

in this solution for one hour. It was then washed, and frozen sections cut from one half of each piece of skin—the other half being dehydrated, embedded in wax and sectioned in the usual way. Sections were afterwards toned in gold chloride to remove the overall brown stain. Granules of reduced silver were present wherever the adrenalin or nor-adrenalin had penetrated.

It was found that even by 5 min. there had been a complete penetration of the skin by both adrenalin and nor-adrenalin and that there was even evidence of their presence in the subcutaneous tissues. They appear to penetrate between the cells rather than through them. Although there are other reducing substances present in tissues which can reduce ammoniacal silver nitrate, treatment of pieces of control skin treated with the emulsion devoid of adrenalin showed only a few black granules.

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### Paper Chromatography in Taxonomic Work

A METHOD for using paper partition chromatography for taxonomic studies on land snails has been described by Kirk *et al.*<sup>1</sup>

In attempting to use the original technique, a number of technical difficulties were encountered, and it was found that exactly reproducible results were not easily obtained. The main source of error lay in the fact that Kirk and his co-workers ran their chromatograms on a glass plate covered by an inverted pie-dish, the solvent being fed to the centre of the disk by a wick passing through a hole in the glass plate. As a result, the chamber (the lumen of the covering dish) only becomes saturated with solvent vapour by evaporation from the surface of the disk during the run. It is also almost impossible to standardize the wick feeding the solvent.

These sources of error are eliminated by the use of the standard circular chromatography apparatus described by Kawerau<sup>2</sup>. By using slotted Whatman No. 3 papers 26 cm. in diameter, and a standard run of six hours at about 20° C., quite easily reproducible results are obtained. The  $R_F$  values of the fluorescent bands resulting from this technique are greater than those obtained by the original method, partly due to the elimination of surface evaporation from the paper and partly because of the differential flow-rate at the solvent front as it moves farther from the centre along the sectors of the paper. Thus the  $R_F$  values obtained from a four-hour run are a little lower than they are after six hours.

The method not only works well with some land snails but also with some aquatic groups, and interesting results are at present being obtained from the genus *Lymnaea*.

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<sup>1</sup> Kirk, R. L., Main, A. R., and Beyler, F. G., *Biochem. J.*, 57 (3), 440 (1954).

<sup>2</sup> Kawerau, E., "Chromatographic Methods", 1 (2) (1956).

### Respiratory Characteristics of Marine Bacteria

BACTERIA which are isolated from marine environments and require for growth a medium containing sea water are classified as true marine organisms. Recent evidence<sup>1</sup> indicates that the inability of marine bacteria to grow in the absence of sea water is due to a requirement for specific inorganic ions. A second, but not as well-defined, characteristic of marine bacteria is their high proteolytic activity and their rapid growth on organic nitrogen compounds<sup>2</sup>.

The majority of studies of the metabolic activities of marine bacteria are concerned with requirements for growth. Seldom have the respiratory characteristics of a marine bacterium been investigated and correlated with growth studies. It was therefore of interest to determine if the criteria used for distinguishing marine bacteria as a group are satisfied by respiratory as well as growth characteristics.

The respiratory activity of a marine bacterium and *Escherichia coli* on endogenous and exogenous substrates, in the absence and presence of sea water, was determined using a Warburg respirometer and conventional manometric procedures<sup>3</sup>. The marine bacterium used in these studies was isolated from sea water collected in the vicinity of Fort Johnson. The growth requirements of the organism fulfil the criteria for its designation as a true marine bacterium. The culture is identified as A-11-3B1 in the Fort Johnson collection.

Table 1 shows that oxygen uptake by the marine bacterium on both endogenous and exogenous substrates was much greater in sea water than in phosphate buffer. With *E. coli*, conditions were reversed, that is, oxygen uptake on both endogenous and exogenous substrates was greater in phosphate buffer than in sea water. Tests showed that the reduced oxidation of glucose by *E. coli* in sea water was not the result of a deficiency of phosphate.

It is evident from Table 2 that the marine bacterium respire preferentially on some component(s) of casamino-acids. Casamino-acids were oxidized rapidly, whereas galactose and glucose were oxidized at a very low rate. It was not possible to replace casamino-acids with asparagine, glutamate, or glycine. Asparagine, citrate, and glycerol reduced endogenous respiration.

While investigation was in progress, Tomlinson and MacLeod<sup>4</sup> reported that the inorganic ion requirements for growth of a marine bacterium were also necessary for its oxidation of exogenous substrates. This provides further evidence that the growth requirements used for defining marine bacteria as a

Table 1. OXYGEN UPTAKE BY A MARINE BACTERIUM AND *E. coli* IN SEA WATER AND PHOSPHATE BUFFER

Suspending medium	Substrate	$\mu$ l. oxygen consumed in 1 hr. at 28° C.	
		Marine bacterium* (A-11-3B1)	<i>E. coli</i> † (Strain B)
Sea water‡	—	48.0	13.0
Buffer§	—	16.7	23.5
Sea water	+	212.4	124.0
Buffer	+	83.5	201.3

\*  $3 \times 10^{10}$  cells of a 24-hr. culture were added per flask, and 0.17 per cent casamino acids added as substrate.

†  $2.7 \times 10^8$  cells of a 24-hr. culture were added per flask and 0.03 M glucose added as substrate.

‡ Fresh sea water collected in the vicinity of Fort Johnson and passed through a millipore filter before use, pH 8.1.

§ M/15 phosphate buffer, pH 8.0.