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 man[6] 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 monkeys[8] 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 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-

[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.

TABLE I.
Influence of Mannide Mono Feeding on Fecal Fat of Rats, 10 Animals.

Day of Exp.	Diet, Purina chow plus	Food consumed, g/3 days	Fecal wt, g/3 days	Fecal fat (as fatty acids) Free fatty acids, %	Neutral fat, %	Soap fat, %	Total fecal fat, %
1	5% cotton seed oil			1.10	1.11	0.56	2.77
2		432	209	0.61	0.54	0.52	1.67
3				0.49	0.76	1.56	2.81
6	5% mannide monoöleate			0.21	0.10	1.49	1.80
7		434	188	0.92	1.49	0.01	2.42
8				0.49	0.64	1.02	2.15

toneally to 5 additional rats; they showed no symptoms other than abdominal distention, but upon autopsy there was a chemical fibrinous and fibrous type of peritonitis.

Feeding Studies (Rats). Young white male rats, 50 to 80 g in groups of 30, were fed a diet of purina chow as a control basal diet. To this were added 3 and 5% quantities of mannide monoöleate respectively. Body weight of control and experimental animals was determined weekly. At the end of 8 weeks, 10 animals in each group were sacrificed and autopsied. The growth curves of the experimental and control animals were identical within experimental error. On gross examination there were no significant abnormalities. Histological sections of the kidneys, liver, spleen and intestinal mucosa were normal.

Excretion Experiments. Over a period of 2 weeks, 8 rats were fed a diet containing 5% of mannide monoöleate. The urine of these animals upon evaporation yielded a syrupy residue, which was extracted with absolute alcohol. Upon evaporation, the alcohol yielded a large crop of crystals, which, by melting point, were proved to be isomannide. No mannitol, mannide or mannitan was found. Previous work has shown[2,3] that isomannide, mannide and mannitan are not utilized, although this ester of mannide is absorbed and hydrolyzed as shown by the appearance of isomannide in the urine.

Feeding Experiments (Monkeys). Five *Rhesus macacus* monkeys were fed 2 cc daily of mannide monoöleate for a period of 8 weeks in addition to a basal diet of apples, carrots, potatoes and sunflower seeds. Two additional monkeys were fed the basal diet. The weights of the animals were determined weekly. The feeding of mannide monooleate produced no abnormalities; there was no significant change in body weight, no diarrhœa and no pathological change in the livers or kidneys, nor were the hemoglobin index or blood smears significantly affected.

Absorption of Mannide Monoöleate. Young white rats were housed in wire-bottom cages and fed a diet of purina chow plus 5% of cotton seed oil for several days. Their fecal fat was determined daily after 2 days feeding. They were then fed purina chow containing 5% mannide monoöleate and their fecal fat again determined after 2 days feeding. The results (Table I) indicate that neither neutral fat nor total fecal fat was significantly changed by the addition of mannide monoöleate to a basal diet of purina chow instead of 5% cotton seed oil. We consider this and the excretion studies to be evidence that mannide monoöleate is absorbed from the intestinal tract of the white rat and is not reëxcreted into the gut.

Summary. In the quantities administered in these experiments mannide monoöleate appears to be neither acutely nor chronically toxic to the white rat and to be innocuous in the diet of the *Rhesus macacus* monkey. Mannide monoöleate is absorbed from the intestinal tract of the white rat. No damage was observed to the important viscera of the white rat or monkey after an 8-week feeding period with mannide monoöleate.

[2] Carr, C. J., Musser, R., Schmidt, J. E., and Krantz, J. C., Jr., *J. Biol. Chem.*, 1933, **102**, 721.

[3] Krantz, J. C., Jr., Evans, W. E., Jr., and Carr, C. J., *Quart. J. Pharm. and Pharmacol.*, 1935, **8**, 213.