function, as for example in monitoring suppressive therapy with the thyroid hormones. Furthermore, very little information is available on the TSH-IRMA response to TRF in these patients (2).

We describe here results obtained from a recently commercialized IRMA for serum TSH (TSH MaiaClone kit™, Serono Diagnostic Ltd., Woking, Surrey, U.K.) in euthyroid subjects and in patients with different grades of thyroid hyperfunction: Group 1: 52 healthy euthyroid persons; Group 2: 27 untreated, overtly hyperthyroid patients (19 with Graves' disease and eight with toxic nodular goitre); Group 3: 14 mildly hyperthyroid patients with relapsed Graves' disease after withdrawal of antithyroid drugs and previous normal TSH responses to TRF; Group 4: 18 patients with autonomously functioning multinodular goiter; and Group 5: 40 clinically euthyroid patients being given suppressive therapy with levothyroxin.

These subjects had previously been classified as euthyroid or as having thyroid hyperfunction on the basis of both clinical examination and results of routine tests of thyroid function: serum free thyroxin, triiodothyronine, and basal and TSH responses (NHS-TSH RIA; Diagnostic Products Corp., Los Angeles, CA) to intravenous administration of 400 μg of TRF. TSH responses to TRF were considered subnormal when results of RIA of TSH 30 min after TRF administration were ≤3.0 milli-international units/L (responses at 30 min post-TRF ranged from 3.7 to 26 milli-int. units/L in our group of euthyroid subjects). Basal and TSH responses to TRF as measured by the TSH-IRMA method were not used to determine the diagnostic categories of the patients.

Figure 1 shows basal and 30-min post-TRF values for TSH as measured by the IRMA procedure in these various groups. Basal and TSH responses to TRF were lower in all patients in groups 2 to 5 than in those in group 1 (euthyroid subjects). Evidently all different grades of thyroid hyperfunction can be distinguished from euthyroidism when basal or TSH responses to TRF are measured by this IRMA method. However, basal and TSH responses to TRF were not sufficient to discriminate among different groups of patients with thyroid hyperfunction. Many patients from these groups showed subnormal but detectable increments of TSH in response to TRF administration, indicating absence of total TSH suppression. Moreover, the finding of an undetectable basal TSH value did not allow one to predict the absence of TSH response to TRF in many of these patients, especially those patients with multinodular goiter and those on the suppressive therapy. Therefore, these findings appear to indicate that measurements of basal concentration of TSH in serum can be insufficient to prove complete suppression of TSH in these cases, and that TRF tests may still be necessary in many of these patients when this IRMA method is used for TSH.

References

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Centrifugation Speeds and the Removal of Platelets from Heparinized Plasma

To the Editor:

It is curious that papers are being published in 1984 (1) and 1985 (2) on the problems generated by platelet contamination of plasma samples. One would have thought that this fundamental aspect of sample preparation would have been conclusively dealt with long ago. Perhaps the explanation lies in the widespread use in the past of serum rather than plasma. All cells are trapped in the fibrin clot and their concentration in serum is negligible.

Our problem arose when it became apparent that the centrifugation procedures we were using in our laboratory, spinning at 1500 × g (2700 rpm) for about 7 min in a Beckman TJ-6R centrifuge with TH-4 72-place rotor, was inadequate for the determination of lactate dehydrogenase (LD, EC 1.1.1.27) activities with the Technicon SMAc continuous-flow analyzer. Scrutiny of the literature (early 1984) turned up very few references concerning centrifugation of heparinized blood samples. It seemed as if the process of centrifugation was so straightforward that no one thought to mention it, even in reference works. In the first (3) there is no mention at all of centrifugation speeds or necessary g values. In another (4) the only reference states (p 133):

"For separating of serum or plasma strong fields of between 1000 and 2000g are generally applied. For very fine sediments fields of up to 10,000g are necessary." The nature of such sediments was not identified. A third (5) states: "Centrifuges are used in the chemistry laboratory primarily to separate clotted blood or cells from serum or plasma and to clarify body fluids. Although the relative centrifugal force necessary to carry out these separations is not critical, a force of at least 1000g for 10 minutes will give a good separation." And finally, in their proposed standard procedures, the NCCLS (6) recommends a time of 10 ± 5 min and an RCF of 1000–1200g. The latitude in the timing specified is surpris-
Inspection of the indexes for *Clinica Chimica Acta* and *Clinical Chemistry* back to 1970 turned up no references to centrifugation of samples specifically. However, Rothwell et al. (7) were the first to address the problem of the contamination of plasma samples with platelets. They found that, under assay conditions where platelets were lysed, the LD activity of plasma decreased with increasing g values unless the samples were centrifuged at 1270 × g for 15 min. Under non-lytic conditions LD activities in platelet-rich and platelet-poor plasma were indistinguishable. Bais et al. (8) reported that, on using heparinized plasma obtained by centrifuging samples at 1800 × g for 5 min, LD activity in the Technicon SMAC was increased by platelet contamination, the assay system consisting of a preliminary dilution with a detergent-containing solution. Only by spinning at 3300 × g for 10 min was the platelet contribution removed.

Subsequently, Peake et al. (1) observed not only leaking of LD from lysing platelets but also considerable optical interference by platelets at 340 nm, sufficient to give an apparent inhibition of the pyruvate-to-lactate reaction.

Interference was also found with the assays for urate at 295 nm and alkaline phosphatase at 405 nm. Using a bench-top centrifuge at 1240 × g for 15 min, they found a mean platelet count of 88 × 10⁶ per liter.

Ours is a moderately busy laboratory, receiving on average 500 to 600 specimens per day. Most of these arrive before 12 noon. Thus we need to use a centrifuge rotor capable of holding 30 to 60 tubes in order to provide an efficient service. Our custom had been to centrifuge samples at full speed, 2700 rpm or 1500 × g, for about 7 min. When apparent rapid fluctuations in some patient LD values were traced to cloudy samples, we monitored 44 selected samples for platelet content as well. The samples were then spun in a refrigerated centrifuge at 16 000 rpm (30 000 × g) for 20 min, LD activities and platelet counts were monitored, and the changes in both were plotted. The relationship found was y (platelet count) = 36.1x (LD activity) - 11, r = 0.94. Thus a contamination of about 50 × 10⁶ platelets per liter increased LD activity by about 1 μkat/L (60 U/L). By prolonging the centrifugation time to 15 min the platelet count (n = 24) was reduced to a mean of 15 × 10⁶ per liter, but we would wish to lower it further. Also the time taken is excessive and cannot always be sustained. Similar results were obtained when we replaced heparin with EDTA.

This Letter is written because (a) there has been insufficient attention in the past to the fundamental problem of platelet contamination of plasma samples, and the experiences of Rothwell et al. (7) and Peake et al. (1) require to be reaffirmed, and (b) apparently many laboratories are now equipped with high-volume-throughput bench-top centrifuges, which do not achieve high enough g values to sediment platelets within a reasonable period of time. We recommend the advice of Rothwell et al. (7) to spin "for 3000 × g for 15 min to assure a platelet-free plasma." This is quite at variance with the few references in the textbooks and means that many hospital laboratories need to upgrade their centrifuges.

References


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High-Density Lipoprotein Phospholipid Concentrations In Serum of Patients with Liver Disease

To the Editor:

It is well known that the concentration of high-density lipoprotein (HDL) cholesterol is decreased in the serum of patients with liver disease, but as yet there have been few reports on what happens to HDL phospholipids.

Rubies-Prat et al. (1) reported that values for HDL phospholipids were normal or slightly above normal in cases of liver cirrhosis and cholestasis, while values for HDL cholesterol were about half those found in these subjects. The subject remains controversial (2), however. We therefore measured HDL phospholipid concentrations in patients with liver disease, to determine whether these values reflect liver function and correlate with the severity of the disease.

Persons included in the study were 18 healthy subjects (HS), 10 patients

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Fig. 1. Decreasing platelet counts and plasma LD activities between first centrifugation (1500 × g for 7 min) and second centrifugation (30 000 × g for 20 min)