markedly hyperemic, large and well-nourished, comparing rather favorably with those of normal full-term females, and showing a marked contrast to the rigid, contracted tissues of untreated oophorectomized females. It is apparent that oestrin exercises some function during pregnancy, in the rat, in maintenance of the uterus and in this respect perhaps operates synergistically with progestin.

A positive response, when elicited, followed upon a continuous, and progressive increase in, oestrin dosage; abortion occurred during treatment and, with one exception, no response was evoked following withdrawal of oestrin administration. The marked variation in response, however, was striking. Thus, in Series A, 4 animals responded to a total of only 72 R.U., and 3 to 112; 3 failed to respond to a total of 160 R.U., however. In B, 5 responded to 140, 5 to 220, and in C, 3 responded to 108 and 2 to 168, whereas 10 animals failed to react to a total of 240 R.U. Variations in Series D and E are shown in the tables, where it will be seen that 4 animals failed to respond to 336 and to 456 R.U. The quantity required to produce an effect, apparently, is subject to marked individual variation, and the manner and the time of oestrin administration would appear to be of greater importance than the amount administered.

The foregoing observations suggest that oestrin in adequate, timely amounts appears to be definitely involved in preparation of the uterine structure for parturition; whether this consists in a sensitization to the action of some other agency or whether oestrin in itself initiates the birth mechanism remains to be determined.

8908 C

Lead Content of the Spinal Fluid with Special Reference to Multiple Sclerosis.

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Lead was suggested as an etiological factor in multiple sclerosis by Putnam.[1] Cone, *et al.*,[2] examined a series of 40 spinal fluids for

[1] Putnam, J. J., *Boston M. and S. J.*, 1883, **109**, 315; *J. Nerv. and Men. Dis.*, 1883, **10**, 446.

[2] Cone, W., Russel, C., Harwood, R. N., *Arch. Neurol. Psych.*, 1934, **31**, 236.

lead, using the qualitative hexanitrite test of Fairhall.[3] In their series were 8 patients with multiple sclerosis. In 6 of these the tests for lead were positive. Rabinowitch, *et al.*,[4] about the same time reported the results of tests of 50 spinal fluids, including those from 27 cases of multiple sclerosis. These authors used the Fairhall hexanitrite method and checked their results by spectographic determinations. These authors found positive tests for lead in only 2 of their 27 cases of multiple sclerosis; in 2 additional cases they obtained positive tests after administering ammonium chloride to their patients. Boshes[5] tested 28 specimens of spinal fluid for lead, by the same method. Sixteen of his patients had multiple sclerosis; in only one of these did the spinal fluid yield a positive test for lead. All the above authors found occasional positive tests for lead among their control cases.

Because the above findings depended on a qualitative test that is difficult to control, we believed it would be worth while to investigate the lead content of a series of spinal fluids, using a precise quantitative micro-chemical method. Such a method has been devised by Wilkins, *et al.*[6] This method involves the titrimetric extraction of lead with a standard solution of diphenylthiocarbazone in the presence of ammoniacal cyanide. The original method was designed for use with blood; we have adapted it to spinal fluids in the manner described below. All the precautions advocated by the last mentioned authors were closely observed.

Lumbar puncture was done with a dry, sterilized needle, and the fluid allowed to drop directly into a lead-free Pyrex flask. A measured volume of fluid, varying from 25-50 cc. was transferred to a Pyrex evaporating dish, and evaporated to dryness on a steam bath. The Pyrex dish was then transferred to an electric muffle furnace and ashed for 16 hours at 450°C. The ashed sample was dissolved in warm 5% nitric acid and transferred to a Pyrex separatory funnel, in which the titrimetric extraction was carried out. Simultaneous blanks were run with each set of determinations.

According to the above mentioned authors, the titrimetic-extraction method has a constant error of ±0.001 mg. of lead. We have been able to reduce the lead in our blank determinations to less than 0.001 mg. We have further tested the method by adding known

[3] Fairhall, L. T., *J. Biol. Chem.*, 1923, **57**, 455.

[4] Rabinowitch, I. M., Dingwall, A., and MacKay, F. H., *J. Biol. Chem.*, 1933, **103**, 707.

[5] Boshes, B., *Arch. Neurol. and Psych.*, 1935, **34**, 994.

[6] Wilkins, E. S., Willoughby, C. E., Kraemer, E. O., and Smith, F. L., *Analytical Indust. and Eng. Chem.*, 1935, **7**, 33.

amounts of lead to fresh normal spinal fluids. The amounts added varied from 0.010 mg. to 0.050 mg. The maximum discrepancy between the amount added and amount recovered was noted in a fluid to which was added 0.050 mg. from which 0.047 mg. were recovered. In other recovery procedures carried out, the error was not more than ±0.002 mg. of lead.

Nearly all our determinations were carried out upon samples, the volume of which exceeded 35 cc. We have classified as containing no lead those samples whose determined lead content, after subtraction of the blank, was less than our maximum error of ±0.003 mg. This assumption we believe to be more than fair from a statistical viewpoint. Nearly all samples actually differed from the blank by less than 0.001 mg.

Using the method described, which we consider highly accurate and reliable, we have analyzed a series of spinal fluids from 42 patients. Our results are shown in Table I.

TABLE I.

Type of Case	No. of Cases	Lead in Sample (mg.)
Multiple Sclerosis	9	0
Lead Poisoning—chronic	1	0
" " recovered	2	0
Control Cases	28	0
Case X*	1	0.010
Case Y*	1	0.005
Total	42	

* Fluids collected without special precautions against lead contamination.

The absence of lead in the spinal fluids obtained from patients with multiple sclerosis does not necessarily eliminate lead as a causative factor of the disease. It is a well established fact that the barrier permeability for other heavy metals is low. That the same is true for lead is suggested by the negative findings in the case of chronic lead poisoning. A delicate test, such as that which we have used, will give a positive reaction for lead unless precautions are taken to avoid contamination.