not taking calciferol, latent tetany was demonstrated in all but three instances. In 27 observations on patients who, notwithstanding calciferol treatment, still had hypocalcaemia the state of latent tetany was demonstrated in only five instances. Calciferol seems to have this effect irrespective of whether the patients have hypoparathyroidism or calcium deficiency. Study of individual patients clearly reveals the effect of calciferol (see Fig. 2).

Discussion

What should be the clinician's criterion of dosage when treating chronic hypocalcaemia with calciferol? The drug appears to influence latent tetany both by raising the serum calcium and by some other independent effect. The doses required to inhibit the signs of latent tetany in the average case of hypoparathyroidism on ordinary diet is between 50,000 and 150,000 units daily. The dose necessary to keep the serum calcium within normal limits may be considerably larger. It would appear more reasonable to base the therapeutic dosage on a biological test than on a purely chemical estimation. The higher dosage necessary to keep the serum calcium around 10 mg. per 100 ml. may not only be unnecessary but may have a deleterious effect on the patient. It seems well established both in animals and in man that the toxic manifestations of vitamin D are not necessarily accompanied by hypercalcaemia (Reed, Struck, and Steck, 1939); while Eaton (1946) reported a hypoparathyroid patient taking calciferol who notwithstanding a normal serum calcium developed metastatic calcification.

The patients considered here, whose dosage and treatment were based on the signs of latent tetany, have so far shown no ill effect of low blood calcium such as lens opacities. The period of observation, however, is not long enough to permit conclusions.

It is difficult to explain the inhibitory action of calciferol on latent tetany that is not due to increased serum calcium. A relative increase in ionized calcium is at least a theoretical possibility. Unfortunately, complete data on serum protein are not available, but in a small number of cases studied there was no appreciable change. Feeny, Sandiland, and Franklin (1947) found no change in the serum protein fractions, except a slight alteration of globulin fractions, in cases of lupus vulgaris receiving large doses of calciferol. Ingram, Anning, and Dawson (1948) recently reported that the high dosage of calciferol used in treatment of lupus vulgaris did not alter the ionized calcium fraction but significantly increased a diffusible calcium complex. It is unlikely that this alteration would have any bearing on the inhibition of latent tetany as described above.

Summary

The clinical tests for detection of latent tetany in adults and adolescents were investigated, using hypocalcaemia as an index of latent tetany. Almost all the patients observed had chronic latent tetany. It was concluded that the well-known tests are unreliable. The tourniquet test was negative in 34% of 89 observations on 32 patients and Chvostek's sign was negative in 73% of observations.

A new test combining the tourniquet test and hyperventilation is described. It is used when the tourniquet test is negative, and is more reliable than either of the two procedures used separately. Of 58 observations on 32 patients 95% were positive. Two other patients whose tourniquet test was negative were physically unable to hyperventilate.

Calciferol has an inhibitory effect on the signs of latent tetany which is independent of the increase in serum calcium. In 22 of 27 observations on patients who had received a total of more than 500,000 units the signs of latent tetany were negated although the serum calcium was still less than 9 mg.

per 100 ml. Calciferol therefore inhibits latent tetany in two ways. The significance of this observation in the treatment of chronic latent tetany is discussed.

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PATHOGENIC STAPHYLOCOCCI

DETECTION OF α -LYSIN PRODUCTION ON RABBIT- AND SHEEP-BLOOD-AGAR PLATES

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The coagulase reaction is the simplest and perhaps the most important test for pathogenicity of the staphylococcus. However, the agreement between pathogenicity and this reaction, although close, is not absolute (Christie, North, and Parkin, 1946), and it is sometimes desirable to use confirmatory tests. This is especially the case when detecting carriers of pathogenic staphylococci. Christie and his co-workers (1946), having examined a very large number of strains, conclude that the production of α -lysin is the property most closely associated with pathogenicity. This conclusion is shared by Schwabacher *et al.* (1945).

Until recently it was commonly believed that the lysis of a blood-agar plate was an unreliable guide to pathogenicity. The relatively tedious detection of α -lysin in supernatants from broth cultures is still often recommended (Knott, 1947). However, several workers have used sheep-blood-agar plates successfully for this purpose. The changes produced by α - and β -lysins on this medium can usually be distinguished with ease (Naidu, 1934; Bryce and Rountree, 1936; Kojima and Kodama, 1939; Christie and North, 1941). Christie, North, and Parkin (1946), using spot inoculation of strains on sheep-blood-agar plates, observed that with nearly all haemolytic strains there was no difficulty in deciding whether they produced α - or β -lysin. However, with strains producing only a trace of lysis on blood agar it was difficult to decide whether this was due to α -lysin or to something else. Williams and Harper (1947) describe a sheep-blood-plate method similar to that of Christie and his co-workers, except that a control plate containing incorporated α -antitoxin is included with each batch of tests, which are incubated in an atmosphere containing 30% CO₂. They found that the method, although reliable, failed to detect about 2% of α -toxigenic strains (positive by broth method) and that β -lysin, when produced, could mask the α -lysin. They recommend that all apparently negative and all evidently β -toxigenic strains should be tested in a tube before being classed as non- α -toxigenic.

The rabbit-blood-plate method of α -lysin detection described here is simple to perform and gives results which are easily interpreted. Other lysins do not interfere with the test.

Method

Two per cent. rabbit-blood-agar plates are used. The blood is obtained with reasonable aseptic precautions from an ear vein. The strain to be tested is streaked with a loop across the surface of the plate, previously dried in the incubator. Three or four strains can be tested on one $3\frac{1}{2}$ -in. (8.75-cm.) plate. The number may be doubled by inoculating different strains on opposite halves of the plate. A strip of sterilized filter-paper about $\frac{1}{2}$ in. (1.25 cm.) wide and containing dried α -antitoxin is laid on the agar at right angles to the inoculation streaks. These papers are prepared by dipping dry-sterilized strips into α -antitoxin (Burroughs Wellcome refined staphylococcus antitoxin, 1,250 units per ml.). The papers are then dried in a Petri dish containing heated plaster-of-Paris and preserved, sealed in a similar dish, in the freezing chamber of an ordinary refrigerator. The method used is that described by Morley (1945) for penicillin-containing test papers. Sufficient papers for many tests may be prepared at one time. They retain their potency unimpaired for at least two months. We have not tested them after longer periods. Their content of dried serum renders the papers somewhat stiff, but when applied closely to the agar they quickly absorb moisture and adhere to its surface.

Staphylococcus α -toxin production on rabbit-blood-agar plate. One negative and two positive strains.

24 hours most of the positive strains give easily read results. If an apparently negative strain is present the plate is incubated similarly for a further 24 hours before the final reading. Strains which do not produce α -toxin give either no lysis or, more often, a sharply bordered zone of lysis the edge of which is parallel to the growth and which extends right up to the edge of the filter-paper. On the other hand, an α -toxigenic strain shows a wide zone of lysis with hazy margins. Near the filter-paper this zone tapers in the form of a spear-head pointing towards the paper (see illustration). This spear-head zone of narrowing, diagnostic of α -toxigenic strains, is sharply defined by remarkably straight borders corresponding to lines of toxin-antitoxin neutralization. In addition, it is often possible to see straight white lines extending outwards at an angle from the filter-paper. These lines correspond closely with the borders of the haemolysed zone, and are apparently formed by toxin-antitoxin precipitation in zones where optimal proportions exist (Elek, 1948). The presence of other lysins in addition to α -toxin makes no difference to the clarity of the result.

Identical appearances are obtained by spreading antitoxin (4 drops) over half a previously dried plate. The plate is dried again, and strains are then streaked across it at right angles to the dividing line between treated and untreated agar. The necessity for a second drying of the plate renders this technique less convenient than the filter-paper method.

Results

Using this method we examined 201 unselected pure strains of staphylococcus freshly isolated from routine hospital specimens over a period of eight months. The strains were all tested at the same time for α -toxin production in broth (Gillespie, Devenish, and Cowan, 1939) and for coagulase production (Gillespie, 1943). The results obtained are shown in the Table.

Table showing Coagulase Reactions and α -Toxin Production by 201 Strains of Staphylococcus

Rabbit-Blood-Plate Test	α -Toxin in Broth		Coagulase	
	Positive	Negative	Positive	Negative
162 α -toxin-positive strains	162	Nil	162	Nil
39 α -toxin-negative strains	1	38	6	33

On purely clinical grounds it was impossible to be certain of the pathogenicity of the majority of the staphylococci examined. However, 37 strains were certainly pathogenic for man (isolated from pus from previously unopened, non-contaminated abscesses, films of which showed clusters of Gram-positive cocci only and which yielded pure cultures of staphylococcus). Of these 37 certainly pathogenic strains 36 were positive by both plate and broth test and 37 were coagulase-positive. Evidently the correlation between pathogenicity and α -toxin production on plates is close.

Plate methods of detecting α -toxin production are much more simple to perform than the broth method. In addition, the interpretation of the results of the latter is sometimes rendered difficult by haemolysis occurring rapidly in the control tube (due to lysins other than α -toxin).

Sheep-Blood-Agar Plates.—We have used the filter-paper-strip method on sheep-blood plates in parallel with rabbit-blood plates in testing 33 strains (20 α -toxin-positive and 13 negative). Identical results were obtained in this small series. The specific spear-head zone of lysis is as clear as with rabbit blood, but presumably there is a danger that small amounts of α -lysin may be obscured by β -lysin if sheep blood is used (Williams and Harper, 1947).

Summary

A plate method of detecting staphylococcus α -toxin production is described. Filter-paper strips containing α -antitoxin are used on 2% rabbit-blood-agar plates incubated in an atmosphere containing 30% CO₂. The interaction of the toxin and antitoxin diffusing into the agar at right angles to one another causes a sharp tapering of the wide zone of lysis surrounding α -toxigenic strains. This appearance is specific for such strains.

The test is easy to perform and to interpret, and the results have shown a close correlation with tube α -toxin tests, coagulase production, and the clinical pathogenicity of the strains examined.

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