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SOME EFFECTS OF HISTAMINE IN THE NORMAL AND *HAEMOPHILUS PERTUSSIS* VACCINATED RAT

BY

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The effects of histamine in the normal and *H. pertussis* vaccinated rat have been investigated. In the normal animal small doses of histamine (from 1 to 30 mg./kg., i.v.) produced significant, reversible alterations in the blood pressure, electrocardiogram and electroencephalogram. Doses of 100 mg./kg. or more caused bronchoconstriction and doses of 200 mg./kg. or more an effect on the muscular contractions after electrical stimulation of the nerve. Acute histamine intoxication resulted on rapid injection of 500 mg./kg. intravenously; death was probably due to cardiovascular collapse. In the vaccinated rat, a distinction could be made between the immediate and delayed effects of histamine. With a sublethal dose of histamine (5 mg./kg.) alterations in the blood pressure, electrocardiogram and electroencephalogram were noted. The neuromuscular system, which in the normal animal was the least reactive to histamine, showed the most marked increase in sensitivity in the vaccinated rat. The delayed effects of histamine observed after an interval of 5 to 30 min. were characteristic and irreversible, leading to death of the animal. The mechanism of death in the vaccinated rat after histamine appears to differ from that of the normal animal and has been discussed in the light of present knowledge.

The ability of the rat and mouse to tolerate large doses of histamine is well known, and stands in sharp contrast to the high sensitivity of other laboratory animals such as the guinea-pig, rabbit, or cat, which succumb when relatively small doses of this substance are injected (Guggenheim, 1940; Bovet and Bovet-Nitti, 1948). Little is known of the mechanism underlying death of the rat and mouse due to histamine intoxication or the reason for this high natural resistance. The induction of anaphylactic sensitivity is more difficult in histamine resistant than in histamine sensitive species (Mayer and Brousseau, 1946; Perry and Darsie, 1946). Antihistamines protect the guinea-pig, rabbit or dog from many lethal doses of histamine but do not protect the mouse against a single lethal dose (Bovet and Walthert, 1944; Halpern and Wood, 1950).

A number of investigators have shown that it is possible by adrenalectomy to render the rat and mouse considerably more sensitive to the action of histamine (Wyman, 1928; Halpern and Wood, 1950; Houssay, 1955). In the adrenalectomized mouse, adrenaline and cortisone increase resistance to histamine, and when both are injected the normal resistance to histamine is restored (Halpern, Benacerraf, and Briot, 1952). Houssay (1955) has shown that the sensitivity of adrenalectomized rats to histamine is largely due to the loss of cortical function.

A further method of increasing the sensitivity of mice to histamine was first described by Parfentjev and Goodline (1948). They made the interesting observation that mice injected with *H. pertussis* vaccine developed a considerable sensitivity to histamine which reached its maximum 5 days after vaccination. This phenomenon has been confirmed by a number of workers (Halpern and Roux, 1950; Kind and Wood, 1953; Maitland, Kohn, and Macdonald, 1955). Experiments carried out with the rat showed that this animal could similarly be rendered more sensitive to histamine after *H. pertussis* vaccine. The guinea-pig failed to become more histamine-sensitive when thus treated (Maitland, Kohn, and Macdonald, 1955). Antihistamines when given before a challenge dose of histamine to the vaccinated animal were effective in preventing the onset of the fatal syndrome (Parfentjev and Goodline, 1948; Halpern and Roux, 1949; Thiele and Schuchardt, 1952). A number of possible explanations have been advanced to account for the effect

of the vaccine. It may exert an injurious effect on the adrenals and render the animal more sensitive to histamine, since protection is obtained with cortisone (Kind, 1953). Mouse lung tissue is normally rich in histaminase, and it has been shown that the vaccinated lung tissue detoxifies considerably less histamine (Kind and Wood, 1953). However, histological examinations of the vaccinated rat failed to reveal pathological changes in the adrenals (Malkiel and Hargis, 1952). Thiele and Schuchardt (1952) demonstrated that histamine sensitization by *H. pertussis* vaccine could be inhibited by repeated treatment with quercitin during the sensitizing period. This substance is known to prolong the action of adrenaline and decrease cutaneous capillary permeability.

To obtain further information on the action of histamine in the normal and *H. pertussis* vaccinated rat, experiments were designed to study such action on the cardiovascular system, smooth muscle, the central nervous system and neuromuscular transmission.

Materials and Methods

The experiments were carried out on normal and vaccinated rats of both sexes weighing 200 to 400 g. An *H. pertussis* vaccine (BV) containing 200,000 million organisms/ml. and resuspended in saline was used. Animals were injected intraperitoneally with 80,000 million organisms/100 g. of body weight. This standard dose of organisms was employed as it has been shown to sensitize appreciably to histamine (Maitland *et al.*, 1955). The height of histamine sensitization was reached 4 days after vaccination and remained at this level for approximately 2 weeks, hence all tests with the treated animals were undertaken during this period of maximum sensitivity. Histamine in the form of the dihydrochloride was used and all doses are stated in terms of this salt. Unless otherwise indicated the animals were under pentobarbitone sodium anaesthesia (40 mg./kg., i.p.) and in some experiments gallamine was given (10 mg./kg., i.v.). All intravenous injections were made into the tail vein of the rat.

Blood Pressure.—The common carotid artery was cannulated and the blood pressure was recorded in some experiments with a mercury manometer and in others by means of an electromanometer (Frank and Longo, 1955). With the latter system large movements of the anticoagulant in the tube during pressure variations were avoided. To reduce coagulation a small dose of heparin was injected.

Electrocardiogram.—The electrical potentials were recorded from the two fore feet and the left hind foot by insertion of 3 needle electrodes into the skin of the animal. The same recording apparatus was used as for the electroencephalographic recordings.

Tidal Air.—The method of Konzett and Rössler (1940) was employed, based on determination of the tidal air maintaining the volume and pressure of ventilation constant.

Cerebral Electrical Activity.—Four stainless steel electrodes were screwed through the skull overlying the frontal and parietal lobes and the cerebral electrical activity was recorded by the bipolar method. In some experiments the fixation of the electrodes was carried out on animals given light ether anaesthesia, immobilized by the intravenous administration of gallamine and maintained under artificial respiration (30 strokes/min.) with air. The recordings were started 1 hr. after completing these preparations. The electrical activity of the brain was recorded by means of a 4 channel Grass Electroencephalograph (Model III D).

Electrical Stimulation of the Masseter Muscle.—The technique described by Eicholtz, Hotovy, and Erdniss (1949) and modified by Marotta (1957) was employed. The rat, after tracheotomy, was maintained under artificial respiration. The animal was fixed on a wooden board, and the upper jaw held up by means of a spring placed under the upper incisors. A rod connected to a writing lever was attached to the lower incisors. The lower jaw was held down by the weight of the rod. Two electrodes were inserted into the left masseter muscle, which was stimulated once every min. At each stimulation the writing lever recorded the movement of the lower jaw on a slowly moving smoked drum.

Gastrocnemius-sciatic Nerve Preparation.—The contractions of the gastrocnemius muscle following electrical stimulation of the cut peripheral end of the sciatic nerve were recorded isometrically. In some experiments contractions of the gastrocnemius and masseter muscles were recorded simultaneously in the same animal.

Phrenic Nerve-diaphragm Preparation.—The technique described by Bülbring (1946) was employed, as modified by Bovet-Nitti (personal communication). The bath was maintained at 30°, and the nerve stimulated every 30 sec. A shock of 2 to 3 V. was applied for approximately 1 m.sec. The solution employed was that described by Taugner and Fleckenstein (1950). The drug under examination was added to the bath of 150 ml. capacity and allowed to remain for 15 min. Traces of the drug were removed by washing 3 times, and the muscle was left to recover for 15 min. before a fresh test was undertaken. Electrical stimulation of the nerve was continued during the recovery period.

Results

Normal unanaesthetized rats when injected with doses of histamine ranging from 30 to 300 mg./kg. intravenously reacted by manifestations of pain and subsequent shock. They sat in a crouched position and breathed with difficulty. Copious salivation, urination, defaecation, cyanosis, and paralysis of the extremities were also observed. When given by rapid intravenous injection 500 mg./kg. or more of histamine caused the imme-

diate death of the animal in convulsions. The intraperitoneal injection of 2.5 to 3 g./kg. of histamine resulted ultimately in death of the animal, but this was noted to occur from 5 min. to several hr. after the injection, with symptoms similar to those described above. The animal usually died in convulsions. No significant protective effect towards histamine intoxication was afforded by anaesthesia.

With the dose of vaccine injected, more than 90% of the animals could be sensitized. Although it was usually possible to kill the sensitized rat with 10 mg./kg. histamine (i.v.), this dose was considered too near to that from which the animal survived (5 mg./kg., i.v.). Hence in most of the sensitization experiments, doses of 20 mg./kg. histamine (i.v.) were used because this proved invariably fatal. When this dose was injected into the vaccinated rat, symptoms identical to those described for the normal animal were observed. However, whereas the normal rat after a certain time completely recovered, the conditions of the vaccinated animal progressively deteriorated until it died. Death occurred from 5 min. to several hours after the injection. No difference in the speed which which the animal died was observed whether histamine was given intravenously or intraperitoneally.

It was clear that, whereas a lethal dose of histamine given intravenously in the normal animal provoked instantaneous death, the smaller lethal dose of histamine in the vaccinated rat, even when given intravenously, required a minimum period of time, which in no instance was less than 5 min., before the animal succumbed.

Blood Pressure.—The average blood pressure in the normal anaesthetized rat was 140 to 160 mm. Hg. Following the intravenous administration of 1 mg./kg. histamine in the normal rat, a significant lowering of the blood pressure was noted (to 80 to 90 mm. Hg), which then slowly rose to its original level. Large doses of histamine (300 mg./kg.) provoked a pronounced lowering in the blood pressure (to 40 to 50 mm. Hg), which remained low for more than an hour. It was generally observed that with small doses of histamine a hypertension of very short duration preceded the subsequent hypotension. The blood pressure of the vaccinated rat was similar to that of the normal animal and the immediate effects of histamine were similar in both cases. However, the administration of not less than 20 mg./kg. of histamine (i.v.) provoked an arterial hypotension which tended to progress until the death of the animal. In two experiments with vaccinated rats, it was noted that 2 min. after the administration of 50 mg./kg. histamine (i.v.) the arterial blood pressure after the usual fall rose slowly. At this point, simultaneously with the appearance of grave alterations in the electrocardiogram, a sudden fall in the blood pressure ensued, and death followed shortly afterwards.

Electrocardiogram. — The electrocardiogram (ECG) was recorded in the rat under pentobarbitone sodium anaesthesia. It was not possible to obtain a satisfactory tracing for the unanaesthetized rat owing to the anxiety and fear of the animal, which led to an increased frequency in the cardiac rhythm. A large number of control experiments were carried out to determine the reproducibility of the tracings, and it was found that the average heart rate varied roughly from 300 to 450/min. depending to some extent on the age of the animal. These figures agree with the findings of Heise and Kimbel (1955). Daily ECG records of the same animal were taken for a period of five days; these were remarkably constant, only minor modifications being noted.

In the normal animal, doses of histamine up to 200 mg./kg. (i.v.) did not produce any significant change in heart rate. With the slow injection of larger doses (500 mg./kg.) there was a significant reduction in the heart rate within 1 min. of the injection. Thus in a typical experiment the frequency was reduced from 342 to 198/min. A rapid return took place and within 2 min. the rate was 282/min. Changes in the form of the ECG were observed when small doses of histamine (10 mg./kg., i.v.) were administered, and consisted of modifications of the S-T segment and enlargement of the T wave (Fig. 1A). A complete return to normal was obtained. With larger doses of histamine (200 mg./kg., i.v.) a complete and reversible change in the ECG was found (Fig. 1B). The rapid intravenous injection of 500 mg./kg. histamine (lethal dose) caused the immediate flattening and failure of the ECG. The intraperitoneal injection of 2.5 g./kg. histamine was followed by a reduction in heart rate and voltage. In some cases arrhythmia was noted (Fig. 1C).

In the vaccinated animal a sublethal dose of histamine (5 mg./kg.) produced some changes in the heart rate, consisting in a slight preliminary rise, followed by a fall (Fig. 9*a*, upper diagram). With 20 mg./kg. histamine (lethal dose) a slight increase in the number of beats was first noted (300 to 318/min.) which may have been related to the brief period of hypertension. The frequency then began to decrease gradually (Fig. 9*b*, upper diagram). With 100 and 200 mg./kg. a fall in the frequency was obtained within the first min. of

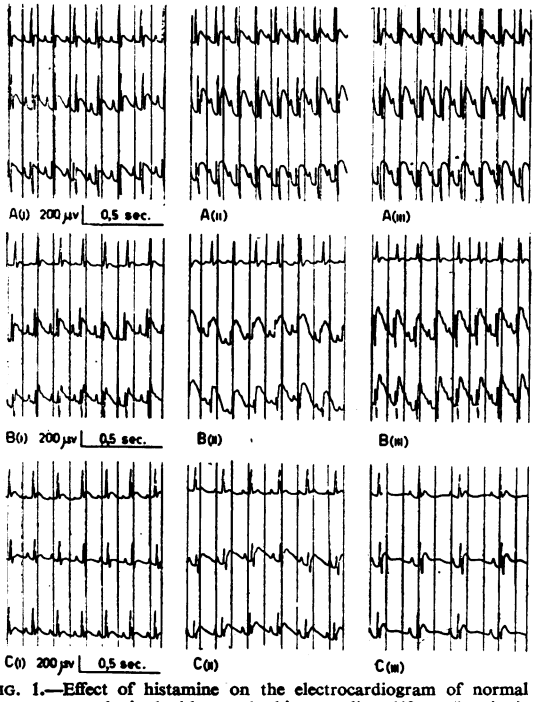

FIG. 1.—Effect of histamine on the electrocardiogram of normal rats anaesthetized with pentobarbitone sodium (40 mg./kg., i.p.). A (I), ECG of a normal rat weighing 230 g.; A (II), 1 min. after 10 mg./kg. histamine (i.v.); and A (III) 8 min. after histamine. B (I), ECG of a normal rat weighing 200 g.; B (II), 4 min. after 200 mg./kg. histamine (i.v.); and B (III), 90 min. after histamine. C (I), ECG of a normal rat weighing 190 g.; C (II), 20 min. after 1·5 g./kg. histamine (i.p.); and C (III), 45 min. after C (II) and 12 min. after a lethal dose of histamine (2·5 g./kg., i.p.).

the injection, which then partly recovered to fall again later (Fig. 9*c*, upper diagram). Fig. 2 (D and E) shows the ECG changes which occurred on injecting histamine in the vaccinated animal. An almost complete arrhythmia and extra-systoles developed and the animal did not recover.

A further series of records were taken from a group of rats which were given daily doses of 50 mg./kg. histamine (i.v.) before vaccination and subsequently during the sensitization period for a total of 5 days. The object of these experiments was to determine the time at which changes in the ECG could be noted. During the first 2 days after vaccination no significant changes occurred (that is, the animals had not become sensitized to histamine) whereas from the 3rd to the 5th day death occurred when the histamine was injected. Rats treated daily with 50 mg./kg. histamine did not become desensitized to the lethal effect of histamine when challenged after the establishment of the hypersensitivity.

Tidal Air.—Registration of the tidal air was selected to test the reactivity of histamine on

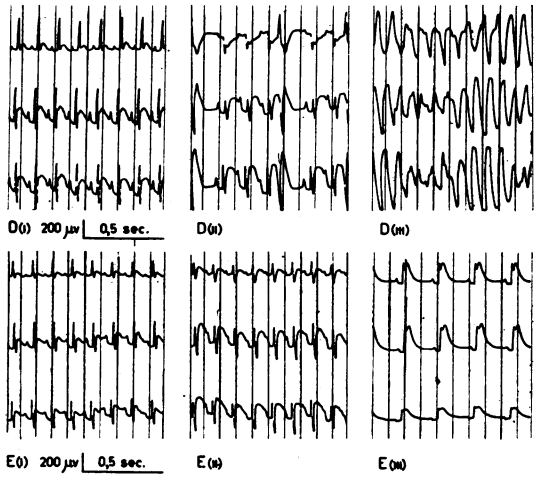

FIG. 2.—Effect of histamine on the electrocardiogram of rats vaccinated with *H. pertussis*. The animals were anaesthetized with pentobarbitone sodium (40 mg./kg., i.p.). D (I), ECG of vaccinated rat weighing 200 g.; D (II), 5 min. after 50 mg./kg. histamine (i.v.); and D (III), 8 min. after histamine. E (I), ECG of vaccinated rat weighing 220 g.; E (II), 20 min. after 200 mg./kg. histamine (i.p.); and E (III), 45 min. after histamine.

smooth muscle. In the rat this organ was found to be only slightly sensitive to the action of histamine. With doses of 100 mg./kg. (i.v.) of histamine a reduction in tidal air, during constant pressure ventilation, attributed to bronchospasm was observed, which lasted for only a short period (Fig. 3). Rapid injection of 500 mg./kg. (i.v.) histamine provoked a marked reduction of tidal air which persisted even after death (Fig. 3).

The actions of histamine on the bronchial musculature of the vaccinated rat may be divided into immediate and delayed effects. The immediate action was similar to that observed in the

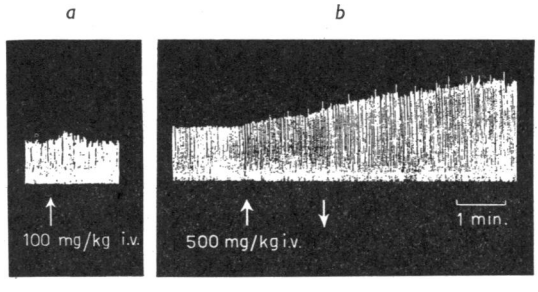

FIG. 3.—Effect of histamine, given intravenously, on the tidal air during constant positive pressure ventilation of the normal rat under pentobarbitone sodium (40 mg./kg., i.p.) and gallamine (10 mg./kg., i.v.). Method of Konzett and Rössler (1940). A rise in the record indicates a fall in tidal air. (*a*) Rat; 250 g. 100 mg./kg. histamine was the smallest dose which produced a decrease in tidal air. (*b*) Rat; 380 g. Rapid administration of 500 mg./kg. histamine. The second arrow indicates cardiac arrest. The decreasei n tidal air continued even after death.

normal animal ; the minimum effective dose which produced a distinct bronchospasm was again 100 mg./kg. (i.v.). In some instances this action was of considerable duration. Administration of 500 mg./kg. histamine (i.v.) once again led to death of the animal with a bronchospasm similar to that observed in the normal animal. The delayed effects of histamine were very characteristic. The injection of 50 mg./kg. (i.v.) histamine in the normal rat was without noticeable effects, but a similar dose given to the vaccinated animal produced a bronchospasm after a period of not less than 5 min. There is thus a delayed effect in the vaccinated rat at a dose substantially less than that which affects the normal animal.

Electroencephalogram.—The electroencephalogram (EEG) of the normal rat was similar to that of the vaccinated animal. Under pentobarbitone sodium anaesthesia, the animal showed a cerebral electrical activity characterized by an irregular basal rhythm with a frequency of 8 to 12 c./sec., upon which spike waves, often organized into spindles, were superimposed (Fig. 4A). On this background it was possible to show the desynchronizing effects on application of external stimuli. The administration of further quantities of pentobarbitone sodium provoked a flattening of the tracing, in which the spike potentials became more apparent. The cerebral electrical activity of the curarized unanaesthetized rat was highly desynchronized and consisted of waves with a voltage generally not more than 100 μV. and a frequency of approximately 20 to 30 c./sec. The arousal reaction could not be elicited. In the normal anaesthetized rat the intravenous administration of histamine in doses of 30 to 300 mg./kg. provoked desynchronization similar to that observed after external stimulation (Fig. 4B). This desynchronization tended to be very prolonged and usually lasted longer than the effects of the anaesthetic on the EEG (Fig. 4C). In the normal, curarized rat the administration of histamine (30 to 300 mg./kg.) was followed by a flattening of the cerebral electrical tracing which lasted for 5 to 15 min. In some cases profound modifications in the ECG took place before these changes of the EEG. In other experiments the modifications proceeded in the reverse order. The diminution in frequency and increase in voltage of cerebral potentials in the rat described by Brandon (1955) with the doses of histamine he employed could not be confirmed by the present authors.

The initial effects of histamine were similar in the vaccinated rat. However, whereas in the normal animal a gradual return to normal was observed after sublethal doses, in the vaccinated rat the modifications in the cerebral electrical activity became more pronounced, as shown by a further flattening of the tracings, and finally gave rise to failure (Fig. 5).

The rapid intravenous administration of 500 mg./kg. histamine both in the normal and vaccinated rat was followed by a general flattening of the EEG tracings and death.

Electrical Stimulation of the Masseter Muscle.—Histamine induced paralysis in the rat, and therefore its effect on neuromuscular transmission was examined. In the normal rat the intravenous injection of histamine (100 mg./kg. or more) gave rise in some experiments to an irregular

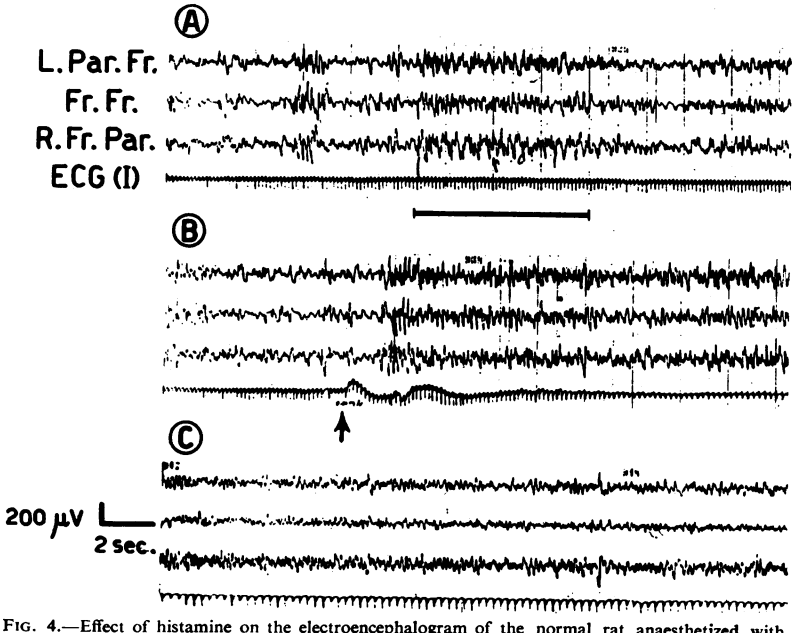

FIG. 4.—Effect of histamine on the electroencephalogram of the normal rat anaesthetized with pentobarbitone sodium (40 mg./kg., i.p.). Leads are as indicated. (A) Rat; 380 g. The EEG of normal animal is synchronized with spikes grouped into brief spindles. The arousal reaction (black line) was elicited by pinching of the tail. (B) Effect of 150 mg./kg. histamine, i.v. (at arrow). The desynchronization of the EEG is similar to that obtained by means of peripheral stimulation but is more prolonged. (C) 15 min. after histamine. The tracing is still desynchronized.

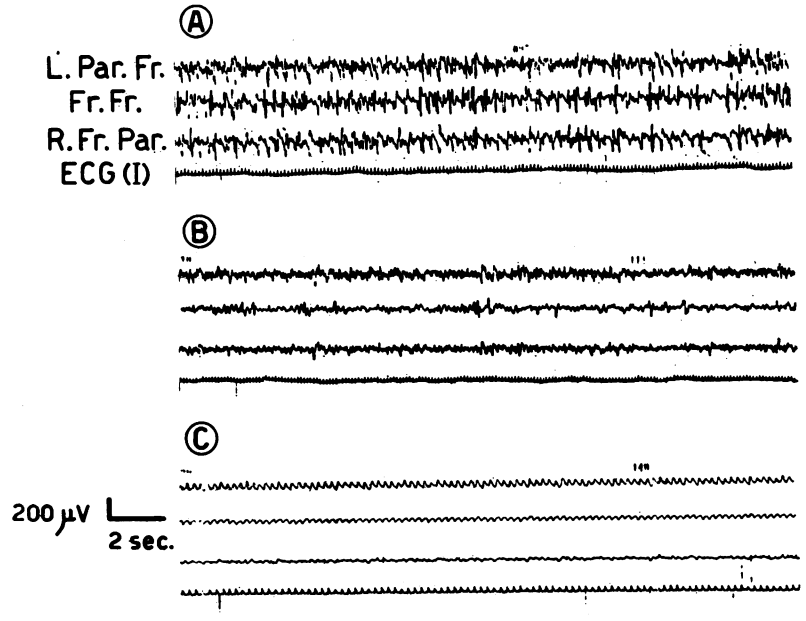

FIG. 5.—Effect of histamine on the electroencephalogram of a rat vaccinated with *H. pertussis*. The animal was anaesthetized with pentobarbitone sodium (40 mg./kg., i.p.). (A) EEG of vaccinated animal is synchronized with spikes which are not grouped as spindles. (B) 2 min. after 50 mg./kg. histamine, i.v. Desynchronization of the tracing is still present. (C) 15 min. after histamine. The tracing is now flattened.

and fairly frequent contracture of the masseter muscle in the absence of electrical stimulation. When a dose of 100 mg./kg. (i.v.) or less of histamine was injected into the normal rat, no effect on the height of the muscular contractions in response to stimuli was noted. With 200 mg./kg. (i.v.) of histamine some effect was sometimes observed (Fig. 6). In two experiments the contractions were 82% and 95% of the original value, and in another two no effect on the muscular contractions was noted. The slow injection of 500 mg./kg. histamine (i.v.) gave rise to a very pronounced reduction in the height of contraction; in four experiments the contractions were reduced to 17, 21, 33, and 94% of the original value (Fig. 6). Generally the maximum degree of paralysis was obtained within 30 min. of injection of histamine and lasted considerably more than 3 hr. Fig. 8 shows the effects of 500 mg./kg. histamine by slow intravenous injection on the heart rate and muscular contractions. It was further observed that a second similar dose of histamine administered after complete recovery produced a far smaller effect on the muscular contractions.

A sublethal dose of histamine in the vaccinated rat (5 mg./kg.) (Fig. 7) produced a significant decrease in the muscular contractions in two experiments (45% and 50% of the original value), but in another two the heights of the contraction were 90% and 100%. In those experiments where a decrease occurred, complete recovery was observed. At the end of the experiments with 5 mg./kg. histamine a challenge dose of 50 mg./kg. histamine was given to establish whether the animals were indeed sensitive. This was confirmed, as all these animals died shortly after injection. With lethal doses of histamine in the vaccinated rat (20 mg./kg., 100 mg./kg., and 200 mg./kg.) a rapid onset in neuromuscular paralysis was observed, leading to complete inhibition of the contraction and death of the animal. It is clear from Fig. 7 that the speed

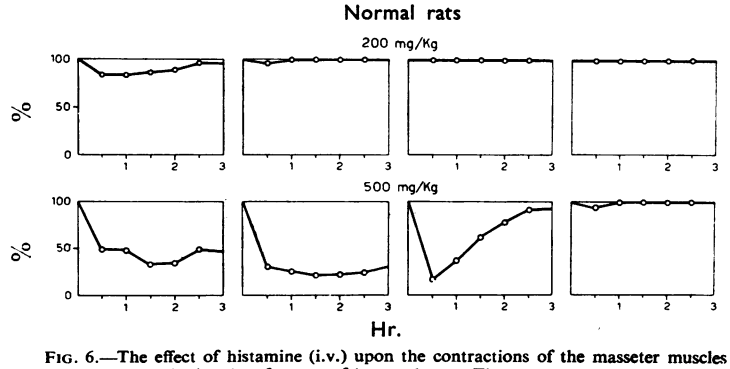

FIG. 6.—The effect of histamine (i.v.) upon the contractions of the masseter muscles (stimulated indirectly) of groups of 4 normal rats. The rats were anaesthetized with pentobarbitone sodium (40 mg./kg., i.p.). Ordinates show % original twitch height.

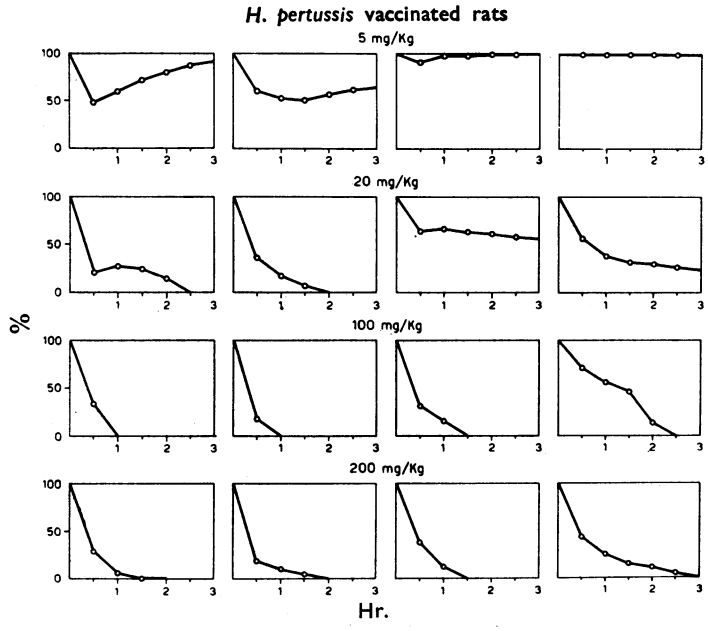

FIG. 7.—The effect of histamine (i.v.) upon the contractions (stimulated indirectly) of the masseter muscles of groups of 4 rats vaccinated with *H. pertussis*. Pentobarbitone sodium (40 mg./kg., i.p.). Ordinates show % original twitch height.

of onset of the neuromuscular paralysis was more pronounced when 100 mg./kg. was given than after 20 mg./kg., but little significant difference was noted between the administration of 100 mg./kg. and 200 mg./kg. histamine. Further, it is of interest to observe from a comparison of Figs. 6 and 7 that the degree of muscular paralysis produced by the injection of 500 mg./kg. histamine in the normal rat was similar to that obtained with 5 mg./kg. in the vaccinated rat. It would thus appear that the vaccinated animal is almost 100 times more sensitive to histamine as judged by this method. Figs. 8 and 9 illustrate the effect of different doses of histamine on the heart rate and muscular contractions of the normal and vaccinated rat. It is clear that there is no relationship between these effects. Thus in the normal animal the intravenous injection of 500 mg./kg. histamine gave rise to a momentary reduction in heart rate which returned to normal and fell again somewhat later, whereas the muscular contractions continued to decrease gradually. After 45 min. when the maximum inhibition of muscular contractions was obtained, the heart rate was still 250/min. (Fig. 8).

In the vaccinated animal, the injection of 5 mg./kg. and 20 mg./kg. of histamine had little effect on the heart rate whereas the muscular contractions were significantly reduced. With 100 mg./kg. and 200 mg./kg. histamine the heart rate very slowly decreased, whereas the inhibition in the muscular contractions was much more rapid (Fig. 9). A close similarity between the sensitivity of the gastrocnemius and masseter muscles to histamine was observed. In most of our experiments we used the masseter.

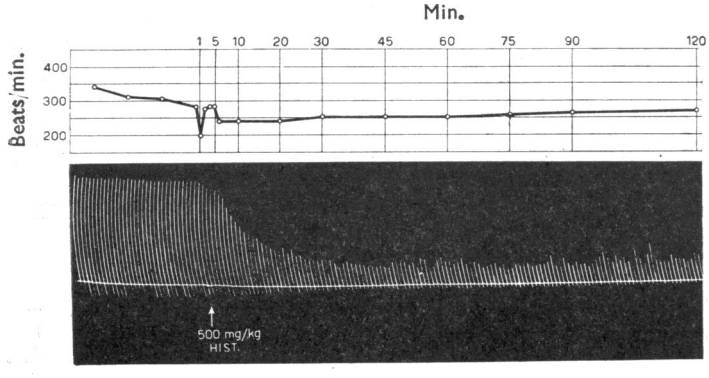

FIG. 8.—Effect of histamine injected intravenously into a normal rat on the heart rate and on the contractions of the masseter muscle stimulated indirectly. Rat (240 g.) anaesthetized with pentobarbitone sodium (40 mg./kg., i.p.).

Phrenic Nerve-diaphragm Preparation.—Various concentrations of histamine were added to the bath containing

nerve-muscle preparations of normal and vaccinated rats. Table I summarizes the results obtained.

It will be noted that the smallest dose of histamine which caused inhibition of the muscular contractions was 10 mg./l. As the dose was increased the degree of paralysis became more pronounced, and with doses of 1 g./l. a paralysis of 40% was obtained. The muscular contractions slowly but usually completely

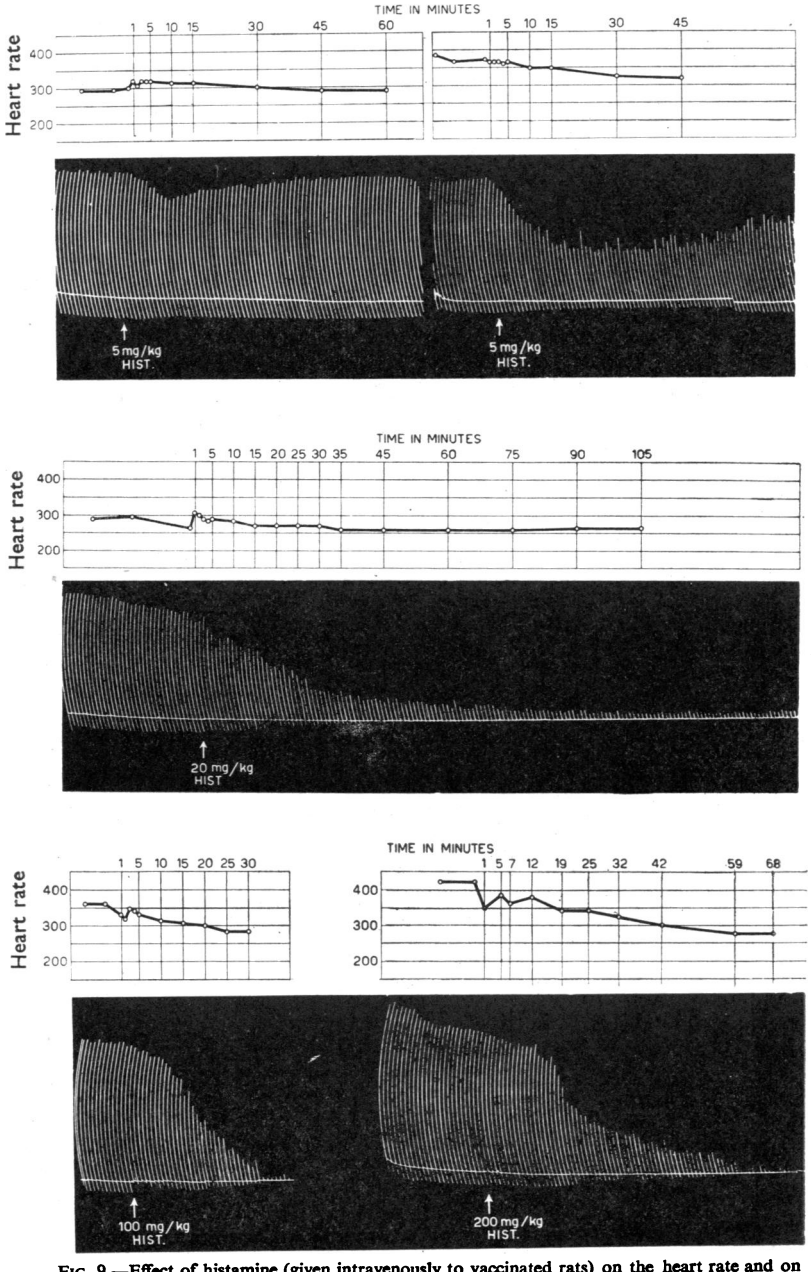

FIG. 9.—Effect of histamine (given intravenously to vaccinated rats) on the heart rate and on the contractions of the masseter muscle stimulated indirectly. Rats anaesthetized with pentobarbitone sodium (40 mg./kg., i.p.).

recovered after repeated washing. It was not possible to show any significant difference between the normal and vaccinated animals by this procedure.

TABLE I

THE EFFECT OF HISTAMINE ON THE PHRENIC NERVE-DIAPHRAGM PREPARATION OF THE NORMAL AND *H. PERTUSSIS* VACCINATED RAT

Conc. of Histamine (mg./l.)	% Paralysis	
	Normal Animal	Vaccinated Animal
1	0	0
10	0–8	4–13
100	8–21	9–23
300	8–44	—
1,000	20–40	—

DISCUSSION

The observed effects of histamine on the heart rate and ECG of the normal rat may result from coronary insufficiency and a change in ionic equilibrium. The action on plain muscle is shown by a bronchoconstriction of relatively minor importance, the central action is characterized by a desynchronization and a flattening of the EEG tracing, and large doses decrease the height of the contractions of the masseter and gastrocnemius muscles.

Under these conditions, the multiplicity of the factors which enter into histamine intoxication was confirmed. Death in the normal animal on rapid intravenous injection of 500 mg./kg. histamine or more took place almost instantaneously and would appear to be due mainly to cardiovascular collapse. Doses of histamine above 500 mg./kg. (i.v.) were invariably lethal as were those of Mayer and Brousseau (1946), Feldberg and Schilf (1930) and Bovet and Bovet-Nitti (1948). It is of particular interest that, whereas the normal animal receiving an intravenous administration of a lethal dose of histamine dies immediately, the vaccinated animal when treated with its lethal dose (20 mg./kg. or more) takes longer to die. Death of the vaccinated rat was not directly due to asphyxia, for the animal was not protected from the lethal effects of histamine by artificial respiration.

The immediate and considerably increased response of the vaccinated rat to the neuromuscular action of histamine was characteristic. Whereas the dose required in the normal animal to produce some effect on the masseter muscle was from 200 to 500 mg./kg. (i.v.), the vaccinated rat was sensitive to a dose of 5 mg./kg. (i.v.). The ratio here (from 40 to 100:1) agreed with other estimates for enhanced sensitivity quoted in the literature. Thus Parfentjev and Goodline (1948) obtained 25-fold and Gauthier, Loew, and Jenkins (1955) a 33-fold increase in the toxicity after pertussis vaccination.

Reports upon the effects of histamine on skeletal muscle and neuromuscular transmission in the rat are very scanty. Sömjén and Uyldert (1955) recently showed that the histamine liberator 48/80 provoked a distinct diminution in the contraction of the gastrocnemius muscle of the rat, but the action of histamine on this preparation was not investigated. These authors were of the opinion that the effects observed were due to the action of the compound itself and not to liberated histamine. Feldberg (1956) has suggested that histamine in the rat may give rise to a pronounced muscular weakness and paralysis, either by direct muscular action or by producing neuromuscular block. It is clear from the experiments here reported that histamine does, in fact, exert an effect on the muscular contraction of the rat, but the mechanism of the block produced is not clear.

In discussing possible explanations for the markedly increased sensitivity of the vaccinated rat to histamine it is of interest to record the recent work of Macmillan and Vane (1956), who examined the effect of histamine on the plasma potassium level of the cat. These authors suggest that the intravenous injection of histamine causes a leak of potassium into the plasma which gives rise to the pharmacological effects, and that potassium may play a significant rôle in histamine intoxication. The possibility cannot therefore be excluded that, in the vaccinated rat, histamine may cause changes in the ionic equilibrium. In this connexion it may be remembered that the vaccinated, like the adrenalectomized animal, may be protected from fatal shock by pretreatment with cortisone. The importance and the influence of the suprarenal hormones on the mineral balance are well known.

An investigation of the effects of histamine on the potassium level in the normal and vaccinated animal is in progress.

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