CCLXVIII. THE CALCIUM OF WHOLE BLOOD, SERUM AND PLASMA IN HUMAN DISEASES, INCLUDING TETANY.

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(Received October 20th, 1933.)

THE work which forms the subject of this paper was suggested by the findings of Cameron and Moorhouse [1925], who stated that in experimental parathyroid tetany the calcium of the whole blood shows proportionately less fall than that of serum. They attributed this to the passage of a large amount of calcium into the fibrin clot in cases of tetany and based this view on the finding of a much higher calcium figure for citrated plasma than for serum in tetany. Sokolovitch [1931] has reported slightly higher figures for whole blood-calcium in human tetany than would be expected if the corpuscles contained no calcium and assumed that the results indicated a slight shift of calcium into the corpuscles in tetany. The effect observed by him was much smaller than that observed by Cameron and Moorhouse. Morgulis and Perley [1930] working on experimental parathyroid tetany, considered both possibilities—a shift of calcium into the corpuscles, and an abnormal passage of calcium into the coagulum—but could find no evidence for either phenomenon. The question of the passage of calcium into the corpuscles in tetany raises again the old controversy as to whether there is normally any calcium in red corpuscles. This point has been under discussion for nearly forty years, but there is still some divergence of opinion even among modern workers using accurate methods.

The present work was planned to determine whether any calcium exists in red corpuscles in human subjects with varying diseases including tetany, and whether any abnormal amount of calcium is to be found in the coagulum in cases of tetany.

Material.

The control observations were made for the most part in cases of renal or prostatic disease and a few other conditions.

Three cases of tetany were examined, *viz.* (1) H. D., age 22, tetany of unknown origin but probably not parathyroid tetany; (2) E. T., age 15, late rickets and tetany, and (3) A. Hi., age 35, chronic diarrhoea and tetany. No case of parathyroid tetany has been investigated from this standpoint.

Plan of experiments.

For experiments on the distribution of calcium between serum and corpuscles, defibrinated blood was used. The corpuscle volume was estimated by the haematocrit and the calcium estimated in serum and whole blood.

For experiments on the passage of calcium into the coagulum, comparisons were made between the plasma from citrated blood and the serum from defibrinated blood. Since citrate causes a shrinkage of corpuscles and a corresponding dilution of the plasma, it was necessary to make haematocrit determinations on both the citrated and defibrinated blood in order to determine the correction

needed for the calcium of citrated plasma. The collection of blood was made as follows. About 20–25 cc. of blood were collected in a flask containing glass beads, and the flask was shaken for a few seconds to ensure even admixture of corpuscles with plasma. Then, before the fibrin had begun to separate, about 10 cc. of blood were poured off into a tube containing not more than 0.1 g. sodium citrate and mixed. The remainder of the sample in the flask was defibrinated by further shaking.

In order to check the validity of the volume correction used in determining calcium in plasma, a few experiments were made in which a sample was first defibrinated and a part of the defibrinated blood subsequently treated with sodium citrate. The usual contraction of corpuscles was observed in the citrated samples. When 0.1 g. sodium citrate was added to 10 cc. blood the corpuscle volume in the citrated blood was, on an average, 38 % as against 45 % in defibrinated blood. The correction to be used was determined for each experiment and the corrected plasma figures agreed well with the serum figures. This result shows that the addition of sodium citrate in this amount causes no precipitation of calcium and no shift of calcium from plasma to corpuscles.

Analytical methods. For serum and plasma the method of direct precipitation of the calcium oxalate from the serum or plasma was used, and the analyses were made by the method of Kramer and Tisdall as described by Harrison [1930], except that at least 18 hours' precipitation was allowed for plasma. The precipitation of calcium is much slower for plasma than for serum. In the earlier part of the work half an hour was usually allowed for the precipitation from serum, and 18 hours at least for plasma. In some later experiments the serum was also left for 18 hours, but the results were the same. G. W. Clark [1921] emphasised the necessity of allowing several hours for the precipitation of calcium from plasma. E. P. Clark and Collip [1925] considered half an hour sufficient for serum.

For whole blood, the modified Alport method of Cameron and Moorhouse [1925] was used. In this method the blood is ashed in a platinum dish, and the iron and phosphate are removed from the ash solution before the calcium is precipitated as oxalate.

In all estimations samples of 2 cc. serum, plasma or whole blood were analysed in duplicate.

Estimations of corpuscle volume were made in capillary haematocrits, which were centrifuged at about 3000 r.p.m. until the corpuscle layer reached constant volume. When constant volume was reached, it was always noted that the corpuscle mass became transparent.

RESULTS.

Serum and corpuscles. Observations were made on fourteen patients with various diseases and the three patients with tetany. Very good agreement was found in most cases between the observed figure for whole blood and the figure for the serum-calcium calculated as mg. per 100 cc. whole blood. Where slight discrepancies occurred, the tendency was for the observed whole blood figures to be slightly lower than the calculated figures. The actual discrepancies between observed and calculated figures for whole blood were as follows (a positive sign indicates that the observed figure was higher than the calculated figure, and a negative sign the reverse discrepancy):

In the 3 cases of tetany, $-0.2, 0, 0$ mg. per 100 cc.

In control cases: ± 0.2 mg. per 100 cc. in 9 experiments.

± 0.2 to 0.5 mg. per 100 cc. in 2 experiments.

-0.5 to -1.0 mg. per 100 cc. in 3 experiments.

Agreement is therefore good in 14 experiments including those in cases of tetany, while three experiments show either a negative error in determining whole blood-calcium (which might be due to loss in the transferences) or a positive error in the serum determinations (which might be due to the use of direct precipitation). The results therefore show that there is no measurable amount

of calcium to be found in corpuscles by the technique adopted, and that no shift of calcium into corpuscles occurs in the types of human tetany examined.

Serum and plasma. The average results in the control experiments were as follows:

Serum-calcium	9.52 mg. per 100 cc.
Plasma-calcium (observed)	9.04 mg. per 100 cc.
Plasma-calcium (corrected for volume change)	10.05 mg. per 100 cc.
Calcium passing into the clot	0.53 mg. per 100 cc.

It will be seen that the serum-calcium and plasma-calcium are approximately equal, but the result is due to a partial balance of opposite effects—the dilution of the plasma due to the addition of citrate tends to lower the plasma figure, and the passage of a trace of calcium into the clot tends to lower the serum figure as compared with plasma.

In the three cases of tetany, no measurable passage of calcium into the clot was observed.

DISCUSSION.

These results in three cases of human tetany, not of parathyroid origin, are similar to the results of Morgulis and Perley [1930] in parathyroid tetany. No calcium passes into the corpuscles and no abnormal amount into the coagulum.

The question of the absence of calcium from normal corpuscles requires further comment. In reading the literature the main impression is of the number and variety of sources of error in the methods for calcium estimation. Almost every detail in every method has been the subject of debate, but these technical points will not be discussed in detail, because it seems impossible to harmonise all the results even when all the sources of error are considered. The technical problems are very fully dealt with by Guillaumin [1930].

There may be differences between different species, and there may be differences between adults and children, but to settle this point more work is needed, applying the same methods to blood from different sources. Almost all the work on human adults shows absence of any appreciable amount of calcium from corpuscles, but sometimes figures of about 3 mg. Ca per 100 cc. corpuscles, or even more, occur among the results of authors whose technical work appears very accurate. Some of these figures have been obtained by direct determinations on unwashed corpuscles and are therefore open to the objection that they include some calcium from the serum remaining in the corpuscle mass, but values from 3 to 6 mg. per 100 cc. corpuscles cannot be accounted for in this way.

SUMMARY.

Calcium was found to be absent from the red blood corpuscles in human subjects with various diseases including tetany.

No abnormal amount of calcium passed into the fibrin coagulum during clotting of the blood of human subjects with tetany.

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