In the latter group residual virus was demonstrable in the brain of a healthy-appearing mouse killed 3 days after inoculation, but was not detected in brains of survivors killed on the last day on which death occurred in this group. It is therefore improbable that the 2 deaths in these mice were related to viral multiplication. Passage of the 3rd day brain tissue known to contain virus failed to kill saline-injected mice, and virus was not demonstrated in the brains of animals so inoculated.

In adult mice dying of infection with either Coxsackie virus employed, paralysis, tremor, and convulsions were not perceived. Conjunctivitis was noted occasionally in mice given cortisone alone, but was almost universally present in animals inoculated with virus as well. Two to 3 days following inoculation some animals became briefly hyperexcitable, but in most mice disease was manifested by huddling, ruffled fur, arched backs, labored respirations, progressive unresponsiveness and lethargy which proceeded to stupor and sudden death. Gross examination of abdominal viscera post mortem has disclosed splenic atrophy and hepatic enlargement and congestion. Histologic studies are in progress.

Discussion. The experiments described show that adult Swiss mice may be lethally infected with Coxsackie virus if such mice are administered a single dose of cortisone prior to the inoculation of virus. Furthermore, viral multiplication in the tissues of the adult mouse, as well as serial passage and specific neutralization of virus have been demonstrated in this ordinarily insusceptible host. Although focal muscle lesions have previously been noted in 10-12-g mice, attempts to propagate Coxsackie virus either in series or by alternate passage through suckling and weaned mice have met with failure in the past(3).

It is a matter of great interest that the administration of a chemically defined substance may convert a state of apparent complete insusceptibility to one of marked susceptibility to a viral infection. It will be important to determine the range and specificity of this phenomenon by the study of allied steroids and other viruses. Elucidation of the mechanism of the phenomenon will obviously be dependent upon the acquisition of further knowledge concerning the metabolic action of cortisone in the murine host.

Summary. 1. Adult mice, ordinarily insusceptible to Coxsackie virus infection, may be lethally infected if preliminarily administered cortisone. 2. Multiplication, serial passage, and specific neutralization of Coxsackie virus in adult mice have been demonstrated. 3. Recovery of Coxsackie virus from a human source has been accomplished in adult mice.

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Nature of Multiple Forms of the *Lactobacillus bulgaricus* Factor (LBF).* (18703)

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In previous reports, the discovery(1,2) and purification(2,3) of a growth factor (designated LBF) required by *Lactobacillus bulgaricus* and many other lactic acid bacteria (4,5) was described. Bioautographic studies

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showed that LBF exists in natural materials in several chromatographically distinct forms (6,7), one of which has recently been isolated(3) and synthesized(8). This compound, an amide of pantothenic acid and β-mercaptoethylamine, exists both in a reduced or thiol form (*pantetheine*) and as the corresponding disulfide (*pantethine*). Both forms show indistinguishable activity for *Lactobacillus helveticus* 80(8).

The ready interconversion of pantetheine and pantethine suggested the possible occurrence of mixed disulfides with LBF activity formed according to equation 1, below:

$$PSSP + RSH \rightleftarrows PSH + RSSP \quad (1)$$

where PSSP represents pantethine, RSH a mercaptan, PSH pantetheine, and RSSP a mixed disulfide. Examination of this possibility forms the subject matter of this article.

Experimental. The formation of mixed disulfides would be favored, if RSH in equation 1 were employed in excess, and the resulting mixture of PSH and excess RSH then reoxidized with iodine. Accordingly, synthetic pantethine (800 μg) was mixed in water with a 20-fold excess of the reducing compound, the solution diluted to 50 ml, and allowed to stand for 3-4 hours. The mixture was then either chromatographed directly (Na_2S or $Na_2S_2O_5$ as reducing agents) or oxidized with iodine to the disulfides(9), neutralized, and chromatographed. For the latter purpose, 0.005 ml (equivalent to about 5 LBF units) of each reaction mixture was spotted near one end of individual 1 x 30 cm strips of Whatman No. 1 filter paper. After drying, these were first equilibrated, then developed by the ascending technic, with water-saturated *n*-butanol. Active zones were located by the bioautograph procedure of Winsten and Eigen (10). *L. helveticus* 80 was used as the test organism with the pantethine-free medium previously described for this organism(5), but solidified with 1.5% of agar.

Mixed disulfides from pantethine. The results (Fig. 1) show that treatment of pantethine with any of the indicated reduced sulfur compounds always resulted in the formation of an active zone differing greatly in R_F value from that due to unchanged pantethine. Controls in which the thiol compounds were first oxidized to their disulfides, then added to pantethine, allowed to stand, and chromatographed, showed only one active zone corresponding to pantethine. Whereas pantethine itself is ninhydrin-negative, chromatograms of the reaction product of pantethine with β-mercaptoethylamine showed a ninhydrin-positive zone that corresponded exactly in outline and R_F value with the "extra" growth-promoting zone revealed by the bioautograph (Fig. 1G).† This behavior would be expected of a mixed disulfide containing a β-mercaptoethylamine residue as one component, since the latter fragment furnishes a ninhydrin-reactive amino group. Thus mixed disulfides are readily formed from pantethine in accordance with equation 1, and these mixed disulfides retain LBF activity. Since many thiols occur naturally (*e.g.* homocysteine, cysteine, cysteine peptides, proteins, glutathione, hydrogen sulfide, ergothioneine, etc.) in amounts far surpassing pantethine, similar mixed disulfides could readily be formed in natural materials in numbers limited only by the number of naturally occurring thiol compounds.

Discussion. In view of the fact that mixed disulfides are readily prepared and stable substances(11), it seems curious that the possible

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† Because of the comparative sensitivities of the growth and ninhydrin tests, larger amounts of the reaction product (up to 1000 units) must be used when the latter procedure is applied.

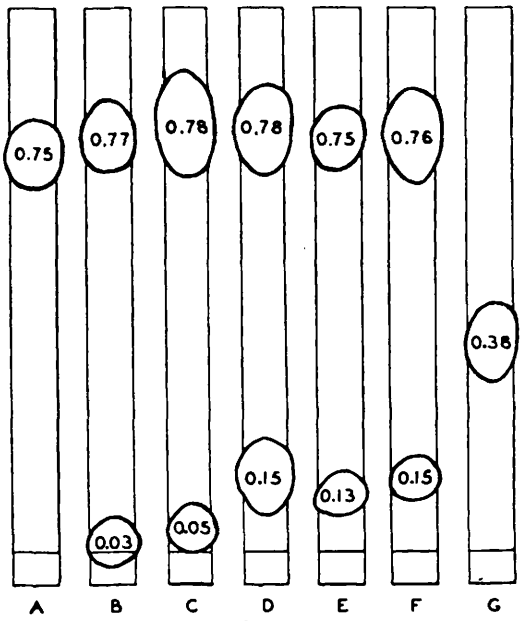

FIG. 1

Tracing of bioautographs showing the formation of mixed disulfides from pantethine and thiol compounds. R_F values of active zones are given within the zone area. A. pantethine, B. pantethine + reduced glutathione, C. pantethine + thiomalic acid, D. pantethine + cysteine, E. pantethine + sodium sulfide, F. pantethine + sodium metabisulfite, G. pantethine + β-mercaptoethylamine. See text for treatment.

natural occurrence of similar low molecular weight compounds has not been emphasized in the past. Although the number of such disulfides that might be formed from pantetheine and other naturally-occurring thiols more than suffice to explain the number of LBF-active zones so far detected in chromatograms of natural materials, it is quite possible that structures other than these may also contribute to the LBF activity of natural materials. From postulated structures for coenzyme A(12,13) and the relationship of the latter to LBF(14), it appears probable that structures intermediate in complexity between pantetheine and coenzyme A may exist, and contribute to the LBF activity of such digests. The occurrence of 3 zones with LBF activity in chromatograms from phosphatase-treated coenzyme A concentrates has been reported(7).

Repeated trials have shown that treatment of appropriate fractions of yeast extract with cysteine, followed by chromatography as described above, results in a pronounced shift in R_F values of certain of the LBF-active zones originally present. This preliminary evidence supports the idea that mixed disulfides contribute to the LBF activity of natural materials.

The possibility that coenzyme A may likewise occur naturally in several forms due to mixed disulfide formation should be considered.‡ The formation of such a disulfide with glutathione, for example, might possibly explain the high glutamic acid content of concentrates of a growth factor (PAC) for *Acetobacter suboxydans*(15), the growth-promoting action of which is duplicated by coenzyme A itself(16).

Summary. Treatment of pantethine with sodium sulfide, cysteine, glutathione, β-mercaptoethylamine, or several other thiols followed, in some cases, by oxidation with iodine resulted in formation of a series of new compounds with growth-promoting activity for *Lactobacillus helveticus* 80. These compounds appear to be mixed disulfides. The natural occurrence of similar mixed disulfides formed from pantetheine and other naturally occurring thiols may explain in part the presence of several chromatographically distinct forms of LBF in natural materials.

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