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RECURRING PHENOMENA EXHIBITED BY
SOME BACTERIA AND FUNGI

EX AEDIBVS ACADEMICIS IN CIVITATE VATICANA



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RESEARCHES ON CERTAIN LONG TERM RECURRING PHENOMENA EXHIBITED BY SOME BACTERIA AND FUNGI

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SUMMARIVM -- Facta quaedam describuntur, quae in nonnullis bacteriis et mycetis ex longis vel longissimis intervallis evenire solent, neque in mutatis loci aut nutrimenti circumstantiis causam habent.

Luculentum exemplum est in lactosio *Bacilli colombensis* Sast., quod, cum primum separatur, negativum (neque acidum neque vapor) est, et post temporis intervallum, quod plerumque longum est, subito fit positivum (et acidum et vapor); post paucos autem dies vel hebdomadas iterum negativum fit subito lactosium, itaque alternis semper vicibus negativo statui, qui fere semper per longum tempus permanet, positivus succedit status per breve plerumque temporis spatium, idque videtur sine fine accidere.

Eius microorganismi culturae haberi possunt ab ipsius Auctoris Instituto (Instituto de Medicina Tropical, Junqueira, Lisboa) et ab American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A.).

The practically continuous observation for several decades of some bacteria and fungi isolated by me long ago has enabled me to note certain long term recurring phenomena which cannot be ascribed to environmental and nutritional factors.

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The present paper is concerned with three such phenomena: 1) the « *Bacillus* » *columbensis* long term recurring lactose fermentation phenomenon; 2) the *Trichophyton balcanicum* (*Trichophyton glabrum* var *balcanicum*) long term recurring pseudopleomorphism phenomenon; 3) the long term recurring *Geotrichum matalense* complete change of cultural appearance.

« *BACILLUS* » *COLUMBENSIS* LONG TERM RECURRING LACTOSE
FERMENTATION PHENOMENON

« *Bacillus* » *columbensis* (for description *vide infra*), a bacterium belonging to the *Enterobacteriaceae* found by me very many years ago in Ceylon, is on first isolation a non-lactose fermenter (neither acid nor gas) and remains a complete non-lactose fermenter for many months, then it suddenly becomes a lactose fermenter (acid and gas) for some days or a few weeks, and then again becomes a non-lactose fermenter (neither acid nor gas) for a long time, then once more a lactose fermenter (acid and gas) and then again a non-lactose fermenter (neither acid nor gas) and so on, it would appear indefinitely, the periods of non-lactose fermentation being usually long, and the period of lactose fermentation, usually brief. The phenomenon occurs also with strains derived from a single cell. Further details are given later in the description of the organism.

« *Bacillus* » *columbensis* being little known and seldom mentioned in text books a short description of it is appended.

« *Bacillus* » *columbensis* Cast. 1905. The term « *bacillus* » is used in this paper not to indicate a special genus but merely as a convenient general term to indicate any rod-like organism the definite classification of which has not been reached.

« *Bacillus* » *columbensis* was first isolated by me in the remote year 1905 from a case of typhoid) like fever in Ceylon and again some years later, 1913, in the same country from

two further cases of typhoid-like fever, one with urinary complications (Central. f. Bact. Ed. 65, Heft. 4-5, 1912; Bd. 74 Heft. 3-4, 1914). The three cases were laboratory negative (agglutination, haemoculture, stool plating) for typhoid, paratyphoid-A, paratyphoid-B. The organism was grown from the faeces in all three cases and also from the blood in one (in the incipient stage of the malady) and from the urine in another. Specific agglutinins were present in the blood. The three strains were identical biochemically and demonstrated to be identical also by agglutination procedures and by using my absorption test, which was still something of a novelty in tropical bacteriological laboratories, although described by me some years previously (« Zeitschrift. f. Hyg. u. Inf. », 40, 17, 1902). They are still extant and their characteristics have remained essentially unchanged during the several decades of continuous cultivation on ordinary plain agar, subculturing being carried out on an average of every two or four weeks.

Since 1913 cases of typhoid-like fever apparently due to *B. columbensis* have been recorded by several workers, among whom SPAAR in Ceylon (« J. Trop. Med. and Hyg. », 1, 281, 1915), by LURIE in the Balkans (« Lancet », 1, 350, 1916), by KELSART in England (« Lancet », 1, 381, 1925), by MARMO in Somalia and Eritrea (« J. Trop. Med. and Hyg. », 35, 20, 1932), by THOMAS and BOSE in India (« Ind. Med. Gaz. », 71, 327, 1936), by SCOTTI in Italy (« Policlinico », 43, 727, 1936), by SERVINO in Italy (« Acta Med. Ital. Inf. », 1, 281, 1946). A joint paper by SERVINO and myself on *B. columbensis* and other little known intestinal bacteria was read before the Sixth International Congress on Microbiology held in Rome in September 1953 (« Atti VI Congr. Intern. Microbiol. », 1, 331-334) which however, did not attract the notice of writers on intestinal bacteriology. Recently a valuable paper has appeared by MUNGELLUZZI giving her results of a prolonged investigation of the organism (three strains), confirming my old

researches and giving important new information on the antigenic structure of the organism and its sensitivity to antibiotics (« Arch. Ital. Scien. Med. Trop. Paras. », 44, 345, 1963).

The organism has been placed in many different genera: *Salmonella*, *Escherichia*, *Enteroides*, *Morganella*, *Proteus*.

Laboratory Data. — « *Bacillus* » *columbensis* is an intestinal rod-like organism motile, aerobic, facultative anaerobic, non-sporigenous, Gram-negative, non-pigment producing, not liquefying gelatine and coagulated serum. It does not clot milk, it produces a slight initial acidity followed by alkalinity. It produces indol. Voges-Proskauer negative. Methyl red positive. Remains viable, and actually multiplies for a time in sterile distilled water for over a year, like many other members of the Enterobacteriaceae (CASTELLANI, 1962, « Annals of the New York Ac. of Sciences », 93, 5, 155). It ferments with production of gas, glucose, levulose, maltose, mannitol, galactose, dulcitol, and glycerol but not saccharose, and these fermentation reaction are stable, they have never changed through the many years of cultivation. Of special interest is its action on lactose; on first isolation the organism is a complete non-lactose fermenter (neither acid nor gas) and remains so for many months, then it abruptly becomes a lactose fermenter (acid and gas) for some days or a few weeks, then again it becomes a complete non-lactose fermenter (neither acid nor gas), then once more a complete lactose fermenter then a complete non-lactose fermenter and so on, it would appear indefinitely the periods of lactose fermentation, usually short, alternating irregularly with the periods of non-lactose fermentation, usually long. The phenomenon occurs also with cultures derived from a single cell. An interesting point is that during the periods of fermentation of lactose in peptone water milk is not coagulated, it undergoes a slight initial acidity followed by alkalinity.

For years I was sceptical about the phenomenon of *recurring* lactose fermentation but after repeatedly carrying out all sorts of controls I had to come to the conclusion that the phenomenon does occur. The phenomenon is of difficult explanation. Is it due to the presence of a symbiont? Plating and replating during the periods of lactose fermentation has never shown the presence of a symbiont or a contaminant, all the colonies were of the same organism. It has been suggested that the phenomenon might be due to different methods of lactose sterilization, but the method of sterilization has always been the same standard one. It has been suggested that the organism is metely one of the so-called late lactose fermenters, but late lactose fermenters once they have become lactose fermenters they remains so, they do not once more become complete non-lactose fermenters (neither acid nor gas). It has been suggested that the organism is the old *Bacillus colimutabilis* of MASSINI but *B. colimutabilis* was unstable not only with regard to lactose but with several other sugars and moreover it coagulated milk and was not pathogenic.

« *Bacillus* » *columbensis* appears to be a homogenous species biochemically and serologically; the strains so far fully investigated have all shown essentially the same biochemical characteristics including the recurrent lactose fermentation. They have also been shown to be serologically identical by agglutinations and by absorption tests.

In recent years further researches on the antigenic structure of *B. columbensis* have been carried out by a few investigators. EWING (private information) has described an antigenic pattern according to which the organism should be placed in the genus *Escherichia* while MUNGELLUZZI has found a pattern of antigenic structure tending to show that the organism should be placed in the genus *Salmonella*, or at any rate nearer *Salmonella* than *Escherichia*.

It is interesting to note that if the original definitions of the genera *Salmonella* an *Escherichia* were strictly adhered to

the organism could not be placed in either, not in *Salmonella* owing to the recurring lactose fermentation and not in *Escherichia* as milk is never clotted.

The following are the original definitions of the genera *Salmonella* and *Escherichia* (CASTELLANI and CHALMERS', *Manual of Tropical Medicine*, pages 538 and 941 respectively).

Genus *Salmonella* (LIGNIERES, 1900) emend. Cast, and Chalm. Ebertheae which completely ferment glucose but do not ferment lactose, and partially or completely ferment mannitol in addition to other carbohydrates. Milk is not clotted. Type species *Salmonella paratyphi* (SCHOTMÜLLER, 1902).

Genus *Escherichia* Cast. and Chalm. 1919 Ebertheae which ferment glucose and lactose completely; milk clotted. Type species *Escherichia coli* (ESCHERICH, 1886).

In accordance to the recommendation made by the *Enterobacteriaceae* sub-committee (VI International Congress of Microbiology, 1953) that the *Salmonellae* and *Escherichiae* and related organisms described before 1953 might be known by name and not only by antigenic formula, it would appear that the specific term *columbensis* should be retained, the organism having been found and described long before that date. As to its taxonomic position whether a *Salmonella* or an *Escherichia* or whether representing a new species opinions vary widely and it might be of advantage in practice to continue to refer to it as « *Bacillus* » *columbensis* using the term « *Bacillus* » not to denote a special genus but merely as a convenient general term — as done by TOPLEY and WILSON in their classic treatise — to indicate any rod-like bacterium the generic position of which is still in a state of flux.

I shall be glad to send cultures of *Bacillus columbensis* to medical and laboratory workers desiring them.

«TRICHOPHYTON BALCANEUM» LONG TERM RECURRING PSEUDO
PLEOMORPHISM PHENOMENON

Trichophyton balcaneum (*Tr. glabrum* Sabouraud var. *balcaneum* Cast.) was isolated by me in the year 1915 in the Balkans from a diffuse scaly condition of the scalp resembling a severe form of pityriasis sicca rather than tinea. It is probably merely a variety of *Trichophyton glabrum* Sabouraud.

It grew on glucose agar, producing a somewhat nodular or crinkled or slightly convoluted glabrous, dirty whitish colony; it liquefied gelatine rapidly; it clotted milk. Complete absence of aerial mycelium. Microscopically no *macro-conidia*, no spirals and no nodular or denticulated bodies were seen; only mycelial threads with a few very doubtful *micro-conidia*. The fungus continued to show these cultural characters for *thirteen years*, until in 1928, when definite changes appeared; the colonies become covered with whitish duvet (aerial mycelium), not very long but quite abundant, and on scraping it off the growth appeared as a smooth, flattish mass, not nodular nor crinkly, not convoluted in parts. I thought the organism had become pleomorphic although it is generally stated that the fungi of the *glabrum* type never so so. These new features remained unchanged for about a year and then, at the end of 1929 the serial mycelium disappeared and the colonies began to show again a glabrous somewhat knobby slightly convoluted aspect. *Five years* later, in 1934 the colonies once more became fluffy and then these totally different appearances of the growth have alternated at long intervals of years *although the medium has always been the same viz* glucose agar 4% until 1930, later 2% and the transplanting has been all along at about a months interval. At present, January 1966 the cultures have the same appearance as on first isolation in 1915.

THE LONG TERM RECURRING « *Geotrichum matalense* » COMPLETE CHANGE OF CULTUREL APPEARANCE

The original strain of *Geotrichum matalense* Cast. isolated in 1914, is still in my possession. When first isolated and for many years thereafter the cultures on glucose agar appeared very fluffy and whitish; when this long duvet was scraped off, the surface of the growth was smooth, not rugose not convoluted. In 1928 after continuous subculturing on glucose agar for 13 years (approximately once a month and using glucose agar prepared with the same formula, 4% glucose up to 1932, since then 2%), the duvet disappeared, and the growth became deeply rugose and somewhat convoluted in parts, with a smooth, glabrous, somewhat moist-looking surface. In 1934 the original characters, with an abundance of duvet, returned. In 1936, the duvet again disappeared, to return a few months later. Eighteen years later in 1964 the long abundant aerial mycelium again disappeared and the growth showed large convolution as in *Geotrichum rotundatum*. In December 1965 once more the white duvet reappeared and on scraping it off the surface the growth was flattish, smooth as when first isolated in 1915.

In this paper describing three peculiar long term recurring phenomena I have contented myself with merely quoting facts and observations without any attempts being made at formulating theoretical explanations, which may perhaps be the subject of a future communication.