6000 feet in all subjects, at which altitudes the question of anoxia can hardly arise. The simplest explanation is that the fall of atmospheric pressure, by removing some of the external support of the pulmonary capillaries, allows dilatation thereof, resulting in a larger volume of blood and a smaller volume of air in the lung. Such capillary dilatation, although increasing the surface of blood exposed to alveolar air, diminishes the surface: mass ratio for pulmonary blood as well as the vital capacity. Blood examinations, however, show that both of these are well compensated by increased ventilation.

It is possible that the increased mass of blood in the lungs may be one of the factors favorably influencing certain forms of pulmonary tuberculosis at moderate altitudes.

Summary and Conclusions. 1. Apparent variations in pulmonary vital capacity are found from day to day and from season to season. When corrections are made for temperature and atmospheric pressure, these variations disappear. 2. True vital capacity diminishes at moderate altitudes, changes appearing even at 3000 feet. The various factors to which these changes have been attributed by different observers were absent in our experiments. Diminished vital capacity at altitudes is believed to be due to dilatation of lung capillaries following the partial loss of external (atmospheric) support, with consequent diminution of air space. Such an increase of blood in the lungs may be a factor in the favorable effect of moderate altitudes on certain forms of pulmonary tuberculosis.

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Variability of Tubercle Bacillus. I. Mucoid Phase of the Human-Type Strain H-37.

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The characteristic attributes of both the rough and the smooth phases of the human tubercle bacillus have been amply recorded, but there has been no indication of the existence of a mucoid phase such as occurs in the dissociative pattern of many other microörganisms.[1] The isolation and subsequent growth of a mucoid phase in the human tubercle strain, H-37, is therefore briefly presented.

[1] Hadley, P. B., *J. Inf. Dis.*, 1937, **60**, 129.

The parent strain was a stock culture of H-37 (intermediate rough phase) on Petragnini egg-medium kept under paraffin seal at room temperature 4 months. The growth was dry, rough, crumbly; the organisms were pleomorphic rods, some being hooked or curved, others showing Y-formation or lateral budding. Many rods contained large granules and all organisms were strongly acid-fast.

The first experimental transfers from this growth were made to 3 liquid mediums: (1) a synthetic medium, pH 7.2, (2) 5% glycerol broth, pH 7.6; (3) a double-strength veal-infusion broth, resterilized, with 2% neopeptone (Difco), 5% glycerol, 0.04% asparagin-l, pH 8.0. The transfers were made, not to the surface, but to the bottom of each flask of liquid. In the first 2 mediums the growth was very slow and finely granular, with no later turbidity or pellicle-formation. The organisms were coherent or discrete beaded acid-fast rods. Repeated transfers from these mediums to a variety of solid mediums produced the dry, rough growth only, typical of culture H-37 containing short acid-fast rods.

The growth in neo-peptone glycerol-broth, pH 8.0, at first resembled that in the other 2 mediums. On the 9th day of incubation at 37°C, however, there developed a slight cloud above the granular growth, and on the 10th day the fluid was turbid. A needle withdrawn from the fluid carried with it material that formed a filament 2 feet long before snapping from the surface of the broth. The organisms in the granular growth were short acid-fast rods with very large terminal granules; those in the supernatant fluid were non-acid-fast, except for a slight retention of the stain (carbol-fuchsin decolorized with 5% nitric-acid-alcohol) in the granules only. Counterstained with Loeffler's methylene blue, the organisms were granular diphtheroid rods, some having clubbed ends. These organisms transferred to Loeffler's serum and to blood agar produced a confluent, almost clear mucoid growth with the consistency of egg-albumen. Most of the individual organisms were composed of large, centrally placed, deeply staining granules in short rods massed together in a matrix of amorphous material. In repeated transfers on Loeffler's serum the growth remained mucoid; the organisms became longer, polar-stained granular, non-acid-fast rods.

The mucoid growth on 2 other solid mediums merits description. One is an agar medium prepared from the filtrate of the growth, in alkaline neo-peptone glycerol-broth, of a "Critical Rough" strain of tubercle bacillus. The peculiar properties of the tuberculin produced by this organism have been described.[2] The pH is determined

[2] Mellon, R. R., Beinhauer, L. G., *Arch. Derm. and Syph.*, 1937, **36**, 515.

(7.2-8.0), 2% agar, 0.04% asparagin-*l*, at times 1% dextrose, are added. This medium is called CR agar. The other medium is a combination of the formulas of Löwenstein[3] and of Herrold,[4] omitting the glycerol in the former and substituting neo-peptone for bacto-peptone in the Herrold formula. This medium also contains dextrose and is referred to as LH agar. These mediums in this and other work have been very useful in enforcing and maintaining distinct coccal phases of the tubercle bacillus, which will be described in detail in another communication.

On the CR agar—and especially with dextrose—the mucoid growth is abundant, occurring typically in a peripheral wall-like formation about the colony. On standard glycerol agar the growth is more scanty, and rubbery in consistency. On the very alkaline CR agar the mucoid phase, after suitable streaking, has given rise to a scantily growing, very minute dry colony which pits the medium. The organisms are not miscible in water and stain poorly. They are non-acid-fast, short diphtheroid rods. The growth of the second diphtheroid in the dry colony is very sparse on the alkaline CR agar, less so on more acid CR agar, absent on standard glycerol agar.

On the LH agar a slow, but definitely spreading growth of the mucoid phase occurs. The center of the growth eventually is viscid and firm—the edge is more fluidly mucoid. The organisms in the two portions differed in the first transfer to this medium. The centrally located organisms were granular, non-acid-fast diphtheroid rods; those in the advancing peripheral growth exhibited remarkable pleomorphism—large, heavily granular organisms, with budding, branching, and terminal clubs—in short, a distinctly fungoid appearance. Moreover, nearly all of the organisms showed a partial return to the acid-fast condition, single structures retaining both acid-fast and non-acid-fast stain, especially noticeable in and near the club-formations which appeared to be the actively growing portion of the organism. On later transfers to the same medium the bacilli have become progressively more acid-fast and approach the pleomorphic picture of the original inoculum.

Repeating the original experimental transfers with freshly growing strains of H-37 on Petragnini medium has resulted in nothing but the usual rough growth in any medium used, either liquid or solid. But an examination of old stock strains of H-37 stored on Petroff's gentian-violet egg-medium in the cold for 6 to 9 months revealed a softening, gummy growth with a markedly pleomorphic

[3] Löwenstein, E., *Deutsche med. Wchnschr.*, 1930, **56**, 1010.

[4] Herrold, R. D., *J. Inf. Dis.*, 1931, **48**, 236.

morphology. *Such cultures only* have produced the mucoid growth in the alkaline neo-peptone glycerol broth, a growth which, so far, has not occurred in any other liquid medium tested. Further work is in progress as to the exact rôle played by this medium and the factors responsible for bringing to maturity the dissociation of the mucoid phase, which appears to be initiated in the aging cultures on Petroff's or other egg-mediums. Steenkin[5] has also noted the relationship between softening growth and pleomorphic rods of old tubercle cultures on egg-mediums; and particularly the eventual outgrowth of variants from the softened areas which, in his experience, however, were not mucoid.

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Magnesium and Calcium in Striated Muscle as Revealed by the Electron Microscope.*

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Several years ago McMillen and Scott[1] described a simple magnetic electron microscope designed for use in localizing certain inorganic salts in biological tissues. After some modifications of this apparatus, mostly in the direction of increasing the magnification,[2] and much experimentation it has been possible for us to localize magnesium and calcium in tissues. Striated muscle is an appropriate tissue for the purpose since it has been shown by other methods that the salt distribution is orderly and precise.[3, 4]

The nearest approach to chemical conditions obtaining in the living is had by examination of tissues prepared for study by the Altmann-Gersh frozen dehydration method. Sections of tissues fixed by a modification of this technic were attached to a barium and strontium coated cathode and placed in the electron microscope. After evacuation of the instrument the cathode was heated slowly and the section vacuum-distilled until only the inorganic salts remained. The

5 Steenkin, W., Jr., *Am. Rev. Tuberc.*, 1938, **38**, 777.

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1 McMillen, J. H., and Scott, Gordon H., *Rev. Scientific Inst.*, 1937, **8**, 288.

2 Scott, Gordon H., and Packer, D. M., *Anat. Rec.*, 1939, in press.

3 Scott, Gordon H., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 349.

4 Scott, Gordon H., *Am. J. Anat.*, 1933, **53**, 243.