trols. On the other hand, one (A) protected against 1,000 to 10,000 intraäbdominal lethal doses. The latter was obtained from a person who has been associated with work on E. E. virus (mostly the Eastern strain) for 6 years; at no time has he passed through an illness resembling encephalomyelitis and his general health has remained excellent. None of the 6 sera showed protective antibodies against the W. E. E. strain.

In view of the recently presented hypothesis that localized barriers develop with increasing age, or are present in particular hosts,[1] which prevent certain viruses from invading the CNS, the positive result herein reported of the presence of protective antibodies in an adult person who has been exposed to the virus in the laboratory, takes on added interest. A suggestion offered[4] is that in man, if the pattern of viral invasion from the periphery to the CNS follows that in the mouse or guinea pig, then the probability exists that in most human adults the virus may perhaps be prevented from invading the CNS by certain localized barriers. Hence adult contacts during an epidemic may have clinically inapparent infection and possibly reveal virus in the circulation. In such instances protective antibodies may be found later in the serum. Proof of this assumption would, of course, depend on further observations in the field.

10625 P

Metabolism of "Sulfapyridine-Fast" and Parent Strains of Pneumococcus Type I.

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The acquisition of "sulfapyridine-fastness" by a strain of Pneumococcus Type I has been described.[1] This induced "fastness" is associated with a fairly stable alteration in metabolism without changes in morphology, type-specificity, or virulence of the pneumococcus. The present communication deals with certain of the biochemical activities of the "drug-fast" and parent strains, together with observations concerning the action of sulfapyridine on the pneumococcus.

On the usual culture media, the drug-fast strain grows as well as the parent strain and ferments the same sugars.

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4 Sabin, A. B., personal communication.

1 MacLeod, C. M., and Daddi, G., PROC. SOC. EXP. BIOL. AND MED., in press.

Hydrogen peroxide is formed in cultures of pneumococcus as a product of aërobic metabolism.[2] Its presence can be detected in cultures by the addition of benzidine and peroxidase; the latter may be supplied in the form of blood or potato.[3]

When grown in a shallow layer of broth exposed to air the parent strain produces an abundance of hydrogen peroxide, while little or no peroxide is formed by the drug-fast strain under the same conditions.

Penfold[4] employed blood-agar plates containing benzidine for the detection of microörganisms which produce peroxide during growth. This method has proved very useful in differentiating the two strains of pneumococcus. The colonies of the parent strain on this medium are jet black after 16-24 hours' incubation due to the production of a relatively large amount of peroxide, whereas the colonies of the drug-fast strain show only moderate browning.

Dehydrogenase-activity of the two strains. The dehydrogenase-activity of both strains was studied by determining the ability of cell suspensions to reduce methylene blue in the presence of various substrates.

Cultures of both strains of pneumococcus, grown for 9-12 hours in

TABLE I.

Dehydrogenase-activity of Parent and "Sulfapyridine-fast" Strains on Various Substrates.*

Substrate (Final concentration)	Reduction of methylene blue after 1 hour at 37°C	
	"Sulfapyridine-fast" strain SV-I/P	Parent strain SV-I
Glucose M/140	++++†	++++
Glycerol M/80	—	++++
Sodium lactate M/80	—	+++
" pyruvate M/80	—	++++
Acetaldehyde M/80	++++	++++
Sodium succinate M/80	—	—
" formate M/80	—	—
" acetate M/80	—	—
0	—	—

*Each tube contained 0.5 cc of 0.002 M methylene blue in M/20 phosphate buffer pH 7.6; 0.1 cc of plain broth as a source of coenzymes; 0.5 cc of the appropriate substrate; 1.0 cc of the suspension of pneumococci. The final volume was brought to 4.0 cc in each case by the addition of M/20 phosphate buffer, pH 7.6. The tubes were sealed with a layer of vaseline and incubated at 37°C.

†++++ indicates complete reduction; — indicates no reduction of the methylene blue.

2 McLeod, J. W., and Gordon, J., *J. Path. and Bact.*, 1922, **25**, 139; *Biochem. J.* (London), 1922, **16**, 499.

3 Avery, O. T., and Morgan, H. J., *J. Exp. Med.*, 1924, **39**, 275.

4 Penfold, W. J., *Med. J. Australia*, 1922, **2**, 120.

plain broth under vaseline seal, were centrifuged under seal and the bacteria resuspended in one-twentieth volume of M/20 phosphate buffer at pH 7.6. When these precautions are observed the cell suspensions retain their dehydrogenase-activity longer than if exposed to air throughout the various manipulations.[5] Cell suspensions of this sort have been termed "resting bacteria".[6,7] To eliminate as far as possible the complicating factors associated with cell multiplication, the conditions were so arranged that reduction of the dye occurred within a 1- to 2-hour period of observation. The dehydrogenase-activity of the 2 strains of pneumococcus on a number of substrates is shown in Table I.

Of the substrates tested, glucose is an active hydrogen donator in the presence of both strains of pneumococcus and no difference in the time required for reduction of the methylene blue is observed. On the other hand, the drug-fast strain shows little dehydrogenase-activity for glycerol, lactate, or pyruvate, whereas the parent strain dehydrogenates these substrates actively. It appears, therefore, that these 2 strains of pneumococcus exhibit distinct differences in their ability to dehydrogenate certain 3-carbon compounds, namely, glycerol, lactate, and pyruvate.

In the preceding experiments the dehydrogenase-activity of the cell suspensions was tested in the absence of sulfapyridine. In the following experiments sulfapyridine was added to the reacting systems

TABLE II.
Effect of Sulfapyridine on Dehydrogenase-activity of Parent and "Sulfapyridine-fast" Strains.*

Substrate (Final concentration)	Sulfapyridine†	Reduction of methylene blue after 1 hour at 37°C: "Sulfapyridine-fast" strain SV-I/P	Parent strain SV-I
Glucose M/140	0	++++	++++
"	1:8,000	++++	++++
Glycerol M/80	0	—	++++
"	1:8,000	—	+
Sodium lactate M/80	0	—	+++
"	1:8,000	—	—
Sodium pyruvate M/80	0	—	++++
"	1:8,000	—	+
0	0	—	—
0	1:8,000	—	—

*System used was the same as described in Table I.
†1.0 cc of a neutral solution of sulfapyridine 1:2,000 added as indicated, making a final concentration of 1:8,000.

[5] Bach, D., and Lambert, J., *Bull. Assn. Diplomes Microb. fac. Pharm. de Nancy*, 1937, **15**, 25.

[6] Quastel, J. H., and Whetham, M. D., *Biochem. J.* (London), 1925, **19**, 520.

[7] Cook, R. P., and Stephenson, M., *Biochem. J.* (London), 1928, **22**, 1368.

in order to determine the direct effect of the drug upon the dehydrogenase activity of resting cells of the parent and drug-fast strains. These results are shown in Table II.

Sulfapyridine, in a final concentration of 1:8,000 in the reacting system, does not inhibit the glucose dehydrogenase of either strain. However, the same concentration of the drug greatly inhibits the dehydrogenase-activity of the parent cells for glycerol, lactate, and pyruvate.

The relation of peroxide formation to the carbohydrate metabolism of pneumococcus is not entirely clear. However, suspensions of the parent cells incubated in a shallow layer in the presence of glycerol and coenzyme produce much more peroxide than if the glycerol is replaced by an equal concentration of glucose, indicating that hydrogen peroxide is produced during the metabolism of glycerol.

Summary. The acquisition of "sulfapyridine-fastness" by a strain of pneumococcus Type I is associated with a marked diminution in the production of hydrogen peroxide in cultures of this strain. Cell suspensions of the parent and drug-fast strains dehydrogenate glucose equally well. On the other hand, the acquisition of sulfapyridine-fastness is associated with a marked loss of dehydrogenase-activity for certain 3-carbon compounds (glycerol, lactate, and pyruvate). When sulfapyridine is added directly to the reacting system the dehydrogenase-activity of the parent cells for the same 3-carbon compounds is likewise much decreased.

10626 P

Specific Absorption of Antibody with Extracts Containing the Rabbit Papilloma Virus (Shope).

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The papilloma virus, or a substance that has proved inseparable from it, reacts with specific immune sera to fix complement, as previous studies have shown;[1] and tests made recently with more than a score of such sera have borne out a finding already obtained, namely that the virus-neutralizing ability of any serum is directly proportional to its complement-fixing capacity. The present work was

[1] Kidd, John G., *J. Exp. Med.*, 1938, **68**, 703, 725, 737.