

It is known that individuals vary within very wide limits in their reactions to injections of foreign proteins. These observations suggest that those individuals who are relatively insusceptible have some protective mechanism either in the serum or in the cells of the body which prevents or delays the union of the antigen, in this case horse serum, with the cells of the body.

ABSTRACTS OF THE COMMUNICATIONS,
PACIFIC COAST BRANCH.

Twenty-fourth meeting.

San Francisco, California, February 11, 1920.

74 (1534)

The determination of calcium in blood and plasma.

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The method consists in a direct precipitation of the calcium without removal of the blood proteins. The only reagents necessary are ammonium chloride (to prevent the precipitation of magnesium), and ammonium oxalate, both adjusted approximately to P_H 7.4.

Blood.—Citrated blood (5 c.c. in a 50 c.c. centrifuge tube), is hemolyzed by the addition of four volumes (20 c.c.) of warm water and after standing 15 to 20 minutes is centrifuged to remove the stroma. An aliquot (20 c.c.) of the clear, red liquid is transferred to a 50 c.c. centrifuge tube. One c.c. of 5 per cent. ammonium chloride is added and, after mixing, 3 c.c. of 3 per cent. ammonium oxalate are added. The oxalate should be added slowly and the contents of the tube must be well mixed. After standing at least 16 hours (the much shorter time recently reported by de Waard¹ results in an incomplete precipitation of the calcium), the mixture is centrifuged and the supernatant liquid siphoned off. The tube is washed by the addition of 25–30 c.c. of cold water

¹ de Waard, D. J. *Biochem. Z.*, 1919, 97–98, p. 186.

and immediately centrifuged. After removal of the wash water the precipitate is dissolved in 5 c.c. of approximately normal sulfuric acid and, after heating in a water bath to 75° C., the contents are titrated with 0.01 normal potassium permanganate. (After precipitation of the calcium the method follows, with minor modifications, that described by Halverson and Bergeim.²

Plasma.—Citrated plasma (5 c.c. in a 50 c.c. centrifuge tube) is diluted with an equal volume (5 c.c.) of one per cent. ammonium chloride and 10 c.c. of one per cent. ammonium oxalate are slowly added. After standing 16 hours the precipitate is centrifuged, washed and titrated as in the procedure for blood.

The method has been checked by:

1. The determination of known amounts of calcium in solutions having approximately the same mixture of salts as is found in the blood.

2. The ashing of whole blood and plasma in platinum dishes and precipitation of the calcium by a modification of McCrudden's³ method.

3. The addition of known amounts of calcium to plasma and recovery by the above method.

The advantages of this method are:

1. The small amount of sample necessary, 5 c.c. blood and 3 to 5 c.c. of plasma.

2. The few reagents necessary and the relative ease of preparing them free from calcium.

3. The precipitation of the calcium in the presence of the proteins. Methods^{2, 4} now in common use require either ashing or removal of the proteins by precipitation, operations which greatly add to the chances for mechanical losses.

The accuracy of this method lies between five and seven per cent.

² Halverson, J. O., and Bergeim, O. *J. Biol. Chem.*, 1917, 32, p. 159.

³ McCrudden, F. H. *J. Biol. Chem.*, 1909-10, 7, p. 83; *J. Biol. Chem.*, 1911-12, 10, p. 187.

⁴ Lamers, A. J. M. *Z. Z. Gebürts. u. Gynäk.*, 1912, 71, p. 393.

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