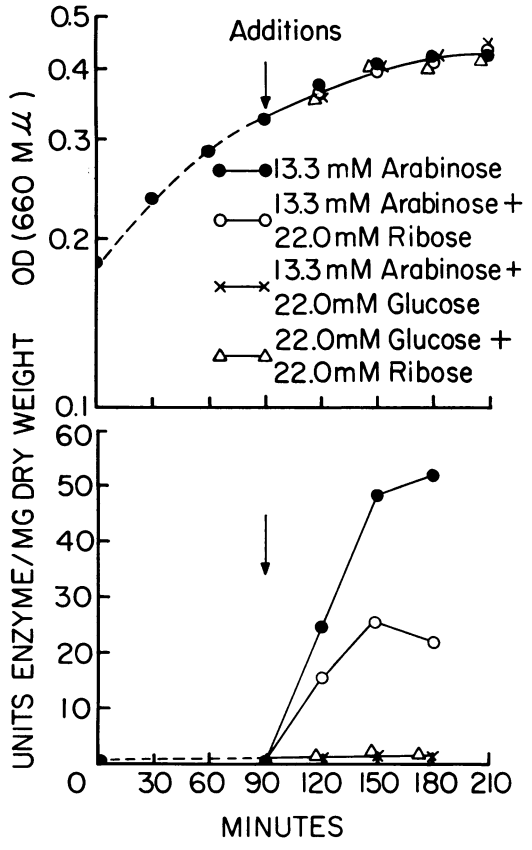

FIG. 2. *Effect of mitomycin C on induction and repression of* L*-arabinose isomerase in Pediococcus pentosaceus.* L*-Arabinose isomerase was measured by use of toluene-treated whole cells as previously described (Dobrogosz and DeMoss, J. Bacteriol.* **85:***1350, 1963). A unit of enzyme was defined as that which produced 1 μmole of* L*-ribulose per hr at 37 C with* L*-arabinose as substrate.*

scribed by Dobrogosz and DeMoss, J. Bacteriol. **85:**1350, 1963) were measured during the subsequent incubation period. It can be seen from these data that the isomerase was formed in response to the addition of arabinose or an arabinose-ribose mixture. In the absence of arabinose or with arabinose plus a catabolite repressor (glucose), no enzyme formation was detected. As indicated by the corresponding optical density data, all these effects were obtained in the absence of significant differences in growth of the cultures. Different growth rates were observed in response to each of the substrate additions when untreated cultures were used in comparable experiments.

These preliminary results suggest that certain cellular phenomena which require the presence of a complete growth environment, i.e., suitable energy and nitrogen sources, may be studied in the absence of gross differences in growth rates by the use of MC.

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ENHANCEMENT OF STAINING INTENSITY IN THE FLUORESCENT-ANTIBODY REACTION

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Fluorescein produces strong fluorescence in alkaline solutions (Pringsheim and Vogel, *Luminescence of Liquids and Solids*, Interscience Publishers, Inc., New York, 1946), and a marked increase to maximal intensity occurs at approximately pH 9.0 to 12.0 (Weber and Teale, Trans. Faraday Soc. **53:**646, 1957; Parker and Rees, Analyst **85:**587, 1960). Solutions prepared in carbonate-bicarbonate buffer (pH 9.6) and in 0.1 N sodium hydroxide possess the same fluorescence efficiency, but a more stable solution is obtained with carbonate buffer (Parker and Rees, 1960). The present study was concerned with evaluating the effect of alkalinity (pH 9.0) on staining brightness in the fluorescent-antibody reaction. The procedure most frequently used consists of washing stained smears in phosphate-buffered saline (pH 7.2) and mounting in buffered

TABLE 1. *Fluorescent intensity of stained smears washed in carbonate-bicarbonate buffer (pH 9.0) and in buffered saline (pH 7.2; mounted in buffered glycerol of corresponding pH)*

Test organism	pH	Undiluted†	Fluorescent intensity of homologous conjugate* at dilutions of										Normal globulin conjugate (1:4)	Unstained smears
			1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024		
Bacillus anthracis	9.0	H4+	H4+	H4+	H4+	H4+	4+	3+	3+	3+	1+	—	—	—
	7.2	4+	4+	4+	4+	3+	2+	1+	1+	—	—	—	—	—
Brucella abortus	9.0	H4+	H4+	H4+	H4+	H4+	H4+	H4+	3+	2+	—	—	—	—
	7.2	4+	4+	4+	4+	4+	4+	4+	2+	—	—	—	—	—
Serratia marcescens	9.0	H4+	H4+	H4+	H4+	H4+	H4+	4+	4+	4+	2+	—	—	—
	7.2	4+	4+	4+	3+	3+	3+	2+	2+	1+	—	—	—	—

* Microscopic reactions are graded by the conventional method for estimating fluorescence: 1+ to H4+, minimal to maximal intensity (H4+, high-intensity fluorescence, more brilliant than the usual 4+); — no fluorescence.

† Dilutions of conjugate prepared in phosphate-buffered saline (pH 7.2); standard macroscopic tube-agglutination tests showed a titer of 1:1280 for both *B. abortus* and *S. marcescens* conjugates.

glycerol of the same or slightly higher pH. Initial studies with bacterial smears showed that stained preparations, washed and mounted in buffered solutions at pH 9.0, exhibited a marked increase in fluorescent brilliance. Staining intensity at pH 9.6 was not significantly different from that observed at pH 9.0.

The following strains were tested for staining reactions: *Bacillus anthracis* CD-3S, *Brucella abortus* CD-476, and *Serratia marcescens* 8UK. Immune sera were obtained from white New Zealand rabbits immunized during a 3-week period. Immune and normal sera were fractionated by the methanol procedure of Dubert et al. (Ann. Inst. Pasteur **84**:370, 1953). The method used for conjugating globulin was essentially that of Marshall et al. (Proc. Soc. Exptl. Biol. Med. **98**:898, 1958) with the following exceptions: (i) crystalline fluorescein isothiocyanate (BBL; 0.04 mg per mg of protein) was used; and (ii) untagged dye was removed by utilizing a Sephadex column (G-25, medium grade) equilibrated and eluted with physiological saline according to the method of Gordon et al. (Proc. Soc. Exptl. Biol. Med. **109**:96, 1962). Air-dried and gently heat-fixed smears were prepared from live suspensions of the test strains grown on appropriate media. Preparations were stained with homologous conjugate for 20 min in a moist chamber at room temperature. After staining, the slides were rinsed and washed in carbonate-bicarbonate buffer (0.5 M, pH 9.0) for 10 min in a Coplin jar, and then wiped free from excess buffer. The smears were mounted in glycerol adjusted to pH 9.0 with carbonate-bicarbonate buffer. Stock preparations of buffered glycerol and carbonate buffer were stored at 4 C, and the pH was again tested prior to use in experimental studies. Slides were examined with a Zeiss fluorescence microscope equipped with a dark-field condenser and an OSRAM HBO 200-w mercury-vapor lamp (Osram Co., Munich, Germany). Filters consisted of a Schott BG-12 exciter filter in combination with barrier filters BG-23 and GG-4 (Schott & Genossen, Jena, Germany).

The effect of alkalinity on the fluorescent-antibody reaction is presented in Table 1. High-intensity fluorescence was obtained with lower dilutions of each conjugate. In all dilutions producing a staining reaction, the fluorescence was distinctly brighter at pH 9.0 than at pH 7.2. Moreover, end-point dilutions exhibiting detectable staining were higher at pH 9.0.

The experimental findings presented in this report may have practical application from several important aspects: (i) upgrading reactions of low fluorescence; (ii) higher working dilutions of conjugate prepared with possible reduction of low-titer, cross-reacting antibodies; and (iii) economy of working materials.

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