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Tuberculin Tests in Guinea Pigs With Purified Protein Derivative.

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Increased purification and the avoidance of denaturation of an antigen would appear to have obvious advantages. Accordingly the purified protein derivative, PPD, has been recommended unreservedly to replace the crude concentration of heterogeneous substances in glycerol broth cultures of the tubercle bacillus, introduced by Koch more than half a century ago under the name of Old Tuberculin. The discovery of the occurrence of non-specific reactions with large doses of PPD¹ seemed only to indicate that smaller doses of this potent material be used. A report that PPD did not give as good reactions in the skin of guinea pigs as did O.T. seemed to be attributable to the low doses of PPD used in that study.²

TABLE I.

Reactions in Different Tuberculous Guinea Pigs.

	Negative	Weak	Strong	Died
PPD	2	19	72	21
O.T.	0	4	31	0
Reactions in Uninfected Controls.				
PPD	40	8	0	0
O.T.	15	1	0	0
Reactions on Opposite Sides of the Same Tuberculous Guinea Pigs.				
PPD (right side)	2	10	24 }	14
O.T. (left side)	1	7	28 }	

In June, 1941, Dr. F. B. Seibert kindly donated a generous amount of her new potent preparation of PPD³ for further study. Comparison of this material with a commercial Old Tuberculin (Cutter) has yielded results which are quite disconcerting.

In general, the local reaction to PPD was less distinct and characteristic than that to

O.T. In known tuberculous animals, the injection of 0.1 mg of PPD, which according to Dr. Seibert corresponds to from 10 to 20 mg O.T., resulted only in 2 or 3 plus reactions in the skin, with erythema and edema of varying extent, disappearing in 3 or 4 days, without the persisting necrosis or ulceration characteristic of the reaction to 5 mg O.T.

The systemic or lethal effect of the PPD, however, was much greater than that of the O.T. Although death from 5 mg O.T. in tuberculous animals is exceedingly rare, the injection of PPD in doses as low as .005 mg which correspond to less than a milligram of O.T. resulted in death of some of the animals. Altogether, about 20% of the animals given less than 0.1 mg PPD died within the following 48 hr.

Further experiments suggested that the greater systemic effect with lessened local reactivity to PPD may be due to the greater solubility and faster absorption and dissemination of the purified material. Thus the addition to the PPD of lampblack, tannic acid, or various oils, to keep it longer *in situ*, increased the local effects.

Large doses of PPD, which give reactions in a larger proportion of tuberculous animals, also gave more non-specific reactions with non-infected animals. The same was true of PPD in oil. Some of these animals had received repeated injections of the PPD, and part of the non-specific responses may have resulted from active sensitization by the earlier doses. Such sensitization has not been observed, however, following repeated use of diagnostic doses of O.T.

This is not the only instance in which purity has not been an unqualified asset in connection with a biological test or agent. The addition of alum to diphtheria toxoid, of protamine to insulin, or of oil to epinephrin, to enhance their effects, are familiar examples. A peptone suspension of a pure

¹ Fureulow, M. L., Hewell, B., Nelsen, W. E., and Palmer, C. E., *Pub. Health Rep.*, 1941, **56**, 1082.

² Medlar, E. M., Sasano, K. T., Caldwell, D. W., and Needham, E. L., *Am. Rev. Tuberc.*, 1941, **43**, 534.

³ Seibert, F. B., and Glenn, J. T., *Am. Rev. Tuberc.*, 1941, **44**, 9.

culture of diphtheria bacilli has been reported to give skin reactions in non-immunized guinea pigs such as are characteristically elicited by field cultures, when pure cultures suspended in water or saline does not.⁴

The particular preparation of PPD used in these experiments had been produced with less heating and denaturing than previously described products. This may account for the more consistent results on skin testing,⁵ the failure to elicit therapeutic focal reactions

in man⁶ and the lessened antigenicity reported elsewhere.⁷

There seems to be a distinct place for PPD in testing for the occurrence of tuberculous infection. In rabbits and monkeys⁸ it is possible to elicit tuberculin reactions with 1 mg PPD or more, where such reactions may not be obtained with O.T. In guinea pigs,⁹ however, O.T. seems to be more sensitive, more specific, less antigenic and less often lethal, as well as less expensive, than the PPD used here.

⁴ Stone, R. V., and Weigel, C., *Am. J. Pub. Health*, 1929, **19**, 1133.

⁵ Seibert, F. B., *Am. Rev. Tuberc.*, Supp., 1935, **30**, 707.

⁶ Thompson, B. C., *Tubercle*, 1936, **18**, 27.

⁷ Seibert, F. B., *Am. Rev. Tuberc.*, 1941, **44**, 1.

⁸ Schroeder, C. R., *Zoologica*, 1938, **21**, 397.

⁹ Negley, J. C., and Bogen, E., *J. Urology*, 1940, **44**, 860.

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Fate of Mannide Monoöleate in the Animal Body.

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Owing to the shortage of glycerin in England toward the close of the world war, fats were prepared without this polyhydric alcohol. Halliburton *et al.*¹ studied the effect of mannitol oleic acid ester as a substitute for butter fat in a balanced ration for rats and observed that it possessed the same nutritional value as butter fat and prolonged feeding did not produce toxic symptoms. In the present war, glycerin is again a product of great importance. Having been interested for many years in the metabolic pattern of the sugar alcohols, we turned our attention to the fate of their fatty acid esters in the animal body. The first compound studied was mannide monoöleate.

Physical and Chemical Characteristics. Mannide monoöleate* is a viscid oily liquid

¹ Halliburton, W. D., *et al.*, *Biochem. J.*, 1919, **13**, 301.

* Generous supplies of mannide monoöleate were furnished by the Atlas Powder Company of Wilmington, Delaware.

with a yellow color. Its solubilities in various common solvents are similar to those of edible fixed oils and its odor is characteristic, resembling that of vegetable fixed oils.

Although this substance is referred to as mannide monoöleate it is actually a mixture composed chiefly of the monoöleic acid esters of the mannitol dianhydrides or mannide together with some minor amounts of mannitol monoanhydride monoöleates, some dioleate esters, and a little free acid. The material used had a hydroxyl number of 120, an acid number of 6.6 and a saponification number of about 165. The mannide fraction has been identified as the 1,4:3,6 dianhydromannitol or isomannide containing some 1,4 mannitan.

Single Dose Administration. Young male white rats were given 2 cc per 100 g of mannide monoöleate by stomach tube. Of the 5 animals receiving the compound none showed symptoms of distress or depression. After 3 weeks no abnormalities were found. This dosage was administered intraperi-