B. Liver of rat sensitized to horse serum and reinjected with horse serum 13 days later (anaphylactic shock). Died 15 min. × 1050. Stained with hematoxylin and eosin.

C. Liver of rat sensitized to horse serum and reinjected with horse serum 13 days later (anaphylactic shock). Died 25 min. ×1050. Stained with hematoxylin and eosin.

Lesion. Except in the case of streptococcus toxin, no definite influence of dietary deficiency (essential amino acids, vitamin B complex or minerals) upon the formation of the bodies could be detected. Dietary deficiencies did seem to favor the development of the inclusions after injection of the streptococcus toxin.

Symptoms. All the substances that produce the liver lesions cause definite prostration lasting varying periods of time; and there appears to be a rough correspondence between the severity of the symptoms and the number of inclusion bodies. However, the bodies may persist after recovery from the prostration.

Description of the Inclusion Bodies. The cytoplasmic masses appear first as agglomerations of granules with fibrin within the liver cells (Fig. 1 A and B) that soon fuse into a roughly spherical or oblong homogeneous body (Fig. 1 C). This lies in a clear vacuole and often pushes the nucleus to one side. In unstained preparations they are greenish and have the same refractivity as erythrocytes. Their reaction to various stains is shown in Table III. Bodies of similar appearance are found also within the Kupffer cells, but they appear only after 2 hours and are smaller in size. Following injection of R-salt-protein,[3] the liver cell inclusions show a varying intensity of red staining; the Kupffer cells are filled with bright red particles. The inclusions do not stain as fat or glycogen.

The interesting problem, that we are studying further, is the chemical relation of these bodies in the liver to the injected protein.

13282

Demonstration of Myelolytic Substances in Disseminated Sclerosis.

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Following the study of the myelolytic action of substances like saponin, bile salts and snake venoms upon the myelin sheaths of rat

[3] Heidelberger, Michael, and Kendall, Forrest E., *J. Exp. Med.*, 1933, **58**, 137.

spinal cords in test tube experiments,[1, 2] the attempt was made to demonstrate the presence of myelolytic substances in disease processes which are characterized pathologically by a selective destruction of myelin in the central nervous system. The classical disease which lends itself best for such a study is disseminated sclerosis, and many authors before had already assumed *a priori* the presence of myelolytic substances in this disease without being able to demonstrate them with certainty.[3]

Weil, Luhan and Balser[4] demonstrated in the urine of about 70% of cases of disseminated sclerosis the presence of substances which acted in test-tube experiments upon fresh rat spinal cord in a similar way as sodium taurocholate. These substances were not identical with the lipolytic ferments which Brickner claimed to have demonstrated in this disease,[5] but the presence of which subsequently could not be confirmed by Richards and Wolff.[6] We did not find myelolytic substances in normal urines or in urines of patients suffering from syphilis of the central nervous system, from subacute combined degeneration or other degenerative diseases of the brain and spinal cord. They were present, however, in cases of post-encephalitic Parkinsonism and in certain diseases of the liver. It appeared that the amount of such substances present in cases of disseminated sclerosis varied with the periods of remissions and exacerbations and that a positive reaction was more frequently found during periods of exacerbations. Since then such experiments have been continued and at present the results are as shown in Table I.

TABLE I.
Myelolytic Substances Present in Urines.

Diagnosis	No. of patients			% of total	
	Total	+	—	+	—
Disseminated sclerosis	165	115	50	70	30
General paresis	29	0	29	0	100
Post enc. Parkinsonism	43	28	15	65	35
Other dis. of c.n.s.	83	4*	79	5	95
Normal controls	25	0	25	0	100

*One case each diagnosed as Huntington's chorea, Wilson's disease, *Dystrophia myotonica* and amyotrophic lateral sclerosis. Nine cases of disseminated sclerosis which had given a positive reaction were verified by autopsy.

1 Weil, A., *Arch. Path.*, 1930, **9**, 828.

2 Weil, A., *Arch. Exp. Path. u. Pharm.*, 1930, **154**, 228.

3 Weil, A., *J. A. M. A.*, 1931, **97**, 1587.

4 Weil, A., Luhan, A. J., and Balser, B. H., *Transact. Am. Neur. Assn.*, 1935, **61**, 142.

5 Brickner, R. M., *Arch. Neurol. and Psych.*, 1930, **23**, 715.

6 Richards, C. H., and Wolff, H. C., *Arch. Neurol. and Psych.*, 1940, **43**, 59.

The original technic which had been used was the following: 200 cc of urine collected in sterilized bottles and which had been preserved in the icebox were evaporated at a temperature not exceeding 50°C *in vacuo*. The residue was redissolved in 5 cc of 0.85% NaCl solution and incubated with a small piece of fresh rat spinal cord which had been removed under aseptic precautions. Following incubation for 16 hours at 37°C the spinal cord was fixed in formaldehyde solution, embedded in paraffin, cut longitudinally (15 micron) and stained for myelin sheaths with the Weil method. In normal controls incubated with rat spinal cord the myelin sheaths are seen under the microscope as narrow dark black staining bands (Fig. A). But in spinal cords incubated with urines from active cases of disseminated sclerosis they appeared broken up into fine granules (Fig. C), resembling somewhat the reaction which is seen following incubation of spinal cords with solutions of sodium taurocholate (Fig. B), though the granulation is much finer after incubation with disseminated sclerosis urines. It becomes coarser and more distinct following incubation with acetone precipitates (Fig. D).

Bacterial contamination of the urines was avoided as much as possible by taking the precautions as described above. But even normal urines which had been kept intentionally at room temperatures for 2 days did not show the myelolytic action after incubation with rat

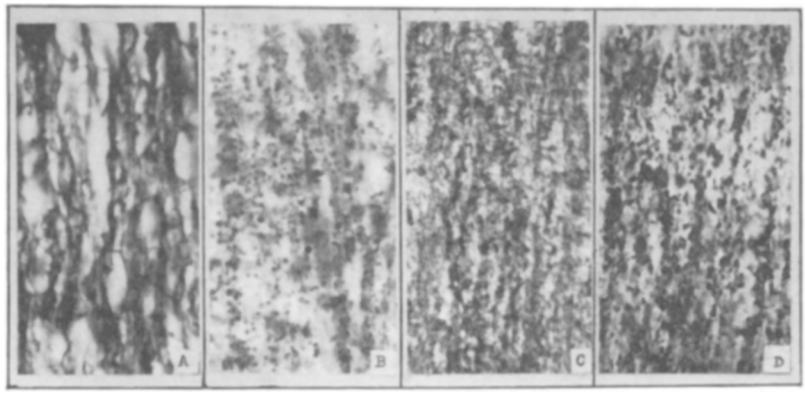

FIG. 1.

Spinal cords of rats after 16 hours' incubation at 37°C were fixed in formalin solution, embedded in paraffin; sections were stained for myelin sheaths with the Weil method. Magnification 910 ×. A. 200 cc of normal urine, concentrated to 5 cc. B. 30 mg of sodium taurocholate dissolved in 3 cc of water. C. 200 cc of urine from a patient suffering from disseminated sclerosis, concentrated to 5 cc. D. 700 mg of acetone precipitate from alcoholic extract of concentrated urine of a case of disseminated sclerosis, dissolved in 7 cc water. For description of the histologic changes compare text.

spinal cord. Active urines from cases of disseminated sclerosis after having been boiled for 5 minutes showed the same myelolytic action as the same urine without sterilization.

Next, the attempt was made to concentrate and to purify these myelolytic substances. They could be extracted from the evaporated urine with 95% alcohol and they were precipitated from this solution by pouring it into about 5 times its volume of acetone (Fig. D). Dioxane extracted them nearly quantitatively from these acetone precipitates, indicated by the fact that the extracted urines, if incubated with rat spinal cord, did not fragment myelin sheaths. Based on these observations the following method is used at present: The residue of one liter of evaporated urine is dissolved in 50 cc of water and shaken with 250 cc of dioxane. After filtration through a Buechner funnel, the dioxane is separated in a separation funnel; the water solution again is shaken with 100 cc of dioxane and both dioxane solutions are combined. They are evaporated *in vacuo* to a thick syrup, redissolved in 5 cc absolute alcohol and slowly poured into 100 cc of water-free ether. The resulting precipitate is separated by centrifugation. In contains about one-half of the myelolytic substances and can be further purified by redissolving it in alcohol and precipitating it again by ether. A similar method has been used by Jones and McNulty for the separation of bile salts.[7] The relative amount of myelolytic substances in the supernatant fluid was determined by incubating varying amounts (200, 300, and 500 mg) of its residue, following evaporation, with spinal cord and by comparing the myelolytic effect with that produced by the same amounts of precipitate. The myelolytic activity was greatly reduced during these different processes, in the same way as exposure to air rapidly diminished the activity of acetone precipitates.

The yellowish precipitate obtained from the dioxane extracts was very hygroscopic. It dissolved readily in alcohol and dioxane; 500 mg of it, dissolved in 5 cc of saline solution and incubated with approximately 30 mg of rat spinal cord gave a positive myelolytic reaction. The substance was strongly surface active, and, compared with sodium taurocholate of equal concentrations, it possessed about one-fifth of the surface reducing activity of this substance; *e. g.*, 58 mg dissolved in 6 cc of water had a surface tension of 65.5%, as measured with the stalagmometric method used by Allen for the measurement of surface tensions of urines.[8] The color reactions for cholic acid and desoxycholic acid and for urobiline were negative.

[7] Jones, K. K., and McNulty, M. C., personal communication.

[8] Allen, G. D., *J. Biol. Chem.*, 1915, **22**, 505.

The attempt will be continued further to purify and to analyze this substance.

Summary. A substance which acted destructively upon the myelin sheaths of rat spinal cord could be demonstrated in the urines of patients suffering from disseminated sclerosis. It was more frequently observed during periods of exacerbations than during periods of remissions. Similar myelolytic substances were also observed in cases of post-encephalitic Parkinsonism, but they were absent in syphilis and in other diseases of the central nervous system. This myelolytic substance could be isolated from the concentrated urines by extraction with dioxane.

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Relation of Blood Lactic Acid and Acetone Bodies to Uric Acid in Pre-Eclampsia and Eclampsia.*

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Pre-eclampsia is a disease of the last trimester of pregnancy characterized by hypertension, albuminuria and edema. It may develop into eclampsia, which is distinguished from the pre-eclampsia by the occurrence of convulsions. Hyperuricemia is the most consistent blood chemical finding in these diseases.[1, 2] It has been attributed to a disturbance of uric acid metabolism in the liver. Quick[3] has suggested that it may result from an elevated blood lactic acid known to occur in eclampsia,[4-7] because lactic acid ingestion causes uric acid retention. Evidence against this theory is that the blood lactic acid in eclampsia rises mainly after the convulsions,[5, 6] whereas the blood uric acid is continuously elevated.

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6 Bokelmann, O., *Arch. Gynäkol.*, 1927, **129**, 802.

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