cork glued to the walls of the block and the retaining plate prevent breaking of the pipette.

The capillary pipette is a 5-mm glass tube, one end of which has been drawn into a long, thin point and bent to form an angle of about 135° with the tube. At the other end of the tube, a small but high nut with a threaded bolt to match is fastened by means of glass cement. It is very important that the bolt should fit exactly into the thread of the nut and that it is greased with a heavy lubricant of the kind used for glass stopcocks of burettes, in order to make a completely air-tight connection. It is desirable to have a set of capillary pipettes, the points of which are 25, 50, 100, and 500 μ thick respectively.

When the apparatus is to be used, the block is fastened to the objective, after which the capillary pipette chosen is placed in such a position that the mouth of the pipette is clearly visible in the center of the field of vision (magnification about 100 times) and the point is nearly horizontal.

If a single microscopic organism is to be selected from a fluid suspension of organisms, it cannot be done by lowering the empty capillary pipette into the drop on the slide. The capillary action, which increases with the diminishing diameter of the pipette, will immediately carry fluid with numerous organisms into the tube. The pipette must be allowed to suck up sterile water and by turning the threaded bolt a convenient column of water remains; if the pipette is then let down into the suspension by lowering the tube of the microscope, no organisms will be sucked up. By means of the mechanical stage the slide is now placed in such a position that the organism selected is situated before the mouth of the pipette; a slight counterclockwise turning of the threaded bolt of the capillary pipette will then carry the cell into the pipette, after which the capillary pipette is removed from the suspension containing other organisms by raising the tube of the microscope.

If an organism is wanted for the cultivation of a clone, the following procedure may be used. After the selection of one cell, the slide with the suspension is replaced with a sterile slide, on which a sterile cover glass is placed. The tube of the microscope is lowered until the point of the capillary pipette touches the cover glass; a slight clockwise turning of the threaded bolt will now eject a droplet of water containing the chosen cell onto the cover glass. At a magnification of, say, 200 times it should now be ascertained that the droplet contains one and only one cell. If this is the case, the cover glass can be slipped from the slide into the sterile fluid in which the species is to be cultivated.

The apparatus can also be used for making permanent slides of new or rare species, where only a few individuals were found among many others, for instance, desmid individuals in a plankton sample. Some drops of glycerine should be added to the sample, which is then placed on a watch glass or in a salt cellar to allow a slow evaporation of the water. The specimens wanted can now be selected from the glycerine suspension and placed in a droplet of pure glycerine on a slide for the usual method of preparing the slide.

Pterin-like Pigment Derived from the Tubercle Bacillus. Fluorescence and Absorption Spectral Data for Erythropterin-like Pigment Isolated by Ultrachromatographic Analysis

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The pterins are pigments having a purine or pyrimidine type of structure that were isolated from the wings of butterflies and from the integument of other insects, first by Hopkins (*5*) and later by H. Wieland and his collaborators (1925–1944), and by Schöpf and Becker (*11*), and others. These pigments usually occur as mixtures, have no melting points, and cannot be transformed into derivatives with definite melting points. Hence, they present exceptional difficulties in their separation and purification. The determination of absorption spectra was one of the criteria used, in the studies cited, as tests in successive stages of purification, and in order to characterize the different pterins derived from different sources (*11*).

We present evidence in this report that pterin-like pigments have been isolated from the tubercle bacillus which, to our knowledge, have not heretofore been reported for this microorganism. Fluorescence and absorption spectral data and other physical characteristics are given for material obtained from the acetone and ether extracts of dried virulent human tubercle bacilli[1] after they had been washed with large quantities of sterile distilled water. This treatment is one of the steps in the preparation of antigens used in the complement-fixation test for tuberculosis (*14b*).

Observations in ultraviolet light were made of zones (some not visible in daylight) formed when a few drops of the extracts were placed on the margin of the thin wedge of an adsorbent such as aluminum oxide, standardized according to Brockmann (*1*) by Merck, hereafter called alumina. These zones yielded fluorescence other than the red fluorescence that may be attributed partly to porphyrin and the yellow fluorescence of flavin reported in our previous studies (*4*). This micromethod (*2*) indicated the practicability of separating some of the materials on chromatographs of alumina.

Numerous exploratory experiments indicated that a better separation of zones was effected by the use of benzine-ether 1:1 and chloroform solutions of the residues of ether and acetone extracts than with the original extracts. In some of the experiments the extracts were partitioned with immiscible solvents prior to placing them on chromatographs. The yield of material from the different zones was in each case very small. Hence, it was not possible to make all of the various characterizations

[1] Human tubercle bacilli strains #13 and #48189 were grown for 3–4 weeks in glycerol broth which consists of beef, 450 g; peptone (Fairchild's), 10 g; sodium chloride, 5 g; glycerol, 50 g; and water, 1000 g (*14a*).

that were reported by Schöpf and Becker for the much greater quantities of pigment that they extracted from the wings of a million butterflies. Our material has been used chiefly for the determination of absorption and fluorescence spectra. These spectroscopic data and observations of the general chromatographic behavior and patterns of the various zones when the chromatographs are developed closely parallel those reported for pterins from butterflies, as do certain other characteristics.

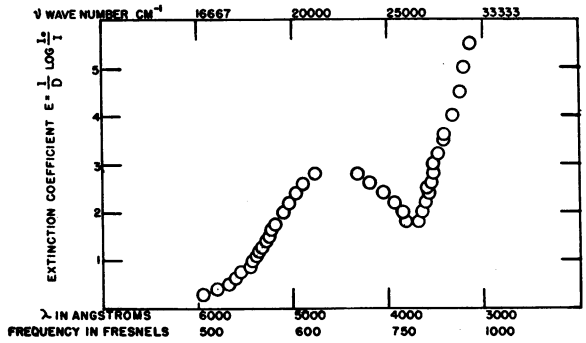

FIG. 1. Ultraviolet absorption spectrum curve. Erythropterin-like pigment (in chloroform) isolated from human tubercle bacillus.

The number of fluorescing pigments revealed on our chromatographs and the reported difficulties of separating them preclude an exhaustive study of all at this time. Observations have been focused mainly on the material from the dark velvet brown fluorescing zone (orange-red in daylight and the only one clearly visible), which has many other characteristics similar to those reported for erythropterin (*11*).

The ultraviolet absorption spectrum curve for this pigment in a limited range (Fig. 1) shows a band at about

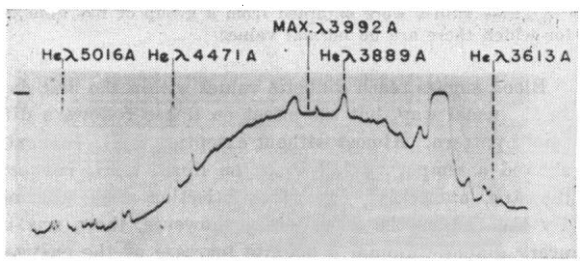

FIG. 2. Microphotometric tracing of a fluorescence spectrogram of erythropterin-like pigment (in chloroform) isolated from human tubercle bacillus.

λ 4540 A. Exploration of the shorter wavelength region revealed a band at about λ 2375 A and an inflection between λ 2900–3200 A, indicating selective absorption in the same wavelength positions reported by Schöpf and Becker for an erythropterin preparation (*11*).

A violet fluorescence in dilute ammoniacal solutions of erythropterin and changes in the fluorescence with changes in pH values have been observed (*11*). We also have noted similar fluorescence with the pigment recovered from the orange-red zone. A microphotometric tracing of the fluorescence spectrogram (Fig. 2) shows an emission band extending from λ 3580 to 4635 A with a maximum at λ 3992 A.

The chromatographic behavior on a column of alumina has been reported (*11*) for a mixture of several pterins —erythropterin, chrysopterin, xanthopterin, etc. The erythropterin is adsorbed at the top of the column as a velvet brown fluorescing zone, chrysopterin as a yellow-green fluorescing zone just below the erythropterin and above the yellow fluorescing xanthopterin zone. Moreover, a brilliant light violet-blue fluorescing zone usually appears in juxtaposition with the velvet brown fluorescing zone and is ascribed by Schöpf and Becker to a transformation product of erythropterin. We also have observed this sequence of zones on our chromatographs. The medium which is used in the cultivation of the tubercle bacillus, when concentrated ten times and passed through columns of alumina, did not show this zone formation. However, the effect of strain differences and of variations in medium are being investigated.

Erythropterin, as reported by Schöpf and Becker (*11*), is precipitated from N ammoniacal solution by treatment with N HCl as a fine red powder that is easily soluble in water. On evaporation of the solution the residue is amorphous. An ammoniacal solution of the pigment, when treated with an ammoniacal solution of silver nitrate, yields a fine dark red precipitate, which when heated turns brown, then black. Erythropterin gives no murexide reaction. It is soluble in concentrated nitric acid; but is quickly destroyed and the dark red solution becomes colorless (*11*). We also have observed the same characteristics for the erythropterin-like pigment isolated from the tubercle bacillus.

The wavelength positions of selective absorption, the fluorescence characterization, chromatographic behavior, and many other reactions observed for our material are similar to those reported by other investigators of pterins (*5, 11*). Hence the presence is indicated of a new group of pigments not previously reported to be isolated from the tubercle bacillus. A more detailed report will be published later.

We also have reported the isolation of pterin-like pigment from the diphtheria bacillus (*3*). Members of the pterin group of pigments have been found in animal and human urine (*8*), mammalian tissue (*9*), and in argentaffine cells in the epithelium of the stomach and intestine, with the same localization as that of the substance active against pernicious anemia (*6, 7*). Studies have been made of the activity of pterin in preventing clinical anemia (*13*), and also in the field of nutrition by Totter and colleagues (*12*), Pritchard (*10*), and numerous other workers. Such investigations, if further substantiated and extended, will result in the pterins' becoming of wide interest and importance in the fields of biological and medical research.

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Serum Lipase and Alloxan Diabetes in Rats[1]

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It was found by Cantor, Tuba, and Capsey (*1*), that the production of alloxan diabetes in the adult male albino rat is paralleled by a pronounced increase in the activity of serum alkaline phosphatase. The development of the diabetic condition is usually accompanied by a marked transient lipemia, which appears about two days after the injection of alloxan and which then decreases after approximately a week. It seemed probable that the existence for several days of a severe lipemia might be reflected in altered serum lipolytic activity, and this paper presents our observations with the enzyme which hydrolyzes tributyrin. This enzyme, given a variety of names in the literature, will be called lipase, although it could equally well be called tributyrinase (*2*).

Twenty adult male rats were starved overnight and in the morning each was given a subcutaneous injection of 16 mg of alloxan monohydrate for each 100 g of body weight. The animals were fed Purina Fox Checkers and water ad lib. As a result of alloxan injection, diabetes was produced in fifteen rats; three of the animals died, while two recovered from the initial hyperglycemia.

Blood for glucose and lipase determinations was taken from the tails of the animals always at 8 A.M. Blood glucose was estimated by the method of Reinecke (*3*). Lipase activity was determined within 24 hr after the serum was obtained although the enzyme was found to be stable for several days in a refrigerator. The enzyme concentration of the sera was determined by a micromethod which will be described fully elsewhere. Essentially it consists of estimating by titration with NaOH the acid set free from tributyrin by the enzyme contained in 0.1 ml serum. Hydrolysis of the finely dispersed substrate proceeds for 30 min at 37° C in an aqueous digest buffered with sodium diethylbarbiturate at pH 7.9. The lipase activity of a serum in units is equivalent to the number of ml of 0.025 N NaOH required to neutralize the butyric acid which would be set free by the enzyme contained in 100 ml of serum under the above experimental conditions. One ml of 0.025 N NaOH is equivalent to one lipase unit. The normal mean value for serum lipase in 30 adult male albino rats was found to be 556 ± 70/100 ml, with a range from 390–690 units/100 ml. Activity of the enzyme in individual rats varies slightly from day to day, but levels are not appreciably altered by starvation for 5 days.

The effect on serum lipase activity of alloxan is indicated in Table 1. Results for the group of 15 diabetic rats are available for 7 days after the onset of hyperglycemia and this information is supplemented for longer periods of time as indicated in Table 1.

TABLE 1

EFFECT OF ALLOXAN ON SERUM LIPASE OF ADULT MALE RATS

Time after alloxan injection	Blood sugar (mg/100 ml)		Serum lipase (units/100 ml)	
	mean	range	mean	range
0	117	90–120	556 ± 70*	390– 690
1 day	369	315–450	536 ± 102	320– 720
3 days	544	230–800	541 ± 80	410– 750
5 days	434	260–670	686 ± 105	510– 890
7 days	429	314–630	800 ± 67	690– 880
21 days†	426	400–450	830 ± 92	760–1000
3 months‡	350	250–400	930 ± 238	930–1400

* The values following the ± sign represent the standard deviation.

† These values were obtained from seven of the group of 15 rats.

‡ These values were obtained from a group of five animals for which there are no normal values.

Blood sugars reach diabetic values within the first day in the usual way, but the effect on lipase follows a different pattern. Almost without exception, individual rats showed a temporary fall in serum lipase levels between the first and third day after injection with alloxan. By the end of the seventh day, however, there was in every diabetic animal a definite increase of the enzyme, with an average increase to about 40–45% above normal levels for the group of 15 rats. The results given in Table 1 for rats diabetic for periods of time up to 3 months indicate that the enhancement in lipase activity of rat serum is persistent and may reach concentrations as much as 65% above normal. The development of increased lipase levels roughly parallels the appearance of high alkaline phosphatase values in alloxan diabetic rats.

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