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Cutaneous Reactivity to Tuberculin in the Dog.*

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The prevailing opinion expressed in the current literature[1-4] is that tuberculous dogs react irregularly or not at all to injections of tuberculin. This opinion is apparently based primarily upon the reports of Cobbett and Griffith,[5] who estimated the sensitivity of tuberculous dogs by determining the thermal changes following the injection of 2 to 5 cc. of tuberculin. The dogs were infected with human, bovine or avian types of tubercle bacilli injected intravenously, intraäbdominally or subcutaneously in doses ranging from 1 to 50 mg. A few of the dogs were infected by feeding.

During the course of studies of experimental pulmonary tuberculosis in dogs we have had the opportunity to observe the cutaneous reactivity to tuberculin from the time of initiation of the lesion throughout the various stages of healing or progression of the disease. Our findings with regard to the reactivity of the skin of tuberculous dogs after intracutaneous injection of tuberculin have been contrary to prevailing opinion. These reactions appear to be more constant than those obtained after subcutaneous injection.

Normal dogs were infected by the intrapulmonary injection of varying doses of virulent human or bovine type† tubercle bacilli sus-

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1 Feldman, W. H., *J. Am. Vet. Med. Assn.*, 1934, **85**, 653.

2 Steiner, P., *Am. Rev. Tuberc.*, 1934, **30**, 683.

3 Caulfeild, A. H. W., Brown, M. H., and Magner, W., *Am. Rev. Tuberc.*, 1930, **21**, 127.

4 Panisset, L., and Verge, J., *Compt. rend. Soc. de Biol.*, 1926, **94**, 815.

5 Cobbett, L., and Griffith, A. S., *Royal Com. on Tuberc. Great Britain, Int. Rep.*, **40**, 1913.

† The strains of human and bovine type employed were isolated in 1930, the human strain from a fatal case of tuberculous meningitis and the bovine from a fatal case of glandular and osseous tuberculosis. The human strain has been shown to possess a high degree of virulence for guinea pigs and the bovine strain for both guinea pigs and rabbits. The organisms are carried on glycerin-egg-yolk medium.

pended in mucin. The injections were made through a ureteral catheter inserted deep in a lower-lobe bronchus by means of a bronchoscope. The dosage of bacilli varied from 0.075 mg. to 0.5 mg. per kilo or total doses of 1 to 5 mg. per dog. The estimation of the quantity of bacilli used for injections was made by centrifuging in a Hopkins tube. Mucin was used in preparing suspensions to insure a more compact inoculum and to reduce the tendency to widespread bronchial dissemination or expulsion of the material by coughing. Comparisons of the pathogenicity for guinea pigs of tubercle bacilli suspended in gastric mucin[6] with saline suspensions have, in the doses employed, demonstrated no significant differences in the course of the disease produced by the two suspensions.[7]

Cutaneous sensitivity was determined by measuring the reaction to 0.1 cc. amounts of varying dilutions of standard "Old Tuberculin" (O.T., Parke Davis) and Purified Protein Derivative (P.P.D., Seibert)‡ injected intracutaneously. The tests were made upon carefully shaved areas of the skin of the neck and upper portions of the hind quarters of the dog after thorough cleansing with soap and water and alcohol. The injections were made with 27 gauge needles and the raised blebs were ringed with a skin-pencil. This greatly facilitated reading the tests especially in pigmented skin or when weak or negative reactions were encountered.

Dilutions of both O.T. and P.P.D. were made with 0.85% saline and in all cases were kept in the refrigerator and employed within 3 days after diluting. One-tenth cc. of a 1:100 dilution was chosen as the standard test-dose. Other dilutions varying from 1:10 to 1:100,000 were also employed when indicated by the reactions to the standard test. Readings of the tests were made at 24, 48, and 72 hours and the 48-hour readings were graded according to the suggestion of Aronson:[8]

Slight redness without edema	—
Slight redness with edema measuring less than 5 mm.	?
Edema measuring 5 to 10 mm.	+
" " 10 to 20 "	++
" " 20 to 30 "	+++
Central necrosis	++++

Results. A series of 30 normal dogs tested before experimental

[6] Nungester, W. J., Wolf, A. A., and Jourdonais, L. F., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 120.

[7] Mills, M. A., and Colwell, C. A., to be published.

‡ The Purified Protein Derivative employed in these experiments was procured from Dr. Florence Seibert through the courtesy of Dr. E. R. Long.

[8] Aronson, J. D., *Am. Rev. Tuberc.*, 1934, **30**, 727.

infection were uniformly negative in their reactions to tuberculin; in no case have reactions in any way similar to the typical tuberculin-reaction been encountered. Forty-two dogs with various types of experimental infection, including 10 dogs inoculated with B.C.G., have given positive reactions with 1:100 tuberculin. This figure represents 93.6% of infected and vaccinated dogs studied. The majority of these dogs became positive early and were definitely and consistently positive (2 to 3 plus) with 1:100 tuberculin. Dogs vaccinated with B.C.G. (2.5 to 10 mg.) became skin-reactive after a longer interval than in the case of the virulent strain and often gave weak or irregular reactions. Weak, irregular or negative reactions when encountered in dogs infected with virulent bacilli have in general been associated with rapidly progressing or far-advanced tuberculosis. Six dogs previously positive to tuberculin became weak or negative before death; necropsies revealed extensive tuberculosis. Three, or 6.4%, of infected or vaccinated dogs studied gave doubtful or negative reactions to tuberculin. One of these, necropsied 98 days after infection, showed no gross evidence of tuberculosis; the other 2 (one injected intravenously with 5 mg. B.C.G., the other with 1 mg. of human tubercle bacilli into the right lower lobe) have not been necropsied.

TABLE I.
Skin Reactivity to Tuberculin in the Dog.

No. tested	Infection and vaccination data: Type of bacilli	Dose (mg.)	Route	Skin sensitivity 1:100 O.T. and P.P.D.
12	Human	1.0	Intrapulmonary	1 to 3 plus
3	"	3.0	"	2 " 3 "
5	"	4.0	"	1 " 3 "
8	"	5.0	"	2 " 3 "
2	Bovine	2.0	"	1 " 3 "
2	"	5.0	"	2 "
1	B. C. G.	2.5	"	1 " 2 "
2	"	4.0	"	1 " 2 "
2	"	4.0	Subcutaneous	1 " 2 "
5	"	5.0 to 10.0	Intravenous	1 " 2 "
2	Human	1.0	Intrapulmonary	Negative*
1	B. C. G.	5.0	Intravenous	" (47 days)
30	Normal controls	0	0	Negative

Summary:

Total No. infected dogs tested	45
" " " " with positive reactions	42, or 93.6%
" " normal control dogs tested (negative reactions)	30

* One of these dogs showed no gross evidence of tuberculosis at autopsy.

Fourteen of the 16 tuberculous dogs which manifested strong (2 or 3 plus) reactions to 1:100 tuberculin were also tuberculin-positive when tested with 1:1000 dilutions. Four of 6 dogs tested

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),

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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.