have reacted positively to dilutions of 1:10,000. No reactions have been elicited in the few cases in which dilutions of 1:100,000 tuberculin have been employed. No reactions showing central necrosis (4 plus) have been seen. In general comparable dilutions of O.T. and P.P.D. as employed in these experiments have elicited similar reactions. Further data concerning the comparison of the two types of tuberculin will be published at a later date.

Summary. Thirty normal dogs tested repeatedly with 1:100 tuberculin have given negative reactions. Forty-two dogs with tuberculous lesions have given positive reactions to the intracutaneous injection of tuberculin in doses of 1:100 to 1:10,000. Three dogs gave doubtful or negative reactions; one of these animals has been necropsied and showed no gross evidence of tuberculosis. Six dogs previously positive to tuberculin have given negative or doubtful reactions from 1 to 3 months before death; necropsies invariably revealed extensive tuberculosis. Other than this there has been no apparent correlation between the degree of cutaneous sensitivity and the extent of the tuberculous lesion.

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Observations on *Bacterium melaninogenicum:* Demonstration of Fibrinolysin, Pathogenicity and Serological Types.*

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A review of the literature on *Bact. melaninogenicum*[1-4] reveals an uncertainty as to its pathogenicity and as to the existence of more than one serological type. Evidence is here presented that the proteins extracted from 2 strains of this organism are different in their chemical and antigenic properties and that pathogenicity can be demonstrated under certain conditions.

Two strains were studied: The first (py) was isolated from the gums of a patient suffering from pyorrhœa and the second (M19),

*Presented before the American Association for Experimental Pathology, Memphis, Tennessee, April 22, 1937.

1 Burdon, K. L., *J. Inf. Dis.*, 1928, **42**, 161.

2 Burdon, K. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1144.

3 Shevky, M., Kohl, C., and Marshall, M. S., *J. Lab. and Clin. Med.*, 1934, **19**, 689.

4 Liebetruth, E., *Z. f. Hyg.*, 1935, **116**, 611.

from the lung of a monkey which had developed a pulmonary abscess after intrabronchial inoculation with infectious material.[5] Both cultures conformed to the description given by Shevky, Kohl and Marshall[3] and Burdon.[1, 2] The first strain differed, however, from the second in its failure to liquefy gelatin and to form indol in peptone-water.

The pathogenicity of pure cultures was tested by injecting dogs intrabronchially, white mice subcutaneously and rabbits intracutaneously.

With the aid of a fluoroscope, 2 dogs were injected intrabronchially with large doses of *Bact. melaninogenicum* suspended in a menstruum of starch.[6] One animal developed a febrile reaction. A rœntgenogram of the lung showed an area of increased density of the infected lobe.† The other had increased bronchial markings which lasted for at least a month.

Mice were inoculated subcutaneously in the groin with 0.05 to 0.1 cc. of broth culture of *Bact. melaninogenicum.* Edema and inflammation developed which persisted for 24 hours. Control animals, injected with heat-killed cultures, showed no demonstrable lesions.

Rabbits were injected intracutaneously with 0.02 cc. of staphylococcal toxin combined with 1/5 unit of Squibbs antitoxin; 48 hours later these sites were inoculated with living or heat-killed cultures of *Bact. melaninogenicum.* Inflammation, exudation and necrosis were observed only in areas infected with living cultures.

Experiments were conducted by the method of Tillett and Garner,[7] employing alcohol for precipitation and concentration and human fibrinogen and thrombin for the demonstration of fibrinolysin. Strain (M19) gave complete lysis in a 1:4 dilution after 40 minutes' incubation, while strain (py) showed partial (2+) lysis only in original concentrations.‡ The presence of fibrinolysin is considered by many authors as evidence of invasiveness or pathogenicity.[8–11]

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[5] Weiss, C., and Shevky, M. C., *Arch. Path.*, 1936, **22**, 770.

[6] Terrell, E., Robertson, O., and Coggeshall, L., *J. Clin. Invest.*, 1933, **12**, 393.

† Dr. J. Levitin, Roentgenologist, interpreted the films.

[7] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

‡ Dr. R. Madison, Department of Bacteriology, Stanford University, collaborated in these tests.

[8] Tillett, W. S., Edwards, L. B., and Garner, R. L., *J. Clin. Invest.*, 1934, **13**, 47.

[9] Hadfield, G., Magee, V., and Perry, C. B., *Lancet*, 1934, April 21, 834.

[10] Morales-Otero, P., and Pomales-Lebrón, A., *Trans. Royal Soc. Trop. Med. and Hyg.*, 1936, **30**, 191.

[11] Schmidt, H., *Z. f. Immunitätsf.*, 1936, **87**, 9.

TABLE I.
Biochemical Properties of Proteins Extracted from Two Strains of *Bacterium melaninogenicum*.

Microörganism	Source	% Total N	Ash %*	Total N (Corrected for ash)	Biuret†	Xantho-Proteic†	Hopkins, Cole†	Ninhydrin†	Millon†	Molisch†	Bial-tollens‡	Precipitin titers§
Bact. melaninogenicum (M 19)	Pulmonary abscess	9.83	4.91	10.35	++	+++	+++	+++	+++	+++	++	1:128,000
Bact. melaninogenicum (py)	Pyorrhœa	14.28	5.17	15.07	+++	+++	+++	+++	+++	+++	—	1:64,000

*Microchemical analyses were performed under the direction of Prof. Paul L. Kirk, by Mr. H. C. Johnson, Department of Biochemistry, University of California, Berkeley.

†Tests performed on 1:1000 dilution.

‡Test for pentose.

§Maximal final dilutions of protein giving strong precipitation when added to an equal volume of homologous antiprotein rabbit serum.

Bacterial proteins, corresponding to fraction D, were extracted from 2 strains of *Bact. melaninogenicum* by the method of Heidelberger and Kendall.[12] The medium[13] was hormone-broth containing 1% cysteine hydrochloride with pieces of autoclaved brain, tied up in a parchment bag, placed at the bottom of the flasks.

The biochemical characteristics of the proteins are given in Table I. Both were antigenic when injected intravenously in repeated doses into rabbits, and non-toxic, when inoculated intra-abdominally into white mice.

Precipitin-tests were performed according to the technic of Lancefield,[14] employing various dilutions of the bacterial proteins and a constant dose of antibacterial and antiprotein sera. Absorptive tests were done, whenever indicated, with concentrated bacterial suspensions as adsorbing agents, since preliminary tests revealed that the proteic solutions were unsatisfactory for this purpose.

It was observed that the proteins extracted from the 2 strains of *Bact. melaninogenicum* are immunologically distinct. Cross-reactions were seen in antibacterial sera for *Strep. hemolyticus,* Group A, but these heterologous precipitins were readily removed by absorption with homologous bacterial suspensions and were not found in sera obtained by immunization with protein-solutions or with suspensions of several other bacterial species: *Fusobacteria,* types I, II and III, *Strep. evolutus, Pneumococcus III* and *Staphylococcus aureus.*

Immune rabbit-sera produced by repeated intravenous injections of the bacterial proteins contain specific anticarbohydrate as well as antiprotein. Absorption with the protein removed both antibodies but absorption with carbohydrate removed only the anticarbohydrate.

These findings are in agreement with those of Heidelberger and Kendall[15] on *Streptococcus hemolyticus* and suggest the existence of a conjugated carbohydrate-protein analogous to the artificial pneumococcus III-polysaccharide-protein synthesized by Avery and Goebel.[16]

Valuable assistance was rendered by Dr. Alex Kaplan, Mrs. D. G. Mercado and Mrs. J. D. Taranik in the chemical and serological investigations. E. R. Squibb & Sons furnished the staphylococcus toxin and antitoxin.

12 Heidelberger, M., and Kendall, F. E., *J. Exp. Med.,* 1931, **54**, 515.

13 Weiss, C., and Mercado, D. G., *J. Exp. Med.,* 1938, **67**, 59.

14 Lancefield, R. C., *J. Exp. Med.,* 1928, **47**, 91.

15 Heidelberger, M., and Kendall, F. E., *J. Immunol.,* 1936, **30**, 267.

16 Avery, O. T., and Goebel, W. F., *J. Exp. Med.,* 1931, **54**, 437.