found to be 1.7%. This is somewhat higher than the figure reported by Folin and Ciocalteau.[8] Of the other proteins given in Table I, the percentage of tryptophane in edestin and egg albumin agrees with the values obtained by the latter workers. The data given in Table I show that such mixed proteins as those of muscle should be incubated for a longer period than the more simple proteins in order to obtain the maximum color intensity. This is probably due to the difficulty of effecting complete solution of the protein material which contains muscle fibre. In this connection Thomas[9] has reported that the tryptophane value of casein rose from 1.3 to 1.6% when the Herzfeld[5] method was used, and from 0.6 to 1.78% when the Fasal[10] method was employed. It is obvious, in order to obtain accurate results, that complete solution of the protein in question is a necessary prerequisite. In the case of finely ground proteins which are difficultly soluble, about 8 days of incubation are necessary in order to reach the maximum color intensity. It is advisable that daily comparisons of the sample solution be made after the sixth day in order to ascertain the point of maximum color intensity.

7719 C

Experimental Hypersensitivity to Undenatured H. Pertussis Protein.*

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It has been observed that hypersensitivity to a solution of undenatured *H. pertussis* protein[1] may occur during whooping cough.[2, 3] It was therefore considered of interest to determine whether anaphylaxis could be provoked with this agent in guinea pigs. Sensitization of these animals was attempted by injection of living *H. pertussis,* undenatured *H. pertussis* protein, and sera of animals immunized against *H. pertussis.*

[8] Folin, O., and Ciocalteau, V., *J. Biol. Chem.*, 1927, **73**, 627.

[9] Thomas, P., *Ann. Inst. Past.*, 1920, **34**, 701.

[10] Fasal, H., *Biochem. Z.*, 1912, **44**, 392.

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† National Research Council Fellow in Medicine.

[1] Kreuger, A. P., Nichols, V. C., and Frawley, J. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1097.

[2] Stallings, M., and Nichols, V. C., *Am. J. Dis. Child.*, in press.

[3] Frawley, J. M., Stallings, M., and Nichols, V. C., *J. Pediatrics*, 1934, **4**, 179.

The effect of undenatured *H. pertussis* protein on normal uterine strips and intracardially *in vivo* was first determined. Five females of 350-750 gm. weight were subjected to hysterectomy under ether anesthesia. (Removal of the uterus was done only between the 6th and 11th days of the oestral cycle.) The strips were washed in Locke's solution and then placed in a Dale bath of Locke's solution. When small rhythmic contractions began the test substance, *H. pertussis* protein in Locke's solution, was added to the bath. It was found that the addition of the test substance to make a final dilution of 1 part of protein in 100,000 regularly stimulated contraction of the strip. The contractions were immediate, at least two-thirds maximal and sustained for 4-8 minutes. They were indistinguishable from contractions produced by histamine. After washing, a second, third and fourth addition of *H. pertussis* protein in this dilution initiated similar contractions. Furthermore if larger doses were given no evidence suggestive of desensitization was obtained.

A day or two later the hysterectomized animals were given intracardial injections of undenatured *H. pertussis* protein in Locke's solution. It was found that doses up to 0.2 mg. could be given without systemic reactions. Larger doses were not given because of the highly dilute state of the protein solution available—1 part in 4,500.

Active sensitization with living H. pertussis. Four female guinea pigs of 300-450 gm. weight were injected with living Phase 1 *H. pertussis* bacilli intraperitoneally (3 day growth on Bordet-Gengou medium). Four injections were done at weekly intervals, the dose being increased from 1 billion to 10 billion organisms. Two days after the last injection one animal was found *in extremis.* Examination disclosed the typical hemorrhagic peritonitis.[4] The uterus failed to contract in the Dale bath on the addition of *H. pertussis* protein and gave only a slight reaction to histamine. Six to 7 weeks later the remaining animals were subjected to hysterectomy. Both uterine horns of one of these gave a positive Schultz-Dale reaction in a dilution of 1 part of *H. pertussis* protein in 7,200,000. The contractions were immediate, almost maximal and sustained 3 and 6 minutes respectively. After washing, subsequent additions of *H. pertussis* protein in this high dilution produced no contraction —indicating desensitization with the primary addition. When progressively larger amounts of the test substance were added no contractions occurred until the oxytocic dose (1 part protein in 100,000) was reached. Here repeated reactions occurred. It is felt that this

[4] Bordet, J., and Gengou, O., *Ann. de l'Inst. Pasteur,* 1909, **23**, 45.

animal's uterus was undoubtedly sensitized to *H. pertussis* protein. Confirmation by intracardial injection of protein was not obtained however. The animal died during a subsequent bronchospasm test.

The uteri of the 2 remaining animals of this group did not contract until a dilution of 1 part *H. pertussis* protein in 240,000 was reached. After washing, second additions of this dilution failed to produce contractions. Desensitization and hence sensitization is here suggested but as the dose was only slightly greater than the usual oxytocic dose (1 in 100,000), the results are equivocal. When these animals were injected intracardially with 0.2 mg. of *H. pertussis* protein anaphylactic death did not occur. Some dyspnea of questionable significance was noted. The intracardial dose of antigen here used is admittedly small but because of the high dilution of the preparation a larger dose could not be given intracardially.

Four other guinea pigs were given 5 injections of living *H. pertussis* intratracheally at 5-day intervals in doses increasing from 1 billion to 10 billion. The established inability of this organism to infect the guinea pig was here well illustrated. All of the animals gained weight during this period. The organism was recovered from the nasal secretions of only one. None developed pulmonary symptoms. Three to 4 weeks after the last injection hysterectomy was performed. Schultz-Dale tests were negative, no contractions occurring in dilution of protein higher than 1:100,000. No pulmonary lesions were found on post-mortem examination of the lungs.

Three other guinea pigs were given single subcutaneous injections of living *H. pertussis* and tested 3 to 8 weeks later. The uterus of one of them contracted and was desensitized at a dilution of 1 part protein in 240,000. The intracardial test was negative.

Sensitization with undenatured H. pertussis protein. To determine whether undenatured *H. pertussis* protein could induce anaphylactic hypersensitiveness 5 guinea pigs were given single subcutaneous injections of 0.06 to 0.2 mg. and tested 3 to 8 weeks later. The uterus of one of these animals tested 3 weeks after the injection gave the typical immediate and prolonged Schultz-Dale reaction to *H. pertussis* protein 1 part in 720,000. No subsequent addition of protein produced a contraction until the oxytocic dose of 1:100,000 was reached. Specific sensitization is therefore inferred. The intracardial injection of 0.2 mg. of *H. pertussis* protein did not, however, produce anaphylactic death. The uteri of the remaining animals of this group reacted only to the oxytocic dose.

Passive Sensitization. Passive sensitization could not be demon-

strated in 5 animals prepared by intraperitoneal injection of rabbit sera high in precipitin and complement fixing antibody titer. Tests were done one to 4 days after injection of the sera.

Summary. A solution of undenatured *H. pertussis* protein was found to be oxytocic in the Dale bath in high dilution. The normal guinea pig uterus reacted with a histamine-like contraction to repeated additions of *H. pertussis* protein—1 part in 100,000.

Active sensitization of the guinea pig uterus to undenatured *H. pertussis* protein was produced by repeated intraperitoneal injections of living Phase 1 *H. pertussis.* In one instance the classical Schultz-Dale reaction was obtained with 1 part of *H. pertussis* protein in 7,200,000. The uterine strips of 3 other animals prepared by injection of living *H. pertussis* contracted and were desensitized in dilution only slightly higher than oxytocic.

Active sensitization of the guinea pig uterus was also produced in one instance by a single subcutaneous injection of undenatured *H. pertussis* protein. The strip contracted and was desensitized by 1 part of *H. pertussis* protein in 720,000. Passive sensitization with *H. pertussis* immune sera of high titer was unsuccessful.

7720 P

Effect of Oestrin and Gonadotropic Hormone Injections upon Hypophysis of the Adult Rat.

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Moore and Price[1] studied the effect of gonad hormones upon the anterior pituitary lobe cells. The writer examined the similarity between the hypophyses of normal adult rats injected with oestrin and those injected with gonad-stimulating preparations. The material includes the hypophyses from 23 males, 21 females, 32 castrated males, and 23 spayed females which had been injected daily with from 15 to 50 R. U. of oestrin, as Theelin in oil,* for 28 to 48 days; 22 males and 29 females injected daily with 50 to 150 R. U. of Antuitrin-S for 14 to 35 days; 11 males and 23 females injected daily with 15 R. U. of a sheep pituitary extract for 12 to 25 days;

[1] Moore, C. R., and Price, D., *Am. J. Anat.*, 1932, **50**, 13.

* All of the hormone preparations used in this study were kindly supplied by Drs. O. Kamm and D. A. McGinty, Parke, Davis and Company.