In order to avoid plasmolysis and to restore turgor to the cells the stain was made up in solution after the formula of Satory, as given by Linder:[1]

Carbolic acid crystals	20	gms.
Lactic acid, syrup	20	"
Glycerine	40	"
Distilled water	20	"
Phenosafranin	0.5	" or less

Mordanting fixed sections for two hours with a 2 per cent. iron alum solution intensifies the stain. Destaining may be accomplished by washing the material in a solution of 0.5 per cent. alum and 0.5 per cent. HCl or by means of alcohol. After destaining it is often desirable to set or intensify the stain by applying a 1 per cent. ammonium hydroxide solution. For use in the examination of mycelia in host tissue, the destaining of the parenchymatous tissue is more rapid than that of the parasite, so that by proper control of this process a differential stain may be attained. Sections of Johnson grass leaves infected with a rust stained for twenty minutes in phenosafranin and then destained for ten minutes in acid alum show a differential stain between host and parasite tissue. In the case of mycelia within woody tissue the contrast is not so pronounced, since the lignified walls retain the stain as well as the mycelia.

This stain has proved particularly adaptable in the study of bacterial and fungal colonies growing on an agar substratum. If these colonies be treated with phenosafranin the medium does not absorb the stain as readily as the cells, so that the latter stand out intensely stained against a lighter background, no destaining being necessary.

Phenosafranin is superior to the cotton blue suggested by Linder, because the red is a more intense stain than the blue and also because of the much greater permanence of the phenosafranin color

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SPECIAL ARTICLES

PROTEIN FRACTIONS OF THE H 37 (HUMAN) STRAIN OF TUBERCLE BACILLUS[1]

THE method of protein fractionation[2] recently used in the case of the timothy grass bacillus[3] has now been applied to a human strain (H 37) of the tubercle bacillus.

A frozen and dried harvest of the organism[4] (grown on Long's medium) was extracted in the cold with acetone and ether, ground in a ball mill and again extracted, as described previously.[3] The cell residues were stirred for about 6 hours in the cold successively with buffer at pH 4.0 (fraction C), buffer at pH 6.5 (fraction D), water containing enough *N* NH_4OH to keep the pH at 8.3–8.5 (E), water made alkaline to about pH 9 (F), and water made alkaline to about pH 11 (G). The residual material was stirred in turn with 0.1, 0.2 and 0.5 *N* NaOH at room temperature (K, K′, K″). The properties of the fractions obtained by acidifying two lots of the extracts are given in the table. Each fraction was redissolved and reprecipitated twice.

Fraction	$[\alpha]_D$ degrees	N Per cent.	P Per cent.	Basic ash Per cent.	Precipitin reaction of 1:2000 solution with: anti-human strain serum[4]	Precipitin reaction of 1:2000 solution with: anti-timothy bacillus serum[4]
701						
D	+ 9	15.7	3.4	0.7	±(±)*	−(+)
E	+11	15.4	2.0	0.5	±(±)	
F	−26	15.0	1.8	1.4	±(±)	±(+)
G	−31				±(±)	±(±)
K	−61	16.2	0.4	0.4	±(±)	+(+±)
K′	−50	14.9	0.04	0.3	±(+)	+(+)
K″	−18	11.2		1.9	+++(++++)	+(+±)
702						
D	+ 9	15.6	3.7	0.8	±(±)	+(+±)
E	−19	15.8	2.1	0.2	±(±)	+(++)
G	−28	15.7	2.1	0.2	±(±)	+(++)
K	−56	15.3	0.6	0.3	±(+)	+(+)
K′	−51	15.3	0.06	0.2	±(+)	+(+)
K″	−46				+++(++++)	+(++)

$[\alpha]_D$, N, P calculated to ash-free basis.
* Values in parentheses obtained after centrifugation.

As in the case of the corresponding fractions of the hemolytic streptococcus,[2] levorotation increases and

[1] David H. Linder, "An Ideal Mounting Medium for Mycologists," SCIENCE, 70: 430, 1929.

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[2] M. Heidelberger and F. E. Kendall, *Jour. Exp. Med.*, 54: 513, 1931.

[3] M. Heidelberger and A. E. O. Menzel, *Proc. Soc. Exp. Biol. and Med.*, 29: 512, 1932.

[4] Kindly supplied by the H. K. Mulford Biological Laboratories of Sharp and Dohme, Glenolden, Pa.

phosphorus decreases in general from the D to the K fraction. The percentage of nitrogen, however, remains nearly constant throughout the present series. The exceptional properties of the very small K″ fraction appear due to contamination with specific polysaccharides,[5] especially since the other fractions are practically inactive toward an immune serum known to be rich in polysaccharide antibodies. The products differ in this respect from Johnson's "water soluble" and "alkali-soluble" tubercle bacillus proteins.[6] The relationship of the new fractions to the three proteins indicated by Levene[7] will be investigated.

The somewhat stronger precipitin reactions given by the fractions in an anti-timothy bacillus serum than in the homologous antiserum indicate that all contain group-specific protein. The only additional biological data available at the present preliminary stage of the study are that a proportion of normal rabbits showing a negative skin test to fraction K respond with a "lighting up" of the original test area during a subsequent course of intravenous injections of K, and, as found by Sabin and Smithburn at the Rockefeller Institute for Medical Research, a distinct difference in the type of skin reaction produced by the D and K fractions in tuberculous guinea pigs.

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INDUCTION OF EXPERIMENTAL GRANULAR CONJUNCTIVITIS BY DIRECT INOCULATION OF TRACHOMATOUS TISSUE

IN a recent review on the causation of human trachoma Dr. Bengtson[1] writes: "If it can be shown that the condition produced in *Macacus rhesus* monkeys by direct transfer from cases of human trachoma is as definite and as easily transmissible as that induced by inoculation with *Bact. granulosis,* then we would feel more certain of the relationship of *Bact. granulosis* to the human disease."

The observations herein reported demonstrate that direct transfer from such human cases is definitely and easily made and no difference exists between the readiness with which the experimental granular conjunctivitis can be induced by means of human tissue and culture of *Bact. granulosis.*

Through the kind cooperation of Dr. Martin Cohen, of New York, we obtained recently the tarsectomized conjunctival tissue removed for curative purposes from a case of florid trachoma of three years' duration, accompanied by bilateral pannus.

The specimen was employed in two ways: (a) For direct subconjunctival injection of one eye of monkeys having smooth lids, and (b) for bacteriological study. A culture of *Bact. granulosis* was isolated and it also was injected subconjunctivally in one lid of normal *Macacus rhesus* monkeys. Thus human trachomatous tissue, on the one hand, and a culture of *Bact. granulosis,* on the other, both having a common origin, were used to inoculate monkeys.[2]

The first two animals injected with the culture showed within seven days characteristic granular conjunctivitis in the inoculated eye. Within another week, the uninoculated conjunctivae became similarly affected, and after three weeks, the experimental disease, previously described in detail,[3] was fully developed. Conjunctival tissue was removed from one of the affected animals two weeks after inoculation, and employed for subconjunctival injection of two fresh monkeys; they in turn were apparently affected in the same way as the preceding animals. In this manner, monkey to monkey transmission was obtained through seven passages. At this point, when we were convinced that transfer could be carried on indefinitely, the experiment was terminated.

The first two animals inoculated with the suspension of human trachomatous tissue exhibited, within seven days, characteristic granular conjunctivitis, and again the tissue of one of them induced the experimental disease in two fresh animals. The affection was thus transmitted through seven consecutive series, at least, of paired animals. The period of incubation, the conveyance of infection from inoculated to uninoculated eye, the appearance of the early and fully developed lesions of the disease and the histopathological changes were identical with those shown by the animals of the culture series.

The activity of the incitant in both series apparently became "fixed" in the consecutive transmissions, that is, the incubation period and the degree of reaction became constant.

Since transfers were made early in the course of the affection, we were able to study the microscopic changes of beginning conjunctival lesions. These consisted of congestion of blood vessels and marked hypertrophy of their endothelium. The vessels were surrounded by a thick layer containing chiefly monocytes, some lymphocytes, and a few polymorphonuclear cells with acidophilic granules. In later stages, the perivascular agglomerations were coalesced to form the large folliculomata characteristic of trachomatous lesions.

[5] M. Heidelberger and A. E. O. Menzel, *Proc. Soc. Exp. Med. and Biol.,* 29: 631, 1932.
[6] T. B. Johnson, *Am. Rev. Tuberc.,* 14: 169, 1926.
[7] P. A. Levene, *Medical Record,* Dec. 17, 1898.
[1] I. A. Bengston, Public Health Rep., 47: 1914, 1932.
[2] All operative procedures were carried on with the aid of ether anesthesia.
[1] H. Noguchi, *Jour. Exp. Med.,* 48, Suppl. 2, 53 pp. 1928; P. K. Olitsky, R. E. Knutti and J. R. Tyler, *Jour. Exp. Med.,* 53, 753, 1931; 54, 31, 1931.