antisera, and in the same way type Rh_2 blood suspensions could be "converted" into type Rh", etc.

The observations on blocking antibodies are of interest in connection with the problem of the nature of agglutination and precipitation reactions in general. According to Marrack's "framework" hypothesis,[6] the second stage as well as the first stage of these reactions is assumed to be specific, in contrast to the classic theory which postulates that the second stage is non-specific. Some observations on mixed agglutination reactions were previously reported which were more readily explained under Marrack's hypothesis than the classic theory.[7] The present observations can also be more easily explained under the framework hypothesis, if one postulates that the blocking antibodies are monovalent antibodies, in contrast to the usual agglutinating and precipitating antibodies which are assumed to be bivalent.[8]

In conclusion, it seems highly improbable that blocking antibodies are peculiar to the Rh factor. Doubtless, study of other antigen-antibody systems will reveal the existence of analogous phenomena.[9]

[6] Marrack, J. R., Report No. 230, Medical Research Council, His Majesty's Stationery Office, London, 1934; second edition, 1938.

[7] Wiener, A. S., and Herman, M., *J. Immunol.*, 1939, **36**, 255.

[8] Pauling, L., Campbell, D. H., and Pressman, D., *Physiol. Rev.*, 1943, **23**, 203.

[9] *Cf.* Jones, F. S., and Orcutt, M., *J. Immunol.*, 1934, **27**, 215; Kleckowski, A., *Brit. J. Exp. Path.*, 1941, **22**, 192; Hooker, S. B., and Boyd, W. C., *Ann. N. Y. Acad. Sci.*, 1942, **43**, 107.

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Effect of Picrotoxin on Electrical Excitability of the Respiratory Center.

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The inspiratory portion of the respiratory center has been accurately located in the ventral reticular formation of the medulla of the cat by means of the Horsley-Clarke stereotaxic instrument.[1] Electrical stimulation of this region results in a marked inspiratory response involving both thorax and diaphragm with fixation of the chest in inspiration. Since there is very little direct evidence as to the precise effect of respiratory stimulating drugs on the respiratory center itself, we have thought it worth while to study the influence of one of these drugs on the electrical excitability of this region.

Cats were anesthetized with sodium phenobarbital, 150 mg per kilo intraperitoneally. A tracheal cannula was inserted and the animal was allowed to breathe through a tube containing soda-lime into a small (400 cc) Krogh-type spirometer calibrated to 5 cc. The Horsley-Clarke stereotaxic instrument was placed on the head, and through a small burr hole in the skull a fine double pole electrode was inserted 12-13 mm posterior to the interaural plane and 1-2 mm lateral to the midline. By means of repeated stimulation as the electrode was lowered, the most sensitive region of the center was located.

The stimulus was applied to the center at the end of a normal expiration by means of a Goodwin stimulator[2] set to deliver a peak voltage of 1-5 volts at a frequency of 100 cycles per sec. Little spread of the current from the tips of the electrode appeared to exist since the response was markedly diminished by moving the electrode 0.5 mm up or

[1] Pitts, R. F., Magoun, H. W., and Ranson, S. W., *Am. J. Physiol.*, 1939, **126**, 673.

[2] Dusser de Barenne, J. G., Garol, H. W., and McCulloch, W. S., *J. Neurophysiol.*, 1941, **4**, 287.

down. The response of the center to a given stimulus was measured in terms of the inspiratory volume, and of course varied with different animals. It was found that a progressively increasing voltage produced an increasing response up to a maximum.

A submaximal response was chosen, usually 50-100 cc, and the voltage and frequency were kept constant for the duration of the experiment. Observations of the response of the center to this standard stimulus were made at intervals of 15 min. or less. Too frequent stimulation showed a tendency toward a decreasing response.

In 3 control cats, in which observations were made at 15 min. intervals for periods of 4-7 hours, it was found that there was a variation in the response (Fig. 1), but the maximum increase was 16%. In a series of 10 cats, after a control period of 1 hour, 0.25 mg of picrotoxin per kilo was injected intravenously. There occurred a somewhat delayed but abrupt and marked increase in the response of the center to the constant stimulus, followed by a very gradual decline (Fig. 1). In general, no change in the sensitivity occurred before 15 min. and in one instance, 23 min. was required. The response increased progressively and reached a maximum 30-135 min. from the time of injection of picrotoxin.

In 4 of the experiments, run for extended periods of time, it was found that the increased sensitivity persisted for from 6 to

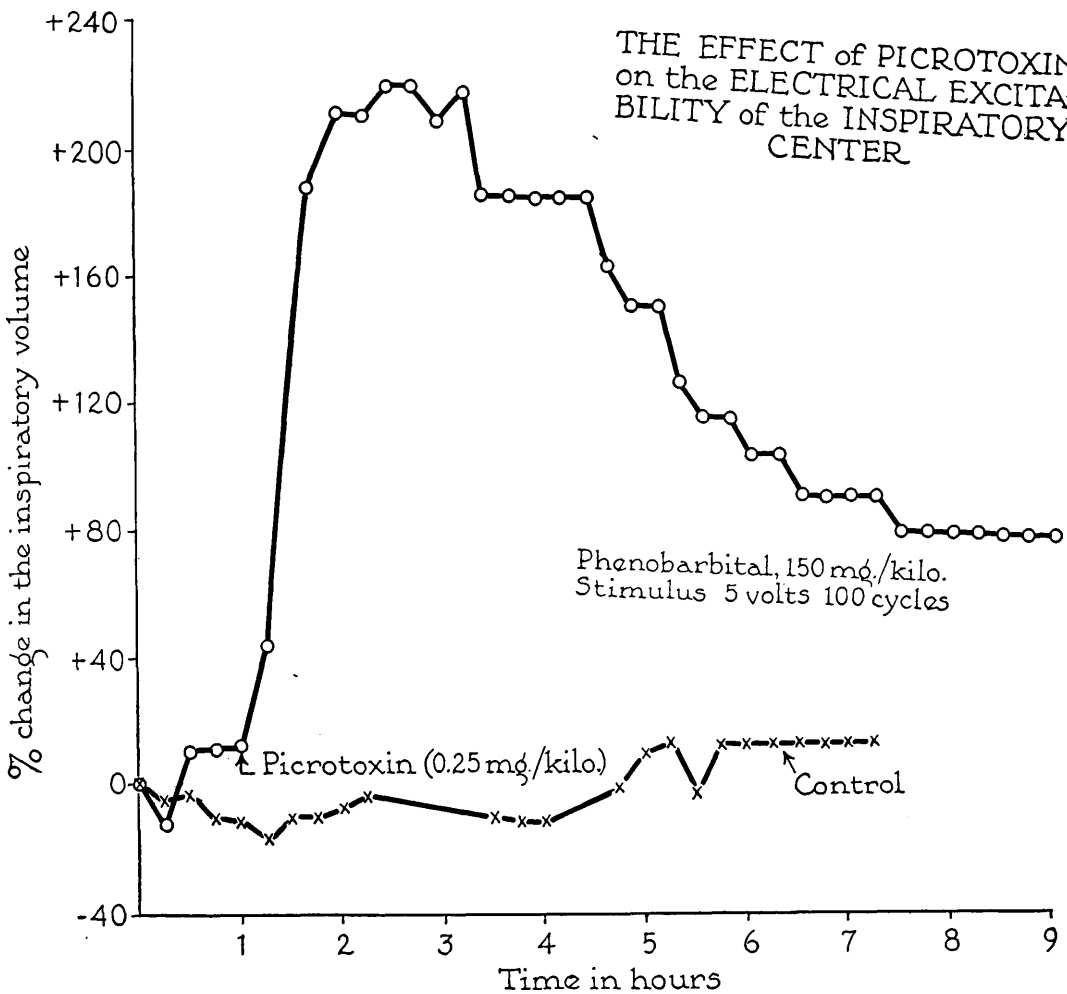

FIG. 1.

8 hours from the time of injection; at the end of this time the response became constant at a level either greater or less than the original. The maximum increase in the respiratory response varied from 30 to 222%. In certain instances the change in respiratory minute volume was also studied at frequent intervals and was found to correlate in a general way with the increased responsiveness of the center to electrical stimulation.

The delay in the onset of action of picrotoxin agrees with the observations of others and may well be due to a delayed passage of this substance into nervous tissue. However, the present prolonged response (average 7.5 hours) is not in accord with the usual beliefs regarding the duration of action of picrotoxin.[3, 4]

Summary. A technique has been described whereby electrical stimulation of the respiratory center can be employed to directly determine the influence on the respiratory center of various drugs affecting respiration. Picrotoxin has been shown to produce a marked and prolonged increase in the sensitivity of the inspiratory center of the cat to direct electrical stimulation. It is believed that the technique which has been described can be used to advantage to contribute additional information to our knowledge of drugs affecting respiration.

[3] Duff, D. M., and Dille, J. M., *J. Pharm. and Exp. Therap.*, 1939, **67**, 353.

[4] Das, S. C., *Quart. J. Exp. Physiol.*, 1939, **29**, 355.

14642

Bacteriostatic Action of Penicillin on Hemolytic Streptococci *in vitro*.*

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In previous communications[1,2,3] preliminary observations on the effect of penicillin on living cultures of hemolytic streptococci were reported. It was shown that the action of penicillin was either bactericidal or bacteriostatic depending on the experimental conditions. Under the influence of relatively large concentrations of penicillin, organisms when present in small numbers decreased in number at a constant rate until approximately 99% of the organisms originally present were destroyed. Penicillin was not inhibited by para-aminobenzoic acid, blood, serum, or peptone. Additional evidence suggested that penicillin was capable of destroying bacteria only when multiplication took place.

In the present communication evidence will be presented which extends these observations.

Experimental. Hemolytic streptococci (strain C203MV) were used throughout. In the first set of experiments the effect of varying amounts of penicillin was observed. A constant number of organisms was incubated in media containing amounts of penicillin varying from 0.0009 to 9 Oxford units per cc. The number of organisms per cc at intervals was determined by colony counts in pour plates. (Graph I).

With concentrations of 0.9 and 9.0 Oxford units of penicillin per cc the number of viable organisms remained unchanged for the first hour following which there was a steady decrease. With 0.09 units per cc there was an initial lag, followed by a slight increase and then a drop in the number of viable organisms.

* This work was presented in part before the New York and New Jersey Branches of the Society of American Bacteriologists, December, 1942.

[1] Dawson, M. H., Hobby, G. L., Meyer, K., and Chaffee, E., *J. Clin. Invest.*, 1941, **20**, 434.

[2] Hobby, G. L., Meyer, K., and Chaffee, E., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 277.

[3] Dawson, M. H., Hobby, G. L., Meyer, K., and Chaffee, E., *Ann. Int. Med.*, 1944, **19**, 707.