DAP by cell-free extracts of *Myco. tuberculosis* was increased by addition of pyridoxal phosphate, although it was not essential for enzyme activity. This is probably because with the methods employed, the decarboxylase present in the extract is still largely undissociated from its coenzyme. DAP decarboxylase activity was markedly inhibited by INH. Inhibition was greater if the drug was preincubated with the cell-free extract before addition of substrate. The results obtained also clearly indicate a relationship between toxicity of INH and pyridoxal phosphate. Further studies, however, are necessary to determine whether the reaction is a strictly competitive one between INH and pyridoxal phosphate for active sites on the enzyme molecule. The protection obtained by preincubation of the preparations with pyridoxal phosphate prior to addition of INH seem to indicate a competitive relationship.

On the basis of our findings it is suggested that the DAP decarboxylase is important in the metabolism of *Myco. tuberculosis*, serving as an important and probably major pathway in lysine synthesis. The finding that DAP decarboxylase is inhibited by INH, and that this inhibition can be overcome by pyridoxal phosphate, is in accordance with the previous observation in our laboratory that lysine synthesis by *Myco. tuberculosis* is markedly inhibited by culture in the presence of subinhibitory concentrations of isoniazid. Because of the nature of the isoniazid molecule the interpretation of any data relating to its specific mode of action is difficult, but the finding of a pyridoxal phosphate-requiring enzyme whose activity is inhibited by the drug lends additional support to the hypothesis that one of the important points of attack of INH in *Myco. tuberculosis* are those enzyme systems requiring pyridoxal phosphate as coenzyme.

Summary. DAP decarboxylase activity has been detected in cell-free extracts of *Myco. tuberculosis*. Enzyme activity was inhibited by INH and this inhibition could be overcome by pyridoxal phosphate.

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Effect of d-Lysergic Acid Diethylamide on Spinal Reflexes. (24287)

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The alterations in mental function induced by d-lysergic acid diethylamide (LSD) are, presumably, paralleled by modifications of the activity of cells or synapses within central nervous system, whether cortical, subcortical or spinal. LSD, when administered to spinal cats, in doses as small as 10 μg/kg i.v. was found to augment polysynaptic withdrawal and crossed extensor reflexes(1). At higher doses (40-80 μg/kg) depression of the flexion reflex was found. The monosynaptic knee jerk was less consistently affected. After administration of LSD in doses sufficient to cause mental symptoms increased activity of stretch reflexes has, however, been a consistent clinical observation(2). In the following experiments, the effects of LSD were evaluated by measurement of changes in magnitude of the reflex volley in ventral roots rather than by recording of muscular contraction.

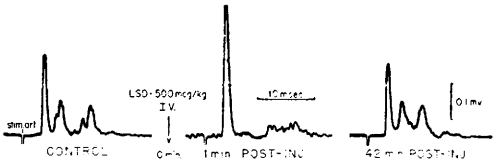

FIG. 1. Oscilloscopic tracing of response in 6th lumbar ventral root in response to stimulation of peroneal nerve.

Methods. The upper cervical spinal cords of adult cats were sectioned through the atlanto-occipital membrane under ether anesthesia. Peripheral nerves and ventral roots were prepared for stimulation and recording utilizing standard technics(3,4). In these experiments a gastrocnemius, tibial or peroneal nerve was stimulated with single square wave stimuli 0.08-3.0 volts intensity and 0.1-0.4 msec. duration. The stimuli were repeated at 2 or 3 second intervals. Temperatures of the animal and the pool of oil surrounding the cord were maintained at 36° and 34°C, respectively. Only those preparations whose baseline responses over a 30 minute control period varied less than 10% are considered in this report (20 out of 37). The criterion of significant change in amplitude of the monosynaptic spike was 20% and the change in area of the polysynaptic complexes, estimated visually, was 50%. Blood pressure was recorded from a common carotid artery with a strain gauge recorder. Thirty-six injections of LSD-25 (Sandoz) dissolved in sterile physiological saline, were administered through a cephalic vein in 20 preparations. Dosage ranged from 4-1000 μg/kg. The usual injection time was 10 seconds. Rate of injection did not influence the results.

Results. The characteristic modification of reflex activity observed in this study is shown in the figure. Increased height of the monosynaptic response was observed after 11 of 16 injections of doses greater than 200 μg/kg. Below this dose only 1 of 20 injections resulted in facilitation. Four instances of failure to respond to doses of LSD greater than 200 μg/kg occurred in cats which had received another dose of LSD less than 2 hours previously. Only one such failure was observed among cats receiving more than 200 μg/kg as the first dose. Increased amplitude of the monosynaptic spike became apparent within 15 seconds after completion of injection and lasted from 4 minutes to 3 hours. Maximum facilitation was observed with 250-300 μg/kg. Higher doses tended only to prolong this effect, but no consistent relation of duration of effect to dose level was observed. For these reasons quantitative analysis of the dose-response relations were not possible. Polysynaptic activity was observed in several preparations where nerves to flexor muscles were stimulated. With one exception, depression of polysynaptic activity accompanied facilitation of the monosynaptic reflex. After injection of doses higher than 100 μg/kg a significant increase in blood pressure was found but facilitation of the monosynaptic spike did not necessarily parallel the hypertensive effects.

Discussion. The foregoing experiments and those previously published on evaluation of LSD action on spinal monosynaptic and polysynaptic pathways(1) are not directly comparable. In the previous studies, muscle reflexes were recorded. In addition to an effect on the central pathway, reflex activity may have been modified by the action of LSD on peripheral structures. Of significance in this respect is the direct peripheral vasoconstriction which LSD induces similarly to its analogue, ergonovine(5). Resulting interference with muscle perfusion may influence contractility and modify the activity of proprioceptive reflexes.

The observation that larger doses were required to produce effects in this series than those reported in previous studies(1) is consistent with the observation of Elder *et al.* (6) that reproducible LSD intoxication in cats is obtained only with intravenous injection of 400 μg/kg or greater.

The magnitudes of the reflexes reported here are dependent primarily upon the integrity and the excitability of motoneurones and, in turn, upon the facilitatory and inhibitory tone of the internuncial systems. Since LSD may act upon any of these components, more precise localization of site of action of LSD is required before interpretation of a central mechanism of action is feasible. At present

significant comparisons can be made, however, with the action of LSD upon other central pathways. Purpura(7) has postulated that those cortical tracts which have predominantly axosomatic synaptic connections are facilitated by LSD. Since the connections of muscle afferents with spinal motoneurones are principally axosomatic(8) facilitation of the monosynaptic spike is to be expected.

Also pertinent is the concept that certain cortical association tracts(9), midbrain reticular pathways(10) and spinal reflexes(11) contain adrenergic links. The present study shows that LSD modifies spinal reflexes in a manner analogous to that of epinephrine(12). Similar evidence has been presented in experiments on the transcallosal preparation(9). LSD may, therefore, interact with proposed adrenaline-like transmitters.

Summary. The amplitude of the monosynaptic reflexes recorded from the ventral roots of unanaesthetized spinal cats was found to be increased after i.v. injection of LSD in doses greater than 200 μg/kg. Polysynaptic activity was depressed coincidentally with facilitation of the monosynaptic spike.

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Zone Electrophoresis of Cerebrospinal Fluid Proteins in Starch Gel.* (24288)

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The observation that total protein in cerebrospinal fluid may increase substantially in many diseases of the nervous system has stimulated considerable investigation of the protein fractions with new methods of protein separation. It has been hoped that when specific changes were found, they would be of diagnostic value and would shed some light on the large number of neurologic diseases of unknown cause. The exceptional resolution of serum protein fractions by electrophoresis in starch gel described by Smithies (1), and our(2) experience with this method, whereby we can obtain 15 to 20 protein fractions in normal sera, suggested that comparable fractionation might be gained by applying this technic to cerebrospinal fluid protein.

Methods and materials. Specimens of spinal fluid and serum were obtained from patients in the hospital. Most of these patients suffered from disease of the nervous system; only a few could be considered normal subjects. After determining concentration of protein of spinal fluid by the sulpho-

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