Kaplow, L. S. and Beck, R. E. (1964). Leukocyte alkaline phosphatase response to surgical stress. *Surg. Gynec. Obstet.*, **119,** 97-102.

Rutenburg, A. M., Rosales, C. L., and Bennett, J. M. (1965). An improved histochemical method for the demonstration of leukocyte alkaline phosphatase activity: clinical application. *J. Lab. clin. Med.*, **65,** 698-705.

Trubowitz, S., Feldman, D., Benante, C., and Hunt, V. M. (1959). The alkaline phosphatase content of the human polymorphonuclear leukocyte in blood and marrow. *Amer. J. clin. Path.*, **31,** 483-486.

Valentine, W. N., Follette, J. H., Hardin, E. B., Beck, W. S., and Lawrence, J. S. (1954). Studies on leukocyte alkaline phosphatase activity: relation to "stress" and pituitary-adrenal activity. *J. Lab. clin. Med.*, **44,** 219-228.

Williams, D. M. (1975). Leucocyte alkaline phosphatase as a marker of cell maturity: a quantitative cytochemical and autoradiographic study. *Brit. J. Haemat.*, **31,** 371-379.

Wyllie R. G. (1962). Neutrophil alkaline phosphatase. Response to acute inflammation. *Aust. Ann. Med.*, **11,** 118-125

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Platelet Counts in Normal Pregnancy

We are pleased to be able to confirm the findings of Sejeny *et al* (*J. clin. Path.*, **28,** 812-813, 1975) that, using a Coulter Counter, the platelet count decreases progressively and significantly in normal pregnancy. Sejeny *et al* suggest, without adducing any evidence, that this decrease in count may be due to an increase in plasma volume. We, however, have evidence that does not prove, but strongly suggests, that this haemodilutional effect is not nearly sufficient to account for the observed drop in platelet count.

We have studied about 30 patients in each trimester and 24 non-pregnant women. The results will be published elsewhere, but in brief we have found a significant progressive decrease in count and increase in the mean platelet volume so that the total platelet volume mass per ml (volume × numbers/ml) remained approximately constant. The concept of a constant total platelet mass has been reported before (O'Brien and Jamieson, 1974; Behrens, 1975). Additionally, we found marked shortening of the heparin thrombin clotting time of platelet-poor plasma. This may perhaps reflect the presence of platelet factor 4 liberated into the plasma as the result of thrombosis that occurs normally and extensively even in the healthy placenta.

From the table it will be seen that, as expected, the haemoglobin falls progressively through pregnancy. The packed cell volume also fell but there was no evidence of iron deficiency developing since the MCHC remained constant. Therefore it seems reasonable to assume that the fall in haemoglobin reflected a relative increase in plasma volume. Assuming that the original total platelet count remained constant, in this case the mean was 272 × 10^9/1, it is then possible to calculate what the count would have been if it had been diluted in the increased plasma volume as had the red cells. It will be seen that the observed count decreased far in excess of the calculated figure due to haemodilution. Thus very probably the total number of circulating platelets per unit volume decreases absolutely.

	Controls	*1st Trimester*	*2nd Trimester*	*3rd Trimester*
Haemoglobin (g/dl)	13·4	12·7	12·5	12·2
Packed cell volume	0·41	0·39	0·38	0·37
Haemoglobin per unit volume (MCHC)	32·7	32·6	32·9	33·0
Observed platelet count (× 10^9/1)	272	249	243	210
Expected count due to haemodilution	—	257	253	247

Table *Possible effect of heamodilution on platelet count: means*

References

Behrens, W. E. von (1975). Mediterranean macrothrombocytopenia. *Blood,* **46,** 199-207.

O'Brien, J. R. and Jamieson, S. (1974). A relationship between platelet volume and platelet number. *Thrombos. Diathes. haemorrh.* (*Stuttg.*), **31,** 363-365.

Sejeny, S. A., Eastham, R. D., and Baker S. R. (1975). Platelet counts during normal pregnancy. *J. clin. Path.*, **28,** 812-813.

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Amylase Assay by the Phadebas Method

In the past we have personally corrected a misconception among some colleagues concerning the assay of amylase by the Phadebas method but now that the erroneous notion has recently appeared in print it becomes necessary to respond likewise. Ojala and Harmoinen (1975), in the discussion on the methodology of the Phadebas amylase kit (Pharmacia AB), confirm the manufacturer's literature that the substrate does not remain suspended but sinks after the initial shaking at the beginning of incubation. They then go on to claim that 'a clearly higher and better reproduced amylase value was obtained by shaking the mixture by hand vigorously during the entire incubation'. We find no significant difference in activity or reproducibility of the assay whether the reaction tubes are (A) shaken or (B) remain unshaken during incubation.

A mean amylase value = 501 ± 12 U/l n = 10

B mean amylase value = 500 ± 12 U/l n = 10.

Higher absorbances were encountered with the shaken tubes (A), as were noted originally by Ceska *et al* (1969), but this was paralleled by a higher absorbance of the blank.

The implication of the statement of Ojala and Harmoinen is that while the substrate sediments the enzyme remains in solution in the supernatant. However it can readily be shown by the experiments detailed below that the amylase is adsorbed on to the solid phase substrate during the initial mixing after addition of the tablet. A further series of reaction tubes containing the same serum was centrifuged immediately after addition of the tablet to the assay mixture. The supernatants were decanted into clean tubes, absorbances were noted, and incubation was carried out after the addition of a further tablet. The increase in absorbance in this series was minimal (ΔA <0·01). Simultaneously with this series, 4 ml of 0·9% saline was added to the residues in the original tubes and the assay procedure was continued. The

values obtained in this case were 503 ± 9 U/l, n = 10. The similarity of this last mean result with those of the earlier assays is fortuitous since it does not represent a valid estimation of amylase activity. Obviously, the reaction continues during centrifugation, and this activity has apparently cancelled out the reduced activity obtained while the reaction mixture was achieving the reaction temperature. However, it does conclusively indicate that the enzyme activity is associated with the insoluble substrate, not the supernatant, and continuous shaking cannot be expected to change the results.

References

Ceska, M., Birath, K., and Brown, B. (1969). A new and rapid method for the clinical determination of α-amylase activities in human serum and urine. Optimal conditions. *Clin. chim. Acta*, **26**, 437-444.

Ojala, K. and Harmoinen, A. (1975). Determination of amylase activity and amylase isoenzymes in serum and urine using a solid phase blue starch substrate. *Scand. J. clin. Lab. Invest.*, **35**, 163-169.

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Dehydrated Test Strip for the Detection of Yeasts

We, too, have evaluated the dehydrated test strip designed for the detection of yeasts (Davies, R. R. and Savage, M. A. *J. clin. Path.*, **28**, 750, 1975).

The selective medium of the strip sustained the growth of various isolates of *Candida albicans* and *Torulopsis glabrata*, as well as that of *C.stellatoidea* (LSHTM 3107), *C.tropicalis* (LSHTM 3111), *C. pseudotropicalis* (LSHTM 3105), *C.parapsilosis* (LSHTM 3104), *C.krusei* (LSHTM 3100), *C.guilliermondii* (LSHTM 3099), *C.pelliculosa* (LSHTM 3115), *C.pulcherrima* (LSHTM 3172), *C.reukaufii* (LSHTM 825), *Pichia guilliermondii* (CBS 2031), *Rhodosporidium toruloides* (CBS 14 and 349), *Saccharomyces cerevisiae* (CBS 1171), *P.ohmeri* CBS 1950), *P.burtonii* (CBS 2352), and *R.diobovatum* (CBS 6084 and 6085). It would not support growth of *S.uvarum* (CBS 395).

Thus, the strip will detect yeasts of non-pathogenic genera as well as non-pathogenic species of the genus *Candida*. It detects perfect fungi, mating types which are the imperfect states of some perfect fungi, and imperfect fungi. Many of these yeasts can be isolated from the vagina (Hurley *et al.*, 1974). Although yeasts other than *C.albicans* or *T.glabrata* will be isolated from only 1-3% of specimens submitted for diagnosis of morbidity of the lower genital tract, if the strip is used without recourse to the traditional methods of isolation and identification of yeasts, misleading reports would occasionally be issued.

Grown on Sabouraud's agar	36
Grown on Microstix Candida (MC)	34
Direct microscopy	23

Table *Number of swabs with yeasts*

We agree with Davies and Savage (1975) that culture on Sabouraud's agar is more sensitive than culture on MC, and that direct microscopy detects less yeast carriage than either culture medium (table).

References

Davies, R. R. and Savage, M. A. (1975). Evaluation of a dehydrated test strip for the detection of yeasts. *J. clin. Path.*, **28**, 750-752.

Hurley, Rosalinde, Stanley, Valerie, C., Leask, Barbara, G. S., and de Louvois, J. (1974). Microflora of the vagina during pregnancy. In *The Normal Microbial Flora of Man*, edited by F. A. Skinner and J. G. Carr, Academic Press, New York.

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Book reviews

An Introduction to Liquid Scintillation Counting. By A. Dyer. (Pp. 111; 21 tables; 26 figures; £3·60.) London: Heyden. 1974.

Since it was first introduced in 1950, the technique of liquid scintillation counting has been of major importance in medical and biological work with β and γ-emitting radioisotopes, and the subject has developed a vast literature and much folklore.

The author has performed a useful service in bringing together in this short book a very wide range of information concerning the techniques and applications of liquid scintillation counting. There are eight chapters, an extensive, selected bibliography, an appendix, and an index. The first three chapters deal with the basic techniques, while the remainder cover in detail specific topics, including sample preparation for materials labelled with ^{3}H, ^{14}C, and other nuclides; the problems of quenching; the choice of sample vials; and the role of Cerenkov counting. Comments on sample preparation techniques are thoughtful: the relative merits and disadvantages of toluene and dioxane as solvents when aqueous samples have to be measured are critically discussed, and the merits of emulsion counting for such samples are clearly set out, together with some of the problems accompanying this technique. There is a clear description of the characteristics of the various primary and secondary solutes. The author supports Fox in urging manufacturers not to promote 'secret recipes', the use of which may result in unsuspected errors arising from the formation of colloids.

Unfortunately, the descriptions of the counting systems form a relatively weak section of the book and appear to rely too heavily on 'manufacturers' jargon' (for example, 'balance point counting'), and too little distinction is made between improvements in the basic counting technique and the important, but surely quite distinct, provision of mechanical systems for handling large numbers of samples. The role and limitations of coincidence counting in reducing the effect of chemiluminescence are discussed only obscurely (Chap. 7, p. 78): 'Lastly, it must be mentioned that instruments are available fitted with photon monitoring which can distinguish between true sample counts and the single photon events occurring in