moment of 2.13 ± 0.04 debye for this compound which is believed to resemble decaborane by being asymmetric and having four hydrogen bridge bonds.[5]

We are continuing measurements on solutions of decaborane in other solvents.

(5) (a) K. Hedberg, M. E. Jones and V. Schomaker, THIS JOURNAL, **73**, 3538 (1951); (b) W. J. Dulmage and W. N. Lipscomb, *ibid.*, **73**, 3539 (1951).

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FRACTIONATION OF AN ACTH PREPARATION BY IONOGRAPHY

Sir:

The technique of ionography,[1,2,3,4] that is, electromigration on wet surfaces, was utilized to fractionate an ACTH preparation[5] obtained from pig pituitaries. This material was prepared by the acid acetone extraction method of Lyons[6] and further purified by treatment with 9% ammonium hydroxide solution and fractional acetone precipitation. The final material was freeze dried from aqueous solution. It was one half as active as Armour's LA la standard. The particular instrument employed was the Precision Ionograph.[7] The paper used was Eaton-Dikeman 613, 8 mm. in width. The experiments were conducted at 24–26° in a helium atmosphere.

The ionogram was dried on a glass plate by a stream of hot air. It was then passed through a saturated mercuric chloride solution of 95% ethyl alcohol containing 1 g. of brom phenol blue per 100 ml. of solution. The ionogram was again dried in a stream of hot air and then passed successively through several beakers containing saturated aqueous mercuric chloride solution until all the excess indicator was washed out of the paper strip. On re-drying the strip, the protein zone appeared as a dull green area which changed to a sharp deep blue color by passing the ionogram over concentrated ammonium hydroxide.

Using a veronal buffer of ionic strength 0.015 at *p*H 5.5, and applying a potential of 6 volts/cm. for three hours across the ends of the filter paper strips, the ACTH preparation separated into three fractions: a heavy-staining fraction "A" which moved to the negative pole, a light-staining fraction "B" which moved to the positive pole and a heavy-staining fraction "C" which did not move. As the *p*H of the buffer used to saturate the paper strips was increased to 6.0–6.6, the mobility of the heavy-staining fraction A was found to approach zero, indicating that its isoelectric point was in this region. This fraction contained 98% of the biological activity as determined by the adrenal ascorbic acid depletion test,[8] but only about 31% of the total input nitrogen. The isoelectric point of the light fraction B, which was found to have only 0.2% activity, but about 45% of the nitrogen was shown to be in the region of 4.2–4.8. The isoelectric point of fraction C, which contained 2% of the activity and 21% of the nitrogen, was shown to be in the region of 5.0–6.0. It would appear from these experiments, and others,[9] that the biological activity of ACTH is not uniformly distributed throughout the whole protein preparation.

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(4) H. J. McDonald, M. C. Urbin and M. B. Williamson, *J. Colloid Sci.*, **6**, 236 (1951).

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(10) Reuben Myron Strong Research Fellow.