conditions generally, so that the burden of the disease should not fall on one class of the community. If it is chiefly the poorer class in the congested centres which is involved can we not by improving social conditions among this class assist in stamping out the disease? Thirdly, realizing that the onset and relapses of rheumatic fever follow regularly on respiratory and throat infections, can we by intelligent directed effort lessen these infections and improve the mouth and throat hygiene of the community. Lastly, and what seems to me most important, surely in all the important centres of population and medical teaching, special facilities should be provided for the intensive study of the disease, facilities for research as to its cause and possible control, and organized effort to assist those stricken and disabled by the disease.

Let me close by a quotation from a well-known authority, giving at least a ray of encouragement, "In its typical clinical form, rheumatic fever has declined in most countries to a remarkable extent, and thus it comes within the category of those diseases of the cause of whose decrease we have no exact or complete knowledge."

No one is more conscious than myself of the vague and inconclusive account of the disease I have presented and the possible methods of its control. However, if I have done anything to call attention to this serious problem and arouse interest in it I will feel my time has been well spent, and that I have made a slight effort to follow in the footsteps of my revered teacher, Dr. Blackader.

VAGUS STIMULATION AND THE PRODUCTION OF MYOCARDIAL DAMAGE

BY G. W. MANNING, G. E. HALL AND F. G. BANTING

Department of Medical Research, Banting Institute, University of Toronto

IN the experiments reported by Hall, Ettinger and Banting[1] it was shown that myocardial and coronary artery damage resulted from long-continued daily administration of acetylcholine to unanæsthetized dogs. Unsuccessful attempts to reproduce these conditions in other dogs by similar injections of choline (Ettinger and Hall[2]), by histamine (Ettinger, Hall and Lang[3]), by the administration of sodium nitrite, by strenuous daily exercise, and by flooding the system with low-grade infection have led us to consider the apparent specificity of acetylcholine in the production of such lesions.

Since the effects of vagus nerve stimulation are due to the action of liberated acetylcholine (Loewi and associates[4, 5, 6]) then, if our supposition is correct, long-continued stimulation of the vagus nerve through liberated acetylcholine should likewise produce myocardial and coronary artery damage. Experiments of this type have been reported by Ettinger, Hall and Banting.[7] They used two methods of stimulation: (*a*) stimulation from an insulated secondary coil, the electrodes of which were in contact with the exposed vagus nerve; implantation of the whole coil in the neck (a modification of the method of Loucks, 1933); (*b*) stimulation by means of shielded electrodes (a modification of Cannon's method, 1933), which were fastened to the vagus nerve in the neck and allowed to protrude through a stab wound in the skin. The stimulations were carried out with the animals under dial or nembutal anæsthesia. Under the conditions of these experiments, Ettinger, Hall and Banting were unable to produce degenerative changes in the heart or its blood-vessels by means of prolonged daily electrical excitation of the vagus nerve. It was thought that under more normal conditions, without deep anæsthesia, prolonged stimulation of the vagus nerve in the dog might still result in cardiac dysfunction. The following groups of experiments were therefore carried out: A. stimulation under light anæsthesia; B. stimulation without anæsthesia.

A. THE EFFECT OF CONTINUOUS STIMULATION OF THE VAGUS NERVE IN THE LIGHTLY ANÆSTHETIZED DOG

Under light nembutal anæsthesia (25 mg. per kilo) the left vagus nerve was separated from the aortic depressor nerve and cervical sympathetic. The electrode-foot was sewn around

the nerve, the lead-in wires brought to the surface, and stimulation carried out continuously in the manner already described. (Ettinger, Hall and Banting, 1936). The left vagus nerve was stimulated in each case.

One animal was given atropine throughout the stimulation period. This was found to be effective in preventing the physiological response of vagal stimulation. At the same time it does not prevent the liberation of acetylcholine following such stimulation. (Loewi and Navratil[4]). In order to accentuate the effects of vagal stimulation another animal was given eserine. This substance decreases the rate of hydrolysis of acetylcholine by inhibiting the activity of the choline-esterase. To supply nourishment and fluid to these animals normal saline and 10 per cent glucose were given occasionally (column 8, Table I).

(22 seconds between stimulating periods) the heart-rate varied from 180 to 220 beats per minute (acceleration due to anæsthesia). During stimulation this was decreased to 60 to 100 beats per minute. Towards the termination of the experiments the heart-rates were difficult to determine owing to respiratory disturbances and weakness of the heart-beat.

Gastro-intestinal.—1. Salivation occurred in all of the animals except the one receiving atropine. The animal receiving eserine secreted very large quantities of frothy saliva.

2. Vomiting occurred frequently, except in the animal receiving atropine. The vomitus at first contained some undigested food, but in the later stages was "coffee-ground" in type (positive benzidine test for presence of blood).

3. Diarrhœa and melæna were common (except in D 73 which received atropine).

TABLE I.

CONTINUOUS STIMULATION EXPERIMENTS—ANÆSTHETIZED ANIMALS

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Dog No.	*Total experimental time*	*Total stimulating time*	*Total off time*	*Total mg. atropine*	*Total mg. eserine*	*Total mg. nembutal*	*Glucose or normal saline intravenously*
D 63	31 hrs.	24½ hrs.	6½ hrs.	0	0	315 mg.	350 c.c. n.s. 300 c.c. 10% gl. 650 c.c.
D 64	36 hrs.	26 hrs.	7 hrs.	0	0	585 mg.	400 c.c. n.s. 400 c.c. 10% gl. 800 c.c.
D 71	33 hrs.	26 hrs.	7 hrs.	0	0	337.5 mg.	300 c.c. n.s. 350 c.c. 10% gl. 650 c.c.
D 72	46 hrs.	36¼ hrs.	9¾ hrs.	0	0	300 mg.	1500 c.c. 10% gl.
D 66	45 hrs.	35½ hrs.	9½ hrs.	0	62.5 mg.	435 mg.	1400 c.c. 10% gl.
D 73	70 hrs.	54½ hrs.	14½ hrs.	51.0 mg.	0	930 mg.	3500 c.c. 10% gl.

The animals were stimulated for 31 to 70 hours (column 3, Table I). Throughout the stimulation small amounts of nembutal were given to maintain light anæsthesia. Heated operating tables were used to keep the animals warm.

Heart-rate.—Inhibition of the heart-rate was taken as evidence of vagal stimulation. During the early hours of stimulation this inhibition was easily detected. When not being stimulated

Autopsy.—The animals were autopsied immediately after death. With the exception of the left auricle the heart was usually filled with blood clot, which suggested complete ventricular fibrillation as the cause of death. Except for a rather large infarcted area at the base of the anterior papillary muscle in the animal which had received eserine, no gross lesions were observed in the heart. The lungs showed some areas of congestion but were otherwise normal.

Numerous hæmorrhagic areas were found throughout the gastro-intestinal tract. In one animal, D 63, which had received eserine, marked ulceration was observed in the pyloric region along the lesser curvature. General congestion of the gastro-intestinal tract was observed except in the animal which had received atropine (D 73). The intestine of D 73 appeared fairly normal, with evidence of slight congestion in the duodenum.

Microscopic examination.—Microscopic examination revealed capillary congestion and hæmorrhage in the heart with the occurrence of some early hyaline degeneration of the cardiac muscle.

The spleen contained large deposits of hæmosiderin. The lungs appeared normal, with no evidence of pneumonia. Capillary congestion was found in some of the kidney sections. All other sections taken from these animals appeared fairly normal.

Summary.—Although these experiments were carried out under light anæsthesia, they differ from those reported by Ettinger, Hall and Banting[7] in that continuous stimulation, rather than daily prolonged stimulation of the vagus nerve, was carried out.

Microscopic examination has shown that myocardial damage has been initiated by such stimulation.

B. The Effect of Continuous Stimulation of the Vagus Nerve in the Unanæsthetized Dog

In order that the animals could be stimulated continuously, without anæsthesia and with perfect freedom to move about the cage, a new type slip-ring-contact described by Manning and Hall[8] was used.

The stimulation experiments were divided into two groups. Animals in the first group received vagus stimulation, and, in addition, one animal received atropine (0.2 mg. per kilo) every hour throughout the period of stimulation. This amount was found to be effective in preventing the physiological response of vagal stimulation. Those animals in the second group received eserine (0.05 mg. per kilo per ½ hour —see discussion) in addition to vagus stimulation. All animals were fed daily, during which time the stimulation was stopped for 45 minutes every 24 hours. Water was available at all times. As the effects of the stimulation became more marked and anorexia was evident, intravenous glucose and saline injections were given.

The animals were placed in the cages two days previous to stimulation, during which time normal electrocardiographs and resting heart-rates were taken. Fairly complete blood analyses were carried out.

Since these experiments were carried out in the unanæsthetized animal, the homolateral recurrent laryngeal nerve was cut. Changes in heart-rate and respiration were taken as the index of effective vagus stimulation. The current was altered by changing the position of the secondary coil in relation to its primary. Eleven dogs were used in these experiments.

Considerable respiratory disturbance and vomiting occurred in the early part of the stimulation. However, by altering the primary in relation to the secondary coil of the inductorium a stimulating current of sufficient strength to produce cardiac slowing without excess respiratory disturbance and vomiting was obtained.

Group I

Vagus stimulation.—During the first hours of stimulation the heart-rate was about normal between stimulating periods (*i.e.*, during the 22 seconds the stimulation was off). During stimulation a decrease of 20 to 30 beats per minute was detected. In some cases the resting heart-rates increased during the progress of the experiments and the degree of inhibition during stimulating periods became greater. The total stimulation time for animals in this group ranged from 70 to 93 hours and in two cases 120 hours.

Atropine produced a cardiac acceleration (160 beats per minute) which was not influenced by vagus stimulation for 160 hours.

Diarrhœa and vomiting were frequently observed, except in the animal which received atropine. The vomitus at first contained much bile-stained mucus and later coffee-ground material. These effects were first observed during the second day and are not to be confused with the preliminary distress which was corrected at the beginning of stimulation. Bouts of dyspnœa and whining occurred which became more pronounced during the latter part of the stimulation experiment. One animal in particular appeared to experience typical attacks of cardiac embarrassment evidenced by marked respiratory

distress, restlessness, whining, etc. Although murmurs were not a constant finding, systolic murmurs were detected many times throughout the experiments.

Exophthalmos of the left eye, which became more pronounced as the experiment progressed, was observed in all the animals. In many cases the nictitating membrane was completely paralyzed.

Electrocardiograph.—Changes in the shape and form of the T-wave were a very constant finding in the electrocardiograph records of these animals. In most cases the normal record showed a negative T II, which later became positive. Some records showed many reversions from negative to positive to diphasic forms. A typical example of these changes is illustrated in the following. In D 81 the normal T II was negative. After 29 hours of stimulation the resting heart-rates became faster and T II reversed. Occasional ventricular extrasystoles occurred at this time. At 58 hours the resting heart-rate had become much faster, with the re-appearance of negative T II.

Autopsy report.—On autopsy the animals appeared to be well nourished. The lungs were fairly normal with no evidence of pneumonia. The gastro-intestinal tract appeared congested with some evidence of intestinal bleeding. The doudenum and upper jejunum were very congested and hæmorrhagic and in some cases showed definite circumscribed pre-ulcerated areas, which could be seen on the serous surface. The spleen and pancreas were congested; the liver appeared fairly normal. In D 81 the heart appeared dark and mottled on both surfaces; in other animals the outer surface of the heart appeared normal. Infarcted areas were observed in some of the papillary muscles. The auriculo-ventricular valves were congested and hæmorrhagic, particularly on the auricular side of the valve. The outer surface of the aorta in the region of the deep and superficial cardiac plexuses appeared congested.

Microscopic examination.—Congestion in the gastric and intestinal mucosa and submucosa was noted. The liver, kidney and pancreas all showed definite evidence of congestion.

Numerous areas of hyaline degeneration were observed in the heart sections taken from these animals. One of the sections showed hæmorrhagic areas about the small blood vessels, while others showed fibrous areas in the myocardium. Scattered areas of hæmorrhage with infarcts were observed in the apex of the heart of D 83. The heart sections taken from D 89, the animal which received atropine in addition to stimulation, appeared normal.

GROUP II

Vagus stimulation in the eserinized dog.—In addition to vagus stimulation these animals were given a continuous injection of dilute eserine through the external jugular vein. A combination of the continuous injection apparatus (Manning and Hall, 1937) with the slip-ring-contact (referred to previously) was used. By means of this apparatus both continuous stimulation and intravenous injection were carried out for 41 to 50 hours, and in one instance for 72 hours, during which time the animal was free to move about its cage. The eserine solution was injected at a rate of 200 c.c. per hour and the concentration regulated so that the animals received 0.05 mg. of eserine every 30 minutes. This amount was found to markedly inhibit the choline-esterase activity.

Throughout the course of these experiments vomiting and diarrhœa occurred. These effects, at first slight, later became more severe. The vomitus became very foul and in one case contained bloody bile-stained material. In addition to the diarrhœa, melæna was observed towards the termination of the experiment. As in the animals in Group I, exophthalmos of the left eye occurred and became more pronounced as the experimental time progressed.

Autopsy report.—The animals appeared to be well nourished. The lungs were fairly normal, with some evidence of slight congestion. The heart appeared dilated, the pericardium œdematous and slightly hæmorrhagic. No gross lesions were observed on the outer surface of the heart except in one case where the apex appeared dark. On opening the heart sub-endocardial hæmorrhage was observed. One animal showed papillary muscle congestion, while another revealed numerous blood clots adherent to the endocardial surface.

In all cases the urinary bladder was small, contracted and empty. The outer surface appeared hæmorrhagic and the mucous membrane œdematous and congested. Congestion was apparent in the kidneys of two animals. There were indications of some fibrotic areas in the

spleen. The liver appeared slightly pale but was otherwise normal.

The upper gastro-intestinal tract appeared congested and hæmorrhagic. In one animal definite ulcerated areas were seen in the pylorus and three similar areas in the duodenum. In another, areas of severe irritation which showed through to the serous surface were observed in the duodenum and stomach. In one animal marked extravasation of blood into the tissue spaces and between the muscle layers (noticed particularly between abdominal muscles) was noted.

Microscopic examination. — Sections taken from these animals showed marked congestion in the liver. Necrosis of the central vein area was observed in some. The kidney and spleen also appeared congested. Marked congestion with hæmorrhage was observed in the pylorus and duodenum, and in some of these sections areas of ulceration were noted.

The heart sections revealed a considerable degree of capillary congestion and hæmorrhage. Hyaline degeneration of the myocardium was noted in all instances. Myocardial degeneration was more marked in this group (vagus stimulation and eserine) than those which had received vagus stimulation alone.

CONCLUSIONS

By means of continuous vagal stimulation (31 to 45 hours) it has been possible to produce a mild degree of myocardial damage in dogs under light nembutal anæsthesia. When the choline-esterase of the blood was inhibited by the frequent injection of eserine more marked myocardial damage was found, and in addition definite peptic ulceration occurred. Since no myocardial or gastro-intestinal damage was noted, atropine apparently, by its effect on the parasympathetic nervous system, protected the animal from the effects of vagus stimulation for long periods of time (70 hours).

When vagal stimulation was carried out on unanæsthetized and unrestrained dogs greater total stimulation was possible (70 to 90 hours, and in 2 instances 120 hours). With this increase in the length of stimulation time more marked myocardial damage was produced. Again, the animal which received atropine in addition to vagus stimulation not only survived the treatment twice as long (160 hours) as the others but showed no evidence of myocardial damage. The group which received eserine in addition to continuous stimulation showed definite evidence of peptic ulceration in addition to more marked evidence of myocardial damage.

When these results are considered together with those of Hall, Ettinger and Banting (1936) the original hypothesis is more definitely established, namely, that parasympathetic over-excitation through liberated acetylcholine results in myocardial damage. Furthermore, the occurrence of gastro-intestinal disturbances appears to be related to an exaggerated vagal tone. Such changes are the result of a more acute disturbance of autonomic equilibrium.

SUMMARY

1. Myocardial damage is initiated by prolonged vagal stimulation under light nembutal anæsthesia.

2. The occurrence of myocardial damage due to prolonged stimulation of the vagus nerve is prevented by atropine and accentuated by eserine.

3. Stimulation of the vagus nerve in the unanæsthetized dog results in a greater degree of myocardial degeneration than in the lightly anæsthetized animal.

4. Areas of duodenum and pylorus show marked congestion and hæmorrhage following prolonged vagal stimulation. These effects are abolished by atropine and accentuated to the extent of ulcer formation by eserine.

REFERENCES

1. HALL, G. E., ETTINGER, G. H. AND BANTING, F. G.: An experimental production of coronary thrombosis and myocardial failure, *Canad. M. Ass. J.*, 1936, 34: 9.
2. ETTINGER, G. H. AND HALL, G. E.: The effect of repeated injections of choline and *B. pyocaneus in* the dog, *Canad. M. Ass. J.*, 1936, 35: 183.
3. ETTINGER, G. H., HALL, G. E. AND LANG, J.: The effect of repeated injections of histamine in the dog. A. On the heart and blood-vessels, *Canad. M. Ass. J.*, 1936, 35: 184.
4. LOEWI, O. AND NAVRATIL, E.: Ueber humorale Uebertragbarkeit der Herznervenwirkung. VI. Mitteilung: Der Angriffspunkt des Atropins, *Pflüger's Archiv*, 1924, 206: 123.
5. LOEWI, O.: Ueber humorale Uebertragbarkeit der Herznervenwirkung. I. Mitteilung, *Pflüger's Archiv*, 1921, 189: 239.
6. LOEWI, O.: II. Mitteilung, *ibid.*, 1921, 193: 201.
7. ETTINGER, G. H., HALL, G. E. AND BANTING, F. G.: Effect of repeated and prolonged stimulation of the vagus nerve in the dog, *Canad. M. Ass. J.*, 1936, 35: 27.
8. MANNING, G. W. AND HALL, G. E.: An apparatus for prolonged stimulation experiments in unanæsthetized animals, *J. Lab. & Clin. Med.*, 1937 (in press).
9. MANNING, G. W. AND HALL, G. E.: A continuous intravenous injection apparatus for unrestrained animals, *J. Lab. & Clin. Med.*, 1937 (in press).