INTERSPECIFIC HYBRIDIZATION: A POSSIBLE MODE OF THE ORIGIN OF "ATYPICAL ACID FAST MYCOBACTERIA.

In a preliminary experiment a strain of chromogenic acid fast mycobacterium was obtained by the mixed cultivation of a BCG strain of M. tuberculosis and a saprophytic mycobacterium: M. phlei * Colonies of the new organism were picked from a selective medium, that contained 50 ug streptomycin per ml and tryptose agar and which did not allow the growth of either of the parent organisms if planted alone.

The streptomycin resistance /S^r/ of the BCG strain, on the one hand, the pigment formation / Pig^+/ and the capability of growth on plain agar medium /Ag^+/ of M. phlei, on the other hand , were selected as marker characteristics /BCG = S^r Pig^- Ag^-, M. phlei = S^s Pig^+ Ag^+/. Other differences in the properties of the parent strains like: growth at room temperature, in liquid medium, peroxydase activity, resistance to INH, PAS, colony appearance and cellular morphology have added to the number of distingishing features as unselected markers /See Plate I/

The descendant organism: $S^r Pig^+$ Ag^+ retained a much larger portion of the main features of M. phlei than those of M. tuberculosis /BCG/, as for instance the capability of growth on agar, pigment formation, colony appearance etc.

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* By a slight modification of the experimental conditions 8 more strains were obtained, but could not yet be examined in all details. A prolonged cultivation of the culture mixture in a medium favoring growth of both parent organisms prior to the exposure to the selective environment was found to yield a more uniform result.

It did inherit on the other hand the streptomycin resistance of the BCG parent organism, displayed it, though at a much higher level than it was established in the BCG strain / resistance to over 1000 ug per ml as compared to 50 ug per ml: 20 fold increase/. In addition to this considerable increase in the streptomycin resistance it showed on first isolation another new feature: a sort of streptomycin semi-dependance which presented itself in the better and faster growth of the micro-organism in the presence of streptomycin both at 37° C and at room temperature. Apart from the emergence of such new properties, and properties which resembled those possessed by one or the other of the parent strain, or even both of them/ e.g. catalase activity /, a considerable number of characters appeared kind of inbetween the properties of the BCG and M. phlei strain / e.g. INH, PAS resistance, peroxydase activity, growth in liquid medium, at room temperature /.

A population study showed evidence that segregation in the new organism occurred at a very high rate when subcultures were made on non-selective medium,- segregants mainly resembled the M. phlei parent organism. Only a minor portion of the population retained after 15 passages on non-selective medium the streptomycin resistance, but even this portion of the population showed properties otherwise similar to those of M. phlei / INH and PAS resistance, growth at room temperature ect. / Streptomycin-dependant individuals were, of course, selectively eliminated of the population in the absence of streptomycin. - If , however, serial transfers were made on media containing streptomycin, not only did the selective environment favor

the outgrowth of streptomycin resistant, and the more so the streptomycin-dependant individuals, but helped at the same time to conserve some of the original traits of the new strain / e.g. growth at room temperature /.- Pure clone studies are on the way.

The clumpy growth of mycobacteria is well know to cause difficulty in making a reliable viable cell count, still a rough estimation would show that the rate of occurrence of the [redacted] organism is somewhat in the proximity of 10^{-5} - 10^{-6} counted per M. phlei cells

No systematical attempt was as yet made at excluding the possibility of transformation or transduction as the genetic mechanism by which the individuals of the new strain are produced. Still, there exists some indirect cytological evidence indicating that a cell-to-cell contact: i.e. conjugation may take place and that this could play a major role in their origin. While the change from the streptomycin sensitivity to the streptomycin resistance per se could be attributed to an additional mutational step, the assumption that mutation alone, as a mere change from the streptomycin sensitivity of the M. phlei organism to the streptomycin resistance may wxplain the whole phenomenon seems unlikely in view of

I. the high frequence of segragation,

2. streptomycin alone could never bring about changes in the M. phlei strain as experienced with large numbers of controls / although the possible inducing role of the BCG organism in the presence of streptomycin at first could not be completely discounted /.

3. finally- and this appears to be of decisive importance-

-it could be shown that the mixed cultivation in a streptomycin free medium of a/ the streptomycin sensitive M. phlei strain, which has never been in any previous contact to streptomycin, and b/ the streptomycin resistant M. tuberculosis / BCG / strain, resulted in the formation of chromogenic strains completely resistant to streptomycin, when plated on tryptose agar containing 500 ug streptomycin per ml.

Conclusion: A new strain of mycobacterium: [redacted] was obtained by means of a mixed cultivation of M. tuberculosis/ BCG / and M. phlei. The progeny appears to possess some new characters, while others resemble the characters of the two parent organisms. The main interest in obtaining such strains lies in the fact that the properties of this [redacted] organism are characteristic for the " atypical " acid fast chromogenic mycobacteria, the origin of which aroused such a big interest in recent years.

TABLE I.

	PARENT M. tb. / BCG /	STRAINS M. phlei	NEW STRAINS [redacted]
SM resistance over 1 ug	+	-	+
over 100 ug per ml	-	-	+ / 1000 ug/
Pigment formation	-	+	+
color of pigment		yellow	yellow which can change to orange
Growth on agar	-	+	+
Growth in Tween-alb.	Deposit	Turbidity, later deposit	Mainly deposit first
Growth at room temp.	-	+	+ /slow/
days required on agar		7-15	20-25*
on SM agar /100ug/		-	5-12
INH resistance over 1 ug	-	+	±
over 10 ug per ml		±	-*
PAS resistance over 1 ug	-	+	+
over 100 ug per ml.		+	-*
Catalase activity	+	+	+
Peroxydase activity	+	-	±
Colony appearance	R	S	S
Cellular morpology	Mainly slender acid fast rods	Pleomorhic forms Many coccoids. Partial acid fast -ness	Pleomorphism. forms varying with culture medium.

* Unstable characters.