Migration was obtained at pH 11.9, 12.0 and 12.1 as a single moiety (Fig. 1).

Discussion. Evidence presented here shows that some isolates of *L. pomona* have considerable amounts of hemolytic activity associated with their cells. In general, the most activity was associated with the culture supernatant fluids; however, the amount of activity contained within the cells of many of the isolates cannot be considered as insignificant.

There is no reason to believe that the hemolytic factor of the cells is a different substance from the hemolysin of the growth supernatant fluids. However, further characterization of the cellular factor is needed in order to ally the 2 factors more closely.

Assuming the 2 factors to be identical, discovery of appreciable hemolytic activity associated with *L. pomona* cells offers an excellent source of the hemolytic factor for characterization and further purification. The SCP is not grossly contaminated by rabbit serum, tryptose and liver extract of the medium that are present in the supernatant fluids.

Summary. Considerable hemolytic activity has been found to be associated with the cells of some isolates of *L. pomona*. Purification of the hemolytic factor by acetone precipitation of a phosphate buffer soluble portion of the sonicated *L. pomona* cells increased the activity per mg of protein 15 times. The 25-30% acetone fraction was found to migrate as a single moiety using paper electrophoresis.

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Continuous Anaerobiosis for Cultivation of Spirochetes.* (29706)

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The anaerobic spirochetes, among which are numbered the causative agents of syphilis and other treponematoses, and "amphibionts" (1) indigenous to man and many animals, remain poorly understood principally because of difficulties of *in vitro* cultivation. No practicable means to grow authentic, virulent *Treponema pallidum* exists today apart from the living animal. The amphibionts, and avirulent "culture pallidum" spirochetes of uncertain provenance, can be grown *in vitro* with varying difficulty. A significant problem is their reluctance to form surface colonies on agar, hence to permit adequate purification. Several workers have reported that stock cultures of anaerobic spirochetes can be grown as surface colonies(2,1). Hardy, Lee and Nell(3) have recently described improved methods for this purpose, and have stressed nutritional factors as the principal determinants of such growth. It is recognized, however, that these microorganisms are exacting anaerobes, and that even brief exposure to atmospheric oxygen may inactivate them.

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Socransky, Macdonald and Sawyer(4), using a glove box and conditions approaching continuous anaerobiosis, reported the growth as surface colonies of treponemes streaked from primary deep cultures(5) of human gingival scrapings. Technical difficulties seem to have prevented routine use of this procedure. We have been able to overcome these difficulties.

Our apparatus comprises a vacuum-tight glove box with interchange lock, especially designed and built for bacteriological work. Constructed of stainless steel with welded seams, the box, of 3/8 inch (0.95 cm) stock, is 30 inches (76.3 cm) wide and 22 inches (55.8 cm) high and deep. A "Herculite" (Pittsburgh Plate Glass Co., Pittsburgh, Pa.) viewing port, 3/8 inch (0.95 cm) thick and 9 inches (22.9 cm) in diameter is set at 45°. The lock, mounted at the left, is made of 1/4 inch (0.64 cm) stock, 15 inches (38.1 cm) long, 10 inches (25.4 cm) wide and 13 inches (33.0 cm) high inside. Bolted joints—lock to chamber, viewing port rings, and a 10 × 20 inch (25.4 × 50.8 cm) rear access plate, are fastened into blind tapped holes in the box and sealed with O rings. Doors on lock and glove ports are 1/2 inch (12.7 cm) stainless steel and close on O-ring seals by means of single knurled screw latches. Electric leads are sealed in plastic rubber. Copper vacuum lines and "Poly-flo" plastic (Imperial Eastman Brass Co., Chicago, Ill.) gas lines are attached via "Poly-flo" fittings to standard pipe fittings, which in turn are sealed to the box by wrapping their threads with 0.001 inch (0.25 mm) Teflon tape. Standard refrigeration valves were modified by inserting Teflon diaphragms. Gas tightness of chamber and lock was verified with a helium leak detector.

To scavenge oxygen remaining after evacuation, H_2 (contained in "forming gas"—an inexpensive mixture of 10% H_2 and 90% N_2 of standard purity) circulates over palladium coated alumina catalyst(6) (Torsion Balance Co., Clifton, N. J.) which requires no pre-heating. Forming gas, and CO_2 (U.S.P.) from a separate tank, enter a manifold which can be connected separately to lock and chamber. Another manifold leads to vacuum pump, glove ports, lock and a hose connector within the chamber. Additional lines permit interconnecting the 2 manifolds, connecting chamber with lock, and connecting the 2 glove ports directly to the chamber to equalize pressure on the gloves when their ports are sealed. Equipment within the box includes fluorescent and UV lamps and a shelf for reagents and instruments.

Packets of catalyst as used in anaerobic jars are suspended, 2 in the lock and 6 in the chamber, the latter in the path of a small fan.

Anaerobic conditions are established, initially or in the presence of dry porous materials such as paper, by evacuating to the full capacity of the pump for 8 hours or more and then refilling once with circulating forming gas. Dry non-porous materials can subsequently be introduced through the lock by evacuating it fully for 5 minutes and refilling. In the presence of reagents, cultures, media or other materials incapable of withstanding full vacuum, repeated evacuation to 27 inches (69 cm) Hg and washing with forming gas have been found effective: in the lock, to introduce these materials, 3 such washings at intervals of 15 and 5 minutes; in the chamber, *e.g.*, when changing gloves, twice with an overnight interval. Minimal O_2 contamination in the chamber, such as may result from introduction of incompletely degassed fluids, is corrected by a single washing.

The presence of anaerobic conditions is verified (a) by persistent reduction of alkaline methylene blue-glucose(7); (b) with a mixture prepared in the chamber of 40% pyrogallol with approximately 10 parts of 1 N NaOH saturated with Na_2CO_3, which remains colorless when reduced; and (c) by means of a redox cell comprising Pt and saturated KCl-HgCl electrodes in a poised O_2-sensitive medium, which gives potentials in the range —0.42 to —0.45 v. Loss of anaerobic conditions evidenced by changes in these indicators has been traceable to O_2 diffusing from inadequately degassed surfaces or fluids or to a punctured glove, and corrected accordingly. Intact gloves have remained fully extended overnight under static positive pressure.

Special bacteriological methods include the

following: overlapping plastic or heavy foil caps on tubes replace cotton or other porous plugs, both to minimize occluded oxygen and to maintain sterile tube lips. A pencil type electrocautery modified to heat a loop made of approximately 7 inches (18 cm) of 24 g nichrome wire to bright redness serves as an inoculating device. By means of the hose connector in the chamber, anaerobic jars carrying cold catalyst can be evacuated in the box and filled with 5% or more of CO_2 in forming gas. We have made convenient use of Mason jars for anaerobic incubation by trapping the chamber atmosphere in them with CO_2 added by appropriate evacuation and refilling of the whole system. A zoom dissecting microscope and focusing lamp mounted over the viewing port permit critical examination of plates in the anaerobic atmosphere.

With the aid of these methods we have been able to isolate spirochetes, apparently of several varieties, routinely by streaking directly from primary cultures(5) and transferring colonies to fresh medium. Such cultures fail to grow under equivalent conditions when streaked in air and incubated in anaerobic jars. Contaminated tube cultures can be purified by streaking in the box. More than 30 pure cultures of spirochetes have now been isolated either directly from human gingival scrapings or from exudates after passage of such scrapings through guinea pigs(8). Initial pure cultures in fluid medium have been stored at −78°C in an effort to prevent the changes, including increased O_2 tolerance, which seem to occur during *in vitro* passage.

Summary. Apparatus and procedures have been developed for bacteriological work based on a vacuum-tight glove box with lock, in which an anaerobic working atmosphere has been maintained by evacuation, followed by removal of residual and diffusing oxygen with hydrogen and a catalyst that does not require pre-heating. By these means anaerobic spirochetes have appeared regularly as surface colonies on agar streaked from primary cultures, and have been isolated routinely.

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Secretion of Nitrogenous Substances by the Pilocarpine-Stimulated Canine Prostate Gland.* (29707)

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Most of the available information on the composition of canine prostatic fluid was derived from studies on samples of fluid obtained by the systemic administration of pilocarpine(1-4). One characteristic of fluid thus obtained is that its composition varies during the course of secretion(2). Since such variation must of necessity reflect the nature of the secretory process, analysis of the composition of serial samples of the prostatic fluid obtained by pilocarpine stimulation should

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