stimuli and less evident with threshold stimuli. As has been stated, the reverse is true. The fact that decrease of the intervals between successive stimulations far within the duration of the optimal extinction interval, and, therefore, much closer to the refractory period, results in facilitation, is important, as it indicates that the phenomenon cannot be explained on the basis of a refractory period of the cortex following stimulation.

Extinction, as described here, is not to be confused with what is called cortical inhibition, which is cessation of a muscular response to stimulation of one focus of the motor cortex when a second, and antagonistic, cortical focus is concomitantly stimulated. Extinction occurs on stimulation of one motor focus following a preceding stimulation of the same focus, and that after the remarkably long interval of 13 to 20 seconds.

In conclusion it should be pointed out that this extinction is a physiological phenomenon, due to a functional change of the motor cortex under investigation or the motor mechanisms involved, and that it cannot be explained by physical inequalities of the various stimulations, because appreciable changes in polarization, resistance or impedance of the cortex are not responsible for the phenomenon. This has been shown by a new method of determining and recording of these various factors during electrical stimulation in living tissues, which will be published in the near future.

7750 C

Specific Factor in H. Pertussis Filtrate and Centrifugate.

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We described[1] a substance found in cultures of veal brain broth medium during the growth of *H. pertussis*. Subsequently,[2] further experiments with this material were reported. The present experiments were done (1) to ascertain the specific element in the filtrate, and (2) to obtain the sticky material that is produced in these cultures in more concentrated form.

[1] Toomey, John A., and McClelland, Joseph E., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 34.

[2] Toomey, John A., McClelland, Joseph E., and Lieder, Louis E., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 403.

After varied experimentation, I found that 4 fractions could be obtained in the following manner: *Fraction I, termed the acid precipitation fraction.* The filtrate obtained as previously described was brought up to pH 4.0 with acetic acid and then left in the icebox overnight. The bulky dark brown precipitate which formed was centrifuged off and was found to be slightly soluble in water or physiological saline. The remaining filtrate was adjusted to pH 8.0 with 25% NaOH and then back to pH 4.0 with acetic acid and put in the icebox overnight. The small precipitate formed was added to the previous one mentioned. Usually all the acid fraction came down after 2 such procedures, but occasionally a small amount could be obtained by a third precipitation. The combined precipitates formed the acid fraction of the filtrate.

Fraction II, termed the alkaline alcohol precipitate. The filtrate remaining after the previous fractionation was brought up to pH 8.0 with 25% NaOH; 2 volumes of 95% alcohol were added and the solution was put in the icebox over night. A heavy precipitate was obtained which was soapy white in appearance and easily soluble in water or physiological saline. The same procedure was repeated once or twice, each time the pH of the remaining filtrate being brought up to 8.0, and 2 volumes of alcohol added. One or 2 such procedures usually brought down all of this fraction. The combined precipitate was the alkaline alcohol fraction of the filtrate.

Fraction III, termed the acid alcohol precipitate. The pH of the filtrate remaining after the previous operation was brought up to 4.0 with acetic acid, 2 volumes of 95% alcohol were added and the solution was kept in the icebox over night. An adherent, sticky, brown precipitate was found which easily dissolved in water or saline. This fractionating procedure was repeated 2 or 3 times, all the precipitates were added together and termed the acid alcohol fraction.

Fraction IV, contained in the residual filtrate. Because of the large alcoholic content, this remaining filtrate was distilled to the original volume of the filtrate and then dried to a powder *in vacuo* or in an oven. This fraction was the residual fraction of the filtrate.

Fractions I, II, III and IV were standardized in physiological saline solution so that 1 cc. of the respective solution or solution suspension contained 4 mg. of the respective fraction. Eight rabbits were injected, each one of the fractions being used in 2 animals. Four separate injections were made at intervals of 4 days, the dosages being 0.5 cc., 1.0 cc., 1.5 cc., and 2.0 cc., respectively. The rabbits were bled 8 days after the last injection. The serum ob-

tained was used to agglutinate organisms, both old and recently isolated. Fraction I, the acid precipitate of the filtrate produced the highest specific agglutinin titer demonstrable by tests with straight and cross agglutinations.

Fractions II and IV produced no agglutinins, while Fraction III, the acid alcohol precipitate, caused the production of a negligible amount of specific agglutinins. Fraction I was not entirely pure, since it contained the acid precipitate that could be obtained from normal controls. The latter, however, when tested, showed no characteristics of specificity.

A great deal of work has been done recently with freshly isolated whooping cough strains grown on blood agar. Vaccines prepared from such cultures are being used in an attempt to prevent or modify the course of the disease. Endo-antigens have also been made from this type of culture. Clinically, the mere presence of *H. pertussis* organisms in the human does not cause a whoop *per se,* since they produce only the symptoms of a cold during the first week or so of the disease. The whoop that appears from 7 to 14 days after the onset of the infection is due to the thick, stringy, adhesive material that is produced by *H. pertussis* as the patient progresses to the later stages of the disease. Freshly isolated organisms do not produce a sticky exudate on placental or any other blood agar, but we have been able to produce material in the artificial media we have previously[1] described in the form of a pearly white, sticky mass, its physical characteristics being the same as those that are seen in the sputum of the human after a coughing spell. This stringy exudate does not consist merely of an increased number of organisms, as can be seen from microscopic examination. The physical characteristics of the growth which appears when these organisms are planted on solid medium (veal brain agar), suggest that this exudate is a product of *H. pertussis* metabolism. It adheres to the media so tenaciously that heavy wire has to be used to scrape it free after it has been growing for 4 or 5 days. It does not, however, penetrate nor break down the medium as some growing organisms do. Just a few surface tension observations with a duNoüy instrument show a cohesiveness of the exudate almost equal to thin glue. It resembles the covering of a Zuckerguss spleen or liver. Most of this stringy exudate has been ignored in the *B. pertussis* filtrate fractionation processes described previously.

H. pertussis growths in liquid cultures were centrifuged at high speed for from 30 to 60 minutes. All of the sticky masses at the bottom of the tubes were combined in one centrifuge tube, were

shaken and washed by repeated centrifugations in physiological saline until the supernatant fluid was clear. The latter was poured off and the remaining material weighed. A few drops of 25% NaOH were then added to the centrifugate and the material was arbitrarily standardized in saline so that 1 cc. contained a certain number of milligrams of wet weighed material, the amount depending upon the experiment. The entire centrifugate was ground in a ball mill for from 2 to 3 hours, and its pH brought up to 7.2; it was then bottled. The resulting mixture was not a true solution, but a finely precipitated substance which had the appearance of ground glass. When 0.1 cc. of a 1/100 dilution of this substance was injected into human subjects, it gave a localized inflammatory reaction that started in 6 and reached its maximum in 24 hours (50 individuals in all). When a 1/100 dilution was boiled for from 10 to 15 minutes, it lost its power to cause a reaction in human subjects (10 observations).

When this same dilution was concentrated to a powder *in vacuo* it lost from 90 to 95% of its wet weight (averages from 6 specimens). For example, the amount obtained from 250 cc. of culture media was 300 mg. of dried powder. The wet weight of the centrifugate in this case was 4.375 gm.

When this powder was injected intravenously into rabbits in the manner and doses described previously, it produced agglutinins specific for old strains of *H. pertussis*.

7751 C

The Effect of Gonadotropic Hormones During Gestation and Lactation.

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Many authors have studied the effect of gonadotropic extracts, prepared from pregnancy urine or pituitary tissue, on the course of gestation, and it is well known now that small doses do not interfere with the development of the embryos, but large doses cause abortion or intra-uterine fetal death.[1-4]

Our previous experiments[5, 6] convinced us that the development

[1] Katzman, P. A., Levin, L., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 873.