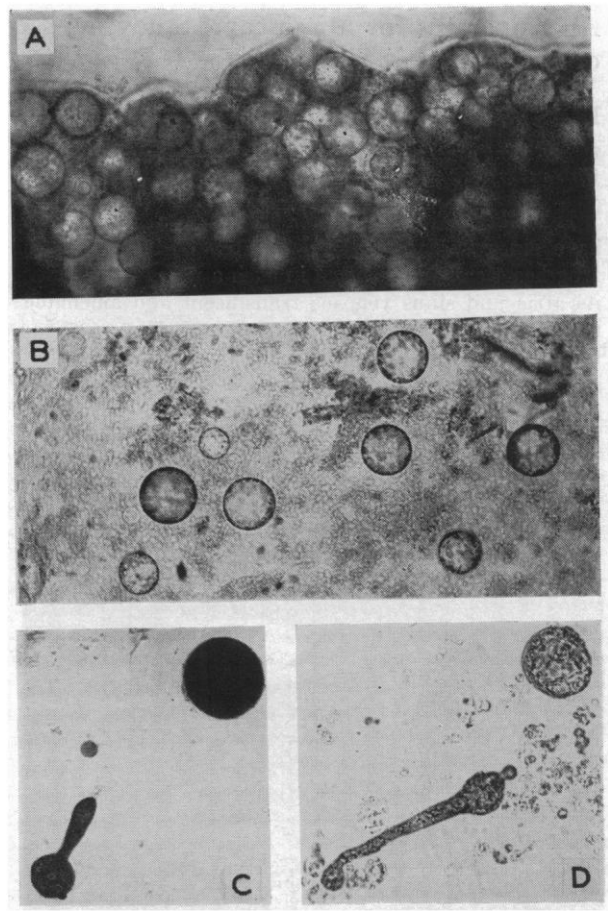

FIG. 1. *A, D. marinum* in oyster gill tissue after 35 days of incubation. × 100. *B,* bodies left on slide after removal of piece of tissue shown in 1 *A*. × 100. *C* and *D,* germinating bodies showing outgrowth of hyphae, iodine-stained. *C* × 200, *D* × 400.

The method here described has advantages over the histological techniques usually employed in economy of time and labor, in amount of equipment and skill required for a reliable diagnosis, and in accuracy in the detection of light infections. At present it is uncertain whether the number of cells increases during incubation; if not, the technique would have the added advantage of indicating intensity of infections as well as incidence. The biological significance of the cystlike bodies has not yet been determined, but investigations on this and other phases of the biology and life history of this organism are in progress.

References

1. MACKIN, J. G., OWEN, H. M., and COLLIER, A. *Science,* **111**, 328 (1950).
2. MACKIN, J. G. *Bull. Marine Sci. of the Gulf and Caribbean,* **1**, 72 (1951).
3. BARGER, G. *Some Applications of Organic Chemistry to Biology and Medicine.* New York: McGraw-Hill, 176 (1930).

Manuscript received March 24, 1952.